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FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2019/2020

Ana Luísa Cabral Rodrigues Magno Leitão

Regulação Autonómica em Pacientes com Doença Arterial Aterosclerótica Intracraniana

Autonomic Regulation in Patients with Intracranial Arterial Atherosclerotic Disease

MARÇO, 2020

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Mestrado Integrado em Medicina

Área: Neurologia

Tipologia: Dissertação

Trabalho efetuado sob a Orientação de:

Professor Doutor Pedro Castro

E sob a Coorientação de:

Professora Doutora Marta Carvalho

Trabalho organizado de acordo com as normas da revista:

Brain Sciences

MARÇO, 2020

U.PORTO

FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Trabalho de acordo com as normas da Revista

Brain Sciences.

(ISSN 2076-3425; CODEN: BSRCCS)

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UC Dissertação/Projeto (6º Ano) – DECLARAÇÃO DE REPRODUÇÃO

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Neurologia

TÍTULO DISSERTAÇÃO

Autonomic Regulation in Patients with Intracranial Arterial Atherosclerotic Disease

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Dedicatória

Aos meus pais que são incansáveis e o meu suporte desde sempre.

Ao meu irmão João Nuno pelo companheirismo constante e cumplicidade incondicional.

Aos amigos que me acompanharam ao longo destes extraordinários 6 anos.

brain sciences

MDPI

Original Article

Autonomic Dysfunction in Patients with Intracranial Arterial Atherosclerotic Disease and its Effects in Cognitive Performance

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Abstract. (1) Background: Intracranial arterial atherosclerotic disease (ICAD) has been pointed by some studies as a contributor to vascular cognitive impairment. However, the pathophysiological mechanisms that play a part in the development of cognitive decline are not clearly understood. It was hypothesized that the autonomic nervous system could have a role in this matter, since it is frequently dysfunctional in neurological diseases. (2) Methods: Autonomic Nervous System was evaluated through parameters related to baroreflex, heart rate and blood pressure variability in ICAD patients, Healthy and Hypertensive Controls. The results were then compared between the 3 groups. The autonomic parameters were also correlated with performance in cognitive tests in ICAD patients. (3) Results: the ICAD group showed lower sympathetic activity in some autonomic tests when compared to both control groups and lower sympathetic activity correlated to a generally worse cognitive performance. (4) Conclusions: The findings of this study indicate that ICAD tends to be associated with autonomic dysfunction, especially at sympathetic level, and that these alterations might possibly be responsible for the vascular cognitive impairment reported in this group of patients.

Keywords: Intracranial Arterial Atherosclerotic Disease, Autonomic Dysfunction, Cognitive Impairment.

1. Introduction

Intracranial arterial atherosclerotic disease (ICAD) represents the luminal narrowing of the large brain arteries, attributable to primary atherosclerosis. ICAD is mostly seen as a cause of ischemic stroke [1]. However, there is growing evidence that ICAD could be also an important contributor to long-term vascular cognitive impairment [2, 3]. In this regard, the pathophysiological mechanisms linking intracranial stenosis and cognition are poorly understood.

There are several caveats in literature. Some studies show that the stroke risk in ICAD could not be fully explained by conventional risk factors, such as arterial hypertension, Diabetes Mellitus and dyslipidemia [4]. More knowledge about the factors that aggravate cerebral cortex perfusion in ICAD territory are of crucial importance to identify the best strategies to manage and treat these patients.

One possible explanation for the vascular cognitive impairment, could be that low blood pressure levels needed to maintain adequate cerebral perfusion could endanger brain at the ICAD territory and cause chronic ischemia. The autonomic nervous system, particularly its sympathetic component, is one of the main regulators of the blood pressure levels. Interestingly, a large number of studies claim that autonomic dysfunction associates with various forms of higher level malfunctions that include cognitive impairment and may also negatively impact patients' clinical outcome. [5] Besides, it is known that dysfunction of the autonomic nervous system is present in many neurological diseases including neurogenic hypertension, stroke, Alzheimer's disease, Parkinson's disease, and depression, and is also observed in metabolic diseases such as diabetes, obesity, and metabolic syndrome. [6] It is plausible that ICAD patients, sharing many of these pathologies, could have some degree of autonomic dysfunction. This could have major impact in ICAD because the autonomic dysfunction particularly, on the sympathetic nervous system can cause orthostatic insufficiency and the lower BP levels decrease the perfusion in downstream vasculature dependent of the large stenotic artery. This was not studied so far.

The classic method to evaluate overt autonomic dysfunction is the Ewing battery which comprises maneuvers such as deep breathing, Valsalva, orthostatic test, and isometric handgrip. However, non-invasive and less cumbersome protocols based on spontaneous fluctuations of heart rate and blood pressure over a couple of minutes at rest can be used to test subclinical autonomic dysfunction. This is usually achieved by the time and frequency domain analysis of the heart rate and systolic blood pressure variabilities (HRV and BPV, respectively).

We sought to evaluate the presence of autonomic dysfunction by the assessment of HRV and BPV in a cohort of patients with ICAD and its correlation with the cognitive performance of these patients.

2. Materials and Methods

This study was conducted in Centro Hospitalar Universitário São João. It was approved by the appropriate local institutional ethical committee and performed in accordance with the Declaration of Helsinki ethical standards. All participants gave written and signed informed consent.

2.1. Population studied

We screened all patients with ICAD with follow-up at our institution between 2013 and 2016. We included patients with unilateral 50-99% stenosis of the middle cerebral artery (MCA) confirmed with cerebral magnetic resonance angiography. MCA irrigates two thirds of the cerebral hemisphere (therefore, most of the cognitive areas) and is not involved in brainstem autonomic control. To minimize the confounding by the ICAD burden on cognition and the affection of autonomic areas of brainstem, we excluded cases with concomitant significant stenosis ≥ 50% in the contralateral MCA, proximal posterior cerebral artery (which is responsible for irrigation of thalamus), vertebral artery, basilar artery and extracranial cerebral vessels. We also excluded patients with brain ischemic disease of large vessel (cortico-subcortical) in the territory of the stenotic MCA or with diffuse and confluent small vessel disease (Fazekas grade 2 or 3), as well as patients with dementia.

We recruited two control groups with similar age and sex to the ICAD group patients: (A) healthy controls selected by from within university and hospital facilities without vascular risk factors (dyslipidemia, hypertension, diabetes mellitus or active tobacco use) or diseases affecting cardiovascular or nervous systems; and (B) hypertensive patients follow-up at hypertension clinic, Hospital Pedro Hispano without diseases affecting cardiovascular or nervous systems except for vascular risk factors. All participants were characterized by age, gender, cardiovascular risk factors, and chronic medication.

2.2. Cognitive assessment

To test cognitive performance in the ICAD group, patients went through several global, dominant and non-dominant hemisphere cognitive evaluations. The global tests applied were the Montreal Cognitive Assessment (MoCA) test, which is sensitive to vascular cognitive impairment and validated in Portuguese population [7], the Mini-Mental State Examination (MMSE), the Dementia Rating Scale 2 (DRS-2), the Frontal Assessment Battery at bedside (FAB), the Learning Evolution test, the Word List Evocation test and the Retention test. To assess cognition in dominant hemisphere we used the Stroop test for Words and Colours (Stroop test W and Stroop test C), the T interference test, a Work Memory test, a Processing Velocity test, Trail A and B test, verbal and animal fluency tests. In non-dominant hemisphere, the tests used were the Wechsler Adult Intelligence Cubes Test (WAIS Cubes), Matrix and Incomplete figures test.

2.3. Monitoring protocol for autonomic assessment

Systolic and diastolic blood pressures were averaged from three measurements in the sitting position with an oscillometric cuff (Omron M6, Japan). Body mass index was calculated. Participants underwent cervical and transcranial ultrasound examination (Vivid e, GE) to exclude hemodynamically significant extracerebral stenosis.

Evaluations were carried out in a dim lighted room, temperature around 20ºC, supine position with bed head at 0º. Subjects were asked to stop alcohol and coffee intake, exercise or vasoactive drugs for at least 12 hours before the monitorization. Continuous blood pressure (BP) was recorded with plethysmography Finometer MIDI (FMS, Amsterdam, Netherlands) at non-dominant side. Heart rate (HR) was assessed from lead II of a standard 3-lead electrocardiogram. All data were synchronized and digitized at 400 Hz with Powerlab (AD Instruments, Oxford, UK) and stored for offline analysis with dedicated software based on MATLAB (Natick, USA). After stabilization, a 5-minute recording was used to further analysis.

By using spontaneous oscillations of beat-to-beat RR intervals and systolic BP along the 5-minute period we could calculate indexes of HRV and BPV. With RR and BP variations over time it is also possible to estimate indexes of baroreflex sensitivity. These reflect in part the influence of the two limbs of the autonomic nervous system and its analysis can be used to assess autonomic function. [8]

HRV was characterized in time domain by the standard deviation of the successive normal RR intervals (SDNN) and in frequency domain by the power spectrum of normal RR intervals, with low frequency (RR_{LF}: 0.04-0.15 Hz), high frequency (RR_{HF}: 0.15-0.4 Hz) and LF/HF ratio (RR_{LFHF} ratio). The LF band of HRV has partial contributions of adrenergic and baroreceptor mechanisms, but the HF spectrum of HRV is highly influenced by parasympathetic vagal function. [9]

BPV was characterized by the power spectrum of beat-to-beat systolic blood pressure values of successive normal RR intervals in low (BP_{LF}; 0.04–0.15 Hz) and high (BP_{HF}; 0.15-0.40 Hz) frequency bands. Concerning BPV, the LF power is again particularly associated with the arterial baroreceptor reflex function, being partly determined by α-adrenergic sympathetic component of vasomotor function. [10] The HF component of BPV is usually ignored because it is mostly determined by the mechanical effects of respiratory movements on cardiac output. [10]

The sensitivity of the baroreflex was assessed in the time domain by the cross-correlation method (xBRS) [11], which is based on the computation of the beat-to-beat correlation coefficients of systolic BP with normal RR intervals in a 10-second window. In the frequency domain, baroreflex is commonly called the α-index [12] and is obtained by calculating the cross-correlation gain between the spectral densities of the systolic BP and the HR in the LF band (0.04–0.15 Hz). [13]

2.4. Statistics

Normality of variables was inferred by Kolmogorov-Smirnoff test. Comparison of baseline characteristics was achieved by Chi-Square, ANOVA or Kruskal-Wallis tests as appropriate. Bonferroni post-hoc tests was used to correct for multiple comparisons. Considering only the ICAD patients, autonomic indexes were compared between subgroups of laterality or severity of stenosis with T-test or Mann-Whitney test as appropriate. The effect of each index of HRV, BPV or baroreflex on cognitive performance was studied by linear regression models adjusted to age and stenosis side. Statistical significance was inferred at $p < 0.05$ level. All statistics were performed using IBM Statistical Package for Social Sciences (SPSS) Statistics.

3. Results

We evaluated 22 patients with ICAD, mostly male and with mean ± standard deviation age of 67±11; left MCA affected in 13 patients (59%); 9 (41%) had MCA stenosis ≥ 70%. One patient had 50-70% stenosis of anterior cerebral artery ipsilateral to MCA stenosis and was included in the study. We also recruited 22 healthy subjects for control group A and a cohort of 22 hypertensive patients without atherosclerotic disease for control group B. Sex and age were similar among groups (**Table 1**). Both ICAD and hypertensive controls had higher systolic BP values ($p<0.001$) and higher BMI ($p=0.002$) compared to healthy controls. In contrast with hypertensive controls, ICAD population had fewer diabetic patients and less frequently medicated with beta-blocker, although these differences were not statistically significant.

Table 1. Demographic and clinical characteristics of ICAD and control groups.

	ICAD (N = 22)	Control A - Healthy subjects (N = 22)	Control B - Hypertensive cohort (N = 22)
Male, n (%)	17 (77)	17 (77)	15 (68)
Age, years	67 ± 11	67 ± 9	68 ± 8
BMI, Kg.m^2	* 28 ± 4	25 ± 3	* 29 ± 4
Systolic BP	* 142 ± 22	130 ± 10	* 141 ± 13
Diastolic BP	77 ± 13	77 ± 17	81 ± 9
Previous stroke/ TIA, n (%)	10 (45)	0	0
Hypertension, n (%)	20 (91)	0	22 (100)
No of antihypertensive drugs	2 ± 1	0	3 ± 1
β-blocker, n (%)	1 (4)	0	5 (23)
ACEI / ARB, n (%)	† 11 (50)	0	21 (95)
CCB, n (%)	12 (55)	0	14 (64)
Diuretic, n (%)	13 (59)	0	16 (73)
Diabetes Mellitus, n (%)	8 (36)	0	14 (64)
Dyslipidemia, n (%)	19 (86)	0	16 (73)
Tobacco, n (%)	5 (23)	0	3 (14)

All variables expressed as mean±SD unless otherwise specified. BMI: body mass index; TIA: transitory ischemic accident; BP: blood pressure; ACEI: Angiotensin converting enzyme inhibitors; ARB: angiotensin receptor blockers; CCB: calcium channel blockers. * $p < 0.05$ for differences to healthy control group. † $p < 0.05$ for differences to hypertensive control group.

In **Table 2**, we show the differences in autonomic parameters between ICAD patients and controls. ICAD patients had lower RR_{LF} variability (nu p=0.001; % p=0.012), higher RR_{HF} variability (nu p=0.001; % p=0.012) and also lower RR_{LFHF} ratio (p=0.001). In what concerns BPV, Systolic BP_{LF} power was significantly lower than in healthy and hypertensive controls (4.0 ± 2.4 vs 7.2 ± 5.4 vs 9.3 ± 9.5 $mmHg^2$, p=0.032). Baroreflex sensitivity was similar in the three groups.

Table 2. Comparison of autonomic tests between the 3 study groups.

	ICAD (N = 22)	Control A - Healthy subjects (N = 22)	Control B - Hypertensive cohort (N = 22)	P
Baroreflex				
xBRS	7.9 ± 7.6	5.6 ± 2.3	6.8 ± 7.4	0.608
α-index	5.5 ± 4.6	4.6 ± 1.9	7.2 ± 6.2	0.599
Heart rate variability				
SDNN	37.0 ± 36.3	26.9 ± 10.1	42.7 ± 44	0.365
RR total power, ms^2	1678 ± 3627	667 ± 553	3184 ± 9998	0.449
RR_{LF} power, ms^2	2675 ± 6654	175 ± 160	954 ± 3146	0.208
RR_{HF} power, ms^2	1075 ± 2896	101 ± 99	1646 ± 6176	0.057
RR_{LF} power, nu	* 0.3 ± 0.1	0.6 ± 0.2	0.5 ± 0.2	0.001
RR_{HF} power, nu	* 0.7 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	0.001
RR_{LF} power, %	* 16 ± 7.0	25 ± 11	25 ± 14	0.012
RR_{HF} power, %	* 38 ± 21	20 ± 15	27 ± 19	0.012
RR_{LFHF} ratio	* 0.6 ± 0.4	3.1 ± 4.0	1.5 ± 1.5	0.001
Systolic BP variability				
BP total power, mm Hg^2	27 ± 14	38 ± 28	39 ± 26	0.322
BP_{LF} power, mm Hg^2	* 4.0 ± 2.4	7.2 ± 5.4	9.3 ± 9.5	0.032
BP_{LF} power, nu	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.130
BP_{LF} power, %	17 ± 7.7	22 ± 14	24 ± 15	0.416
BP_{LFHF} ratio	1.4 ± 1.5	3.9 ± 6.1	1.6 ± 2.4	0.119

BP: blood pressure; LF: low frequency; HF: high frequency.
* $p < 0.05$ for differences to healthy control group.

The severity of the stenosis was not related to baroreflex, HRV or BPV parameters (**Table 3**).

Table 3. Comparison of autonomic tests between 50-70% and ≥ 70 % stenosis within ICAD population.

	50 – 70% stenosis subjects (N = 13)	≥ 70 % stenosis subjects (N = 9)	P
Baroreflex			
xBRS	8.9 ± 9.5	9.5 ± 5.5	0.123
α-index	6.4 ± 5.9	5.9 ± 5.4	0.356
Heart rate variability			
SDNN	42.6 ± 44.5	44.5 ± 31.1	0.234
RR total power, ms2	2298 ± 4475	4475.3 ± 1055.7	0.203
RR_{LF} power, ms2	394 ± 833	833.2 ± 120.8	0.974
RR_{HF} power, ms2	1567 ± 3581	3580.9 ± 116.3	0.238
RR_{LF} power, nu	0.4 ± 0.2	0.2 ± 0.5	0.862
RR_{HF} power, nu	0.6 ± 0.2	0.2 ± 0.5	0.538
RR_{LF} power, %	21 ± 17	17 ± 17	0.357
RR_{HF} power, %	34 ± 25	25 ± 21	0.124
RR_{LFHF} ratio	1.1 ± 1.3	1.3 ± 1.2	0.987
Systolic BP variability			
BP_{LF} power, mm Hg^2	4.5 ± 2.7	2.7 ± 3.4	0.832
BP_{LF} power, nu	0.5 ± 0.2	0.2 ± 0.5	0.380
BP_{LF} power, %	19 ± 9.3	9.3 ± 17	0.478
BP_{LFHF} ratio	1.6 ± 1.7	1.7 ± 1.5	0.456

BP: blood pressure; LF: low frequency; HF: high frequency.
* $p < 0.05$ for differences between the two groups.

Within ICAD group, the significant relationships between HRV and BPV parameters and performance in global, non-dominant hemisphere and dominant hemisphere cognitive tests are presented in **Table 4**. A complete crossed analysis between autonomic parameters and cognitive performance is shown in **Appendixes A**, **B** and **C**.

In global cognitive tests, a better performance in MoCA was associated with a greater BPV at LF band (ms^2, β=0.7 (95%CI 0.1-1.3)) and a better performance in MMSE significantly associates with a HRV greater at LF band (nu, β=6.5 (95%CI 2.8 – 10.3)) and lower at HF band (nu, β=-6.54 (95%CI -10.3 – -2.8)), which translates in a greater LF to HF ratio (β=1.4 (95%CI 0.4 – 0.24)). The performance in Word List Evocation test was also positively associated with BPV at LF band ($mmHg^2$, β=0.36 (95%CI 0.1 – 0.6)). In terms of dominant hemisphere tests, we found a positive association between Stroop Test C performance and RR_{LF} power (nu, β=24.8 (95%CI 0.9 – 48.7)) and a negative one with RR_{HF} power (nu, β=-24.8 (95%CI -48.7 – -0.9)). In addition to this, Stroop Test C also positively correlated with BPV at LF band (nu, β=23.9 (95%CI 2.7 – 45)) and a negatively correlated with BPV at HF band (nu, β=-23.9 (95%CI -45 – -2.7)). The Interference test performance was associated with higher HRV at LF power (ms^2, β=0.03 (95%CI 0.01 – 0.1)) and with higher BPV at HF power ($mmHg^2$, β=0.7 (95%CI 0.1 – 1.3) and %, β=0.2 (95%CI 0.01 – 0.4)). In terms of non-dominant hemisphere, a better performance on the WAIS Cubes test was associated with a relatively higher BPV at LF band (LF to HF ratio, β=0.6 (95%CI 0.5 – 1.6)). There were no associations found between BRS and cognitive performance.

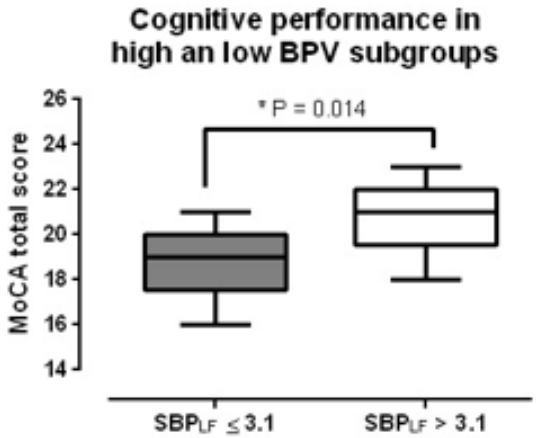
Table 4. Association of autonomic parameters with performance in cognitive tests.

	Global			Dominant hemisphere		Non-dominant hemisphere
	MoCA	MMSE	Word List Evocation	Stroop Test C	Interference T	WAIS Cubes
Heart rate variability						
RR_{LF} power, ms^2	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	* 0.03 (0.01; 0.1)	0 (0; 0)
RR_{LF} power, nu	5.6 (-4.8; 16)	* 6.5 (2.8; 10.3)	-0.8 (-4.6; 2.8)	* 24.8 (0.9; 48.7)	-11.2 (-38.5; 16)	6.6 (-1.1; 14.2)
RR_{HF} power, nu	-5.6 (-16; 4.8)	* -6.5 (-10.3; -2.8)	0.8 (-2.8; 4.5)	* -24.8 (-48.7; -0.9)	11.2 (-16; 38.5)	-6.6 (-14.2; 1.1)
RR_{LFHF} ratio	0.8 (-1.9; 3.6)	* 1.4 (0.4; 2.4)	-0.4 (-1.2; 0.5)	4.7 (-1.3; 10.8)	-1.8 (-9.7; 6)	1.5 (-0.4; 3.4)
Systolic BP variability						
BP_{LF} power, $mm\ Hg^2$	* 0.7 (0.1; 1.3)	0.2 (-0.2; 0.5)	* 0.4 (0.1; 0.6)	0 (-1.1; 0.3)	* 0.7 (0.1; 1.3)	0.5 (-0.1; 1.1)
BP_{LF} power, nu	4.4 (-5.9; 14.8)	1.6 (-2.6; 5.8)	2.1 (-1; 5.3)	* 23.9 (2.7; 45)	-7.3 (-29.9; 15.2)	2.2 (-5.3; 9.7)
BP_{HF} power, nu	-4.4 (-14.8; 5.9)	2.1 (-2.1; 6.3)	-2.1 (-5.3; 1)	* -23.9 (-45; -2.7)	7.3 (-15.2; 29.9)	-2.2 (-9.7; 5.3)
BP_{LF} power, %	0.2 (0; 0.48)	0 (-0.1; 0.1)	0 (-0.1; 0.1)	-0.1 (-0.4; 0.1)	* 0.2 (0.01; 0.4)	0.1 (-0.1; 0.3)
BP_{LFHF} ratio	0.8 (-0.4; 1)	-0.3 (-0.9; 0.3)	0.4 (0; 0.8)	2.1 (-1.1; 5.3)	0.5 (-2.6; 3.7)	* 0.6 (0.5; 1.6)

BP: blood pressure; LF: low frequency; HF: high frequency. Values are β coefficients and 95% confidence intervals obtained by multivariate linear regression analysis adjusted to age and stenosis side. * $p < 0.05$

The systolic BP at LF power (BP_{LF} power, $mmHg^2$) was an autonomic parameter that showed to be significantly lower in the ICAD group, when compared to control groups, and also correlated with a statistically significant higher performance in some tests, such as the MoCA test, Word List Evocation and Interference test. In **Figure 1** it is possible to see more accurately how the BP_{LF} influences the MoCA test performance. Patients with a greater score on MoCA test tend to have a higher activity of BPV at LF power (β = 4.53 (95% CI 1.21 - 7.98), p=0.014).

Figure 1. Box and whisker plots representing the median and interquartile ranges of total score of MoCA test between low and high systolic blood pressure variability (BPV) subgroups, accordingly to BP_{LF} mm Hg^2.



SBP: systolic blood pressure; LF: low frequency. P value to differences between groups, accordingly to linear regression model adjusted to age and stenosis side. * $p < 0.05$

4. Discussion

This study found evidence of sympathetic dysfunction in ICAD patients when compared to healthy subjects and some degree of cognitive impairment in those with a greater autonomic dysfunction. Data obtained by both the HRV and the BPV show that the sympathetic system activity is decreased in the ICAD patients when compared to healthy subjects of patients with similar age. This decreased sympathetic system activity is associated with a generally lower cognitive performance in ICAD patients.

4.1. Autonomic Dysfunction in ICAD patients

The parameters used in this study to assess autonomic dysfunction (HRV, SBP and baroreflex) are becoming increasingly popular indexes for the assessment of autonomic nervous system [10].

Systolic BP variability is a marker of peripheral autonomic nervous system (ANS) activity. In frequency domain, the LF oscillations of BPV are reported to be mediated by sympathetic activity, while the HF component has not been totally understood, but it is thought to be a mechanical consequence of respiration. [14] An example of this, is after severe hemorrhage in rats, LF fluctuations of BPV do not occur if α-adrenergic activity is blocked by prazosin but they are present despite inhibition of angiotensin II or vasopressin activities. [15] In addition to this, the LF peak disappears and the LF band decreases after chronic lesion of the sympathetic nervous system fibers by guanethidine and LF oscillations of BP increase after atropine, which reflects the lack of direct influence of the parasympathetic system on these oscillations. [16] In this study, ICAD patients tended to lower values of BPV at LF band when compared to the control groups, which suggests a decreased sympathetic activity in this group.

HRV is also considered an index for assessment of ANS dysfunction [14], and is a powerful marker of bad prognosis (mortality and arrhythmic complications) when it is depressed. [17] The time domain of HRV (SDNN) is associated with parasympathetic ANS function, while the frequency domain is associated with both parasympathetic (HF band) and sympathetic (LF band) ANS function. [18] This is supported by the fact that, in physiological conditions, the sympathetic excitation that leads to tachycardia is accompanied by a reduction in the total power and in the SD of HRV and during vagal activity the reverse is true. [19] Also, the power of the LF component is percentually increased during maneuvers exciting sympathetic activity while HF is decreased. [9] LF component of heart rate variability is increased by tilt, mental stress, or moderate physical exercise. In all these conditions, which are characterised by a shift of the sympatho-vagal balance towards sympathetic predominance, the increase in the LF component of HRV is accompanied by a decrease in the HF component. [19] In this study, ICAD patients showed lower values of HRV at LF band, which is again consistent with decreased sympathetic activity.

There is a significantly decreased sympathetic activity in ICAD patients when compared to healthy subjects, and this decrease is not present in the hypertensive control group. This finding suggests that ANS dysfunction is related to ICAD. One possible explanation for this is that the ICAD group tend to have a great incidence of metabolic syndrome [1, 20], and metabolic syndrome has been proved to be associated with ANS dysfunction, being a condition that causes often ANS hyperactivity in small peripheral nerve fibers. [21] In fact, it has been proved that autonomic influences are important regulatory mechanisms of the metabolic homeostasis, contributing to the control of blood pressure, glucose and insulin levels. [22] The existence of dysfunction in this system develops and worsens metabolic syndrome, which can also drive to an aggravation in autonomic impairment.

Another explanation could be that the lower sympathetic activity in ICAD patients might be caused by an impairment in cerebral blood flow and damage in the brain ANS regulatory areas [23] due to the narrowing of the arteries in ICAD. In fact, heart failure is accompanied by loss of tissue and other indications of neural injury in specific brain sites that include areas with significant autonomic modulation roles. This has been proved to be associated with reduced perfusion of brain tissue, and poorer perfusion may trigger or enhance brain tissue injury, especially to autonomic areas, contributing to further deterioration [23]. The same can happen in ICAD, since the narrowing of the intracranial arteries reduce the blood flow to brain tissue. Further studies could elucidate this issue. We do not have knowledge of other studies that correlate ICAD with autonomic dysfunction that can confirm or deny this hypothesis.

4.2. Autonomic Dysfunction effect in Cognitive Performance

Some bilateral performance tests (MoCA, MMSE and Word List Evocation) show a better performance with higher levels of sympathetic activity (evidenced by higher values of BP_{LF} and $RR_{LF}nu$) and lower levels of parasympathetic activity ($RR_{HF}nu$). Some dominant hemisphere cognitive tests were associated with sympathetic function, particularly the StroopTest C and the T Interference test. In fact, a greater sympathetic activity seems to be associated with a better performance on these tests. The increased LF to HF (ratio) in BPV was also linked to better performance in non-dominant hemisphere cognitive tests.

Few studies relate autonomic dysfunction with cognitive impairment. Interestingly, orthostatic hypotension (a sign of autonomic dysfunction) is more prevalent in patients with mild CI. [8] It has been hypothesized that since orthostatic hypotension increases vulnerability to cerebral hypoperfusion when in orthostatic position, it leads to brain damage that contributes to progressive cognitive decline. [8] CI in turn can contribute to dysautonomia through disruption in central autonomic control and create a vicious cycle, and there is a preferential dysfunction of the sympathetic system in these patients. [8] The decrease in HRV or BPV at LF band could be caused by mild sympathetic dysfunction which agrees with previous results.

On another perspective, autonomic dysfunction and poor cognitive performance might be a co-occurring phenomenon of a central nervous system malfunction and not have a causal relation. It is studied that ANS main structures are located in the brainstem (mediates vasomotor activity and specific reflexes), diencephalon (hypothalamus) and limbic system (supports a number of higher level functions, including memory, emotion processing, behavior and motivation). These brain structures are known to be also implicated in cognitive functions. [5] If there is a lesion or malfunction in any of these structures, it will affect both the CI and the autonomic function. This study also supports this evidence, since bilateral cognitive performance seems to be slightly affected when there is sympathetic autonomic dysfunction. [5]

4.3. Limitations

Small number of subjects and cross section nature of the study prevents us from drawing conclusions about a causal relation between BPV and HRV and cognitive impairment. Besides, some studies claim that provocative tests are necessary for the autonomic dysfunction to be detected, since it doesn't exist in baseline conditions. [8] This might be particularly true for the study of sympathetic nervous system function. Nevertheless, this study suggests that it might be worthwhile studying autonomic dysfunction with other methods in ICAD patients.

5. Conclusions

The presence of mild, predominantly sympathetic, autonomic dysfunction in patients with ICAD can impair their cardiovascular pressor responses to everyday activity and contribute to induce damage in a chronically ill-perfused brain. In this sense, there might be benefits with the use of drugs with autonomic effects in these cases. This novel finding opens roads for further understanding of the link between intracranial atherosclerotic disease and cognitive dysfunction and new perspectives on how to optimize medication on this particular group of patients.

Author Contributions: Conceptualization, Pedro Castro and Ana Luisa Magno; methodology, Pedro Castro and Ana Luisa Magno; software, Pedro Castro; validation, Marta Carvalho, Ana Monteiro, Jorge Polónia and Elsa Azevedo; formal analysis, Pedro Castro and Ana Luisa Magno; investigation, Pedro Castro and Ana Luisa Magno; resources, Marta Carvalho and Jorge Polónia; data curation, Marta Carvalho; writing—original draft preparation, Ana Luisa Magno; writing—review and editing, Pedro Castro; visualization, Ana Monteiro and Jorge Polónia; supervision, Elsa Azevedo; project administration, Pedro Castro. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A - Bilateral cognitive performance

	MoCA	DRS2	Learning evolution	MMSE	FAB	Word List Evocation	Word List Total	Evocation Total	Retention
Baroreflex									
xBRS	-0.38 (-0.91; 0.15)	-0.54 (-1.17; 0.1)	0.02 (-0.11; 0.15)	-0.12 (-1.78; 1.54)	-0.17 (-0.37; 0.03)	0 (0;0)	0 (-0.33; 0.3)	0 (-0.14; 0.16)	-0.43 (-2.42; 1.56)
α-index	0.27 (-0.53; 1.09)	-0.42 (-1.53; 0.69)	-0.03 (-0.24; 0.19)	-0.17 (-0.38; 0.03)	-0.22 (-0.56; 0.12)	0 (-0.17; 0.17)	-0.08 (-0.60; 0.44)	0.04 (-0.21; 0.29)	-0.14 (-3.46; 3.18)
Heart Rate Variability									
SDNN	-0.02 (-0.18; 0.13)	-0.07 (-0.21; 0.07)	0 (-0.03; 0.03)	-0.03 (-0.05; 0)	-0.03 (-0.07; 0.02)	0 (-0.02; 0.02)	0 (-0.07; 0.07)	0 (-0.03; 0.03)	-0.11 (-0.55; 0.32)
RR total power, ms^2	0 (0;0)	0 (0;0)	0 (0;0)	0 (0;0)	0 (0; 0)	0 (0;0)	0 (0;0)	0 (0;0)	0 (-0.01; 0)
RR_{LF} power, ms^2	0.01 (-0.01; 0.05)	0 (-0.01; 0)	0 (0;0)	0 (0;0)	0 (0;0)	0 (0;0)	0 (0;0)	0 (0;0)	-0.01 (-0.03; 0.02)
RR_{HF} power, ms^2	0 (0;0)	0 (0;0)	0 (0;0)	0 (0;0)	0 (0;0)	0 (0; 0)	0 (0;0)	0(0;0)	0 (0;0)
RR_{LF} power, nu	5.59 (-4.81; 15.99)	3.11 (-21.26; 27,48)	-1.75 (-6.46; 2.96)	6.54 (2.76; 10,31)	1.36 (-6.19; 8.92)	-0.81 (-4.46; 2.84)	-4.38 (-15.6; 6.84)	-0.27 (-5.72; 5.19)	27.65 (-43.58; 98.89)
RR_{HF} power, nu	-5.59 (-15.99; 4.81)	-3.11 (-27.48; 21.26)	1.75 (-2.96; 6.46)	-6.54 (-10.31; -2.76)	-1.36 (-8.92; 6.19)	0.81 (-2.84; 4.46)	4.38 (-6,.4; 15.6)	0.27 (-5,19; 5,72)	-27,65 (-98.89; 43,58)
RR_{LFHF} ratio	0.82 (-1.94; 3.58)	0.25 (-5.74; 6.24)	-0.48 (-1.63; 0.67)	1.39 (0.38; 2.39)	0.02 (-1.86; 1.9)	-0.37 (-1.25; 0,51)	-1.75 (-4.44; 0.93)	-0.07 (-1.41; 1,27)	7.71 (-9.69; 25.11)
Systolic BP variability									
BP total power, mm Hg^2	0.01 (-0.13; 0.16)	0.09 (-0.25; 0.42)	-0.03 (-0.09; 0.04)	-0.03 (-0.10; 0.03)	0.05 (-0.06; 0.15)	0.05 (0.004; 0.10)	0.13 (-0.01; 0.28)	-0.04 (-0,12; 0.03)	-0.82 (-1.76; 0.12)
BP_{LF} power, mm Hg^2	0.72 (0.09; 1.35)	0.28 (-1.63; 2.19)	-0.33 (-0.67; 0.02)	0.17 (-0.19; 0.54)	0.32 (-0.26; 0.89)	0.36 (0.12; 0.6)	0.6 (-0.25; 1.46)	-0.07 (-0.50; 0.36)	-0.78 (-6.44; 4.88)
BP_{HF} power, mm Hg^2	0.09 (-0.43; 0.61)	0.31 (-0.33; 0.95)	0.09 (-0.03; 0.21)	-0.05 (-0.18; 0.08)	-0.01 (-0.22; 0.19)	-0.03 (-0.13; 0,07)	0.09 (-0.21; 0.39)	0.06 (-0.08; 0.20)	-0.09 (-2.02; 1.85)
BP_{LF} power, nu	4.44 (-5.95; 14.84)	-7.5 (-29.12; 14.11)	-2.11 (-6.29; 2.06)	1.57 (-2.65; 5.79)	2.00 (-4.82; 8.82)	2.12 (-1.03; 5,27)	2.27 (-7,89; 12,42)	-0.26 (-5.15; 4.63)	4.83 (-59.91; 69.57)
BP_{HF} power, nu	-4.44 (-14.84; 5.95)	7.5 (-14.11; 29.12)	2.11 (-2.06; 6.29)	-1.57 (-5.79; 2.65)	-2.00 (-8.82; 4.82)	-2.12 (-5.27; 1.03)	-2.27 (-12.42; 7.89)	0.26 (-4.63; 5.15)	-4.83 (-69.57; 59.91)
BP_{LF} power, %	0.2 (-0.07; 0.48)	-0.29 (-0.82; 0.24)	-0.03 (-0.14; 0.07)	0.05 (-0.05; 0.15)	-0.04 (-0.21; 0.12)	0.01 (-0.07; 0.09)	-0.08 (-0.33; 0.17)	0.04 (-0.08; 0.16)	0.87 (-0.69; 2.44)
BP_{HF} power, %	0.01 (-0.16; 0.18)	0.08 (-0.15; 0.31)	0.04 (-0.01; 0.08)	-0.01 (-0.06; 0.03)	-0.01 (-0.08; 0.06)	-0.03 (-0.06; 0.01)	-0.01 (-0.12; 0.1)	0.03 (-0.02; 0.08)	0.15 (-0.53; 0.83)
BP_{LFHF} ratio	0.77 (-0.4; 1.04)	0.76 (-2.41; 3.93)	-0.26 (-0.87; 0.35)	0.3 (-0.32; 0.91)	0.69 (-0.27; 1.65)	0.41 (-0.03; 0.85)	0.88 (-0.55; 2.31)	0.25 (-0,46; 0.95)	3.59 (-5.71; 12.88)

BP: blood pressure; LF: low frequency; HF: high frequency. Values are β coefficients and 95% confidence intervals obtained by multivariate linear regression analysis adjusted to age and stenosis side. * $p < 0.05$

Appendix B - Dominant hemisphere cognitive performance

	Stroop Test W	Stroop Test C	Stroop Test PC	Inter-ference T	Work memory	Processing velocity	Trail A	Trail B	Fluency MRPE	Fluency animal PE
Baroreflex										
xBRS	-0.45 (-1.43; 0.53)	-0.14 (-1.43; 1.16)	-0.08 (-1.3; 1.14)	1.03 (-0.06; 2.11)	-0.53 (-1.41; 0.35)	-0.69 (-2.02; 0.63)	0.03 (-0.10; 0.16)	0.05 (-0.11; 0.20)	-0.02 (-0.18; 0.13)	-0.01 (-0.2; 0.18)
α-index	-0.9 (-2.31; 0.51)	-0.29 (-2.13; 1.56)	0.74 (-1.02; 2.5)	1.48 (-0.13; 3.09)	-0.48 (-1.98; 1.03)	-0.4 (-2.33; 1.53)	0 (-0.19; 0.19)	0.09 (-0.14; 0.31)	-0.1 (-0.35; 0.16)	-0.01 (-0.33; 0.32)
Heart Rate Variability										
SDNN	-0.12 (-0.31; 0.08)	-0.03 (-0.29; 0.23)	0.01 (-0.24; 0.26)	0.23 (0.01; 0.45)	-0.09 (-0.29; 0.1)	-0.11 (-0.38; 0.15)	0 (-0.03; 0.03)	-0.01 (-0.05; 0.04)	0 (-0.04; 0.03)	0 (-0.04; 0.05)
RR total power, ms^2	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)
RR_{LF} power, ms^2	-0,14 (-0,04; 0,01)	0 (-0,03; 0,03)	0 (-0,02; 0,04)	0.03 (0.01; 0.06)	0 (-0.01; 0.01)	-0.01 (-0.05; 0.02)	0 (0; 0)	0 (0; 0,01)	0 (0; 0)	0 (0; 0)
RR_{HF} power, ms^2	0 (0; 0)	0 (-0.03; 0.03)	0 (0; 0)	0 (0; 0.01)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)
RR_{LF} power, nu	12.08 (-10.23; 34.39)	24.8 (0.87; 48.74)	6.04 (-21.88; 33.96)	-11.24 (-38.5; 16.02)	23.48 (-7.84; 54.80)	15.03 (-11.73; 41.79)	0.2 (-2.57; 2.96)	1.33 (-1.41; 4.07)	-5.76 (-10.88; -0.64)	-4.45 (-11.16; 2.27)
RR_{HF} power, nu	-12.08 (-34.39; 10.23)	-24.8 (-48.74; -0.87)	-6.04 (-33.96; 21.88)	11.24 (-16.02; 38.5)	-23.48 (-54.8; 7.84)	-15.03 (-41.79; 11.73)	-0.2 (-2.96; 2.57)	-1.33 (-4.07; 1.41)	5.76 (0.64; 10.88)	4.45 (-2.27; 11.16)
RR_{LFHF} ratio	1.36 (-5.18; 7.9)	4.72 (-1.33; 10.76)	-0.02 (-7.98; 7.95)	-1.81 (-9.66; 6.04)	3.10 (-4.88; 11.08)	1.09 (-5.66; 7.83)	0.14 (-0.53; 0.81)	0.47 (-0.11; 1.06)	-1.73 (-2.89; -0.57)	-1.55 (-3.12; 0.02)
Systolic BP variability										
BP total power, mm Hg^2	-0.14 (-0.45; 0.16)	-0.11 (-0.51; 0.29)	0.20 (-0.17; 0.57)	0.10 (-0.27; 0.48)	-0.32 (-0.76; 0.11)	-0.19 (-0.57; 0.19)	0.02 (-0.01; 0.06)	0 (-0.03; 0,05)	0.05 (-0.03; 0.12)	0.05 (-0.05; 0.14)
BP_{LF} power, mm Hg^2	1.71 (-0.12; 3.54)	2.62 (0.43; 4.81)	1.73 (-0.56; 4.02)	0.70 (-1.72; 3.13)	0.60 (-1.97; 3.18)	0.35 (-2.02; 2.71)	0.12 (-0.09; 0.34)	0.02 (-0.23; 0.28)	0.04 (-0.41; 0.48)	-0.04 (-0.62; 0.54)
BP_{HF} power, mm Hg^2	-0.36 (-0.90; 0.17)	-0.38 (-1.08; 0.31)	0.18 (-0.50; 0.86)	0.72 (0.15; 1.28)	-0.33 (-1.2; 0.55)	-0.39 (-1.11; 0.33)	0 (-0.07; 0.07)	-0.03 (-0.14; 0.08)	0.09 (-0.05; 0.24)	0.07 (-0.11; 0.26)
BP_{LF} power, nu	14.57 (-2.84; 31.99)	23.86 (2.69; 45.04)	5.11 (-17.79; 28.01)	-7.34 (-29.9; 15.23)	6.35 (-23.08; 35.79)	12.49 (-12.4; 37.38)	0 (-2.55; 2.55)	0.71 (-1.73; 3.15)	-0.49 (-5.55; 4.57)	-2.66 (-8.84; 3.52)
BP_{HF} power, nu	-14.57 (-31,99; 2.84)	-23.86 (-45.04; -2,69)	-5.12 (-28.01; 17.79)	7.34 (-15.23; 29.9)	-6.35 (-35.79; 23.08)	-12.49 (-37.38; 12.4)	0 (-2.55; 2.55)	-0.71 (-3.15; 1.73)	0.49 (-4.57; 5.55)	2.66 (-3.52; 8,84)
BP_{LF} power, %	0.75 (0.3; 1.2)	0.96 (0.45; 1.46)	-0.02 (-0.75; 0.7)	-0.02 (-0.74; 0.72)	0.47 (-0.24; 1.17)	0.36 (-0.34; 1.06)	-0.02 (-0.09; 0.06)	-0.01 (-0.08; 0.06)	-0.04 (-0.17; 0.08)	-0.1 (-0.25; 0.05)
BP_{HF} power, %	-0.1 (-0.29; 0.09)	-0.14 (-0.38; 0.1)	0.04 (-0.19; 0.28)	0.21 (0.01; 0.42)	-0.04 (-0.35; 0.27)	-0.08 (-0.34; 0.18)	0 (-0.03; 0.02)	-0.01 (-0.04; 0.02)	0.03 (-0.03; 0.08)	0.02 (-0.04; 0.09)
BP_{LFHF} ratio	1,64 (-0.83; 4.11)	2.10 (-1.08; 5.28)	2.22 (-0.71; 5.16)	0.54 (-2.60; 3.67)	1.09 (-3.19; 5.37)	2.08 (-1.39; 5.54)	-0.13 (-0.48; 0.23)	0.07 (-0.25; 0.4)	0.21 (-0.52; 0.94)	0.06 (-0.85; 0.97)

BP: blood pressure; LF: low frequency; HF: high frequency. Values are β coefficients and 95% confidence intervals obtained by multivariate linear regression analysis adjusted to age and stenosis side. * $p < 0.05$

Appendix C - Non dominant hemisphere cognitive performance

	WAIS Cubes	Matrix	Incomplete figures
Baroreflex			
xBRS	-0.37 (-0.74; 0)	-0.24 (-0.55; 0.07)	-0.31 (-0.67; 0.05)
α-index	-0.12 (-0.69; 0.46)	-0.26 (-0.71; 0.2)	-0.17 (-0.72; 0.37)
Heart Rate Variability			
SDNN	-0.03 (-0.11; 0.04)	-0.03 (-0.1; 0.03)	-0.04 (-0.11; 0.04)
RR total power, ms^2	0 (0; 0)	0 (0; 0)	0 (0; 0)
RR_{LF} power, ms^2	0 (0; 0)	0 (0; 0)	0 (0; 0)
RR_{HF} power, ms^2	0 (0; 0)	0 (0; 0)	0 (0; 0)
RR_{LF} power, nu	6.56 (-1.12; 14.23)	1.72 (-4.9; 8.35)	1.05 (-6.78; 8.88)
RR_{HF} power, nu	-6.56 (-14.23; 1.12)	-1.72 (-8.35; 4.9)	-1.05 (-8.88; 6.78)
RR_{LFHF} ratio	1.53 (-0.36; 3.45)	-0.04 (-1.67; 1.59)	-0.25 (-2.17; 1.67)
Systolic BP variability			
BP total power, mm Hg^2	-0.02 (-0.13; 0.1)	-0.01 (-0.1; 0.08)	-0.07 (-0.19; 0.04)
BP_{LF} power, mm Hg^2	0.49 (-0.13; 1.11)	0.10 (-0.43; 0.64)	-0.12 (-0.75; 0.51)
BP_{HF} power, mm Hg^2	-0.05 (-0.27; 0.17)	-0.02 (-0.2; 0.16)	-0.04 (-0.25; 0.16)
BP_{LF} power, nu	2.24 (-5.27; 9.75)	0.76 (-5.37; 6.89)	0.25 (-7.15; 7.65)
BP_{HF} power, nu	-2.24 (-9.75; 5.27)	-0.76 (-6.89; 5.37)	-0.25 (-7.65; 7.15)
BP_{LF} power, %	0.12 (-0.09; 0.32)	0.02 (-0.15; 0.19)	0.03 (-0.18; 0.25)
BP_{HF} power, %	-0.01 (-0.09; 0.06)	0 (-0.07; 0.06)	0 (-0.07; 0.08)
BP_{LFHF} ratio	0.58 (0.46; 1.62)	0.48 (-0.36; 1.32)	0.48 (-0.55; 1.51)

BP: blood pressure; LF: low frequency; HF: high frequency. Values are β coefficients and 95% confidence intervals obtained by multivariate linear regression analysis adjusted to age and stenosis side. * $p < 0.05$

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23. Serber, S.L., et al., *Cerebral blood flow velocity and vasomotor reactivity during autonomic challenges in heart failure.* Nurs Res, 2014. **63**(3): p. 194-202.

ANEXO 1

Normas de Publicação da Revista *Brain Sciences.*

brain sciences

Type of the Paper (Article, Review, Communication, etc.)

Title

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Abstract: A single paragraph of about 200 words maximum. For research articles, abstracts should give a pertinent overview of the work. We strongly encourage authors to use the following style of structured abstracts, but without headings: (1) Background: Place the question addressed in a broad context and highlight the purpose of the study; (2) Methods: Describe briefly the main methods or treatments applied; (3) Results: Summarize the article's main findings; and (4) Conclusions: Indicate the main conclusions or interpretations. The abstract should be an objective representation of the article, it must not contain results which are not presented and substantiated in the main text and should not exaggerate the main conclusions.

Keywords: keyword 1; keyword 2; keyword 3 (List three to ten pertinent keywords specific to the article; yet reasonably common within the subject discipline.)

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0. How to Use This Template

The template details the sections that can be used in a manuscript. Note that each section has a corresponding style, which can be found in the 'Styles' menu of Word. Sections that are not mandatory are listed as such. The section titles given are for Articles. Review papers and other article types have a more flexible structure.

Remove this paragraph and start section numbering with 1. For any questions, please contact the editorial office of the journal or support@mdpi.com.

1. Introduction

The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance. The current state of the research field should be reviewed carefully and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the principal conclusions. As far as possible, please keep the introduction comprehensible to scientists outside your particular field of research. References should be numbered in order of appearance and indicated by a numeral or numerals in square brackets, e.g., [1] or [2,3], or [4–6]. See the end of the document for further details on references.

2. Materials and Methods

Materials and Methods should be described with sufficient details to allow others to replicate and build on published results. Please note that publication of your manuscript implicates that you must make all materials, data, computer code, and protocols associated with the publication available to readers. Please disclose at the submission stage any restrictions on the availability of materials or information. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited.

Research manuscripts reporting large datasets that are deposited in a publicly available database should specify where the data have been deposited and provide the relevant accession numbers. If the accession numbers have not yet been obtained at the time of submission, please state that they will be provided during review. They must be provided prior to publication.

Interventionary studies involving animals or humans, and other studies require ethical approval must list the authority that provided approval and the corresponding ethical approval code.

3. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

3.1. Subsection

3.1.1. Subsubsection

Bulleted lists look like this:

- First bullet
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Numbered lists can be added as follows:

1. First item
2. Second item
3. Third item

The text continues here.

3.2. Figures, Tables and Schemes

All figures and tables should be cited in the main text as Figure 1, Table 1, etc.

(**a**) (**b**)

Figure 1. This is a figure, Schemes follow the same formatting. If there are multiple panels, they should be listed as: (**a**) Description of what is contained in the first panel; (**b**) Description of what is contained in the second panel. Figures should be placed in the main text near to the first time they are cited. A caption on a single line should be centered.

Table 1. This is a table. Tables should be placed in the main text near to the first time they are cited.

Title 1	Title 2	Title 3
entry 1	data	data
entry 2	data	data [1]

[1] Tables may have a footer.

3.3. Formatting of Mathematical Components

This is an example of an equation:

$$a = 1, \tag{1}$$

the text following an equation need not be a new paragraph. Please punctuate equations as regular text.

4. Discussion

Authors should discuss the results and how they can be interpreted in perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

5. Conclusions

This section is not mandatory, but can be added to the manuscript if the discussion is unusually long or complex.

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Appendix A

The appendix is an optional section that can contain details and data supplemental to the main text. For example, explanations of experimental details that would disrupt the flow of the main text, but nonetheless remain crucial to understanding and reproducing the research shown; figures of replicates for experiments of which representative data is shown in the main text can be added here if brief, or as Supplementary data. Mathematical proofs of results not central to the paper can be added as an appendix.Appendix B

All appendix sections must be cited in the main text. In the appendixes, Figures, Tables, etc. should be labeled starting with 'A', e.g., Figure A1, Figure A2, etc.

References

References must be numbered in order of appearance in the text (including citations in tables and legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as EndNote, ReferenceManager or Zotero to avoid typing mistakes and duplicated references. Include the digital object identifier (DOI) for all references where available.

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1. Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* **Year**, *Volume*, page range.
2. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A., Editor 2, B., Eds.; Publisher: Publisher Location, Country, 2007; Volume 3, pp. 154–196.
3. Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, 2008; pp. 154–196.
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5. Author 1, A.B. (University, City, State, Country); Author 2, C. (Institute, City, State, Country). Personal communication, 2012.
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Agradecimentos

Gostaria de expressar o meu profundo agradecimento a todos os intervenientes neste projeto pela oportunidade que me proporcionaram e pela valiosa contribuição prestada.

Queria agradecer em particular ao Prof. Pedro Castro por toda a disponibilidade e paciência demonstradas de forma constante ao longo da elaboração deste artigo. Sem dúvida que as suas orientações e conhecimentos foram imprescindíveis, não só para a realização desta tese, mas também para a minha formação profissional. Obrigada por ter confiado em mim para a construção deste trabalho e, acima de tudo, por todo o apoio, compreensão e colaboração.