UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE
GRIGORE T. POPA IAȘI

ASSESSMENT OF CARDIAC AUTONOMIC CONTROL BY LINEAR AND NONLINEAR ANALYSES OF THE HEART RATE

HABILITATION THESIS

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CONTENTS

ABBREVIATIONS

ACC - Anterior Cingulate Cortex
Ach - Acetylcholine
AD - Alzheimer's disease
ANS - Autonomic Nervous System
ApEn - approximate entropy
ATLOs- artery tertiary lymphoid organ
BP- blood pressure
CAN - cardiac autonomic neuropathy
CMAP- Compound muscle action potential
CT- computer tomography
DA – Dopamine
DBP – diastolic blood pressure
DFA- Detrended fluctuation analysis
DM- Diabetes Mellitus
E – Epinephrine
FES- Functional electrical stimulation
HDL- high density lipoprotein cholesterol
HF - high-frequency component
HPA - Hypothalamic–Pituitary–Adrenal axis
HHD- handheld dynamometry
HR- Heart rate
HRDB- heart rate deep breathing
HRV - Heart Rate Variability
IL- Interleukins
LDH - lumbar disc herniation
LDL - low density lipoprotein cholesterol
LF- low-frequency component
LF/HF - index of cardiac sympathetic/parasympathetic balance
M - Migraine without aura
MA - Migraine with aura
MCA - right middle cerebral artery
MMSE - Mini-Mental Status Examination
MRC- Medical Research Council
MRI- magnetic resonance imaging
MTRS - Multiple Trigonometric Regressive Spectral analysis
NCV- Nerve conduction velocity
NE – Norepinephrine
NTS- Nucleus of the Solitary Tract
ODI- Oswestry Disability Index
OH - orthostatic hypotension
PAG- Periaqueductal Gray Matter
PBN - Parabrachial Nucleus
PFC - Prefrontal Cortex
PE - Physical exercise
PNS - Parasympathetic Nervous System
pNN50%- Percentage of differences between adjacent NN intervals differing more than 50 ms
RMSSD - Square root of the mean of the sum of the squares of differences between adjacent NN interval
ROS - Reactive Oxygen Species
RNS- Reactive Nitrogen Species
SampEn - Sample entropy
SBP- systolic blood pressure
SNS - Sympathetic Nervous System
TP- total power
TMS- Transcranial magnetic stimulation
TNF-α - Tumor necrosis factor alpha
ULF - ultra-low-frequency
VaD- Vascular dementia
VLF - very low-frequency component
VLM - Reticular formation of the Ventrolateral Medulla
VMM - Reticular formation of the Ventromedial Medulla
VNS- Vagus nerve stimulation
VR - Valsalva ratio
30/15 - lying-to-standing tests

THESIS SUMMARY

The habilitation thesis entitled "**ASSESSMENT OF CARDIAC AUTONOMIC CONTROL BY LINEAR AND NONLINEAR ANALYSES OF THE HEART RATE**" presents my clinical research in the field of normal and pathological physiology, and also some others subject related with Biomedical Sciences.

The whole didactic activity I carried out within the Faculty of Medical Bioengineering "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, which train specialists in two fields: Bioengineering, Balneo-physio-kinetotherapy and Rehabilitation. This unique dual degree programs try to bridge communication and collaboration between bioengineers, physical therapist and doctors for a clear benefit to maintain and improve health. Teaching and pedagogy are complex professional challenges for academic stuff. Without professionalism, continuous training and involvement in the contemporary scientific world it cannot be achieved a higher quality of teaching and an improvement in the learning process.

Before I have started to write the present thesis I have studied the recommendations of The National Council for Attestation of University Titles, Diplomas and Certificates (CNATDCU). I have followed precisely the mentioned instructions and I have structured it into three main sections:

Section I - Professional, scientific and academic achievements from the postdoctoral period;

Section II - Future projects in the professional, academic and scientific field;

Section III - References.

A short overview of my professional, academic and scientific activities has been introduced before section I, where I reviewed my studies and the main direction I have followed after my PhD thesis. In this section, are presented the elements of the national and international visibility of my activity, such as the publication of chapters of books (including 6 international book chapters as first author). Participation in national and international congresses, with 137 scientific papers published in full or in summary in scientific journals or proceedings of international or national conferences: 37 articles published in journal rated or indexed by Thomson ISI Web of Science Core Collection; 33 articles published in international databases and 67 articles in the volumes of international or national scientific events. I have also pointed the projects in which I have been involved (1 as a director and 4 as a member of the project

team), involvement in research activities carried out in collaboration with research teams from the University of Medicine and Pharmacy "Grigore T. Popa " from Iasi. Publication of articles in Thomson ISI databases leading to a Hirsch-index of 7 in the Thomson ISI Web of Science Core Collection.

In the first section of the Habilitation thesis entitled **"**Professional, scientific and academic achievements from the postdoctoral period**"** I have illustrated the publications accumulated during the post-doctoral period in three main themes of study:

1. Heart and Brain interaction

2. Rehabilitation and its significance in injury recovery

3. Prevention is better than cure

In the first subchapter of Section I, autonomic nervous system network and the analysis of heart rate variability, were discussed at the opening of the chapter. Then I covered topics like autonomic dysfunction in type 2 diabetes mellitus with and without vascular dementia, autonomic impairment in patients with migraine and heart rate variability in stroke patients.

The second subchapter of Section brings into discussion the different rehabilitation methods used in stroke, lower back pain due to lumbar disc herniation, pharmacoresistant epilepsy such as mirror therapy, functional electric stimulation, vagus nerve stimulation.

In the third subchapter of Section I, I discussed about the links between autonomic nervous system and immune system, oxidative stress, and atherosclerosis. Also I mentioned the importance of maintaining a normal balance between the sympathetic and parasympathetic nervous system here detailing the effects of physical exercise in maintaining a balanced autonomic nervous system.

Section II Future projects in the professional, academic and scientific field, I described academic and research development plan for the next years, in which I set out to focus on building up a multidisciplinary research team, developing the logistics base and also increasing dissemination and visibility of research results. I want to address new clinical research topics that might be presented in national and international scientific projects competitions, involving young doctors and PhD students.

Having the opportunity to teach students of both programs Medical Bioengineering and Balneo-physio-kinetotherapy and Rehabilitation together with my team, I am always preoccupied with development of solid skills in the field of rehabilitation. The increasing

incidence of chronic diseases, as well as the numerical increase of the third-age population with different degrees of disability have brought into discussion the importance of the reintegration of the individual, in the family and socio-professional environment, in the direction of promoting the patient's autonomy in everyday life, the final goal being the optimization of the quality of life, by increasing the degree of functional independence.

The rehabilitation process is a process that involves a multidisciplinary team in which, in addition to doctors of various specialties, physiotherapists and bioengineers cannot be missing. The future projects will include intelligent systems for diagnosis and monitoring the control of rehabilitation processes based on functional electrical stimulation and vagal nerve stimulation; software for monitoring parameters involved in rehabilitation processes; complex robotic recovery systems, virtual reality. Another area of interest would be analysis of biomedical signals that will help to create functional models, improve the performance of existing medical devices, design new techniques and methods of investigation and data analysis useful in diagnosis and monitoring. We also proposed to monitor various physical activities. Using wireless systems we can acquire electrocardiographic signals and monitor heart rate variability of each stage of physical effort, in different types of physical activities. In this way, we can contribute to the practice of physical effort in safe conditions and to prevent the occurrence of cardiac arrhythmias and sudden death associated with physical exercise.

The third section contains the most representative bibliographic references of my currently knowledge and for future projects.

In order to continue my research activity in the future, I plan to make more efficient the professional interaction between the current and future collaborations, between my ability to coordinate original researches and the need to supervise young students and PhD students.

The experience and professional skills gained so far and proven through the publication of articles, participation in national research projects represent a real proof of personal possibilities to fulfill the objectives of this professional, academic and scientific development plan, in accordance with future opportunities and challenges.

REZUMATUL TEZEI

Teza de abilitare intitulată „**ASSESSMENT OF CARDIAC AUTONOMIC CONTROL BY LINEAR AND NONLINEAR ANALYSES OF THE HEART RATE**“ prezintă cercetările mele clinice în domeniul fiziologiei normale și patologice, precum și alte subiecte legate de Științele Biomedicale.

Întreaga activitate didactică am desfășurat-o în cadrul Facultății de Bioinginerie Medicală, Universitatea de Medicină și Farmacie „Grigore T. Popa” din Iași, care formează specialiști în două domenii: Bioinginerie, Balneo-fizio-kinetoterapie și Reabilitare. Aceste programe unice încearcă să creeze o punte de comunicare și colaborare între bioingineri, kinetoterapeuţi și medici pentru un beneficiu clar de a menține și îmbunătăți sănătatea. Predarea și pedagogia sunt provocări profesionale complexe pentru structurile academice. Fără profesionalism, formare continuă și implicare în lumea științifică contemporană nu se poate realiza o calitate superioară a predării și o îmbunătățire a procesului de învățare.

Înainte de a începe redactarea prezentei teze, am studiat recomandările Consiliului Naţional de Atestare a Titlurilor, Diplomelor şi Certificatelor Universitare (CNATDCU). Am urmat întocmai instrucțiunile menționate și le-am structurat în trei secțiuni principale:
Secțiunea I - Realizări profesionale, științifice și academice din perioada postdoctorală;
Secțiunea II - Proiecte de viitor în domeniul profesional, academic și științific;
Secțiunea III - Referințe.

O scurtă prezentare generală a activităților mele profesionale, academice și științifice a fost introdusă la inceputul secțiunii I, unde mi-am trecut în revistă studiile și direcția principală pe care am urmat-o după teza mea de doctorat. În această secțiune, sunt prezentate elementele de vizibilitate națională și internațională a activității mele, precum publicarea de cărţi şi capitole de cărți (inclusiv 6 capitole internaționale de carte ca prim autor). Participarea la congrese naționale și internaționale, cu 137 de lucrări științifice publicate integral sau rezumat în reviste științifice sau lucrări ale conferințelor internaționale sau naționale: 37 articole publicate în reviste cotate sau indexate de Thomson ISI Web of Science Core Collection; 33 de articole publicate în baze de date internaționale și 67 de articole în volumele evenimentelor științifice internaționale sau naționale. De asemenea, am punctat proiectele în care am fost implicată (1 ca director și 4 ca membru al echipei de proiect), implicarea în activități de cercetare desfășurate în colaborare cu echipele de cercetare din cadrul Universității de Medicină și Farmacie „Grigore T. Popa" din

Iaşi. Publicarea articolelor în bazele de date Thomson ISI conducând la un indice Hirsch de 7 în Thomson ISI Web of Science Core Collection.

În prima secțiune a tezei de abilitare intitulată „Realizări profesionale, științifice și academice din perioada postdoctorală" am ilustrat publicațiile acumulate în perioada postdoctorală în trei teme principale de studiu:

1. Interacțiunea dintre inimă şi creier
2. Reabilitarea și semnificația ei în recuperarea leziunilor
3. Este mai bine să previi decât să vindeci

În primul subcapitol al Secțiunii I, rețeaua sistemului nervos autonom și analiza variabilității ritmului cardiac, au fost discutate la deschiderea capitolului. Apoi am abordat subiecte precum disfuncția autonomă în diabetul zaharat de tip 2 cu și fără demență vasculară, afectarea autonomă la pacienții cu migrenă și variabilitatea ritmului cardiac la pacienții cu accident vascular cerebral.

Al doilea subcapitol al Secțiunii I aduce în discuție diferitele metode de reabilitare utilizate în accidentul vascular cerebral, durerii lombare datorate herniei de disc lombare, epilepsie farmacorezistentă precum terapia în oglindă, stimularea electrică funcțională, stimularea vagală.

În al treilea subcapitol al Secțiunii I, am discutat despre legăturile dintre sistemul nervos autonom și sistemul imunitar, stresul oxidativ și ateroscleroză. De asemenea, am menționat importanța menținerii unui echilibru normal între sistemul nervos simpatic și parasimpatic aici detaliind efectele exercițiului fizic în menținerea unui sistem nervos autonom echilibrat.

Secțiunea II - Proiecte viitoare în domeniul profesional, academic și științific, am descris planul de dezvoltare academică și de cercetare pentru următorii ani, în care mi-am propus să mă concentrez pe formarea unei echipe multidisciplinare de cercetare, dezvoltarea bazei logistice și, de asemenea, creșterea diseminării și vizibilității a rezultatelor cercetării. Doresc să abordez noi subiecte de cercetare clinică care ar putea fi prezentate în concursuri de proiecte științifice naționale și internaționale, care implică tineri doctori și doctoranzi.

Având ocazia să predau studenților de la ambele programe ale facultăţii noastre Bioinginerie Medicală și Fizio-kinetoterapie și Reabilitare împreună cu echipa mea, sunt mereu preocupată de dezvoltarea unor abilități solide în domeniul reabilitării. Incidența tot mai mare a bolilor cronice, precum și creșterea numerică a populației de vârstă a treia cu diferite grade de

handicap au adus în discuție importanța reintegrării individului, în mediul familial și socio-profesional, în direcția promovării autonomiei pacientului în viaţa de zi cu zi, scopul final fiind optimizarea calităţii vieţii, prin creşterea gradului de independenţă funcţională.

Procesul de reabilitare este un proces care implică o echipă multidisciplinară din care nu pot lipsi, pe lângă medici de diverse specialități, kinetoterapeuții și bioinginerii. Proiectele viitoare vor include sisteme inteligente de diagnosticare și monitorizare a controlului proceselor de reabilitare bazate pe stimularea electrică funcțională și stimularea nervului vag; software pentru monitorizarea parametrilor implicați în procesele de reabilitare; sisteme complexe de recuperare robotică, realitate virtuală. Un alt domeniu de interes ar fi analiza semnalelor biomedicale care va ajuta la crearea modelelor funcționale, la îmbunătățirea performanței dispozitivelor medicale existente, la proiectarea de noi tehnici și metode de investigare și analiză a datelor utile în diagnostic și monitorizare. De asemenea, ne-am propus să monitorizăm diverse activități fizice. Cu ajutorul sistemelor wireless putem achiziționa semnale electrocardiografice și monitoriza variabilitatea ritmului cardiac al fiecărei etape a efortului fizic, în diferite tipuri de activități fizice. În acest fel, putem contribui la practicarea efortului fizic în condiții de siguranță și la prevenirea apariției aritmiilor cardiace și a morții subite asociate exercițiului fizic.

A treia secțiune conține cele mai reprezentative referințe bibliografice din cunoștințele mele actuale și pentru proiectele viitoare.

Pentru a-mi continua activitatea de cercetare în viitor, îmi propun să eficientizez interacțiunea profesională dintre colaborările actuale și viitoare, între capacitatea mea de a coordona cercetări originale și nevoia de a supraveghea tineri studenți și doctoranzi.

Experiența şi competențele profesionale căpătate până acum şi dovedite prin publicarea de articole, participarea în cadrul proiectelor de cercetare națională reprezintă o dovadă reală a posibilităţilor personale de a îndeplini obiectivele acestui plan de dezvoltare profesională, academică și științifică, în concordanţă cu oportunităţile şi provocările viitoare.

SECTION I
PROFESSIONAL, SCIENTIFIC AND ACADEMIC ACHIEVEMENTS

Didactic activity/ Academic activity/ Professional achievements

The teaching profession requires continuous training in order to transmit the necessary knowledge to students. Without professionalism, continuous training and involvement in the contemporary scientific world it cannot be achieved a higher quality of teaching and an improvement in the learning process.

My professional career has started immediately after graduating from the University of Medicine and Pharmacy „Grigore T. Popa" Iași in 1994. In the same year I participated at the national residency examination and was admitted as to the specialty General Medicine for Adults. From 1995 to 1997 I went through all the internships during my residency. In november 1997 I sustained the exam for the degree of Specialist in the specialty General Medicine for Adults.

Then, in 1998, I gained through contest the post of Assistant Professor between 1998-2013, at the Department of Biomedical Sciences, Faculty of Medical Bioengineering, University of Medicine and Pharmacy "Grigore T. Popa" Iasi, România.

I competed for every academic step :

- Lecturer from 2013, at the Department of Biomedical Sciences, Faculty of Medical Bioengineering, University of Medicine and Pharmacy "Grigore T. Popa" Iasi, România
- Associate Professor from 2019 until now at the Department of Biomedical Sciences, Faculty of Medical Bioengineering, University of Medicine and Pharmacy "Grigore T. Popa" Iasi, România

I have a professional experience that covers 22 years of academic activity. The whole didactic activity I carried out within the Faculty of Medical Bioengineering "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, which train specialists in two fields: Bioengineering, Balneo-physio-kinetotherapy and rehabilitation.

Bioengineering, as a defined field, is relatively new, but its position at the interface between life science and engineering makes it an exciting and fast developing field. Balneo-physio-

kinetotherapy and rehabilitation is a profession which incorporates theoretical base and widespread clinical application in the preservation, development and restoration of physical function. This unique dual degree programs try to bridge communication and collaboration between bioengineers, physical therapist and doctors for one clear benefit to improved rehabilitation technologies and therapies for restoration and maintaining human functions.

My didactic experience materialized in practical works and courses in the field of Physiology, Ergophysiology, Paraclinical Function Testing, Osteoarticular and muscle assessment.

I chose to be a member of Department of Biomedical Sciences, because the faculty staff comes from diverse academic disciplines including all main branches of life sciences, medicine, engineering creating a rich collaborative environment which promotes new concepts in the field of modern bioengineering and physical therapy.

I must mention that since 2016 I am the director of Department of Biomedical Sciences which mobilized me to continue the mission of the Faculty of Medical Bioengineering. The Faculty's mission is to pursue excellence in biomedical education, research, and innovation.
From this position I try to increase the quality of teaching activity by promoting a high-performing academic environment, of mutual respect between teachers as well as in relations with students, which stimulates the achievement of the professional and personal potential of each member of the academic community. Also I try to increase the performance of the teaching act by adapting, restructuring and harmonizing the curricula with those at national and international level. Introduction of new study objects according to the new requirements of knowledge, technical development and constantly evolving requirements of the labor market; stimulating the practical training of future specialists, through internships, workshops with specialists in the field; boosting research activities and increasing the scientific and professional prestige of our Department. I pay a major attention to the endowment and modernization of laboratories for the teaching activity from own funds; accessing additional funds by submitting different projects.

I have had the opportunity to experience each of the teaching positions one at a time, from Assistant Professor to Associate Professor, and that led to the development of a powerful ability to interact with students in practical classes, in lectures and during research activity.

The entire experience gained in all these years made me realize the importance of getting students to be attracted to classes, raising their interest, especially by personal example and by emphasizing the practical importance of what they are studying. I have permanently strived to upgrade and improve the teaching methods. Interactive teaching has and will always be an essential part of my relationship with the students, and their appreciation of my methods has always been reflected in the annual performance feed-back given by the students. This has helped me in forming a connection based on respect and mutual trust with students, master's students and young researchers I have encountered throughout the years.

I try to stimulate student's creativity and promote their ability to think independently; to continue improving and expanding their technical and professional skills through formal or informal means (e.g., continuing education and training, attending conferences); to conduct research leading to significant discoveries in medical sciences; and to engage with scientific community worldwide for knowledge dissemination.

From this period on my professional life developed as a highly stimulating mixture between bioengineering (methods and techniques for collection, analysis and processing of biomedical signals) and physical therapy (examination, evaluation, diagnosis, alleviation of impairment and functional limitation, and also promotion of positive health behavior).

Gradually, I developed skills in the majority of noninvasive cardiovascular, respiratory and neuromuscular diagnostic methods through continuous professional training courses such as " Electrophysiological Bases of ECG and EEG Investigation" , " Electrophysiological Bases of Neuro-Muscular Exploration", "Neuro-Muscular Exploration in Effort and Sleep", "Monitoring Techniques for Cardio-Respiratory Parameters under Effort and Sleep Conditions", "Modern Techniques of Electrodiagnosis", "Functional Electrical Stimulation in the Recovery of Neurological Patient". I followed the graduate specialization studies in the Management of Public Institutions (two semesters).

As a department director I tried to increase the quality of scientific research activity by identification and establishment together with the members of the Department of the priority directions of interdisciplinary research; and by stimulating the collaboration between disciplines in order to be able to use material and technical resources in common.

Throughout my career, I have been permanently interested to integrate the research activities with teaching, academic and professional development. I have disseminated our outcomes by

scientific papers presented at congresses, symposiums, national and international conferences and scientific papers published in ISI-rated journals. I have been constantly involved in coordinating the teaching and scientific activities of students by my tutor position for many series of students, coordinating and supervising some original papers presented at student congresses, some of these works being awarded.

I have constantly tried to systematize the theoretical notions and to update the knowledge with medical news from the literature. I and my colleges developed the course support that was materialized in four books "A Practical Guide of Paraclinical Function Testing " , “Computerized Applications of Paraclinical Function Testing”, Neurology for medical students ”, and “Assessment of joint amplitudes”.

The emergence of the e-learning platform marked a new stage in didactic activity not only through the new way of managing the activities and the results of the students but also by providing the course support which was updated and adapted for each specialization.

Since 1999 I have been conducting more than 120 license theses both at the Bioengineering, Balneo-physio-kinetotherapy and rehabilitation specialization.

I was part of the professional commission of biology at the admission to the University and which materialized with the publication of test books for admission as: The human anatomy and physiology. Admission tests for 2017, 2018, 2020, 2021, 2022. Also I became member of the curricular, scientific, and promotion commission of the Faculty of Medical Bioengineering.

Scientific activities

My research activity began with admission to PhD in 2003. In 2009 I have finished my PhD Thesis, entitled "The utility of monitoring microvascular, cardio-respiratory and neuromotor reactivity in type 2 diabetes mellitus", Scientific Advisor - Prof.Dr. Ana Stratone, which conferred me the title of Doctor of Medical Sciences, Medicine domain.

I chose an ambitious theme because Diabetes mellitus has a great medical impact due to the complexity of long-term medical care for the affected persons, the increase of mortality, particularly in subjects over 45 years old. The economic impact of the disease is evaluated through the costs resulted from the screening, diagnosis, treatment. The chronic complications of

the diabetic patient are the ones that decide on long term the life quality, health and life expectation of the subjects with diabetes mellitus.

The objectives of the thesis consisted in the description of type II diabetes mellitus from the clinical, biological, etiopathological and function testing point of view. Most difficult aspect was the need for multidisciplinary approach of these patients. The research envisaged an comparative evaluation of metabolic modifications, of cardio-vascular, respiratory and neuro-sensitive status in the study groups, using different methods of investigation: bioumoral, imagistic (cardio-thorax x-ray, abdominal ultrasound image, 2D echocardiography, vascular Doppler imaging) and functional (standard electrocardiography, ambulatory monitoring of blood pressure, Holter monitoring of electrocardiography signal with time and frequency domain analysis, photopletismography, electromiography and nerve conduction velocity etc.). The original character of the thesis is supported by the complex research directions, highlighting some pathogenic mechanisms involved in the onset of type 2 diabetes mellitus, evaluation of endothelial function, detecting the presence of the autonomic nervous system dysfunctions and somatic neuropathy.

The theme that I approached gives new research perspectives and opens new opportunities for early elaboration of a modern screening-type investigation algorithm with the aim of improving the early diagnosis of metabolic disorders and of some criteria for complication prognosis with the aim of early implementation of prophylactic measures.

In addition to diabetes mellitus, I looked for new research topics such as autonomic dysfunction in Vascular Dementia, Migraine, Stroke, Parkinson and Alzheimer diseases. I was also attracted to research topics on the recovery of various diseases such as Stroke rehabilitation using Mirror therapy, Biofeedback versus switch-triggered functional electrical stimulation on sciatica-related foot drop, and Vagus Nerve Stimulation. Other research topics have focused on physical effort, especially antioxidant and anti-inflammatory changes in exercise. All these research topics allowed me to acquire the necessary skills in performing clinical and cohort studies, and statistical analyzes.

The scientific and research activity has materialized in the publication of articles indexed by the Web of Science Core Collection and other international databases, participation in national and international congresses and conferences, application and winning by competition as a member or coordinator of research teams of grants, participation as investigator in clinical trials.

I have published international book chapters in prestigious collections such as “New Aspects of Therapeutic Management in Alzheimer’s Disease. In: Frontiers in Clinical Drug Research – Alzheimer Disorders. Atta-ur-Rahman (Ed). Bentham Science Publishers, 2014; “Natural Compounds from Plants Targeting Alzheimer’s Disease. In: Frontiers in Clinical Drug Research - Alzheimer Disorders. Atta-ur-Rahman (Ed). Bentham Science Publishers, 2016”, “Triggering early neuroplasticity mechanisms: new perspectives in ischemic stroke management. Avid Science”; “Primary headaches and their relationship with the autonomic nervous system. In Tech. Medicine » ″Current Perspectives on Less-known Aspects *of* Headache″, book edited by Hande Turker, 2017; “Analytical Design of Two-Dimensional Filters and Applications in Biomedical Image Processing”, chapter in the book "Digital Filters and Signal Processing", edited by Fausto Pedro García Márquez, IN-TECH, Vienna, Austria, 2012.

Over the years of clinical activity I was involved in the development of some competition funded research projects:

As the main investigator:

- Internal Research Grant No. 29241 / 20.12.2013. Study of Hemodynamic and Neurophysiological Changes in Patients with Alzheimer's Disease, Vascular Dementia and Mixed Dementia.

As sub-investigator

• Grant of Romanian Academy (GAR): “Nonlinear Analysis Methods for Pattern Recognition in Polysomnography Recordings for Patients with Sleep Respiratory Disorders and Cardiovascular Risk” (Principal Investigator: Lect. Grigoraş Carmen Liliana - contract no. 295, 2008-2009.

• “Investigation, Assistance and Control System for Neurological Disorders, Based on BCISIS Brain-Computer Interface” - contract no. 12115 / 01.10.2000 - (Principal Investigator: Assoc. Prof. Dr. Eng. Lazăr Anca Mihaela), 2008 – 2011.

• Implantable Magnetic Microsensors For Medical Applications ”- MEDISENS - contract - 12110/2008 - (Principal Investigator: Prof. Dr. Iacob Gheorghe, 2008-2011)

• Internal research grant 17084 / 30.09.2010: “Analysis of some hemodynamic parameters using electrical impedance plethysmography” (Principal Investigator: Assistant professor Corciovă Călin, PhD.

Other projects I participated was:

1. Responsible for internships - POCU/90/6.13/6.14/108943 “Facilitating the transition from education to the labor market in health care - balneophysiokinetotherapy ".
2. Project CNFIS-FDI-2020-0249 – “Supporting research excellence through the development of medical technologies and biotechnologies (FizioBiotech) ”Project co-financed by the Institutional Development Fund 2020 Domain 6: Supporting research excellence in universities
3. Project CNFIS-FDI-2021 –“Supporting research excellence through the development of medical technologies and biotechnologies (FizioBiotech) ”Project co-financed by the Institutional Development Fund 2021 Domain 6: Supporting research excellence in universities”
4. Physiology/anatomy course expert – ROSE Project = “Support for first year students from the Faculty of Medical Bioengineering at risk of dropping out of school, to improve academic performance” - BioRemedis Grant Agreement no.359/SGU/SS/III din 10.09.2020
5. Physiology/anatomy course expert –ROSE Project = “Support for first year students from the Faculty of Medical Bioengineering at risk of dropping out of school, to improve academic performance” - BioRemedis Grant Agreement no.359/SGU/SS/III din 27.01.2022

As a result of the improvement in my research skills, over the years I have managed to publish original review articles as first author or co-author. Early in my career I published several articles in B and B+ Romanian journals or conference and congress proceedings.

At that time I also had the opportunity to familiarize myself with the requirements for writing article and publication criteria as I was member of the scientific groups or committees of scientific events such as: eLearning and software for education conference, IEEE International Conference on e-Health and Bioengineering-EHB, also peer reviewer for ISI journal such as: PLOS ONE, Journal of International Medical Research, Scientific Reports, Journal of Novel Physiotherapy and Rehabilitation, Diabetes/Metabolism Research and Reviews, Frontiers Neurology. Also I was peer reviewer for BDI journal such as: Engineering Science and Technology International Journal (Springer), Medical Exploration, International Journal of

Neurology and Neurotherapy. I am member of professional associations: College of Physicians, Romanian Society of Medical Bioengineering.

The scientific portfolio currently includes:

- 14 Books and Chapters of books; of which 6 chapters of an international book;
- 137 scientific papers published in full or in summary in scientific journals or proceedings of international or national conferences:

- 37 articles published in journal rated or indexed by Thomson ISI Web of Science Core Collection;
- 33 articles published in international databases
- 67 articles in the volumes of international or national scientific events

In conclusion to this overview of my activity, I believe that national and international visibility could be highlighted by:

- participation in continuing education and educational courses;
- improvement of teaching and student evaluation methods;
- participation in original collaborative researches, with the publication of research results in journals rate or indexed by Thomson ISI Web of Science Core Collection and presentation of papers at international congresses;
- engaging in research programs obtained by competition;
- Author of 6 chapters published in international textbooks;
- citations in international databases: 175 citations in Thomson ISI Web of Science Core Collection
- ISI Web of Science Core Collection Hirsch-index of 7.
- All Databases H- index: 8;
- Using the ORCID platform

CHAPTER 1

HEART AND BRAIN INTERACTION

1.1. STATE OF THE ART

The autonomic nervous system (ANS) has important functions in maintaining homeostasis by adjustment of the body to internal and environmental demands. Beside key functions controlled by the ANS such as respiration, blood pressure, heart rate, hormonal regulation, etc. ANS is also involved in regulating emotional behavior and cognitive functions.

The ANS acts through two main systems: the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS).

Stimulation of the SNS brings the body to a state of increased activity called the “fight or flight” response (heart rate and blood pressure increase, temporary inhibition of the gastrointestinal tract peristalsis, etc.) [1]. Preganglionic and postganglionic sympathetic neurons facilitate communication between the SNS and the peripheral organs. The point at which the axon of the preganglionic neuron connects with the postganglionic neuron is also the place where the cell body of the preganglionic neuron can be found, between the segments of the first thoracic and third lumbar spinal cord. Subsequently, the postganglionic neuron axon reaches the target organ. Norepinephrine (NE), epinephrine (E) and dopamine (DA) are neurotransmitters.

The PNS regulates the body’s unconscious actions, which can be summarized as the “rest and digest” response, as these return the body functions back to normal: blood pressure lowers, heart rate slows down, intestinal and gland activity increases, sphincter muscles in the gastrointestinal tract relax, etc. [1]. The structures in which the parasympathetic preganglionic neurons can be found are the sacral spinal cord and the brainstem. The parasympathetic nuclei of the glossopharyngeal, oculomotor, vagus and facial nerves and the cardiac preganglionic neurons are located in the nucleus ambiguus. The primary parasympathetic neurotransmitter is acetylcholine (Ach), which has been shown to regulate various processes such as arousal, learning and memory, cognition and modulation of sensory information [2].

If the peripheral pathways of the ANS are relatively distinct and very well documented, the central control of the ANS involving several areas throughout the spine, bulbopontine, pontomesencephalic and forebrain is still discussed.

The lower brainstem level includes the nucleus of the solitary tract (NTS), the reticular formation of the ventrolateral and ventromedial medulla (VLM, VMM) and the parabrachial nucleus (PBN) and is involved in the reflex control of circulation, respiration, gastrointestinal function and micturition [3].

The upper brainstem level includes the periaqueductal gray matter (PAG), which integrates autonomic control with pain modulation and behavioral responses to stress [3].

The forebrain level includes the paraventricular and related nuclei of the hypothalamus, thalamus, amygdala, anterior cingulate, the insular and the medial prefrontal cortex, which are involved in the integration of autonomic and endocrine responses [1]. The anterior limbic circuit (insula, the anterior cingulate cortex, and amygdala) realize the integration of specific sensations with emotional and goal-related autonomic responses [1]. The hypothalamus is the major homeostatic center of the brain. It regulates endocrine activity and controls glucose, lipid metabolism, food and water intake, body temperature, blood flow and composition; it also drives behaviors related to feeding, emotional responses, autonomic function control in relation to sleep, arousal and motivated behavior [4]. The rostral and caudal hypothalamus is responsible for the control of the ANS. The rostral controls parasympathetic activity while the caudal controls sympathetic activity.

The amygdala provides affective or emotional value to incoming sensory information [5] and generates responses that include autonomic function modulation. The basolateral complex of the amygdala has reciprocal connections with cortical association areas and the hippocampus, thus participating in learning and conditioned responses to aversive stimuli (fear) [5].

The insular cortex, anterior cingulate cortex (ACC) and amygdala are connected with the prefrontal cortex, which regulates decision-making and emotional behavior [6]. The left anterior insula is activated predominantly by parasympathetic functions and the right anterior insula is activated by pathways associated with sympathetic functions [7]. Imagistic studies show that the caudal ACC is activated during tasks that involve awareness and attention and is associated with an increase in sympathetic drive [6]. The rostral ACC becomes inactivated during such tasks [8] and is involved in the parasympathetic control of the heart [6].

The prefrontal cortex (PFC) coordinates autonomic and neuroendocrine functions with cognitive and affective processes. The dorsomedial PFC suppresses the hypothalamic–pituitary–adrenal axis (HPA) response to acute psychological stress, while the ventromedial PFC serves to

activate the HPA axis [9]. The dorsomedial PFC shares connections with the primary motor and somatosensory cortices, premotor area and somatosensory association areas and coordinates goal-directed actions [10]. The ventromedial PFC has reciprocal projections with subcortical limbic structures contribute to the regulation of stress and ANS [11]. Dysfunction within the PFC can produce disturbances in cognitive performance, emotional responses, autonomic regulation, neurotransmission and neuroendocrine responses that are associated with stress disorders [11].

The SNS controls of the heart coming from the upper thoracic region of the spinal cord. Preganglionic fibers synapse with postganglionic sympathetic fibers and release acetylcholine, which binds to nicotinic receptors on the postganglionic fibers. Through sympathetic adrenergic efferent fibers extend to the sinoatrial and atrioventricular nodes in the heart where they release norepinephrine at synapses with beta-adrenergic receptors [12]. Stimulation of the SNS increases heart rate (positive chronotropy), ventricular contraction (positive inotropy), conduction velocity (positive dromotropy), and rate of relaxation (positive lusitropy).

The PNS control of the heart coming from vagal nuclei within the medulla oblongata in the brainstem, and efferent nervous outflow occurs via the 10th cranial nerve (vagus nerve). The long preganglionic efferent nerve fibers extent to the heart and synapse with a ganglia located near the sinoatrial and atrioventricular nodes. Acetylcholine is released, binds to nicotinic receptors, and activates short postganglionic efferent nerve fibers. These postganglionic fibers synapses with muscarinic receptors in the sinoatrial and atrioventricular nodes, and is activated by acetylcholine. For heart PNS decreases heart rate (negative chronotropy), force of atrial contraction (negative inotropy), rate of relaxation (negative lusitropy), and negative dromotropy [12].

The actions of the SNS and PNS are often opposing in their effects and normally the SNS and PNS activities are in dynamic balance thus indicating a healthy and flexible physiological system [13]. The autonomic imbalance described by increased SNS activity and suppressed PNS activity is associated with an increased risk of diseases [13]. Electrical stimulation of the prefrontal and cingulate cortex, left insula, lateral nucleus of hypothalamus decreased heart rate and blood pressure, whereas electrical stimulation of right insula, ventromedial nucleus of hypothalamus increased heart rate and blood pressure [14]. Stimulation of the basolateral nucleus of amygdala increases blood pressure and decreases heart rate; stimulation of the rostral nucleus of amygdala results in depressor effects and variable changes in heart rate [14].

The normal sympathovagal regulation induces an increase in heart rate during inspiration and decrease during expiration, and this physiological phenomenon is known as respiratory arrhythmia. The intrinsic heart rate is 105 beats/minute while resting heart rate is only 60–80 beats/minute, indicating that the heart is under "vagal dominance" [15]. The electrical signal produced by the heart can be measured with an electrocardiogram. Heart rate (HR) measures the numbers of consecutive heart beats in 1 min (beats per min). The analysis of consecutive sinus rhythm RR intervals is known as heart rate variability (HRV), a noninvasive electrocardiographic marker reflecting the activity of the ANS on sinus node function [16].

HRV parameters can be calculated in time domain (statistical and geometrical), frequency domain (power spectral density), and nonlinear measures. In time domain methods HRV parameters are standard deviation between normal intervals during recording—SDNN (ms), square root of the mean of the sum of the squares of differences between adjacent NN intervals—RMSSD (ms), percentage of differences between adjacent NN intervals differing more than 50 ms—pNN50% [16]. A lot of studies indicate that SDNN, RMSSD, and pNN50%, time domain indicators of the HRV, represent the activity of the vagal nerve.

Using simultaneously the Fast Fourier transform method and parametric– autoregressive method, HRV can be analyzed in frequency domain (power spectral analyses of HRV) in which can be measured low-frequency component (LF < 0.15 Hz) taken as an indicator of both vagal and sympathetic functions, high-frequency component (HF ≥ 0.15 Hz) as an indicator of parasympathetic function, very low-frequency component (VLF—the frequency band in the range 0.003–0.04 Hz), ultra-low-frequency (ULF—the frequency band below 0.003 Hz), and the total power (TP) [16]. The ratio of LF/HF is considered as an index of cardiac sympathetic/parasympathetic tone balance.

In addition to linear parameters, nonlinear parameters of HRV might be useful to identify patients prone to cardiac arrhythmia, thus a prognostic marker of cardiac function. The Poincaré plot is a visual representation of the dependence between successive RR intervals, first used as a qualitative tool [17] by fitting an ellipse to the shape of the Poincaré plot in order to calculate HRV indices [18]. This geometrical technique can be used to assess the dynamics of HRV by a representation of the values of each pair of RR intervals into a simplified phase space, describing the dynamics of a phenomenon that can recognize the hidden correlation patterns of a time series signal [19]. Each pair of successive elements in a time series (tachogram) is pictured into a

simplified Cartesian plane [20]. Series of these points at successive times outline a trajectory. This describes the system's evolution and therefore is commonly applied to assess the dynamics of HRV. Using this technique, SD1 and SD2 are the semi-axis of this ellipse. SD1 is related to the fast beat-to-beat variability, while SD2 describes the longer-term variability, SD1/SD2 showing the ratio of short-term to long-term interval variation. This quantitative method of analysis is based on the notion of different temporal effects of changes in the vagal and sympathetic modulation of the HR on the subsequent RR intervals without a requirement for a stationary quality of the data [21]. SD1 is considered a parasympathetic index of sinus node control being a measure of rapid changes in RR intervals, because vagal effects on the sinus node are known to develop faster than sympathetically mediated effects [22, 23] and SD2 is influenced by both parasympathetic and sympathetic tonus [24].

Other useful parameter is approximate entropy (ApEn), a measure of the disorder in the HR signal which quantifies the regularity and complexity of time series. Sample entropy (SampEn) is a less biased measure derived from approximate entropy [25], which quantifies signal complexity robustly within short time segments [26].

Detrended fluctuation analysis (DFA) is used to quantify the fractal scaling properties of R–R interval and has been validated for time series data [27]. DFA calculates the root-mean-square fluctuation of integrated and detrended time series, permits the detection of intrinsic self-similarity embedded in a non-stationary time series, and also avoids the spurious detection of apparent self-similarity [27]. The scaling exponent, the self-similarity a parameter represents the autocorrelation properties of the signal: <0.5 anti-correlated signal, large and small values of the time series are more likely to alternate; a = 0.5 corresponds to white noise, an a greater than 0.5 and less than 1.0 indicates positive autocorrelation in the signal such that a large inter-beat interval is more likely to be followed by large interval and vice versa. a = 1 represents 1/f noise; for a ≥ 1, correlations exist but cease to be of a power-law form and a = 1.5 indicates Brownian noise or random walk [28]. DFA plot is not strictly linear but rather consisted of two distinct regions of different slopes, there is a short range scaling exponent (a1) over periods of 4–11 beats (or 4–13), and a long-range exponent (a2), over longer periods (larger than 11 beats) [28].

Cardiovascular reflex tests based on HRV and blood pressure (BP) changes with stress are the most commonly used methods to detect cardiovascular autonomic neuropathy (CAN) [29]. The clinical autonomic function tests are carried out according to Ewing's battery, three

tests for heart rate variations which depend mainly on parasympathetic activity — heart rate deep breathing (HRDB), Valsalva ratio (VR) and lying-to-standing tests (30/15), and two tests for blood pressure (BP) response which depend mainly on sympathetic activity — diastolic blood pressure rise with sustained hand grip (ΔDBP) and postural hypotension on standing (ΔSBP). Each of these 5 tests was assigned a score of 0 for normal, 0.5 for borderline, and 1 for abnormal results, and the sum of these 5 scores made up the Ewing score, which was used to assess severity of cardiac autonomic neuropathy (CAN). A score of 2 or larger denoted CAN [29].

Autonomic dysfunction is underestimated and is found in a wide range of diseases such as Diabetes Mellitus, Parkinson Disease, Vascular Dementia, Alzheimer Disease, Multiple Sclerosis, etc. Medical examination is not effective for the early detection of the autonomic dysfunction, therefore it is important to identify the early stages of CAN with non-invasive and inexpensive techniques such as HRV and cardiovascular reflex test. These techniques should be part of the family doctor's consultation.

Epidemiological studies provide evidence that CAN determine a high mortality rate due to silent myocardial infarction, cardiac arrhythmia, cardiovascular and cardiorespiratory instability, stroke and sudden death [30, 31]. Patients with CAN may have intra- and perioperative cardiovascular instability that results in much higher perioperative cardiovascular mortality and an increased risk of anesthesia [33,34].

CAN is one of the main drivers of orthostatic hypotension (OH) and contributes to symptoms of orthostatic intolerance which can directly or indirectly increase the risk of falling [32]. Orthostatic hypotension may cause dizziness, weakness, visual disturbances and even syncope when switching from clino to orthostatism. Another common manifestation of CAN is coexistent orthostatic hypotension and supine hypertension which has been associated with target end-organ damage (eg, left ventricular hypertrophy and kidney dysfunction) and negative cardiovascular outcomes, such as the occurrence of stroke [32-34].

Vasovagal syncope and loss of consciousness is preceded by pallor, diaphoresis, nausea, abdominal discomfort, yawning, sighing, and hyperventilation, during emotional stress, or pain is another manifestation of CAN. This leads to cerebral and retinal hypoperfusion, auditory and cognitive difficulties [39].

Postural orthostatic tachycardia syndrome is found in autonomic denervation, recent viral illness, chronic fatigue syndrome, hypovolemia, hyperadrenergic stimulation, or hypervigilance

can produce lightheadedness, palpitations, tremulousness, generalized weakness, blurred vision, exercise intolerance, fatigue, presyncope, and syncope [40].

In middle-aged patients who participated in the American Atherosclerosis Risk in Communities Study (ARIC, 1987–2008), the presence of OH at baseline was associated with the increased risk of heart failure and stroke [41].

The autonomic failure or OH was associated with reverse or nondipping of nocturnal BP, arterial stiffness, left ventricular hypertrophy, peripheral arterial disease, attenuated HR variability, increased carotid intima-media thickness [42-46]. Impairment of autonomic sympathetic and parasympathetic function was an independent predictor of stroke [47].

During brain injury the heart is affected even in subjects with a previous normal heart functions. It is known that the stroke in right middle cerebral artery, affecting the right insula, is accompanied by increased incidence of cardiac arrhythmias, high catecholamine levels all of which can cause sudden death.

Reduced PNS and increased SNS activity can lower the threshold for myocardial ischemia, ventricular tachycardia, ventricular fibrillation, and sudden cardiac death in patients with coronary heart disease. On the other hand, heart failure was associated with altered sympathetic and parasympathetic activity, and that these dysfunctions might contribute to the progression of heart failure [48].

High heart rate variability (HRV) is associated with highly functional PFC inhibitory activity over subcortical structures which make the body to well adapt to the environment [49]. Low HRV is associated with reduced prefrontal inhibitory control over subcortical structures and failure to recognize safety signals [49]. Failure of inhibition leads to continue to process fear information and is linked with anxiety, and depression [50].

The blood flow distribution to different body regions is primarily regulated by the ANS through its control of vascular dilation and constriction. Sympathetic nerves fibers are found in the muscular and cholinergic nerve in the muscular and endothelial layers of vessel walls. It has been demonstrated that ANS denervation alters endothelial function in animal studies [51].

The SNS is influenced by factors regulating vascular function: NO, reactive oxygen species (ROS), endothelin (ET), the renin-angiotensin system [52].

NO has vasodilator, anti-inflammatory and anti-oxidant functions and acts as a sympathoinhibitory substance within the central nervous system [53]. NO released from the

endothelium inhibits central and peripheral SNS activity and increase central and peripheral PNS activity [52]. This suggests that NO released from endothelial cells may play a role in modulating the balance between the SNS and PNS branches of the ANS.

Normal endothelin levels may suppress SNS activity, whereas endothelin excess may enhance central and peripheral SNS and influence hemodynamic regulation by the baroreflex, chemoreflexes and vascular tone [52]. Also SNS stimulation can increase endothelin release.

Exaggerated SNS activity may impair endothelial function and enhance endothelium-mediated atherosclerosis. On the other hand since blood vessels provide nutrients for neurons and synapses endothelial dysfunction has a major impact on the autonomic nervous system. ANS dysfunctions can contribute to endothelial dysfunction and this leads to worsening ANS functions, creating a vicious circle that will aggravate endothelial functions and impair angiogenesis.

The renin-angiotensin aldosterone system (RAAS) through angiotensin II produce vasoconstriction, SNS activation, decrease of the baroreflex function, sodium and water reabsorption, inflammation, release of aldosterone, vasopressin and noradrenaline. Angiotensin II increases SNS activity and decreases parasympathetic drive [54]. Angiotensin II reduces NO and increase endothelin production. IL-1b, IL-6, TNF-alpha are able to stimulate renin and noradrenaline.

Recent studies suggest that sympathetic activity may influence coagulation factors and platelet activation and thereby increase the risk for further vascular events, such as myocardial infarctions, recurrent stroke, and deep venous thrombosis. The SNS could contribute to atherosclerosis by increased vasoconstriction or by stimulating platelet aggregation.

It is a well know that hypertensive patients are at high risk for coronary disease, less known is the fact that patients presenting with high sympathetic tone have a similar risk of developing coronary disease. Increase numbers and mean platelet volume, are important determinants of both the first myocardial infarction [53] and recurrent myocardial infarction [54]. Immediately after myocardial infarction large and hyperaggregable platelets have been found.

Elevated catecholamine may promote pro-coagulant processes by potentiating platelet activation, by increasing hemodynamic stress walls, or by inhibiting vascular eicosanoid synthesis. Adrenergic stimulation may not only lead to increased coagulation, but also to

increased fibrinolysis. In a study using thrombelastography were demonstrated changing from normal to hypercoagulable, to hypocoagulable and finally hyperfibrinolytic in severely trauma patients with high circulating levels of catecholamine that influence hemostasis [55].

It is interesting that in hypertensive patients, during and after myocardial infarctions patients, in current and recurrent stroke, were reported increased serum epinephrine levels [56]. Emotional stress increased blood levels of fibrinogen, von Willebrand factors, factors VII, VIII, and fibrin D-dimer, all of which can due to coronary occlusion [57].

Experimental studies in animals have shown that estrogens act centrally to modulate the ANS, increasing vagal and decreasing sympathetic activity [58]. Also they decreased endothelin levels and increased NO release from endothelium thus providing a cardiovascular protective function. Progesterone, on the other hand, appears to have an opposing effect, elevating central noradrenaline release. Given these effects, changes in progesterone and estradiol across the menstrual cycle may be associated with changes in ANS functions. Most studies have found decreased vagal activity in the mid-luteal compared with the mid-follicular phase [59].

Studies in the last decades demonstrate that the heart has an extensive intrinsic nervous system therefore was entitled as a “little brain” and is not just a pump but also an endocrine organ. Heart synthesizes the atrial natriuretic peptide, B-type natriuretic peptide, secrete oxytocin, and release of noradrenaline and dopamine [60]. Atrial natriuretic peptide or the “balance hormone,” plays an important role in fluid and electrolyte homeostasis. Also ANP inhibits the release of stress hormones and reduces SNS activity.

The ANS is a vast network of nerves affecting every organ in the body and is responsible for maintaining the balance between body and mind. Health is a result of the harmonic interchange between the SNS and PNS branches of ANS.

Acute stress response with predominant SNS activity is important for survival, and for achieving performance and various goals. But when this activation becomes chronic it can be detrimental to our health and well-being. Negative emotions are followed by dysregulation of the ANS with SNS predominance and withdraw of the PNS. If this negative emotional state is maintained for a long time, an exhaustion response with reduction of both sympathetic and parasympathetic systems activity was observed.

Emotional and cognitive functions result in ANS activation as proven using imagistic studies. Positive emotions and approach-related behavior are commonly associated with left

hemisphere activity, whereas the right hemisphere is more involved in the processing of negative emotions and withdrawal behaviors [61]. Positive states were also associated with lower cortisol levels, enhanced immune activity, and reduced risk of coronary heart disease, with high cardiac vagal tone and more adaptive patterns of emotional responding [62]..

Negative affect (stress, anxiety, depression) were associated with cardiovascular diseases, low HRV, inflammation, cancer progression and with shorter telomere length which can lead to replication mistakes and diseases [63-65].

This research direction has been materialized by publishing the following articles:

1. **Daniela Matei,** Bogdan Ignat, Cristian Dinu Popescu – "Autonomic dysfunction in type 2 diabetes mellitus with and without vascular dementia", The Journal of Neurological Sciences, Volume 325, issues 1-2, 2013, pages 6-9.
2. **Matei Daniela**, V. Constantinescu, C. Corciova, B. Ignat, R. Matei, CD.Popescu. Autonomic impairment in patients with migraine. European Review for Medical and Pharmacological Sciences 2015; 19:3922- 3927**.**
3. Victor Constantinescu, **Daniela Matei**, Dan Cuciureanu, Calin Corciova, Bogdan Ignat & Cristian Dinu Popescu. Cortical modulation of cardiac autonomic activity in ischemic stroke patients. Acta Neurol Belg., 2016; 116:473-480.
4. Constantinescu V., **Matei D**, Costache V et al. Linear and nonlinear parameters of heart rate variability in ischemic stroke patients. Neurologia i Neurochirurgia Polska, 2018; 52(2): 194-206.
5. Victor Constantinescu, Catalina Arsenescu-Georgescu, **Matei Daniela**, Mihaela Moscalu, Calin Corciova, Dan Cuciureanu. Heart rate variability analysis and cardiac dysautonomia in ischemic stroke patients. Clinical Neurology and Neurosurgery 186 (2019) 105528.
6. Constantinescu V., **Matei D,** Ignat B, Hodorog D; Cuciureanu DI Heart rate variability a useful tool to assess poststroke cardiac dysautonomia. Neurologist, 2020, Volume: 25, Issue 3, pag. 49-54.
7. **Matei D**, Luca C, Andritoi D, Fuior R, Corciova C. Autonomic dysfunction and peripheral nerve involvement in patients with Parkinson's disease. Balneo Research Journal, 2019; 10(1):55-61.

1.2. Autonomic dysfunction in type 2 diabetes mellitus with and without vascular dementia

1.2.1. Introduction

Vascular dementia (VaD) is the second most common form of dementia, after Alzheimer's disease (AD) [66]. Several studies suggest that the risk of developing dementia is increased when a patient is exposed to vascular risk factors such as hypertension, diabetes mellitus (DM), peripheral arterial disease, and smoking, which usually are associated with cerebrovascular disease and vascular dementia [67]. Cardiovascular autonomic neuropathy (CAN) is one of the most common complications of DM, but detection of CAN is not a practical screening method for a large number of diabetic patients [68]. Research has shown that increased activity of the sympathetic nervous system (SNS) is associated with an increased risk of cardiovascular events, such as myocardial infarction, stroke or sudden cardiac death [68].

1.2.2. Aim

The objectives of the study were to examine the associations between vascular,metabolic risk factors, autonomic and cognitive function in patients with type 2 DM.

1.2.3. Patients and Methods

45 participants with type 2 DM and 23 age related normal subjects were investigated in our study. Diagnosis and classification of diabetes were based on guidelines of the Expert Committee Report of the American Diabetes Association [69]. The subjects under study were in the age group of 65–85 years and the duration of diabetes was 10–25 years. Patients with myocardial infarction, acute brain injury, arrhythmias, atrioventricular block or bundle branch block and frequent extrasystoles were excluded from the study. Inclusion criteria for the controls were the absence of any history of diabetes, normal levels of fasting serum glucose, normal cognitive status and with two normal consecutive electrocardiograms in the course of one month. Any individuals who were taking drugs known to affect autonomic nervous system activity (ANS) were also excluded from the study. The study was carried out in accordance with the Helsinki Declaration.

Height, weight and body circumferences, systolic and diastolic blood pressure were measured in all subjects; body mass index (BMI, kg/m2) was calculated as weight divided by height squared; waist-to-hip ratio (WHR) was defined as waist circumference divided by hip circumference. History and evidence of cardiovascular disease, hypertension, past history of heart attacks, peripheral vascular disease, and strokes were recorded. In all patients there were measured: fasting blood glucose, total cholesterol, high density lipoprotein cholesterol (HDL), and triglycerides.

Mini-Mental Status Examination (MMSE) was used in screening cognitive status of DM patients. MMSE assesses a broader range of functions, such as the examination of attention and concentration, the evaluation of the orientation capacity to time and place, instantaneous recall, short term memory, writing and constructional capacities, the use of language and executive functions. A score of less than 23 out of 30 were considered evidence of significant cognitive impairment. In 15 patients with DM we found cognitive impairment. For diagnosed VaD all suspected dementia cases were analyzed according to the criteria of the: NINDS-AIREN (National Institute for Neurological Disorders and Stroke – Association Internationale pour la Recherche et l'Enseignement en Neurosciences [70], Ischemic Score of Hachinski [71] and modified ischemic score (including computer tomography — CT or magnetic resonance imaging — MRI). Diagnosis of VaD according to NINDS-AIREN criteria implies a diagnosis of dementia plus a diagnosis of cerebrovascular disease with history of cerebro-vascular disease (over the last 3 months), neurological examination and neuroimaging. The Hachinski ischaemic score is based on the multi-infarct concept of VaD and may not perform as well in detecting other subtypes of VaD. It has been modified to include CT or MRI findings. VaD clinically manifests through: history of vascular disease, abrupt onset, stepwise course, preservation of judgment, focal neurological signs, mixed cortical-subcortical features, emotional incontinence.

The absence of cerebral vascular lesions on CT or MRI excludes the diagnosis of VaD. Features on CT or MRI that are suggestive of VaD include cortical or subcortical infarctions, multiple lacunar strokes and white matter hyperintensities. After these examinations were performed, 11 DM patients were diagnosed with VaD. 4 DM patients were diagnosed with other forms of dementia and they were excluded from the study.

Using BIOPAC Acquisition System, we monitored the HRV in basal condition and during Ewing's tests [29]. HRV was analyzed following the recommendations of the Task Force

of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [16]. Clinical autonomic function tests were carried out according to Ewing's battery, three tests for heart rate variations which depend mainly on parasympathetic activity — heart rate deep breathing (HRDB), Valsalva ratio (VR) and lying-to-standing tests (30/15), and two tests for blood pressure (BP) response which depend mainly on sympathetic activity — diastolic blood pressure rise with sustained hand grip (ΔDBP) and postural hypotension on standing (ΔSBP). A score of 2 or larger denoted CAN. Evaluation of the tests, which depend on changes in heart rate, was performed using published tables based on age [16]. Short time ECG data were digitized and stored on computer for subsequent off-line analysis.

From these measurements using simultaneously fast Fourier and Wavelet transform, HRV parameters were calculated. The ectopic bits or artifacts were manually edited. Time-domain parameters used were Mean–R–R, SDNN, RMSSD, and pNN50 %. Frequency Domain HRV measured were LFb, HF (as an indicator of parasympathetic function), VLF associated with the slow regulation mechanism such as thermoregulations and Total Power (TP) [16]. We analyzed LF and HF power, LF/HF ratio (considered an index of cardiac sympathetic/ parasympathetic tone balance).

Statistical analyses were performed using SPSS, version 4.0.1 (SPSS, USA) and EPI INFO V 6.01 program. The results were expressed as mean±standard deviation. Test t — Student or variance analysis (ANOVA) was used to determine the differences between the groups.
The standard linear regression analysis and the Pearson correlation coefficient r were used for determining relationship between parameters. The values <0.05 were considered statistically significant.

1.2.4. Results

The patients were divided as follows: group N (n=23) included normal controls, group DM (n=30) included DM patients without VaD and group VaD (n=11) included DM patients with VaD. The demographic and clinical data of subjects are shown in Table 1. The diabetes duration was much longer in VaD than in DM patients ($p<0.05$). The BMI ($p<0.05$), fasting blood sugar, HbA1c, triglyceride ($p<0.001$), total cholesterol ($p<0.01$), systolic blood pressure ($p<0.05$) and resting heart rate ($p<0.01$) for the DM patients were much elevated compared with

control group but more reduced in VaD patients ($p<0.05$). VaD subjects had HDL-cholesterol more reduced ($p<0.001$) compared with control group (Table 1).

Parameters	**N** n=23	**DM** n=30	**VaD** n=11
Age (years)	74.2±2.9	72.5 ±3.08	74.1±2.47
Diabetes duration (years)	-	15.4±3.28	18.4±2.1•
BMI (Kg/m²)	25.38±2.11	28.5±3.97*	26.85±2.27
Waist/hip ratio	0.83±0.04	0.88±0.03*	0.85±0.04
Fasting blood sugar (mg/dl)	86.8±14.1▪▪	138.9±32.6***	123.5±9.52•
HbA1c (%)	3.92±0.44▪▪	7.84±0.44***	6.91±0.72•
Total cholesterol (mg/dl)	173.2±9.5▪	211.2±21.1**	189.1±17.82•
HDL-cholesterol (mg/dl)	50.5±2.29▪▪▪	46.6±2.91**	43.5±3.35
Triglyceride (mg/dl)	124.3±21.3▪▪	184.2±31.2***	161.3±14.36•
SBP (mmHg)	121.2±10.18▪	136.7±17.83*	129.6±8.31
DBP (mmHg)	65.7±12.1	72.18±15.98	68.1±12.4
Resting HR (beat/min)	62.1±7.85▪	69.3±10.8**	67.8±11.7

BMI – body mass index, glycosylated haemoglobin -HbA1c, SBP - systolic blood pressure, DBP - diastolic blood pressure, HR-heart rate
Data: expressed as mean ± standard deviation;
*- p< 0.05; ** - p< 0.01; *** - p< 0.001 for difference between controls and DM without VaD;
•- p< 0.05; •• - p< 0.01; ••• - p< 0.001 for difference between DM without and with VaD
▪ - p< 0.05; ▪▪ - p< 0.01; ▪▪▪ - p< 0.001 for difference between controls and DM with VaD

Table 1 Clinical and biochemical features of the groups

The prevalence of CAN was 56.6% in DM patients and 81.8% in VaD patients. The averages of results obtained in the parasympathetic tests (HRDB, 30/15, VR) for VaD patients are statistically significant lower than the averages for the control group ($p<0.001$, $p<0.01$, $p<0.001$ respectively) (Table 2). Patients with VaD had impaired HRDB ($p<0.001$), 30/15 ($p<0.01$) and VR ($p<0.01$) in comparison with DM patients in univariate ANOVA. VaD patients had a greater fall in blood pressure on standing than controls ($p<0.001$) and DM patients ($p<0.05$). Patients with VaD had reduced blood pressure responses to isometric exercise in comparison with controls ($p<0.001$).

In Pearson correlation the HRDB test correlated negatively with body mass index (BMI) in VaD ($r=-0.78$, $p<0.001$). HRDB, VR correlated negatively with diabetes duration ($r=-0.63$, $p<0.002$) and ($r=-0.35$, $p<0.017$). 30/15 correlated negatively with systolic blood pressure ($r=-0.75$, $p<0.001$) in VaD patients.

	N n=23	DM n=30	VaD n=11
PARASYMPATHETIC CLINICAL AUTONOMIC FUNCTION TESTS			
Mean change in HR during deep breathing (HRDB)	9 ±1.58▪▪▪	7.6±1.32	5.8±1.45•••
Mean HR response to standing (30/15 ratio)	1.2±0.2▪▪	1.07±0.04*	1.01±0.02••
Mean Valsalva ratio (VR)	1.42±0.08▪▪▪	1.3±0.08*	1.21±0.06••
SYMPATHETIC CLINICAL AUTONOMIC FUNCTION TESTS			
Mean fall in SBP on standing (ΔSBP) (mmHg)	29.9±2.65▪▪▪	38.16±3.75***	42.72±4.69•
Mean change in DBP on isometric exercise (ΔDBP) (mmHg)	25.3±5▪▪▪	21.56±3.34	16.4±3.3••

HR-heart rate, SBP-systolic blood pressure, DBP-diastolic blood pressure
Data: expressed as mean ± standard deviation;
*- p< 0.05; ** - p< 0.01; *** - p< 0.001 for difference between controls and DM without VaD;
•- p< 0.05; •• - p< 0.01; ••• - p< 0.001 for difference between DM without and with VaD
▪ - p< 0.05; ▪▪ - p< 0.01; ▪▪▪ - p< 0.001 for difference between controls and DM with VaD

Table 2. Clinical autonomic function tests

Table 3 shows the comparison of measures in time and frequency domain analysis of HRV between the three groups. The SDNN, pNN50% showed statistically significant reduction in the VaD groups when compared to the control group ($p<0.001$). Also we found a reduction in SDNN ($p<0.05$) and pNN50% ($p<0.01$) in VaD patients compared with DM group. The systolic blood pressure correlated significantly negatively with SDNN ($r=-0.40$, $p<0.01$). There was a significant positive correlation between systolic blood pressure and the RMSSD ($r=0.44$, $p<0.01$) and the pNN50 ($r=0.39$, $p<0.05$). Low and high frequency power were reduced in patients with VaD ($p<0.01$, $p<0.0001$, respectively) in comparison with healthy controls. There were no differences between the VaD and DM patients in low frequency power. The LF/HF ratio was lower in the healthy subjects than in the DM ($p<0.001$) and in VaD ($p<0.0001$) subjects (Fig. 1). Increase in the LF/HF component ratio in VaD, DM patients indicated a vagal–sympathetic dysfunction; VaD patients ($p<0.05$) were more affected in comparison with DM patients. Analyzing the frequency domain measures of heart rate variability, diminished heart rate variability correlated significantly negatively with mean systolic blood pressure (LF: $r=-0.39$,

p<0.05). In the VaD subjects, the LF/HF ratio was correlated significantly with the BMI (r=0.41, p<0.01) and with HbA1c (r=0.45, p<0.02).

Parameters	**N** n=23	**DM** n=30	**VaD** n=11
R-R (ms)	918±84.02▪▪	836.7±125.7*	757.5±98.2
SDNN (ms)	163.6±16.4▪▪▪	154.1±17.42**	144.7±11.8•
RMSSD (ms)	34.9±3.2▪	32.28±3.8	27.9±2.12•
pNN50%	23.7±1.49▪▪▪	22.7±2.7	18.5±2.24••
LF (ms²)	616.7±54.37▪▪	560.8±49.64*	527.6±61.68
HF (ms²)	824.8±94.15▪▪▪	661.4±56.1**	575.7±58.4••
LF/HF	0.74±0.06▪▪▪	0.83±0.03***	0.91±0.07•

SDNN- Standard deviation of all NN intervals, RMSSD- square root of the mean of the sum of the squares of differences between adjacent NN intervals , pNN50%-percentage of differences between adjacent NN intervals differing more than 50 msec, LH- low frequency component, HF- high frequency component.
Data: expressed as mean ± standard deviation;
*- p< 0.05; ** - p< 0.01; *** - p< 0.001 for difference between controls and DM without VaD;
•- p< 0.05; •• - p< 0.01; ••• - p< 0.001 for difference between DM without and with VaD
▪ - p< 0.05; ▪▪ - p< 0.01; ▪▪▪ - p< 0.001 for difference between controls and DM with VaD

Table 3 Time and frequency domain analysis of heart rate variability

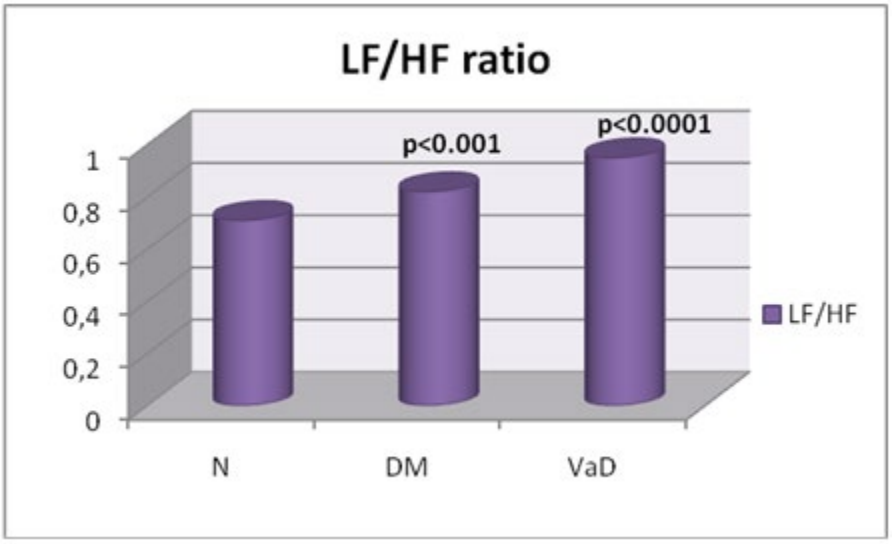

Fig.1 LF/HF ratio in study groups

1.2.5. Discussion

Diabetes mellitus is associated with a large number of chronic complications which finally result in a premature mortality. Type-2 diabetes is associated with increased inflammation, increased oxidative stress, advanced glycosylated end products, macro and microvascular injury, decreased neurogenesis, reduced neuronal repair, neuronal damage [69, 72]. DM predisposes to impairments in autonomic nervous system (ANS) regulation or endothelial function. Impairments in ANS regulation may contribute to abnormal changes in endothelial cells, resulting in endothelial dysfunction, or impairments in endothelial function may lead to dysfunction in ANS regulation. Endothelial dysfunction is an important early event in the pathogenesis of atherosclerosis, contributing to plaque initiation and progression. Nitric oxide (NO), relaxing factors released by the endothelium, contributes to cerebral arterial and arteriolar dilatation, increases in cerebral blood flow (CBF), and decreases in cerebral vascular resistance. Endothelin-1 is one of the contracting factors released by the endothelium. Insulin resistance decreases NO and increases endothelin-1 activity, favoring vasoconstriction and reducing capillary recruitment [73].

Vascular dementia can be defined as a dementia syndrome likely to be the consequence of lesions of the brain, vascular in origin, irrespective of their ischemic, hemorrhagic or hypoxic nature. Subcortical ischemic vascular dementia refers to lesions that involve the basal ganglia, cerebral white matter and the brainstem and is the most common cause of cognitive decline and VaD in the elderly [74]. Decreased CBF destabilizes synaptic connections and neuronal activity in regions involved in cognitive function (limbic regions, association areas, white matter that links association areas), also in regions involved in autonomic control. White matter lesions can disrupt efferent projections from the nucleus basalis of Meynert resulting in cortical cholinergic denervation [75].

It has been shown that an increase in short-term BP variability is associated with cognitive dysfunction [76]. In patients with CAN, vagal impairment can lead to a predominance of SNS activity which stimulates the renin–angiotensin–aldosterone system and increases heart rate and peripheral vascular resistance.

Reduced HRV is the earliest indicator of CAN [77]. A recent study reported that presence of CAN, assessed by standard HRV testing, was one of the strongest predictors of ischemic stroke together with age and hypertension [47]. Other studies have demonstrated that an

unbalanced sympathetic/parasympathetic tone, with a prevalence of sympathetic activity, is associated with higher cardiovascular mortality in type 2 diabetic patients [78, 79]. In our study the prevalence of CAN in VaD patients was high (81.8 %), cardiovascular reflex tests have shown both sympathetic and parasympathetic failure in VaD disease.

A possible limitation of our study is the presence of a small number of subjects. This decreases the statistical power of our study to detect differences between the groups. These results should be confirmed in larger studies specifically addressing the relationship between vascular, metabolic risk factors, autonomic nervous activity and cognitive function. Several subsequent autopsy series have confirmed that patients with dementia have both AD and vascular changes, and pure vascular dementia or pure AD is almost non-existent particularly among the elderly [80]. Other studies suggest that brain ischemia promotes the production of beta-amyloid, a key player in AD pathogenesis [81]. This could be another limitation of our study because we agree that most elderly subjects have multiple brain pathologies. A lot of studies indicate that SDNN, pNN50%, time domain indicators of the HRV, represent the activity of the vagal nerve. In the frequency domain, HF is an index of cardiac vagal nerve tone, while LF represents the SNS activity with vagal modulation. It has been established that LF/ HF ratio is a more sensitive measure of increased of sympathetic activity because the vagal modulation affects LF significantly [29]. The analysis of HRV demonstrated that both parasympathetic and sympathetic nerve functions were impaired in DM and VaD patients because the measures of SDNN, pNN50%, HF and LH were significantly lower in DM, VaD groups than in controls. Also significant difference of LF/HF ratio between the three groups suggested that great sympathetic dysfunction was found in VaD patients compared with DM ($p<0.01$) and controls ($p<0.001$). The data reported in the present study indicate that the sympathovagal balance (expressed by the LF/HF ratio) remains consistently altered with a sympathetic overactivity in all VaD subjects.

1.2.6. Conclusions

Using standard cardiovascular reflex tests and analysis of HRV we demonstrated an impairment of the autonomic nervous system in VaD patients with marked parasympathetic dysfunction and sympathetic predominance.

1.3. Autonomic impairment in patients with migraine

1.3.1. Introduction

Migraine is a chronic neurovascular disorder characterized by intermittent attacks of severe headache with or without aura. Typically the headache is unilateral and lasts from 2 to 72 hours [82]. The autonomic nervous system (ANS) involvement is suggested by many symptoms and signs including nausea, diarrhea, cold extremities, light and sound sensitivity or dizziness during attacks. Some people with migraine headaches perceive an aura: a transient visual, sensory, language, or motor disturbance which signals that the headache will occur [83]. Migraine, and specifically migraine with aura, has been associated with increased risk of ischemic stroke, particularly among young women [84]. Abnormalities in the SNS or PNS have been found in migraine patients during the headache-free phase [85].

1.3.2. Aim

The aim of the study was to investigate cardiac autonomic function in teenagers with and without migraine by using 24-hour ambulatory ECG monitoring with HRV evaluation while also assessing the severity of this dysfunction and its relation to the type of migraine.

1.3.3. Patients and Methods

Twenty-seven subjects (mean age of 26.7 ± 2.12 years) with migraine were evaluated during the pain-free period; 10 with migraine with aura (MA) and 17 with migraine without aura (M). We confined the study to women aged 20 to 35 years who had suffered from migraine for more than 1 year and had at least one migraine attack per month. The diagnosis of migraine was made using criteria of the International Classification of Headache Disorders 2nd Edition (ICHD-II) [86]. The control group (C) consisted of 10 age and sex-matched healthy control subjects. Patients who were on any prophylactic headache treatment or had a systemic disease that might interfere with heart rate such as: cardiovascular or neurological diseases, endocrine dysfunction or pregnancy were excluded.

All the subjects underwent detailed history taking and neurological examination. Migraine cases underwent all examinations in a headache free period (> 3 days after a migraine attack). Subjects were assessed by interview upon smoking, alcohol intake, oral contraceptives

use. The average alcohol intake in the past year was based on question inquiry upon drinking frequency and quantity of drinks per occasion and categorized into none, moderate (1-3 drinks/day) and high (>3 drinks/day).

Weight and height were used to calculate body mass index (BMI – weight [kg]/height^2 [m]). In all subjects there were measured: fasting plasma glucose, total cholesterol, high density lipoprotein cholesterol and triglycerides. Blood pressure (BP) was measured using an electronic device.

The psychological evaluation included two self-reporting questionnaires aimed at assessing anxiety and depression. The questionnaire to assess anxiety consisted of Beck Anxiety Inventory (BAI), which is a 21-item scale with a scoring range from 0 to 63 points, high scores indicating a more severe anxiety. In order to measure the severity of depression we used Beck depression inventory (BDI-II) which is composed of a 21-item rated on a 4-points scale and a total score results after ratings summation for the individual items. The total score ranges from 0 to 63 points. Higher scores indicate greater depressive symptoms [87].

Autonomic nervous system function was evaluated by HRV analysis during 24-hour ambulatory electrocardiographic (ECG) recording. The ectopic bits or artifacts were manually edited. We measure time-domain and frequency domain parameters.

Statistical analyses was performed using STATISTICA 6.0. The comparison of demographic data between the groups of migraine patients and control was performed using Chi-square test (for dichotomous and categorical data, e.g. alcohol consumption, cigarette smoking) and Student's *t*test or variance analysis (ANOVA) for continuous data. The results were expressed as mean ± standard deviation. The Pearson correlation coefficient r was used for determining the relationship between parameters. The $p < 0.05$ value was considered statistically significant.

1.3.4. Results

The patients were divided as follows: group C (n=10) included controls, group M (n=17) included migraine without aura patients and group MA (n=10) included migraine with aura patients. The demographic, clinical and biochemical data of subjects is shown in Table 4. There was no significant differences regarding age between the three groups (F=0.3, p=0.681). The average age was 26.1±2.5 years in the control group, 26.7±2.12 years in patients with M, and

25.8±2.3 years in MA. Body mass index was 24.8±2.11 kg/m² in C, 24.2±2.7 kg/m² in M patients, and 23.5±2.5 kg/m² in MA patients with no significant differences between groups, p=0.772. Positive family history of migraine was reported in both groups of migraine patients (M 35.2%, MA 40%) with p<0.05 compare with control group.

Medical family history proved that patients with migraine with aura had more hypertension cases (p<0.01) in their family compared with control group. Alcohol consumption and smoking were reduced with no significant difference between groups (alcohol p = 0.726, smoking p=0.562). The length of migraine attack (h) was more prolonged in migraine with aura compared to migraine without aura (16.3±11.2 h vs. 9.4 ± 6.1 h, p<0.05). Migraine with aura sufferers had increased triglycerides value (p<0.05) compared to control group. There was no significant difference in fasting blood sugar, total cholesterol and HDL cholesterol values between the study groups. Systolic blood pressure levels and heart rate did not differ within the groups. Diastolic blood pressure was increased in subjects with migraine with aura compared to normal controls (89.7±11.8 mmHg vs. 77.2±11.1 mmHg, p<0.05).

BAI was 9.88±5.01 in C vs. 14.93±9.23 in M patients, p<0.152 and 25.88±14.92 in MA patients with p<0.007 when compared to C group and p<0.050 when compared to M group. Minimal or mild anxiety (score 0-15) was found in 9 patients (90%) in control group, 12 patients in M group (70.5%) and in 5 patients with MA (50%). Moderate anxiety (score 16-25) was found in 1 patient of C group (10%), in 5 patients of M group (29.5%) and in 3 MA patients (30%). Severe anxiety (scores 26-63) was found in 2 MA patients (20%). BDI was 9.11±4.67 in controls vs. 10.06±7.23 in M patients with p=0.727 and 19.3±11.85 in MA, with p<0.028 when compared to controls and p<0.022 when compared to M patients. Minimal or mild depression (score 0-19) was found in all patients in control group, in 16 migraine patients without aura (94.1%) and in 7 (70%) migraine with aura patients. Moderate depression was found in 1 patient of M group and in 3 patients of MA. We found an increased frequency of anxiety and depressive symptoms in migraine patients, especially in migraine with aura group.

Time-domain and frequency parameters of HRV can be observed in Table 5. Mean heart rate (HR) beat/minute and RR during daytime did not differ between groups, but the values of night recordings showed increased HR among migraine with aura patients when compare with control group (MA: 70.1±9.1 beat/min vs. C: 64.2±9.4 beat/min, p<0.05) and RR reduction among the same patients (MA: 859.1±108.37 ms vs. C: 923.6±84.5 ms, p<0.02). SDNN during

day time was found to be lower in migraine with aura than controls (MA: 87.11±35.2 ms vs. C: 92.6±34.7 ms, $p<0.05$). During night period SDNN was decreased in both migraine groups (MA: 69.5±37.6 ms vs. C: 83.4±23.6 ms, $p<0.01$; M: 76.7±38.1 ms vs. MA: 69.5±37.6 ms, $p<0.05$). Migraine with aura had lower RMSSD than controls both day and night periods (day-MA: 43.6±8.3 ms vs. C: 49.6±11.7 ms, $p<0.03$; night- MA: 51.6±7.3 ms vs. C: 63.2±16.2 ms, $p<0.001$), and also was lower than in migraine group without aura during night ($p<0.05$).

Parameter	**C** N=10	**M** N=17	**MA** N=10
Age (years)	26.1±2.5	26.7±2.12	25.8±2.3
BMI (Kg/m²)	24.8±2.11	24.2±2.7	23.5±2.5
Medical family history (%)			
- migraine	10 †	35.2	40*
- diabetes mellitus	20	23.5	20
- hypertension	20 †	47.05	60*
High alcohol use (>3 drinks/day) (%)	10	5.88	10
Smoking (%)	20	29.4	20
Duration of migraine attack (h)	-	9.4±6.1‡	16.3±11.2
Fasting blood sugar (mg/dl)	83.8±16.1	88.1±18.3	76.2±14.1
Total cholesterol (mg/dl)	169.2±9.5	167.2±11.5	171.2±8.3
HDL-cholesterol (mg/dl)	52.5±2.2	52.1±2.7	49.7±2.2
Triglyceride (mg/dl)	109.1±12.7	113.1±17.3	142.5±23.8*
SBP (mmHg)	126.2±21.2	131.2±29.1	128.1±.21.5
DBP (mmHg)	77.2±11.1	78.1±10.7‡	89.7±11.8*
HR (beat/min)	69.1±7.2	68.5±9.1	70.4±7.5
BAI	9.88±5.01	14.93±9.23‡	25.88±14.92*
BDI	9.11±4.67	10.06±7.23‡	19.3±11.85*

Data are expressed as number (%) or as means ± standard deviation; BMI - Body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, HR – heart rate, BAI- Beck anxiety inventory, BDI- Beck depression inventory.
† $p<0.05$ for difference between migraine without aura groups and controls
* $p< 0.05$ for difference between controls and migraine with aura groups
‡ $p<0.05$ for difference between migraine with and without aura groups

Table 4. Clinical and biochemical features of the groups

LF in migraine groups was increased during night period compared with normal subjects (MA: 59.11±12.1 nu vs. C: 47.3±11.2 nu, $p<0.01$) and in migraine without aura (51.3±5.5 nu, $p<0.05$). HF during day period was reduced in MA group when compared with control group (MA: 32.6±11.4 nu vs. C: 36.4±11.7 nu, $p<0.05$). HF during night was 41.5±10.1 nu in migraine

with aura vs. 54.6±10.2 nu in control with $p<0.01$ and 49.7±11.2 nu in migraine without aura with $p<0.05$. LF/HF was increased during night in MA patients 1.7±0.5 vs. 1.2±0.5 in controls, $p<0.04$.

Using Pearson correlation analysis the SBP was correlated with BMI (r= 0.85, $p<0.003$) and with LF (r= 0.42, $p<0.02$). HF spectral value of HRV was negatively correlated to triglycerides (r= -0.45, $p<0.05$), with BDI score (r= -0.79, $p<0.006$), and BAD score (r= -0.81, $p<0.001$). BDI score was also correlated with LF/HF (r= 0.73, $p<0.003$).

Parameter	**C** N=10	**M** N=17	**MA** N=10
Mean HR (beat/min)			
- day	76.4±11.6	78.1±7.91	77.1±11.8
- night	64.2±9.4	65.5±10.7	70.1±9.1*
Mean RR (ms)			
- day	797.3±124.4	829.2±95.5	820.6±74.06
- night	923.6±84.5	897.5±76.2	859.1±108.3*
SDNN (ms)			
- day	92.6±34.7	89.3±24.9	87.11±35.2*
- night	83.4±23.6†	76.7±38.1‡	69.5±37.6*
RMSSD (ms)			
- day	49.6±11.7	47.7±10.9	43.6±8.3*
- night	63.2±16.2 †	56.5±11.7‡	51.6±7.3*
pNN50%			
- day	11.7±8.6	9.9±8.2	9.2±6.4
- night	22.7±14.1	21.3±6.1	19.7±11.7
LF (nu)			
- day	68.1±10.4	65.6±8.21	67.3±8.8
- night	47.3±11.2 †	51.3±5.5‡	59.11±12.1*
HF (nu)			
- day	36.4±11.7	37.6±13.1	32.6±11.4*
- night	54.6±10.2 †	49.7±11.2‡	41.5±10.1*
LF/HF			
- day	2.1±0.7	2.1±0.5	2.2±0.5
- night	1.2±0.5	1.3±0.5	1.7±0.5*

Data: expressed as means ± standard deviation;
† p<0.05 for difference between migraine without aura groups and controls
*- P< 0.05 for difference between controls and patients with migraine with aura;
‡ - P< 0.05 for difference between migraine with aura and migraine without aura

Table 5. Time and frequency domain HRV parameters

1.3.5. Discussion

The migraine pathogenesis is not completely understood, several theories, such as the vascular theory, neuronal excitation, neurotransmitter levels variations, trigeminal sensory–

parasympathetic reflex or autonomic dysfunction have been proposed as possible disease pathways.

The autonomic nervous system imbalance, characterized by sympathetic hyperfunction and parasympathetic hypofunction was reported in patients with migraine [88, 89]. Low HRV is associated with reduced prefrontal inhibitory control over subcortical structures such as the amygdala and is linked with anxiety and depression [15].

In our study we found an increased frequency of anxiety and depressive symptoms in migraine patients, especially in migraine with aura group. In this study, we tried to analyze the ANS involvement in migraine using HRV on long-term 24-hour ECG. Time and frequency-domain analysis of HRV was achieved for two periods: diurnal (7-12 a.m.) and nocturnal (0-6 a.m.). The significant decrease of SDNN, RMSSD and HF indicates parasympathetic dysfunction in migraine groups during night headache free periods, most affected were migraine with aura patients. Also LF in both migraine groups was increased during the night period compared with normal subjects ($p<0.01$ for MA and $p<0.05$ for M group). LF/HF was increased during night in MA patients 1.7±0.5 vs. 1.2±0.5 controls, $p<0.01$. In both groups of migraine patients we discovered an autonomic nervous system dysfunction, the most marked SNS and PNS impairment being present in the group of migraine with aura sufferers. In these groups we showed the highest BDA and BDI scores. In MA patients we pointed out the sympathetic component predominance associated with parasympathetic hypo-activation especially at night with loss of circadian rhythms.

Stress, depression and poor sleep quality can contribute to the occurrence of migraine [90]. Most migraine attacks start or end at night. It is not clear why but serotoninergic and dopaminergic dysfunction, hormonal fluctuations, central sensitization or drugs over-use are some of the discussed pathophysiological mechanisms [91].

When asked about sleep quality and dreaming, migraine patients complained about bad sleep quality. The vast majority also experience negative sensations such as anxiety, fear or terror and contents such as perception of fall, unsuccessful efforts to do various things, fights, death of relatives, etc. These observations suggest that there is some malfunction in the prefrontal cortex, limbic system, amygdala, and hypothalamus, elements involved in dream and migraine pathophysiology [92]. Activation of the limbic system, amygdala, and anterior cingulate cortex observed in rapid eye movement sleep are involved in cardiovascular regulation

and could reflect responses to intense emotions suck as fear and anxiety found in migraine patients during night [93]. Individuals with high level of stress, anxiety and depression display an imbalance between PNS and SNS activity what was revealed in our study.

A possible limitation of our study is the presence of a small number of subjects. This decreases the statistical power to detect differences between the groups, not allowing us to generalize our results. These results should be confirmed in larger studies specifically addressing the relationship between autonomic functions and migraine. The use of self-report instruments in order to diagnose depression and anxiety was another shortcoming.

1.3.6. Conclusions

Reduced parasympathetic activity with sympathetic predominance was found in migraine patients more pronounced during the night period, most affected were those with migraine with aura. The current findings suggest that anxiety and depression are more likely to be associated with reduced cardiac vagal modulation.

1.4. **Heart rate variability in stroke patients**

1.4.1. **Introduction**

Stroke represents a major public health issue, being worldwide, the second cause of death, after ischemic heart disease and the first cause of long term acquired disability [94]. In Europe, the incidence of stroke varies, being estimated between 100 and 200 new cases for each 100,000 inhabitants annually, variations that depend on the importance of risk factors, among which hypertension plays a major role [95].

Approximately, 40 % of stroke patients present a risk of recurrence in the first 5 years and a higher risk for myocardial infarction, uncontrolled hypertension, cardiac arrhythmias and cardiogenic shock [96]. Cardiac arrhythmias, especially malignant ventricular arrhythmias, are frequently in acute stroke due to autonomic dysfunctions, triggered by the impairment of the central autonomic nervous system structures and catecholamine storm [97]. Increased sympathetic nervous system and reduced parasympathetic nervous system activity is common in acute stroke patients [98].

Animal studies support the asymmetry in central nervous control of cardiac function showing that experimental stroke in right hemisphere induced more pronounced sympathetic

effects than lesions on the left side [99, 100]. Both human studies and experimental data revealed that insular cortex, anterior cingulate gyrus, hypothalamus and amygdala may be involved in central autonomic nervous system regulation [6, 101]. Lesions at these levels might be held responsible of cardiac arrhythmias. After acute cerebrovascular events arrhythmias and electrocardiographic abnormalities are common, even in the absence of structural heart disease, with a high incidence of dysautonomia [102, 103].

Identifying high risk patients prone to develop neurogenic cardiac complications, by better understanding dysautonomia pathophysiology, and further implementation of adequate prophylactic and therapeutic measures, may significantly reduce mortality rate in stroke patients. The influence of stroke's hemispheric lateralization in cardiovascular autonomic dysregulation has been illustrated using modern neuroimaging data. An acute ischemic lesion involving the cortical network controlling the activity of the autonomic nervous system may imbalance autonomic responses at cardiac level and lead to an increased risk of arrhythmia.

Quantification of HRV represents a non-invasive method of sympathovagal balance evaluation. HRV evaluation may be completed by conventional time and frequency- domain analysis methods, by analyzing the spectral power, or using non-linear analysis that may indicate sensitive adjustments in the dynamics of heart rate. The increase of sympathetic and the decrease of parasympathetic activity are closely interrelated, raising the risk of cardiac arrhythmia. A low HRV is associated with an increased risk of cardiac arrhythmias and sudden death, being a mortality predictor [104].

After an ischemic stroke in the right middle cerebral artery (MCA) territory, particularly with right insular cortex involvement, the total spectral power of the variability of the heart rate is reduced [105]. Right hemisphere stroke, compared to left hemisphere stroke, may reduce the circadian variability of blood pressure, increasing nocturnal blood pressure levels. Several other alterations have been described in right hemispheric strokes such as an increase of plasma noradrenaline, prolongation of QTc and recurrent cardiac arrhythmias [106].

The sympathetic hyperactivity represents an independent risk factor for long-term cardio and cerebrovascular events [107]. Other studies revealed a higher risk for fatal and non-fatal cardiac events such as myocardial infarction in patients with left insular ischemic stroke versus non-insular stroke, especially for those without other comorbidities, like coronary artery disease [108].

Besides HRV, as we mention, Rüdiger and colleagues proposed an algorithm to detect physiological oscillations of the heart rate on the basis of R wave to R wave (RR) intervals measurements from the ECG recordings—the trigonometric regressive spectral analysis [109] The HRV parameters are often analyzed by Fourier Transform. The mathematical approach using trigonometric regressions excluded the RR intervals equidistance issue, arising with the method of Fourier Transform, whereas the heart rate is irregular with a high degree of beat to beat variability. The dynamic assessment of HRV by Multiple Trigonometric Regressive Spectral (MTRS) analysis allows a precise evaluation of cardiovascular modulation under different conditions.

1.4.2. Aim

Study 1 - Cortical modulation of cardiac autonomic activity in ischemic stroke patients

The purpose of this study was to investigate ANS function in patients with monofocal ischemic stroke in middle cerebral artery (MCA) territory. Another objective was to determine, using Ewing's battery of autonomic function tests and power spectral analysis of HRV, whether autonomic function is impaired depending on cortical localization of the ischemic stroke.

Study 2 - Linear and nonlinear parameters of heart rate variability in ischemic stroke patients

The aim of this research is to illustrate autonomic nervous system dysregulation in middle cerebral artery ischemic stroke patients.

Study 3 - Heart rate variability analysis and cardiac dysautonomia in ischemic stroke patients

This study aims to evaluate the sympathovagal balance using HRV analysis in MCA ischemic stroke patients applying different autonomic activation tests in the first six months after the acute event.

Study 4 - Heart Rate Variability Analysis A Useful Tool to Assess Poststroke Cardiac Dysautonomia

This research aims to evaluate the impact of the MCA ischemic stroke on cardiac autonomic function, during sympathetic and parasympathetic activation tests, using MTRS analysis of HRV. The hypothesis is that right MCA ischemic stroke leads to sympathetic hyperactivation,

less present in left MCA ischemic stroke and absent in controls, underlined by MTRS analysis of HRV.

1.4.3. Patients and Methods

Study 1 - 40 consecutive ischemic stroke patients were recruited from Department of Neurology Rehabilitation Hospital Iasi, Romania, between June 2014 and May 2015. Inclusion criteria were as follows: age between 40 and 75 years old, right handed patients, clinical assessment suggestive for stroke, evaluated in the first 6 months after the acute event, computed tomography (CT) or magnetic resonance imaging (MRI) showing a single ischemic lesion within the left or right hemisphere (superficial and/or profound MCA territory), cardiologic evaluation prior to stroke. Exclusion criteria were: congestive heart failure, moderate- to-severe valvular dysfunction, any cardiomyopathy, previous acute myocardial infarction and left ventricular hypertrophy, arrhythmia on current admission (including atrial fibrillation), dementia, any major concurrent illness (including renal failure and malignancies), diabetes mellitus or other dysmetabolic pathologies generating polyneuropathy, no present medication interfering with heart rate (medication with beta blockers), fever, hypoxia, alterations in consciousness or any relevant hemodynamic compromise on admission.

Every patient was clinically assessed, the motor deficit being scored upon the Medical Research Council (MRC) scale from 0 (no contraction) to 5 (normal strength and movement of the limb). We also analyzed the anthropometric data (body weight, body mass index), bioclinical parameters, such as blood count, glycemia, cholesterol with fractions low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), triglyceride, hepatic function—ALAT, ASAT, renal function—urea, creatinine. Systolic and diastolic blood pressure (SBP and DBP) were measured in supine and upright positions using an aneroid sphygmomanometer with an adult cuff.

Using Biopac MP150 Acquisition System, we monitored HRV in resting condition and during Ewing's tests (Valsalva maneuver, heart rate difference during six deep breaths, changing heart rate after standing, blood pressure measurement after 5 min of supine position and sustained handgrip). Data were afterwards processed using Kubios HRV 2.2—Heart Rate Variability Analysis Software (Biosignal Analysis and Medical Imaging Group—University of Eastern Finland). We used the time domain and frequency domain methods.

The statistical analysis was performed using the MaxStat Lite software. The values are presented as mean values and standard deviation. Test t Student or variance analysis (ANOVA) was used to determine the differences between the groups. The values p\0.05 were considered statistically significant.

Study 2- The study included 30 ischemic stroke patients, within 6 months post stroke and 15 age- and sex-matched healthy controls, volunteers from community dwelling people, with no previous history of cerebrovascular pathology. All the subjects were divided into three groups. In the first group there were 15 patients with right MCA ischemic stroke (8 men and 7 women), in the second group there were 15 patients with left MCA ischemic stroke (7 men and 8 women), and the control group was based on healthy volunteers (8 men and 7 women).

Inclusion criteria were as follows: age between 40 and 75 years old, right handed patients, clinical assessment suggestive of stroke, evaluation in the first 6 months after the acute event, computed tomography (CT) or magnetic resonance imaging (MRI) showing a single ischemic lesion within left or right hemisphere (superficial and/or profound MCA territory) and cardiologic evaluation prior to stroke. For the stroke group, the patients were under specific medication for their associated cardiovascular co-morbidities, including statins (in case of hypercholesterolemia), platelet antiaggregants and antihypertensive drugs (in case of arterial hypertension) in different associations, including angiotensin- converting-enzyme inhibitors, thiazide-like diuretics, calcium channel blockers or angiotensin receptor blockers, but not beta-blockers which could influence the presented data.

Sympathetic and parasympathetic modulation of the HR was assessed in MCA ischemic stroke patients by analyzing HRV parameters in resting condition and during challenge, using BIOPAC® Acquisition System. ECG recordings were performed at least 5 min in each subject in a 22 C atmosphere, in resting state, after 20 min of supine position, in the absence of any anterior physical effort. A minimum of 264 RR intervals for each recording were analyzed.

Autonomic function was also evaluated by ‘‘deep breathing test’’, which assesses parasympathetic response, measures HR changes after 6 deep breaths during 1minute and ‘‘standing test’’ that measure HR changes during passive movement from supine position to orthostatic position, evaluating sympathetic response. Orthostatic hypotension was defined as a decrease in systolic blood pressure of 20 mmHg or a decrease in diastolic blood pressure of 10

mmHg within three minutes of standing when compared with blood pressure in the supine position.

Data were analyzed using the SPSS version 22.0 (IBM Corporation, Chicago, IL, USA). The results of the univariate analysis were reported as mean ± standard deviation for continuous variables. Having into consideration the small sample size, series normalization is very difficult. This was evaluated using Kolmogorov–Smirnov test. Applied comparative tests were specific to the characteristics of the analyzed series. In the case of pretest analysis, the homogeneity of variance for the studied parameters was analyzed using Levene test, this being the mandatory condition for applying the ANOVA analysis. In the case of nonhomogeneous series, the groups were compared using Kruskal–Wallis. In order to identify differences in the three studied groups' parameters, we used post hoc multiple comparison analysis tests. In the case of homogenous series we applied Duncan Test because, when compared to Newman–Keuls or Tukey, there are the lowest chances of type I error results. When in the three groups there were statistically significant differences between the variance of analyzed parameters, we used Dunnett's test. Total count and percent were reported for categorical variables. Chi-square test (Maximum-Likelihood and Yates) was performed for categorical variables. The significance level (p-value), which represents the maximum error probability, was considered to be 0.05 (5%); a confidence interval of 95% shows that the decision is correct.

Study 3 - we evaluated 71 ischemic stroke patients from the Neurology Unit with right and left MCA ischemic stroke and a control group of 30 healthy volunteers, without cardiovascular or cerebrovascular disorders. All patients included in our study presented the acute ischemic stroke four to six months prior to the HRV evaluation. Forty-eight patients had subcortical stroke (NIHSS 5.56 ± 1.70) while twenty-three patients had corticosubcortical stroke (NIHSS 8.43 ± 2.21). All patients and healthy volunteers were duly informed according to the study protocol and consented to the assessment in agreement with ethical principles. The study was carried out in accordance with the Helsinki Declaration. The following inclusion criteria were considered: age ranging from 39 to 79 years, right-handed subjects, clinical assessment suggesting stroke, imagistic confirmation by cerebral CT scan or cerebral MRI, single ischemic lesion, first stroke in the medical history and cardiologic assessment before inclusion. The following exclusion criteria were considered: cardiac arrhythmia present upon the current

admission (including atrial fibrillation), heart failure, moderate or severe valvular dysfunction, history of myocardial infarction or left ventricular hypertrophy, febrile status, oxygen desaturation, impaired consciousness, renal insufficiency, oncologic pathology, dementia, diagnosed diabetes mellitus with polyneuropathy, current beta-blocker medication.

The autonomic control over heart rate was assessed in resting state and during four autonomic activation tests (Ewing tests), each test entailing a 5 min ECG recording. BIOPAC® acquisition system was used for data collection and analysis. A manual data correction of ECG artifacts was carried out before each analysis. A second correction was performed automatically using BIOPAC®, indicating and selecting the NN-intervals (Normal-to-Normal intervals), supplementary artifacts on the ECG recordings being removed from the final processing.

HRV time-domain parameters and frequency-domain were analyzed from the ECG recordings together with non-linear parameters SD1 and DFAα1, that reflect the variability of the heart rate. The test sequence was standardized: resting state, deep breathing test, handgrip test, standing test and Valsalva maneuver for all the patients and the healthy subjects.

The data were analyzed using the SPSS software V.24. If the analyzed data showed a normal distribution, we used the parametric inferential method – respectively One Way Anova and for data without a normal distribution nonparametric inferential methods, respectively Kruskal – Wallis test were applied. The One Way Anova test (95% CI) was applied for the comparative analysis. The "t Student", Pearson-χ2, Fisher, ANOVA, linear regression, logistic regression, and multivariate analysis test were also applied, using generalized linear models. To identify differences in the three studied groups' parameters, we used post hoc multiple comparison analysis tests (Dunnett's test). Receiver Operating Characteristic Curves (ROC curve) were performed to evaluate the discriminative power of the HRV parameters.

Study 4 - We evaluated a group of 40 patients who had an ischemic stroke, which was divided into 2 subgroups: the first subgroup of 20 patients (12 men and 8 women, mean age 62.5 ± 9.6 years) with ischemic stroke in the right MCA territory and the second subgroup of 20 patients (10 men and 10 women, mean age 63.5 ± 7.5 years) with ischemic stroke located in the left MCA territory. The features of this group of patients were compared with a control group consisting of 20 healthy volunteers (8 men and 12 women, mean age 56.2± 2.7 years), without

cardiovascular or cerebrovascular disorders. The patients were recruited from the Neurology Department and were evaluated 3 months after the acute ischemic stroke.

Patients were included in the study according to the following criteria: right-handed subjects, older than 18 years, clinical examination evocative for stroke, evidence of left or right MCA ischemic stroke on imagistic investigations, single ischemic lesion, and cardiologic assessment before being included in the study. Some of the patients enrolled in our study were taking specific medication for their associated pathologies, such as statins or fenofibrate for dyslipidemia, antiplatelet agents, antihypertensive treatment. Patients under beta-blockers, anticholinergic drugs, or amiodarone were excluded.

The evaluation of a 5-minute ECG recording in the each of the following conditions: resting state (supine position), "deep breathing" test (6 complete cycles of deep inhale and exhale over 60 s with timing, 10 s for each cycle), and "standing" test (orthostatic position).

BIOPAC acquisition system was used for collecting and processing biological signals. The data gathered were subsequently processed using MTRS software version 7.3.2.0 (UniversitätsKlinikum, Zentrum für Klinische Neurowissenschaften, Dresden, Germany). This software assesses the HRV time domain and frequency domain parameters, on the basis of the trigonometric regressive analysis. All oscillations of the biosignals are analyzed using the following condition: Σ (RRI(t(i)) − Reg(t(i)))2= >minimum, with RRI(t(i)) being the original RR intervals and Reg(t(i))=A* sin (ωt(i) +φ(i)) being a trigonometric function of the parameters A (amplitude), ω (frequency), and ϕ (phase shift).

We used the same local data segment settings of 30 seconds for each recording, with a minimum variance reduction of 1%, a shift of the local data segment of 1 and delta frequency 0.006 Hz. Time and frequency domain HRV parameters were analyzed from the ECG recordings in the 3 conditions mentioned above (resting state and the 2 autonomic activation tests).

Data were analyzed using GraphPad Prism version 8.0.2 (GraphPad Software Inc.). The results of descriptive statistics were reported as mean ± standard deviation. Taking into consideration the small sample size, series normalization is very difficult. When the assumption of normal distribution was not met, we applied a nonparametric test. Analysis of the 3 groups of patients was performed using the Kruskal-Wallis test. When comparing the patient's group with the control group and between stroke groups, the Mann-Whitney test was applied. Analysis

between different autonomic tests in the same group was performed applying the Wilcoxon matched-pairs signed rank test. The significance was met when $p<0.05$.

1.4.4. Results

Study 1- The subjects were divided in two groups: 20 right hemisphere (first group) and 20 left hemisphere (second group) ischemic stroke patients, in the MCA territory, in both groups.

In resting state, heart rate was higher in right hemisphere stroke patients compared both to control group ($p<0.01$) and left hemisphere stroke patients ($p<0.05$). Time domain analysis in resting state showed low values for HF in normalized units (HF nu) (expressing the parasympathetic activity) in right hemisphere stroke patients compared to left hemisphere stroke patients ($p<0.01$) and to control patients ($p<0.01$) suggesting low parasympathetic activity in right MCA stroke patients. The sympathetic tone (LF/HF ratio) was more pronounced in right sided MCA stroke patients ($p<0.01$) compared to contralateral stroke patients (Table 6). Moreover, RMSSD values in resting state showed predominant parasympathetic activity in left MCA infarcts compared to right MCA infarcts ($p<0.05$) and to control group ($p<0.05$).

HRV parameters in resting state	Right hemisphere stroke N=20	Left hemisphere stroke N=20	Control group N=20
RR (msec)	777,1±87,8	789,2±99	823,2±74
SDNN	33,9±17,6	44,9±5,2	37,5±7,8
Heart rate (b/min)	77,4±7,4	74,3±8,8	68,7±6,4
RMSSD	37±35,2†	54,8±38,2•	38,8±11,3
pNN50	3,54±3,2	4,2±1,34	6,05±1,5
VLF (ms²)	245,1±226,5	96,6±86,5••	362,3±83,8*
LF (ms²)	427,1±410,8††	496,5±107,1	539,4±175,1
HF (ms²)	210,7±187,4††	513,6±226,1	526,7±67,8**
LFnu	65,9±17,5††	40,4±21,5•	56,8±14,1
HFnu	34,2±18,56††	59,7±21,5	51,4±12,2**
LF/HF	2,07±2,21††	0,97±0,82	1,14±0,29*

Values are expressed as a mean ± standard deviation
†p<0.05, †† p<0.01 when comparing stroke patients
*p<0.05, **p<0,01 right hemispheric stroke patients compared with control patients
•p<0.05, ••p<0.01 left hemispheric stroke patients compared with control patients

Table 6 Heart rate variability parameters in resting state

HRV parameters in deep breathing test illustrated predominant parasympathetic tone in left hemisphere stroke patients compared to control group Frequency domain analysis for HRV parameters in deep breathing showed high parasympathetic predominance in left hemisphere stroke patients (HF nu) $p<0.05$ when compared to control (Table 7). Sympathetic activity, evaluated by LF/HF ratio was predominant in right hemisphere stroke group when compared to left MCA stroke group ($p<0.01$) and to controls ($p<0.05$).

Valsalva maneuver revealed lower frequency domain values, with HF and HFnu lower in right hemisphere stroke ($p<0.01$ for HFnu) when compared to left hemisphere stroke and increased LF/HF ratio in right MCA stroke group compared to contralateral stroke patients ($p<0.05$).

HRV parameters in deep breathing test (inhale/exhale)	**Right hemisphere stroke N=20**	**Left hemisphere stroke N=20**	**Control group N=20**
RR (msec)	773,7±79,2	751,29±103,5	806,7±81,3
SDNN	48,9±5,6	56,5±4,7•	33,9±5,33
Heart rate (b/min)	79,28±7,9	71,1±11,3•	67,3±5,24**
RMSSD	42±25,1	69,8±32,5•	49,5±12,8
pNN50%	4,47±3,1	3,66±3,35	5,01±3,2
VLF (ms²)	184,7±159,1	199,2±156,2••	441±162,4**
LF (ms²)	612,9±466,7†	498,2±130,7••	556,7±123,5**
HF (ms²)	378,7±327,2†	545±331,4•	882,6±426,8**
LFnu	65,2±12,4††	38,3±16,3	31,6±12,2**
HFnu	35,12±10,20††	60,9±15,29•	42,9±11,6
LF/HF	1,93±0,73††	0,76±0,42	0,83±1,08*
HRV parameters in Valsalva test			
RR (msec)	774±61,8	765,9±112,5	799,2±66,9
SDNN	63,3±25,1	83,2±58,4•	35,7±7,95*
Heart rate (b/min)	78,4±6,2	71,9±12,47	69,8±9,7*
RMSSD	53,9±43,3	82,1±64,3	45,7±13,3
pNN50%	5,21±6,6	6,3±5,03	8,85±11,4
VLF (ms²)	166,3±127,9†	271,7±158,2	417,2±42,2**
LF (ms²)	979,4±861,3	935,8±826•	752,7±318*
HF (ms²)	660,4±510,1	945,8±754	727,5±340,6
LFnu	69,5±18,4†	45,02±21,3	44,4±14,3*
HFnu	31,1±16,7††	47,8±21,9•	38,4±14,8
LF/HF	2,4±1,92†	0,98±0,61	1,03±0,76*

Values are expressed as a mean ± standard deviation
†$p<0.05$, †† $p<0.01$ when comparing stroke patients
*$p<0.05$, **$p<0,01$ right hemispheric stroke patients compared with control group
•$p<0.05$, ••$p<0.01$ left hemispheric stroke patients compared with control group

Table 7 Heart rate variability parameters in deep breathing test and Valsalva test

Analysis of HRV time and frequency domain measures in orthostatic position, revealed that right hemisphere stroke patients displayed a decreased parasympathetic tone compared to control group (higher HF in absolute values, $p<0.01$). Moreover, they showed predominant sympathetic activity compared to left hemisphere stroke, illustrated by elevated LF/HF ratio ($p<0.01$). Higher time domain parameters in left hemispheric stroke patients, were consistent with parasympathetic tone dominance in these patients, when compared to other groups (Table 8). In ‘‘hand grip’’ test, most of the HRV parameters in time domain showed a clear difference between right and left hemisphere stroke patients, with better represented parasympathetic activity in left hemisphere stroke group (SDNN, RMSSD, HF).

HRV parameters in orthostatism	**Right hemisphere stroke N=20**	**Left hemisphere stroke N=20**	**Control group N=20**
RR (msec)	714±69,5	721,2±134,2	765,7±75,2
SDNN	57,3±26,4	65,7±23,7	42,5±7,35
Heart rate (b/min)	89,6±13,1	83,6±8,77	78,7±5,07*
RMSSD	36,7±32,9	60,9±41,7	32,4±5,28
pNN50%	4,95±2,45	6,01±4,93	6,89±4,2
VLF (ms²)	142,7±129,5	126,8±112,7••	419,2±44,4**
LF (ms²)	359,5±312,6	278,2±130••	742,4±359,5*
HF (ms²)	131,2±112,6	295,8±251•	514,1±240,4**
LFnu	72,5±22,6††	44,7±14,8•	65,5±17,8
HFnu	31,15±10,20†	49,2±19,3	46,4±17,26*
LF/HF	2,74±1,59††	1,06±0,72	1,62±0,68*
HRV parameters “hand grip” test			
RR (msec)	725±59,2	705,5±106,2	763,4±85,2
SDNN	52,5±18,4	63,2±56,4	51,9±28,5
Heart rate (b/min)	82,4±7,1	81,4±18,4	77,3±8,6
RMSSD	27,5±25,3	47,2±42,5	32,3±17,7
pNN50%	4,22±3,6	5,3±5	7,3±6,4
VLF (ms²)	191,1±166	308,8±256,2	283,4±193,9
LF (ms²)	519,8±490,3	462,6±431,2	881,4±708
HF (ms²)	341,5±200,1	694,2±654	276,9±248,6
LFnu	78,2±17,7†	54,6±25,7•	78,8±4,8
HFnu	32,6±19,2	44,6±25,4•	30,3±4,1
LF/HF	3,72±3,65††	1,12±1,04•	2,7±1,04

Values are calculated as mean ± standard deviation
† $p<0.05$, †† $p<0.01$ when comparing stroke patients
* $p<0.05$, **$p<0,01$ when comparing right hemisphere stroke group to control group
• $p<0.05$, ••$p<0.01$ when comparing left hemisphere stroke group to control group

Table 8 HRV parameters in orthostatism and during “hand grip” test

Patients with left hemisphere ischemic stroke showed predominant parasympathetic activity in deep breathing test ($p<0.01$), while right hemisphere ischemic stroke patients showed impairment in two parasympathetic activation tests (heart rate response to deep breathing and Valsalva) when compared to controls. Consistent with the findings, in resting state and in all tests, right hemispheric stroke patients presented higher values of LF/HF ratio illustrating greater sympathetic tonus ($p<0.01$) (Fig. 2).

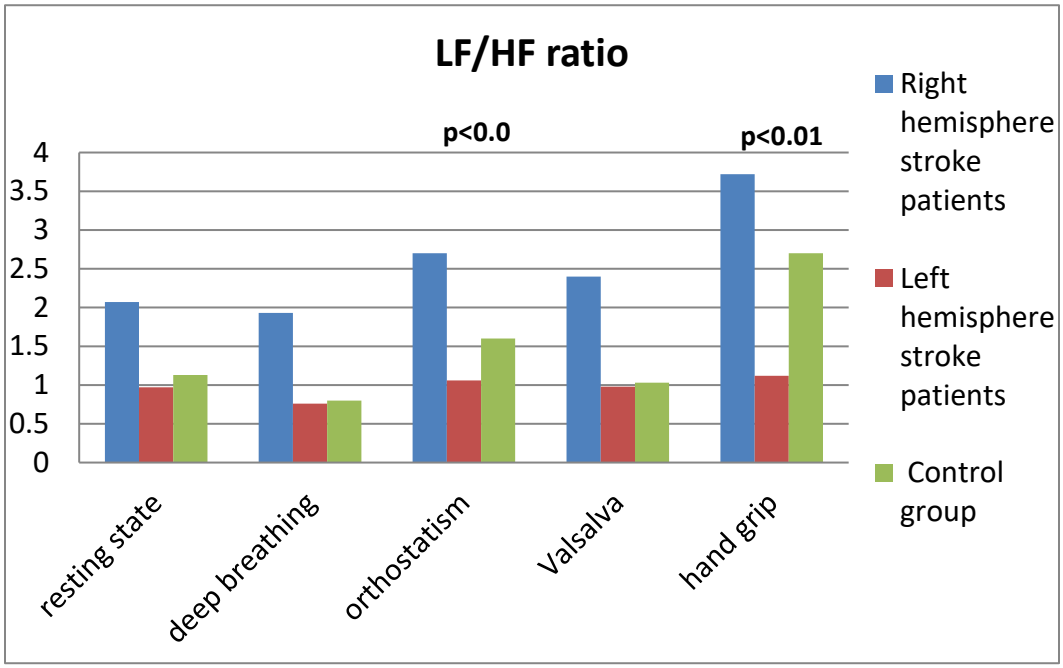

Fig 2.LF/HF ratio in all groups

Study 2- Left hemisphere stroke patients presented enhanced parasympathetic control of the HR, evidenced by higher RMSSD and pNN50 values in resting state when compared to right hemisphere stroke patients ($p < 0.05$) (Table 9). Moreover, frequency domain parameters (HFnu) also showed enhanced parasympathetic control of the HR in left MCA infarction compared to right MCA infarction ($p < 0.05$) and to control group ($p < 0.05$) and lower LF/HF ratio values ($p < 0.05$). The increased vagal influence on the HR in left hemisphere stroke patients is also confirmed by high SD1 levels in resting state when compared to controls ($p < 0.05$). On the other hand, in patients with right MCA stroke we found a higher sympathetic control of the HR, when compared to left MCA strokes ($p < 0.05$) and to controls ($p < 0.05$).

HRV parameters in resting state	Group 1 – Right hemisphere stroke (n=15)	Group 2 – Left hemisphere stroke (n=15)	Control group (n=15)	Levene Test df=2	Group 1 vs. Group 2	Group 1 vs. Control	Group 2 vs. Control
				p value			
RR (msec)	874.4±108.4	782±92.3	797.9±85.7	0.546	*0.0135*	*0.031*	0.645
SDNN	47.1±19.8	66.7±46.8	65.5±34.1	0.290	0.152	0.154	0.926
Heart rate	74.8±9.7	74.3±7.5	69.8±6.9	0.422	0.884	0.112	0.124
RMSSD	37.7±22.4	81.7±64.1	65.3±34.3	0.100	*0.010*	0.087	0.304
pNN50	3.2±3.8	12.2±13.7	17.2±16.8	0.0003*	0.052	*0.004*	0.282
LFnu	67.1±12.5	32.4±15.7	57±8.7	0.010*	*0.0001*	*0.032*	*0.0001*
HFnu	32.7±12.4	66.1±15.3	42.6±8.4	0.016*	*0.0001*	*0.040*	*0.0001*
LF/HF	2.6±1.6	0.5±0.3	1.3±0.3	0.000*	*0.0001*	*0.001*	*0.049*
SD1	26.7±15.8	58.1±45.4	43.4±20.8	0.020*	*0.005*	*0.013*	*0.018*
SD2	58.7±24.2	70.1±51.9	71.2±41.3	0.253	0.442	0.426	0.936
ApEn	0.8±0.2	0.6±0.2	0.8±0.1	0.331	0.097	0.770	0.066
SampEn	1.1±0.3	0.8±0.5	0.9±0.4	0.280	0.224	0.704	0.359
DFA α1	1.1±0.3	0.5±0.2	0.9±0.2*	0.329	*0.00006*	*0.023*	*0.003*
DFA α2	0.9±0.2	0.8±0.3	0.5±0.2*	0.206	0.314	*0.0004*	*0.005*

Values are expressed as a mean ± standard deviation
p-value <0.05 was considered to be statistically significant; df - degrees of freedom
* p-value <0.05 for Levene test →the Dunnett's test was used for comparison

Table 9. Heart rate variability parameters in resting state.

During deep breathing test, left hemisphere stroke patients described more pronounced vagal influence on the HR expressed by RMSSD and HF nu parameters compared to right hemisphere stroke patients. During standing test we noticed a decreased parasympathetic control of the HR in right MCA ischemic stroke patients (lower pNN50, SDNN values) compared to controls ($p < 0.05$). The frequency-domain parameters (LFnu, HFnu) underline the same results toward a more decreased parasympathetic control of the HR in right MCA ischemic stroke patients vs. controls. Nonlinear parameters showed difference between right and left hemisphere stroke patients, as shown in Table 10.

In right hemisphere stroke patients, in resting state, we found a very strong correlation between SD1 and SDNN ($r = 0.9$, $p < 0.05$) and between SD1 and RMSSD ($r = 0.9$, $p < 0.05$) (Table 4). We found a negative correlation between SD1 and DFA a1 ($r = \_0.7$, $p < 0.05$) (Table 11). SDNN presented a negative correlation with DFA a1 ($r = \_0.6$, $p < 0.05$) and with RMSSD ($r = 0.9$, $p < 0.05$). DFA a1 presented a negative correlation with HFnu ($r = –0.5$, $p < 0.05$) and a positive correlation with LFnu ($r = 0.6$, $p < 0.05$) and LF/HF ratio ($r = 0.5$, $p < 0.05$) (Table 11).

HRV parameters in deep breathing test	Group 1 – Right hemisphere stroke (n=15)	Group 2 – Left hemisphere stroke (n=15)	Control group (n=15)	Levene Test df=2	Group 1 vs. Group 2	Group 1 vs. Control	Group 2 vs. Control
				p value			
RR (msec)	862.3±111.3	788.5±98.1	733.2±70.7	0.141	*0.036*	*0.0007*	0.112
SDNN	69.3±24.9	93.8±62.6	34.9±22.3	0.002*	0.104	0.024	*0.0004*
Heart rate	73.1±7.9	69.8±4.2	68.7±6.8	0.011*	0.054	0.021	0.905
RMSSD	38.3±23.8	117.6±85.2	74.8±46.2	0.001*	*0.001*	0.086	*0.045*
pNN50	6.1±5.4	13.9±16.9	5.8±3.8	0.00002*	0.103	0.998	0.106
LFnu	47.4±1	29.9±11.7	47.0±11.1	0.291	*0.001*	0.996	*0.002*
HFnu	52.3±15.8	69.6±11.6	52.9±11.2	0.317	*0.001*	0.992	*0.002*
LF/HF	1.1±0.7	0.5±0.2	1.1±0.5	0.005*	*0.01*	0.954	*0.027*
SD1	53.1±32.8	83.4±60.4	28.6±15.5	0.0004*	0.102	0.246	*0.002*
SD2	79.3±24.6	100.8±68.2	46.8±24.1	0.0009*	0.183	*0.047*	*0.002*
ApEn	0.6±0.1	0.5±0.1	0.7±0.2	0.457	0.179	0.336	0.061
SampEn	0.8±0.4	0.7±0.4	1.0±0.5	0.664	0.489	0.279	0.184
DFA α1	0.9±0.3	0.7±0.2	0.9±0.2	0.163	0.071	0.693	0.128
DFA α2	0.9±0.2	0.9±0.3	0.7±0.4*	0.466	0.611	0.053	0.121

Values are expressed as a mean ± standard deviation
p-value <0.05 was considered to be statistically significant; df - degrees of freedom
* p-value <0.05 for Levene test →the Dunnett's test was used for comparison

HRV parameters in standing test	Group 1 – Right hemisphere stroke (n=15)	Group 2 – Left hemisphere stroke (n=15)	Control group (n=15)	Levene Test df=2	Group 1 vs. Group 2	Group 1 vs. Control	Group 2 vs. Control
				p value			
RR (msec)	823.0±99.2	767.7±106.0	759.3±80.1	0.259	0.114	0.085	0.808
SDNN	43.6±29.4	56.4±33.3	68.5±22.1	0.245	0.240	*0.024*	0.217
Heart rate	83.6±8.1	81.0±10.9	74.4±8.6	0.763	0.056	*0.012*	0.450
RMSSD	32.4±19.6	49.2±46.7	35.2±22.2	0.0002*	0.320	0.814	0.228
pNN50	3.4±3.6	5.7±9.9	10.1±8.1	0.211	0.402	*0.024*	0.119
LFnu	73.60±11.8	67.3±18.9	58.2±6.1	0.007*	0.201	*0.003*	0.067
HFnu	26.3±11.7	32.4±18.7	41.5±6.1	0.007*	0.403	*0.007*	0.153
LF/HF	4.0±3.4	2.7±1.9	2.3±0.7	0.015*	0.139	0.069	0.649
SD1	23.3±13.9	37.9±32.4	31.4±19.8	0.002*	0.099	0.323	0.442
SD2	93.0±30.1	67.2±39.3	57.9±34.2	0.584	*0.044*	*0.009*	0.457
ApEn	0.6±0.1	0.6±0.2	0.6±0.2	0.753	0.710	0.821	0.576
SampEn	0.6±0.2	0.7±0.4	0.8±0.5	0.016*	0.735	0.239	0.359
DFA α1	1.2±0.2	1.0±0.4	1.1±0.3	0.328	0.087	0.298	0.802
DFA α2	1.2±0.2	1.1±0.3	0.9±0.4	0.021*	0.892	0.063	0.159

Values are expressed as a mean ± standard deviation
p-value <0.05 was considered to be statistically significant; df - degrees of freedom
* p-value <0.05 for Levene test →the Dunnett's test was used for comparison

Table 10. HRV parameters in standing and deep breathing tests

Using the algorithm described in Methods, we found in right hemisphere stroke patients attenuated responses to Ewing tests indicated by low HRV based on RR interval variation (resting state 5.4 ± 2.2%, standing test 8.4 ± 2.9% and deep breathing test 8.1 ± 3.1%, $p < 0.05$), thus suggesting a predominant sympathetic control of the heart rate. In left hemisphere stroke patients we observed a tendency for intensified HRV responses after applied activation tests

(resting state 8.7±6.2%, standing test 7.3 ± 4.1% and deep breathing test 11.9 ± 8.4%, p = 0.06). This data is in line with our previous results using time and frequency domain parameters, characterizing the asymmetrical involvement of the autonomic nervous system in the central control of the HR (Figure 3).

Correlation coefficients calculated based on the Spearman Rank Order test; Include condition: Group 1 - Right hemisphere stroke										
	RMSSD	**LF/HF**	**LF nu**	**HF nu**	**SD1**	**SD2**	**ApEn**	**SampEn**	**DFA α1**	**DFA α2**
SDNN	0.908*	-0.169	-0.169	0.177	0.908*	0.914*	-0.238	-0.371	-0.611*	-0.315
RMSSD		-0.344	-0.341	0.352	0.995*	0.779*	-0.247	-0.484	-0.769*	-0.353
LF/HF			0.997*	-0.999*	-0.344	0.006	-0.024	0.341	0.596*	-0.091
LF nu				-0.995*	-0.341	0.003	-0.035	0.330	0.601*	-0.115
HF nu					0.352	0.009	0.012	-0.349	-0.590*	0.075
SD1						0.779*	-0.247	-0.484	-0.769*	-0.353
SD2							-0.188	-0.328	-0.406	-0.300
ApEn								0.524*	0.278	0.159
SampEn									0.428	-0.206
DFA a1										0.082
Include condition: Group 2 - Left hemisphere stroke										
SDNN	0.667*	0.153	0.165	-0.132	0.644*	0.918*	-0.571*	-0.571*	-0.003	-0.179
RMSSD		-0.408	-0.398	0.411	0.996*	0.561*	-0.493	-0.375	-0.595*	-0.515*
LF/HF			0.999*	-0.997*	-0.432	0.253	-0.218	-0.326	0.726*	0.332
LF nu				-0.995*	-0.424	0.271	-0.233	-0.343	0.731*	0.336
HF nu					0.435	-0.221	0.221	0.321	-0.712*	-0.315
SD1						0.526*	-0.468	-0.329	-0.597*	-0.524*
SD2							-0.662*	-0.706*	0.135	0.026
ApEn								0.924*	-0.044	0.100
SampEn									-0.053	0.059
DFA a1										0.562*
*p-value <0.05 was considered to be statistically significant;										

Table 11. Relationships between linear (SDNN, RMSSD, LFnu, HFnu and LF/HF) and nonlinear indexes (SD1, SD2, SampEn, ApEn, DFA a1 and DFA a2) in resting state.

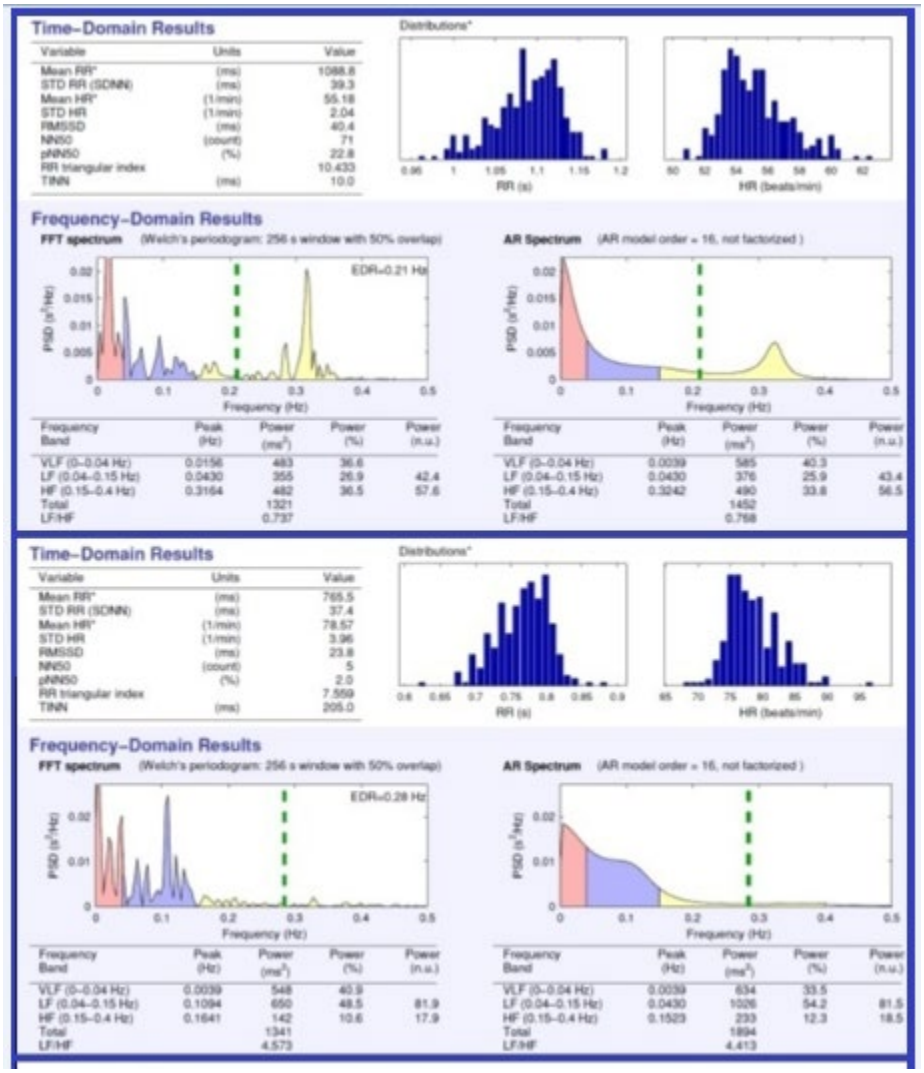

Figure 3. HRV parameters using Kubios software
Above: Patient 1 – ischemic stroke with left insular involvement (parasympathetic predominance)
Below: Patients 2 - ischemic stroke with right insular involvement (sympathetic predominance)

Study 3- Study population diagram can be seen in Figure 4. Corticosubcortical ischemic strokes were associated with an increased clinical severity score (NIHSS) compared to subcortical strokes ($p < 0.001$). Comparing ipsilesional ischemic strokes, NIHSS values were higher in the right MCA corticosubcortical compared to subcortical strokes ($p < 0.05$), while for the left MCA infarctions there was no significant difference.

Patients with right MCA infarctions presented a decreased vagal modulation, reflected by lower values of RMSSD, HFnu, SD1 during the autonomic activation tests in both

corticosubcortical and subcortical stroke groups. Right MCA ischemic stroke patients presented diminished vagal tonus in resting state, expressed by lower values of the RMSSD, pNN50 and HF compared to the other two studied groups. We observed normal cardiac autonomic responses during vagal activation tests in both groups of patients and controls, suggested by increased RMSSD, pNN50, HF values (Figure. 5) and decreased LF/HF. After the vagal activation maneuvers, we noticed a tendency towards rebalancing the sympathovagal activity. The sympathetic activation tests led to a more pronounced sympathetic response in the right MCA ischemic stroke group ($p < 0.001$ for the handgrip and standing tests), therefore pointing towards a predisposition for rapid sympathetic activation in these patients.

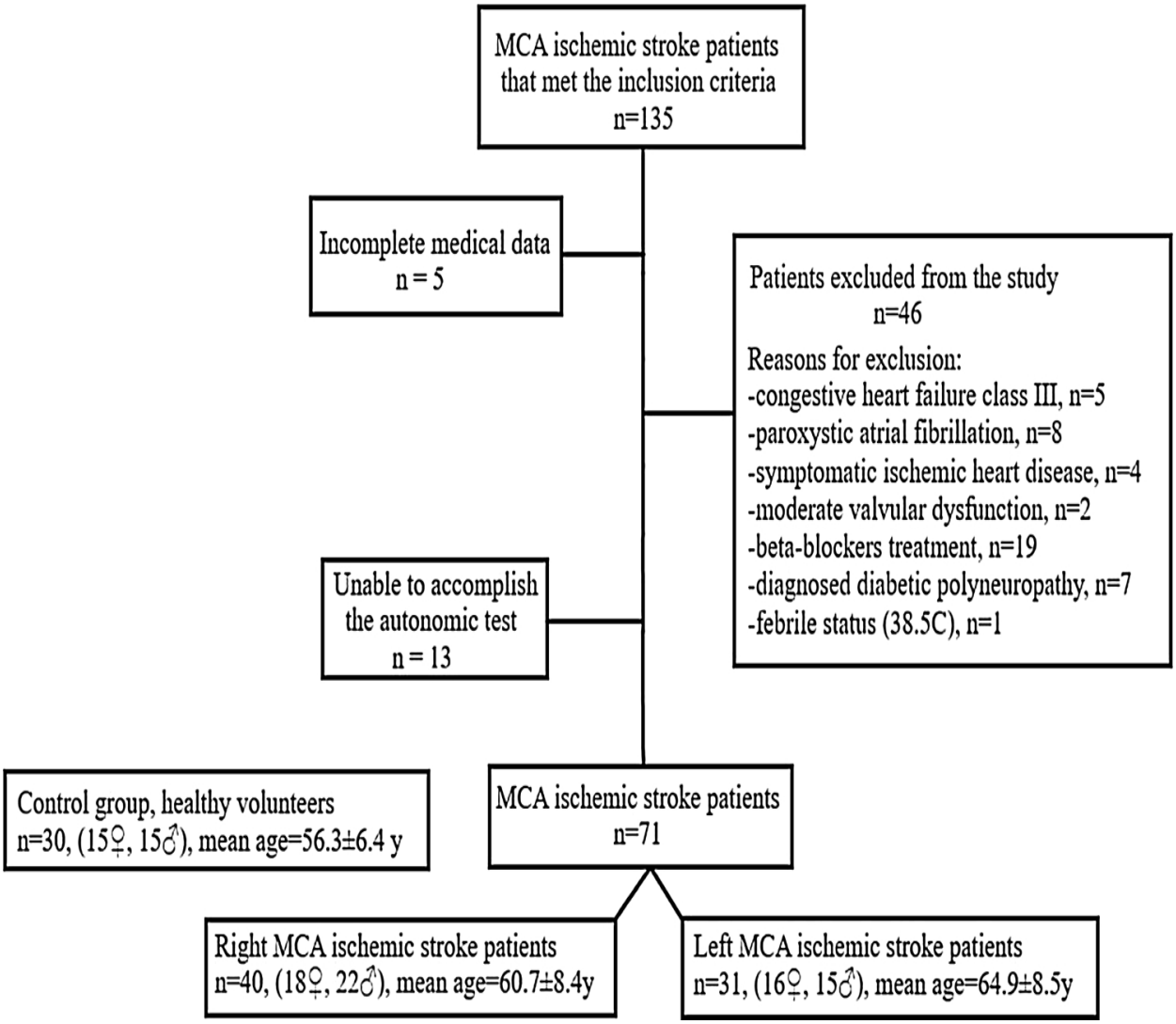

Figure. 4. Study population diagram.

We evaluated HRV linear and non-linear parameters (SD1, DFAα1) in resting state and during the Ewing tests, correspondingly to the linear parameters ahead discussed (Figure. 6). Our results showed differential values for SD1 between the two groups of patients: vagal predominance in patients with left MCA ischemic stroke, intermediate values, within normal range for the control group and lower values indicating a diminished vagal influence on the heart rate and a decreased variability of the heart rate in patients with right MCA ischemic stroke. For this last group, the non-linear HRV parameter DFA α1 reflects a decreased complexity and loss of the fractal properties when the values of the exponent α are higher than 1, and, in this case, is correlated with a reduced vagal influence and an augmented sympathetic control over the heart rate.

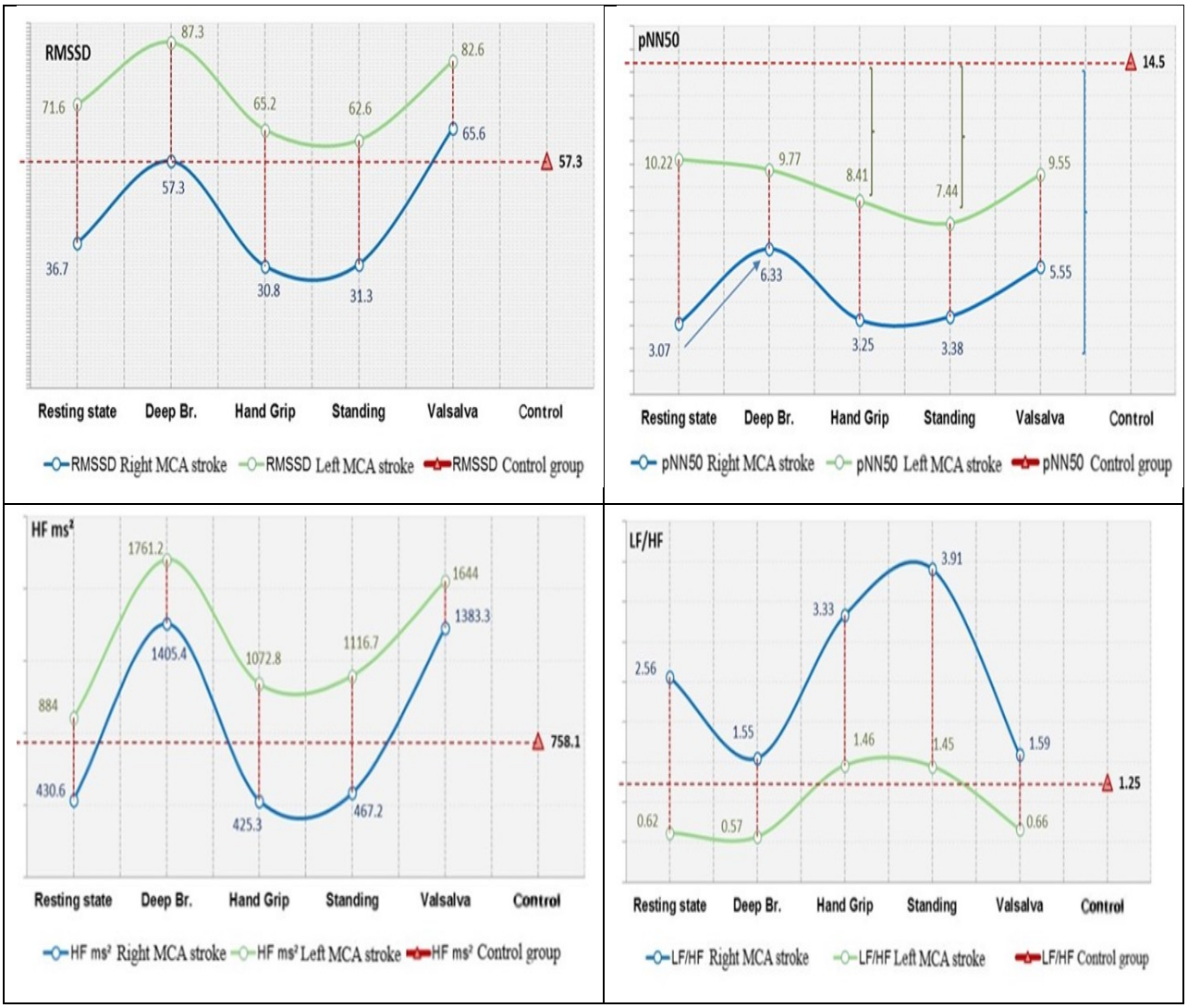

Figure.5. Comparative assessment of RMSSD, pNN50, HF(ms2) and LF/HF values in dynamics.

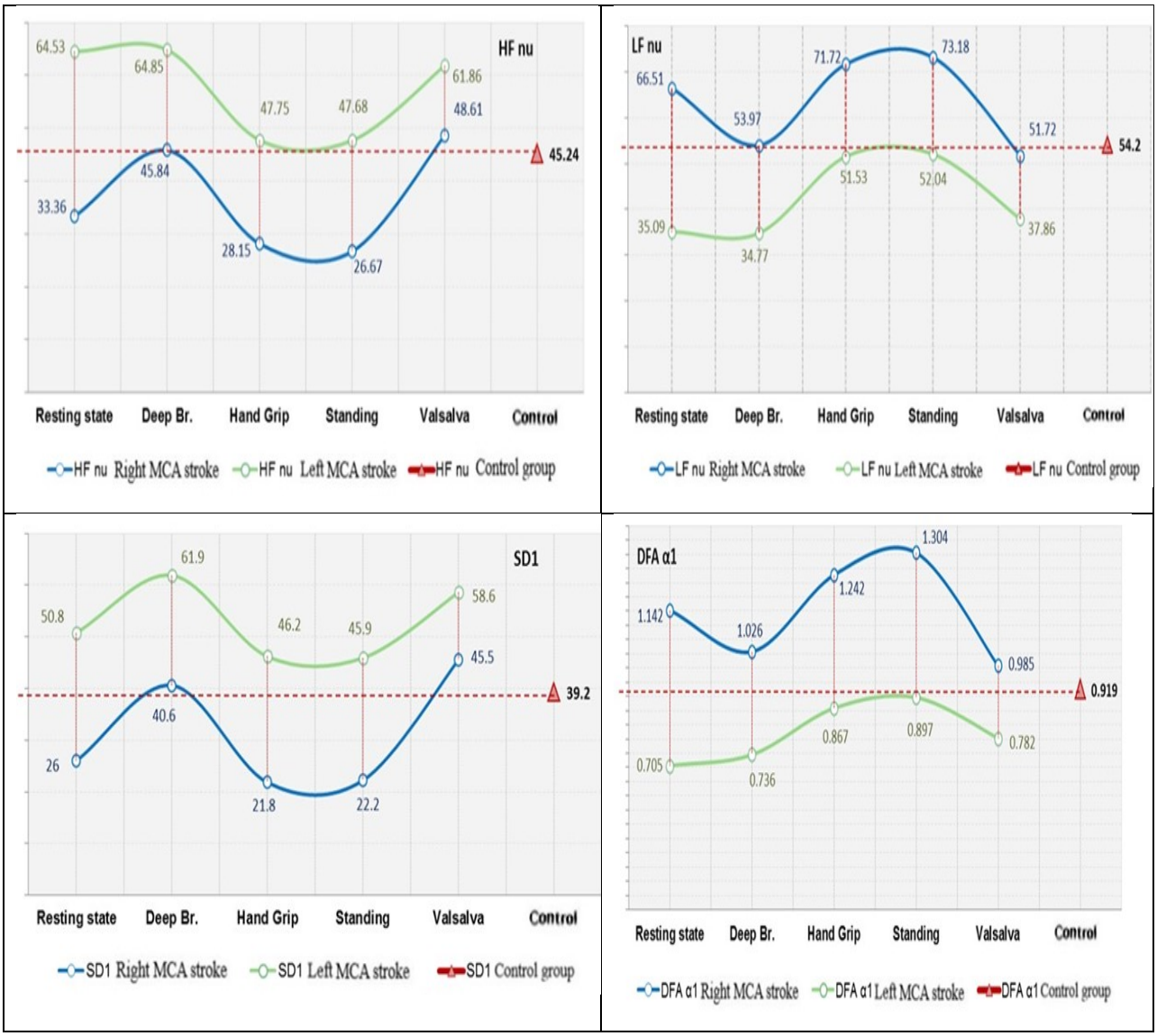

Figure 6. Comparative assessment of LF(nu), HF(nu), DFA α1 and SD1 values in dynamics.

Study 4

Patients who had a right MCA ischemic stroke displayed a decreased parasympathetic control of the heart rate in resting state, illustrated by lower values of RMSSD, pNN50, HF, and HFnu parameters, compared with left MCA ischemic stroke group and the healthy control group (Tables 12). After autonomic activation tests, patients who had a right MCA ischemic stroke maintained lower values of the parasympathetic specific parameters mentioned above, compared with the left MCA ischemic stroke group and the control group.

HRV Parameter	Mann-Whitney Test (*P*-value)			All Groups
	Right vs. Left MCA Ischemic Stroke	Right MCA Ischemic Stroke vs. Controls	Left MCA Ischemic Stroke vs. Controls	Kruskal-Wallis Test
RMSSD (RS)	0.021	<0.001	0.182	0.002
RMSSD (DB)	0.004	<0.001	0.301	0.001
RMSSD (ST)	<0.001	<0.001	0.461	<0.001
pNN50 (RS)	0.037	<0.001	0.062	0.001
pNN50 (DB)	0.002	<0.001	0.139	<0.001
pNN50 (ST)	<0.001	<0.001	0.380	<0.001
HF (RS)	0.059	0.040	0.883	0.072
HF (DB)	<0.001	0.006	0.211	0.001
HF (ST)	0.157	0.001	0.129	0.008
HFnu (RS)	0.040	0.040	0.738	0.056
HFnu (DB)	0.001	0.019	0.157	0.002
HFnu (ST)	0.201	0.005	0.141	0.020
LF (RS)	0.013	0.383	0.108	0.041
LF (DB)	0.001	0.511	0.006	0.003
LF (ST)	0.081	0.583	0.288	0.214
LF/HF (RS)	0.043	0.015	0.722	0.037
LF/HF (DB)	0.015	0.026	0.383	0.022
LF/HF (ST)	0.046	0.002	0.596	0.013

DB indicates deep breathing test; HF, high frequency; HFnu, high-frequency normalized unit; LF, low frequency; MCA, middle cerebral artery; RMSSD, root mean square of the successive differences; RS, resting state; ST, standing test.

Table 12. Differences in HRV Parameters Between the 3 Groups

Comparing the values of the time and frequency domain parameters RMSSD, pNN50, HF, HFnu, LF, LF/HF for the right MCA ischemic stroke group, between resting state and the 2 autonomic activation tests, we observed a consistent sympathetic activation response after standing test (Table 13). Patients who had a left MCA ischemic stroke presented a decrease of the parasympathetic specific parameters RMSSD, pNN50, HF, HFnu after the standing test and an increase of the LF/HF ratio, indicating a sympathetic activation in accordance to the autonomic test (Table 13).

HRV Parameter	Autonomic Activation Tests	Wilcoxon Matched-Pairs Signed-Rank Test (*P*-value): Right MCA Ischemic Stroke	Wilcoxon Matched-Pairs Signed-Rank Test (*P*-value): Left MCA Ischemic Stroke
RMSSD	RS vs. DB	0.189	0.013*
	RS vs. ST	0.005*	0.132
	DB vs. ST	<0.001*	<0.001*
pNN50	RS vs. DB	0.552	0.015*
	RS vs. ST	0.151	0.611
	DB vs. ST	0.052	0.014*
HF	RS vs. DB	0.756	0.202
	RS vs. ST	0.006*	0.001*
	DB vs. ST	0.010*	<0.001*
HFnu	RS vs. DB	0.927	0.330
	RS vs. ST	0.004*	<0.001*
	DB vs. ST	0.017*	<0.001*
LF	RS vs. DB	0.956	0.756
	RS vs. ST	0.069	0.002*
	DB vs. ST	0.053	0.003*
LF/HF	RS vs. DB	0.589	0.819
	RS vs. ST	0.005*	0.002*
	DB vs. ST	<0.001*	<0.001*

DB indicates deep breathing test; HF, high frequency; HFnu, high-frequency normalized unit; LF, low frequency; MCA, middle cerebral artery; RMSSD, root mean square of the successive differences; RS, resting state; ST, standing test.
*$P < 0.05$.

Table 13. Differences in HRV Parameters between Tests for Stroke Patients

1.4.5. Discussion

The cardiac dysfunctions secondary to destruction of cerebral structures involved in central control, particularly the insular cortex, located in the vascular territory of the MCA, and amplified by catecholaminergic storm, may induce cardiac ventricular arrhythmias or myocardial detriment, which are often associated with sudden death.

Patients with right insular cortex ischemia had increased incidence of ECG abnormalities (atrial fibrillation, A–V block, ectopic beats and inverted T wave), higher blood pressure values compared to left insular cortex ischemia [110]. Atrial fibrillation is the most frequent cardiac

arrhythmia after stroke, as well as a risk factor for secondary cardiac complications such as ventricular tachycardia or fibrillation and heart failure, which can increase the risk of sudden death [111].

Patients from the studied groups presented higher sympathetic activation in right hemisphere stroke (MCA territory). These data are according to other results of similar studies, where it was proved that right insular cortex seems to modulate the sympathetic tone and left insular cortex the parasympathetic activity in these patients [112].

HRV study in the first 6 months after stroke may be useful to highlight the cardiac dysfunctions with potential clinical involvement. The risk of developing cardiac arrhythmias such as atrial fibrillation or other ECG abnormalities (QT prolongation, A–V block, T wave inversion) in ischemic stroke patients is higher in those with predominant sympathetic activity, with poorer prognostic for cardiac or cerebral events on long term. The cardiac arrhythmias following stroke may be related to the degree of sympathetic predominance. The presence of electrocardiographic abnormalities and cardiac arrhythmias is very common after acute cerebrovascular events, even in the absence of structural heart disease, but determining a causal relationship has been difficult as patients with stroke associate usually risk factors for coronary artery disease, such as hypertension, diabetes mellitus and smoking.

As suggested by Ozdemir and Hachinski [113], awareness of a ‘‘neurogenic heart syndrome’’ and recognition of right hemispheric involvement, especially the insular cortex, is important. The risk of developing cardiac arrhythmia in post stroke patients is higher when associated to raised sympathetic activity and low HRV. Several investigators have reported decreased HRV in stroke patients, not only in the acute phase, but also within the next six months [114].

Early cardiac monitoring may change long-term prognosis, since sympathetic overactivity predisposes to secondary cerebro- and cardiovascular events. Therefore, it is highly important to manage dysautonomic imbalance, cardiac causes being held responsible for 2 up to 6% of total mortality three months after acute ischemic stroke [115]. As proven by previous studies, early cardiac monitoring of the stroke patients using HRV linear and nonlinear parameters in order to identify a sympathetic overactivation on the control of the heart rate, may improve the therapeutically approach and impact the short-term prognostic.

The autonomic dysregulation measured by HRV is not routinely assessed in everyday practice in stroke patients, but HRV might be a useful tool to predict and prevent secondary vascular events.

Recognizing the "neurogenic cardiac syndrome" [113] as well as establishing a personalized therapeutic strategy in ischemic stroke patients with altered sympathovagal balance represents an important management point because of the elevated risk of cardiac arrhythmias, especially in patients with sympathetic hyperactivity.

We also observed a tendency to normalize the values of the linear parameters following vagal activation tests in patients with sympathetic hyperactivity. This opens new therapeutic perspectives, such as the vagal nerve stimulation (VNS).

1.4.6. Conclusions

HRV represents a simple method of monitoring post ischemic stroke cardiac autonomic activity. Our results indicate that in ischemic stroke the autonomic nervous system dispose asymmetric responses to different stimulation autonomic tests between right and left hemisphere.

The right hemisphere stroke has a more pronounced sympathetic tonus than left hemisphere in right handed patients. This should provide new cues for the stratification of the outcome in stroke patients, and thus, contribute to the development of new clinical evaluation algorithms based on dysautonomic changes in these patients.

CHAPTER 2
REHABILITATION AND ITS SIGNIFICANCE IN INJURY RECOVERY

2.1. State of Art

By 2030, 1 in 6 people in the world will be aged 60 years or over. Moreover, people aged over 80 will represent 13% of the population over 60 years old; in industrialized countries, the value of this indicator will increase by 2050 from 19% to 29% [116].

Functional ability, independence and quality of life issues are of greater concern for old population. Due to a constant growth in the global population and, specifically, in the older population, a more comprehensive understanding of ageing is a must in order to be able to implement measures for ensuring and maintaining a successfully active ageing. If people can experience these extra years of life in good health and if they live in a supportive environment, their ability to do the things they value will be little different from that of a younger person [116]. Common conditions in older age include declines in physical and mental capacity, hearing loss, cataracts, stroke, vascular dementia, osteoarthritis, chronic obstructive pulmonary disease, diabetes, depression, etc.

Stroke represents the leading cause of long-term disability and the leading preventable cause of disability in adult population worldwide [117]. In industrialized countries it represents the third most common cause of death and a major burden with increasing clinical, economic and social impact. Although mortality has decreased significantly in the past decades, it is the disability that plays an important role in the future social outcome. Many patients have severe disability after stroke and only few gain full recovery and independence. The level of disability can vary from weakness, paralysis to cognitive impairment, including vascular dementia. Stroke leaves worldwide 5 million people permanently disabled [118].

Ischemic strokes occur as a result of an obstruction within a blood vessel supplying a local region of the brain, except when there is general circulatory failure due to cardiac arrest or systemic hypotension of various reasons (cardiac insufficiency, sepsis, etc.) involving diminished perfusion in a vascular territory, potentate by vascular stenosis.

Ischemic strokes are due to brutal decreases in blood supply and glucose to the brain parenchyma, involving either embolic obstruction of a brain vessel (cardiac, arteryto- artery embolic source) or initiation of the coagulation cascade at the level of ulcerated atherosclerotic

plaques (thrombotic mechanism), the underlying condition for this type of obstruction is the development of fatty deposits on the vessel walls.

Studies have shown that “time is brain”- neural plasticity is a complex phenomenon initiated after brain injury (vascular, traumatic, etc.) is based on the same fundamental mechanisms characterizing learning processes and development in healthy brains, and has been intensively studied by different approaches, from clinical to neuro-molecular level. It is well known that time window after stroke is crucial for effective therapeutically intervention, therefore rehabilitation protocols should critically address besides well-documented and individualized interventions, the timing issue, in order to promote recovery.

It has been shown that prognosis after stroke largely depends on a number of factors such as size and localization of the lesion, age of the patient, co-morbidities like atrial fibrillation, hypertension, diabetes, etc. Therefore, post-stroke management is currently still based on secondary prevention of cerebral vascular risk factors and physical therapeutic approach of deficits.

Usually, rehabilitation strategies are initiated after the subacute phase. In a clinical trial by Ronning and Guldvog [119], the authors reported that initiation of rehabilitation program in subacute phase post stroke facilitates recovery, patients with moderate and severe lesions showing the best functional outcome. Yet, there is an increasing need to better understand pathological mechanisms underlying early recovery post stroke and promotion of more targeted therapeutically approaches both in terms of mechanisms and time related efficacy following vascular injury.

One of the fundamental principles of neurorehabilitation in stroke is that repetitive and specific learning programs may promote mechanisms of neural plasticity underlying improved function. It is well established that following different brain destructive events, such as stroke or trauma, a cascade of regenerative events is initiated, lasting from weeks to months. Brain connections can be reshaped and reorganized by training strategies, implying repetition and temporal coherence.

This research direction has been materialized by publishing the following articles:

1. Cuciureanu I.D., Constantinescu I., Constantinescu V., **Matei D**. Triggering early neuroplasticity mechanisms: new perspectives in ischemic stroke management. Avid Science cfbc.ebooks@avidsciences.com,
2. Mirela Cristina L, **Matei D**, Ignat B, Popescu CD. Mirror therapy enhances upper extremity motor recovery in stroke patients. Acta Neurol Belg. 2015, Volume 115, Issue 4, pp597-603.
3. **Matei Daniela**, Corciovă Călin, Ignat Bogdan, Matei Radu. Transcranial magnetic stimulation in stroke rehabilitation. Balneo Research Journal. 2018;9(3):264–269.
4. Grigoras A.V., **Matei D**., Ignat E.B. Non-Immersive Virtual Reality for Upper Limb Rehabilitation in Stroke Survivors. - A Feasibility Study. Balneo Research Journal, Vol.9, No.3, September 2018 p:232 –239.
5. Constantinescu V, **Matei D**, Constantinescu I, Cuciureanu DI. Heart Rate Variability and Vagus Nerve Stimulation in Epilepsy. Transl Neurosci. 2019;10:223-232. doi: 10.1515/tnsci-2019-0036. eCollection 2019.
6. Constantinescu, V, **Matei D**, Constantinescu I; Cuciureanu, DI. Cardiac autonomic modulation in drug-resistant epilepsy patients after vagus nerve stimulation therapy. Neurologia I Neurochirurgia Polska, 2020, Volume: 54, Issue 4, pag. 329-336.
7. Matei Daniela, Carmen Grigoras, Dan Cuciureanu, Victor Constantinescu. The circadian rhythm of arterial blood pressure in Alzheimer's disease and vascular dementia. Acta Neurologica Belgica 2021.

2.2.1. **Mirror therapy enhances upper extremity motor recovery in stroke patients**
Introduction

Mirror therapy (MT) has been developed by Ramachandran and Roger-Ramachandran [120] in an attempt to control abnormal sensation in phantom limb syndrome. It acts by providing a falseinput to the brain, that in turn integrates it into a multimodal sensation or action: the patient has the illusion that the healthy limb he sees moving in the mirror is the affected one (that is hidden behind it), and associates correct movement with his intention/tentative.

Presence of the mirror did not increase excitability in the opposite M1 area in the absence of motor training in normal subjects, but it has modulated interhemispheric transcallosal inhibition, increasing the effect the active hemisphere has on the contralateral one [121].

An overview of Cochrane and other database reviews on different interventions that are thought to improve upper limb function in stroke provided moderate-quality evidence for a beneficial effect of MT on impairment, upper limb function and activities of daily living, suggesting that it may be effective. The same review concludes that in present, no high-quality evidence can be found in support of any of the approaches (either specialized interventions or part of routine practice), and also that evidence is insufficient to enable comparison of the relative effectiveness of interventions [122].

2.2.2. **Aim**

In this study, we try to evaluate the efficacy of MT combined with classical treatment in improving motor recovery of the upper limb in subacute stroke patients and also we try to evaluate if this simple, cost-effective therapy can be beneficial for patients recovering from the early phase after stroke.

2.2.3. **Patients and Methods**

Twenty-three patients with hemiparesis following a first stroke (documented by CT scan), time from stroke (between 1 and 3 months) and without severe attention deficit, were selected for the study. After clinical and paraclinical evaluation, 5 patients were excluded. Exclusion criteria were global aphasia, cognitive impairments that might interfere with understanding instructions for testing, concomitant progressive central or peripheral nervous

system disorders. 15 participants with subacute ischemic stroke, both women and men aged between 56 and 68 years, agreed and signed an informed consent form before joining the study.

The computer randomly assigns patients in two groups: mirror therapy group (MT) and control group (CT). This was a single-blinded trial. All the patients received a 6-week conventional stroke rehabilitation program for the upper limb, consisting of five—half an hour sessions a week. The control therapy consisted in neurorehabilitation techniques, electrical stimulation and occupational therapy.

The MT group received both, control therapy and 30-min mirror therapy sessions (5 session/week, 6 weeks) consisting of mirror seen unaffected upper limb movements. Patients were seated on a chair, with the mirror positioned between the upper limbs perpendicular to the subject's midline and with the unaffected upper limb facing the reflective surface. Under the supervision of the physiotherapist, the patients observed the reflection of their unaffected limb while performing the following movements with the both arms, the affected one as good as possible: flexion and extension of the shoulder, elbow, wrist and finger, prone supination of the forearm (Fig. 7).

Motor recovery was measured using the Brunnstrom stages, the Fugl–Meyer Assessment (upper extremity), the Ashworth Scale and the Bhakta test. Initial and final evaluations were made 1 day before and 1 day after the treatment period. None of the patients missed a session during the study and all of them finished the treatment period.

The statistical analysis of the results was performed using the software package STATISTICA 6.0 (StatSoft Inc., USA).

2.2.4. **Results**

We compared the baseline characteristics among 7 patients of the MT group and 8 patients of the control group, who received treatments for 6 weeks. The baseline assessments had no statistically significant difference between the groups. All parameters in the MT combined with conventional stroke rehabilitation program group showed significant improvements when compared with control group.

The Brunnstrom stage initial was 3.16 ± 0.71 and after therapy 4.52 ± 0.54 with $p<0.005$ for patients in MT group. Also in CT was 3.28 ± 0.75 before and 4.33 ± 0.89 after treatment with $p<0.05$. The Fugl–Meyer Assessment upper extremity score was 34.1 ± 8.4 before and 46.5 ± 7.5

after 6 weeks of rehabilitation, with $p<0.01$ in MT group. In the control group, there were small statistically significant improvements in the Fugl–Meyer Assessment, $p<0.05$ (Table 14).

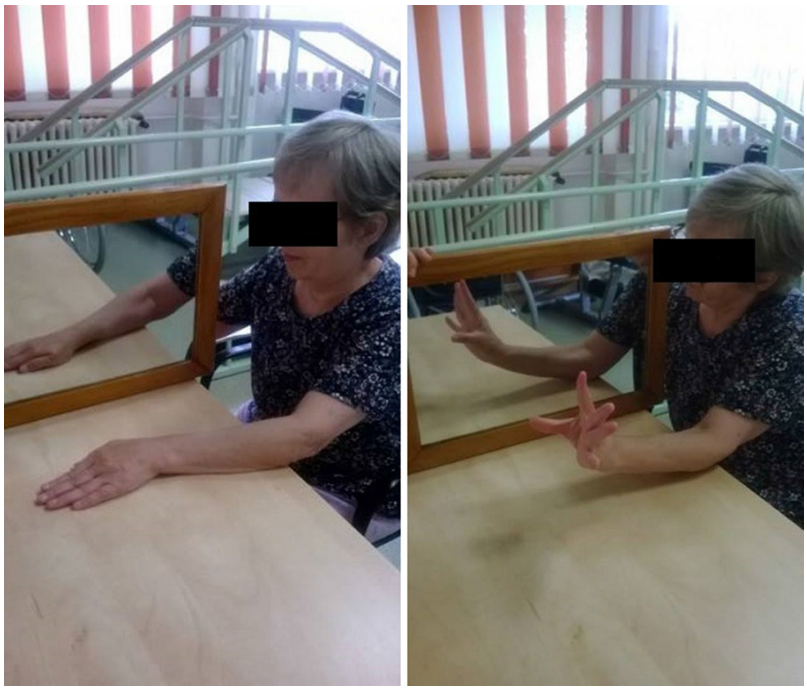

Fig. 7. Patient following mirror therapy

When we compare initial and final values using the Ashworth Scale, we see improvements in elbow ($p<0.02$), wrist ($p<0.04$) for the MT group and for the CT group, improvements were only in wrist level ($p<0.05$). Finger flexion scale was statistically significant in MT group with values before 3.44 ± 0.52 and after 3.88 ± 0.33 therapy, $p<0.04$. For the CT, we did not find any statistically significant improvement.

After 6 weeks of treatment, patients of MT showed improvements in the Fugl–Meyer Assessment ($p<0.01$), in the Ashworth Scale only for elbow ($p<0.02$), wrist ($p<0.04$) and the Bhakta test ($p<0.04$) compared to the CT group. If painful symptoms generally improved, according to patients, the statistical processing has not achieved significant results. Both MT and CT were well tolerated and no relevant adverse event was recorded during the study.

The most important results that we found are in the wrist articulation. As a result, the initial and the final measurements showed that extension increased from $40 \pm 7.07°$ to $56.6 \pm$

7.06° in MT group ($p<0.02$); flexion increased from 50.5 ± 9.5° to 63.8 ± 15.5° ($p<0.02$); pronation from 38.5 ± 7.5° to 57.1 ± 11.2°, $p<0.05$; supination from 41.4± 7.3 ° to 48.5 ± 9.7° $p<0.08$ in the same group. In the CT group, we found small improvements, but only in pronation ($p<0.05$). Elbow joint testing shows improvements in flexion in MT group ($p<0.05$). For shoulder, we did not find any statistically significant improvement in any groups.

	MT N=7	**CT N=8**	**p**
Mean age(years)	58.2±7.2	56.8±8.3	0.72
Gender (female/ male)	4/3	4/4	0.41
Affected side (right/left)	5/2	5/3	0.73
Stroke duration (days)	54.3±7.9	52.2±12.7	0.38
Brunnstrom stage			
initial	3.16±0.71	3.28±0.75	0.72
final	4.52±0.54	4.33±0.89	0.68
p	**0.005**	**0.05**	
Fugl-Meyer Assessment			
Upper extremity initial	34.1±8.4	38.6±6.2	0.22
Upper extremity final	46.5±7.5	47.3±6.3	0.84
p	**0.01**	**0.04**	
Asworth Scale			
Shoulder initial	1.72±0.36	1.83±1.44	0.34
Shoulder final	1.53±0.35	1.61±0.41	0.14
p	0.21	0.32	
Elbow initial	1.83±0.55	1.55±0.46	0.26
Elbow final	1.27±0.36	1.33±0.43	0.77
p	**0.02**	0.30	
Wrist initial	1.55±0.48	1.61±0.33	0.19
Wrist final	1.11±0.22	1.38±0.22	**0.01**
p	**0.04**	**0.05**	
Bhakta Test			
initial	3.44±0.52	3.22±0.19	0.34
final	3.88±0.33	3.55±0.52	**0.05**
p	**0.04**	0.16	

Table 14. Patients characteristic before and after therapy

$p<0.05$ means statistically significant (the values are in bold

2.2.5. **Discussion**

In our study, all the patients received a conventional stroke rehabilitation program for the upper limb, consisting in neurorehabilitation techniques, electrical stimulation and occupational therapy (5 half-hour sessions a week, for 6 weeks). The MT group received beside this program 30 min of mirror therapy. After 6 weeks of treatment, patients of MT showed improvements in

the Brunnstrom stage ($p<0.005$), in the Fugl–Meyer Assessment ($p<0.01$), in the Ashworth Scale only for elbow ($p<0.02$), wrist ($p<0.04$) and the Bhakta test ($p<0.04$) compared to the CT group. Both MT and CT were well tolerated and no relevant adverse event was recorded during the study.

On a cortical reactivity level, the initial dysbalance of hemispheric activity may be followed by an inhibition of the lesion side by the healthy hemisphere, thus preventing it from fully reaching its potential [123]. Temporary non-functional areas tend to lose their functional connections and in time may become permanently mute/non-functional [124]. 'Proper visual input' provided by MT may substitute for some of the missing proprioceptive input from the affected body side [125]. Facilitation of self-awareness, increased spatial attention, and intense concentration required to complete the bimanual task might contribute to better resource utilization and improved quality of movement [124].

It has been shown that incorporating mirror therapy into the conventional stroke rehabilitation program during the early stages [126] of treatment, but also in early chronic stroke [127] and applying it for a sufficiently long period might generate a supplementary improvement of the upper limb function.

Based on the data in the literature and on previous experience, we have appreciated that half an hour rehabilitation sessions suffice, avoiding excessive stress on the patient—mental effort that was required during the MT training was very tiring for the patients, as some of them stated that ''I feel less tired when I'm walking for 1 h'' or ''I feel that something it's happening in my brain, I feel that it works and it is tired.'' The therapist had the task to ensure the full collaboration of the patient. As we have stated before, attention and complexity of the task are important characteristics that are crucial for the ability of a procedure to induce long-term neuroplasticity [125].

In our study, there was a tendency for better results in patients that had started rehabilitation (and MT) immediately after the stroke, but the size of the study group was not large enough to allow a clear conclusion. As stroke survivors acquire incorrect movement patterns, it is more difficult to replace them with normal motor behavior, and MT could help in correcting these issues. One of the limitations of our study is the lack of a comprehensive cognitive evaluation (as the impact of attention- dependent techniques might reversely correlate with the patient's cognitive loss). Since we have anticipated an important role for patients'

degree of involvement, initial patient selection has discarded patients with major cognitive loss, and none of the included patients showed signs of significant depression. Adding MT to classical rehabilitation had beneficial effects in terms of motor abilities.

2.2.6. Conclusions

This study shows that 30 min 5 days of week for 6 weeks of mirror therapy in addition to a conventional stroke rehabilitation program was beneficial in terms of motor recovery of upper limb.

2.3. Other non-invasive methods for stroke rehabilitation

In recent years, there is an increasing interest in developing multi target therapeutically strategies to modulate pathophysiological complex processes post stroke. Neuroprotective agents such as Cerebrolysin, Edaravone are pharmacological agents which may repair damaged brain circuits by mediating endogenous brain-derived neurotrophic factor. Activation of intrinsic neural stem cells or transplantation of extrinsic neural stem cells or neural cells derived from stem cells such as embryonic stem cells and induced pluripotent stem may be future alternatives to“correct” vascular damage [128].

Other non-pharmacological strategies to target multi-level “healing” processes are still to be clarified such as: Transcranial magnetic stimulation, Functional Electrical Stimulation, Virtual Reality.

Transcranial magnetic stimulation (TMS) it is a novel noninvasive method externally modulates cortical excitability, by inducing brief and pulsed magnetic field and causing localized depolarization of superficial cortical and subcortical neurons, located between 1.5 and 2 cm below the cranial bone [129]. TMS can be applied as a single-pulse, paired-pulse or in trains (repetitive TMS). Single-pulseTMS is used to analyse different aspects of sensorimotor cortex and pyramidal tract function by measuring the motor threshold (MT) and motor evoked potential parameters [129].

Repetitive TMS can use stimulation at 1 Hertz or less (slow rTMS) or stimulation at a frequency higher than 1 Hz (fast rTMS). Studies have shown that rTMS at low frequency produces long-lasting inhibition, which is called as long-term depression, whereas repeated high-frequency stimulation can produce excitation through long-term potentiation [130].

TMS short-term effects are due to changes in neuronal excitability caused by shifts in ionic balance of active neurons. Longer-lasting effects of TMS appear to depend on synaptic changes among cortical neurons, also known as long-term depression and long-term potentiation. Experiments using fMRI and TMS have revealed evidence of extremely rapid plasticity. rTMS may have neuroprotective effects by reducing oxidative stress, inflammation, and by increasing levels of neurotrophic factors. Experimental evidence suggests that focal brain stimulation can improve motor and cognitive processes, such as working memory. Elevations of mood are associated with right-sided excitation and depression with left-sided excitation. The disadvantage might be the short duration of this effect.

Using TMS after stroke, it has been shown that excitability of the peri-infarct cortex is reduced in the vicinity of the vascular injury [131]. It is already well-known that peri-infarct areas play a role in neurological recovery via plasticity mechanisms [131]. Recent data postulated that stroke may affect the balance of transcallosal inhibitory pathways between motor primary areas in both hemispheres: the affected hemisphere may be disrupted not only by the infarct itself but also by the resulting asymmetric inhibition from the unaffected hemisphere. Low-frequency stimulation (≤1 Hz) delivered to the unaffected hemisphere may diminish hyperactivity of the intact brain, while high-frequency rTMS (>1 Hz) delivered to the affected cortex promotes recovery mechanisms [132].

This differential modulation, “upregulating” the lesion hemisphere or “downregulating” the intact hemisphere, being associated with improved motor performance [132]. Therefore, rTMS could be used as a therapeutically tool to restore the balance of interhemispheric inhibition after stroke.

TMS might provide a new insight into the pathophysiology of the nervous system, and can be used in all areas of cognitive neuroscience. All these are supported by the available studies, but more investigations are needed to establish the clinical indication as a diagnostic or therapeutic tool in any neurological or psychiatric disease.

Functional electrical stimulation (FES) is a form of electrical stimulation applied in rehabilitation practices on a nerve pathway or motor point to produce a muscle contraction that has the ability to be assimilated in the normal motor engram. The patient uses the stimulation to execute a functional movement and depending on the severity of the paresis can be used as a functional substitute. Studies have shown that FES is able to improve axonal conduction velocities, axonal growth, and the myelination of peripheral nerves [133]. It has been shown that

FES device enhances peripheral nerve activity (efferent activation) and corresponding muscle and joint proprioceptive feedback (afferent activation).

In the case of foot drop, the stimulation is made on the common peroneal nerve. The forefoot is raised by electrical stimulation during the dynamic swing phase of the leg, and the patient's gait is thus improved. The literature abounds in clinical studies regarding utilization of FES for patients with upper neuron problems such as multiple sclerosis, Parkinson and stroke.

Virtual reality is a computer-generated, interactive simulation that maps the real environment by affecting human senses, and shows all activity in real time and with real speed. Virtual reality sends the user a great number of sensory information comparable to authentic experiences. This modern computer technology emulates learning process in the real world, while allowing the addition of extrinsic feedback and increasing the frequency, duration, and even intensity of an exercise. Virtual environment enables the user to have the opportunity to interact with objects and situations produced by the hardware [134]. A distinctive platform creates well-defined and customized activities, combining factors such as intensity, variation and specificity of the tasks described as significant for increasing plasticity of the brain. Virtual reality technologies allow creation of a simulated environment so that proper adjustment of exercise intensity and feedback would provide the patient with safe and effective training and rehabilitation. Virtual reality has now emerged as a promising tool in many domains of therapy and rehabilitation.

Virtual reality therapy for stroke victims has infinite possibly and potential. It was proven to help improve motor impairment as mentioned in a 2011 study published by the American Stroke Association found. In the 2011 study found that 11 out of 12 studies previous studies showed a significant benefit from rehabilitation with virtual reality. Another benefit of using Virtual reality therapy in patients post stroke is being able to offer many different treatment options for each patient as no stroke survivor has the same impairment. Stroke physical therapy can be individualized to render the most effective treatment which will optimize rehabilitation and help the patient get close to where they were physically and motor skill wise before suffering from a stroke.

Experimental data showed that the use of virtual reality and interactive video gaming may be beneficial in improving upper limb functions and activities of daily living (ADL) outcome, when compared with the same amount of conventional therapy [135]. Virtual reality

may be easily used in hospital settings where global motor activity and gait training may be more difficult to apply on a regular basis.

2.4. Rehabilitation of lower back pain due to lumbar disc herniation (LDH) is an important and enormous public health and social issue. Although the discectomy is a relative common surgical procedure for lumbar disc problems, in this moment in Romania we do not find studies that treat this problem in its socio-economical context. In European Union's western countries and in the United States of America there are surveys made by the social security services in order to understand the impact of this health problem on social context and economic costs in the effort to minimize the burden on the society. A few epidemiological data reveals that 75% - 85% of the population will experience an episode of lower back pain in their life and national statistics from USA report prevalence between 15% and 20%. Back pain is the most frequent cause of job activity limitation for people under 45 years old, the fifth most frequent reason for hospitalization and the third place for surgical procedures. About 2% of the population is chronically and temporarily disabled [136]. In the medico-social decision making situation in which various clinical studies stated that in long terms surgical treatment is no more and no less efficient than classical rehabilitation or physiotherapy treatment the costs of the medical services in treating the patients with LDH must be carefully addressed.

This research direction has been materialized by publishing the following articles:

1. Sardaru, Dragos Petrica; **Matei, Daniela**; Zaharia-Kezdi, Dan; et al. Effects of biofeedback versus switch-triggered functional electrical stimulation on sciatica-related foot drop. Journal of Back and Musculoskeletal Rehabilitation, 2018; 31(2): 239-245.
2. Sardaru, D., Boldureanu, D., Andruseac, G., **Matei, D**., Zaharia-Kezdi, D., Poeata, I. Paralytic Lumbar Disc Herniation. A Four Years Social and Economic Impact Study for North-East Region of Romania. Revista de Cercetare si Interventie Sociala, 2017; 57: 67-77. ISSN: 1583-3410.

2.4.1. Introduction

Study 1. Effects of biofeedback versus switch-triggered functional electrical stimulation on sciatica-related foot drop.

Foot drop refers to a significant weakness of ankle and toe dorsiflexion that can be caused by a number of clinical disorders. In the specialized literature foot drop was defined as a significant decrease of tibialis anterior and extensor halluces longus muscles force. Using manual muscle testing (MMT), foot drop was categorized as the ankle dorsiflexion power at MMT grade 3 or less [137]. It can be present in paretic/paralytic lower limb determined by peripheral pathologies such as degenerative conditions of the spine as well as central nervous system pathology.

Functional electric stimulation (FES) is a form of electrical stimulation applied in physiotherapy practices on a nerve pathway or motor point to produce muscle contraction that can be assimilated in the normal motor engram. In the case of foot drop, the stimulation is made on the common peroneal nerve. The forefoot is raised by electrical stimulation during the dynamic swing phase of the leg, and the patient's gait is thus improved [138].

The literature abounds in clinical studies regarding utilization of FES for patients with upper neuron problems such as multiple sclerosis, Parkinson and stroke but is utterly silent in what concerns the investigation of the effects of FES on peripheral neuronal regeneration

Study 2 - Paralytic lumbar disc herniation. a four years social and economic impact study for north-east region of Romania

The complications of the LDH are not to be minimized in what concerns the social and economical costs. This can begin with the recurrence of the health problem, hyperalgesic sciatica, loss of different degrees in the capacity of movement in the lower limb (paralytic sciatica) that can produce serious difficulties in ambulation and social interaction and the risk of the condition in becoming chronic. The chronic state of the LDH is a real factor to take in consideration because from this point the patients is surely going to access every year rehabilitation or physiotherapy services which in long term will increase the economic costs and the missing days from work.

2.4.2. **Aim**

Study 1 - the study wants to clarify if FES is potentially efficient in the functional re-education in foot drop and muscle re-innervation due to peripheral nerve injuries, and to compare two different techniques of employing FES.

Study 2- The current study aims to clarify and approximate the economic costs that are necessary for the treatment of patients suffering from LDH. The subject will be treated cumulating the total costs of hospitalization for surgery and rehabilitation treatment in the idea of obtaining an approximate total cost for the reinsertion/reintegration in the line of work.

2.4.3. **Patients and Methods**

Study 1 - fifty patients suffering from foot drop were enrolled in our study. The participants were randomly assigned to two groups. The first group of 25 patients received FES triggered by electromyography (EMG-FES) and the second group of 25 patients received FES triggered by a foot-floor contact transmitter placed bellow the heel (heel switch) (SWITCH-FES). FES was applied in both subgroups in 20 physiotherapy sessions as follows: once a day for 30 minutes during 5 consecutive days, over a period of 4 weeks. Stimulation was performed on the affected limb by using surface electrodes according to the principle of transcutaneous electrical nerve stimulation (TENS). The scientific literature provides information about the frequency and phase duration on static electrical stimulation for peripheral nerve regeneration and axonal repair (20 Hz) but it does not provide these parameters for use of FES in peripheral nerve problems [139]. sEMG triggered muscle stimulation is a protocol that requires a patient to voluntarily contract a muscle in order to initiate a certain movement. The patient is asked to generate a maximum contraction during the 10 second capture time; the software will then identify the maximum muscle effort automatically. The sEMG evaluation is done through the detection of peak to peak signal and is returned as a graphic numerical scale.

For the SWITCH-FES group electrical stimulation was done with ODFS – Pace 1.0 (Odstock Dropped Foot Stimulator) from Odstock medical Ltd. U.K. FES was administered percutaneously via round electrodes measuring 4 cm in diameter. The cathode electrode was placed immediately under the proximal head of the peroneus bone and the anode was placed on the anterior face of the tibialis anterior muscle. In the SWITCH-FES group the electrical

contraction was determined by a foot-floor sensor mounted under the heel. When the heel is elevated during the gait cycle the electrical stimulation is transmitted (Fig 8, 9)

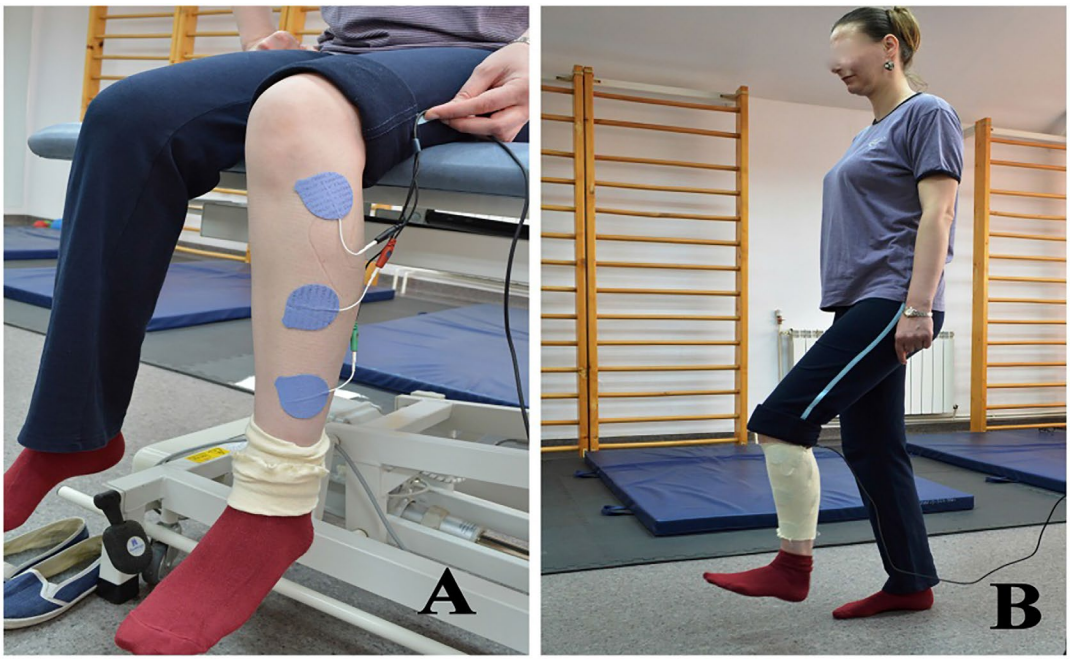

Fig. 8. EMG triggered functional electric stimulation technique. A – Placement of the electrodes for EMG signals capturing. Patient in siting position is asked to contract tibialis anterior muscle during 10 seconds. B – Patient during walking with the aid of the FES. During the swing phase is required for the patient to achieve a minimal active contraction to trigger the electrical stimulation.

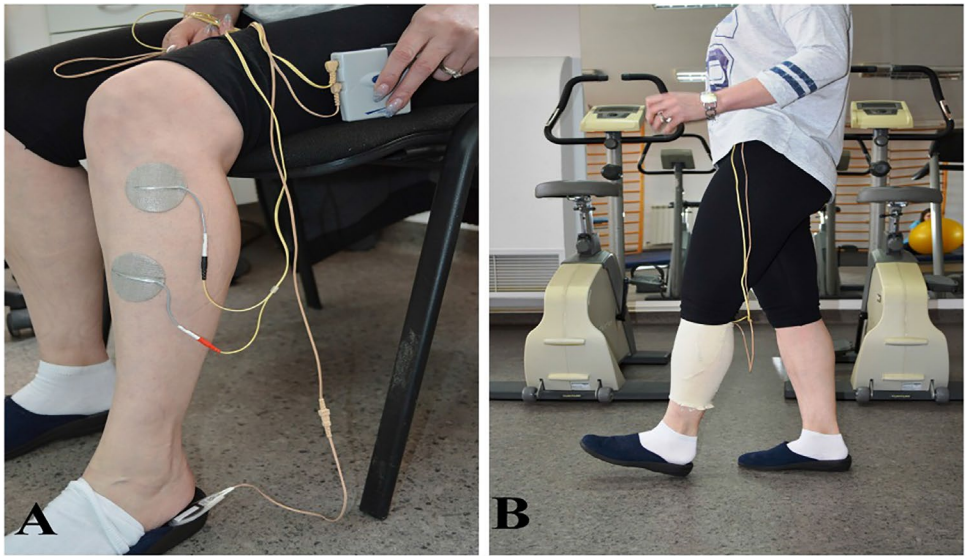

Fig. 9. Switch triggered technique. A – Placement of the electrodes and the heel-floor sensor. B – Patient walking with the aid of the FES. At the beginning of the swing phase of the gait the patient elevates the heel and the sensor from the underneath is released and in turn the device triggers the electrical stimulation.

For electrophysiological investigations the electromyograph Neuro – MEP Micro was used. The stimulodetection examination was realized in the motor fibers of peroneal nerve. We took into consideration the nerve conduction velocity (NCV-m/s) to evaluate the demyelination and the amplitude of compound muscle action potential (CMAPmV) to evaluate the axonal loss. The surface electrodes were used to obtain muscular response according to the principle "belly-tendon". Proximal site of stimulation was placed behind the fibular head and distal site was ankle (between the extensor digitorum longus and extensor hallucis longus tendons. The extensor digitorum brevis muscle represented the collection point of the recording.

Muscle force was tested also in order to correlate the functional restoration of the link between the nerve and muscle, we used the "make test" from the handheld dynamometry technique (HHD). The muscle force test was performed twice and we took into consideration the sum of the two muscle force values.

The overall functional status was evaluated by the Oswestry Disability Index (ODI) because foot drop caused by radicular compression is closely related to lumbar column status. ODI is an index derived from the Oswestry Low Back Pain Questionnaire used by clinicians and researchers to quantify disability for low back pain and its related dysfunctions.

The statistical analysis of the results was performed using the BioStatAnalystSoft software package. To determine the differences between the groups we used Test t-Student for normally distributed continuous variables, and the chi-square test for categorical variables.

Study 2- We have collected data from two hospital centers from two different counties in the North-East region of Romania. The data about the costs and patients were received after the approval of the administration and the ethics committee of each of the hospital center. We took in consideration two specialized hospital centers in order to be more representatives for our research as follows: Clinical Emergency Hospital "N. Oblu" from Iasi (First center) and County Emergency Hospital from Vaslui (Second center). The data were collected from for years consecutively from 2013 until 2016.

In total a number of 7438 patients were found to have received surgical treatment in the neurosurgery departments for lumbar disc herniation from these only 252 patients have received physiotherapy and rehabilitation treatment. This is also due to the fact that either the patients are not guided to the rehabilitation centers by the medical staff or they decide to undertake their rehabilitation treatment in the private sector.

From this data we have analyzed for each year taking in consideration the specific of each medical branch department: total days per year of hospitalization for neurosurgery and rehabilitation treatment, average number of days for each patient, total cost per year per department, average cost for each day of hospitalization, incidence of the LDH from the total number of patients admitted in the neurosurgical departments.

2.4.4. Results

Study 1- In our study the first group was made up of 25 patients (14 males and 11 females), with mean age of 44.40 ± 7.69 years old, received FES triggered by electromyography and the second group of 25 patients (12 males and 13 females), with mean age of 42.92 ±6.73 years old, received FES triggered by a foot-floor contact transmitter placed bellow the heel.
The results for both groups of nerve conduction velocity were not statistically significant. However, in the analysis made on CMAP we found differences between the two groups. For the EMG-FES group CMAP registered initially 1.252 ± 0.357 mV, and after the therapy it went to 1.894 ± 0.490 mV. The CMAP for SWITCH-FES group initially was 1.134 ± 0.273 mV, and after the treatment it was 1.615 ± 0.435 mV. At the end of therapy, CMAP increased for the EMG-FES group with 0.642 ± 0.180 microV (52%), and for SWITCH-FES group with 0.481 ±0.199 microV (43%), with statistical difference between groups ($p < 0.004$).

The Dynamometry results were at the beginning 11.04 ± 2.57 Kgf and 15.72 _±2.96 Kgf at the end of the treatment for the EMG-FES group. For the SWITCH-FES group, the Dynamometry results were 12.12 ± 3.50 Kgf initially and 14.92 ±4.04 Kgf at the end. Dynamometry values improved by 4.44 ± 1.26 Kgf (41%) for the EMG-FES group and by 2.840 ±1.028 Kgf (26%) for the SWITCH-FES group with $p < 0.0001$. The ODI initial score (35.84 ± 6.90 EMG-FES vs. 34.36 ± 4.70 SWITCH-FES) and final score (19.72± 5.38 EMG-FES vs. 22.08 ± 4.58 SWITCH-FES) registered a decrease of 16.12 ± 3.46 points (45%) for EMG-FES vs. 12.28 ± 1.51 points (36%) for SWITCH-FES, differences with statistical signification, $p < 0.0001$.

Study 2 - The mean age of the patients in the neurosurgery departments were in 2013 – 48.2 years old, in 2014 – 46.9 years old, in 2015 – 50.1 years old and in 2016 46.4 years old. For department of neurology the patients with LDH had a mean age in 2013 of 60.2, 2014 – 59.8, 2015 – 60.8 and in 2016 was 61.3 years old. For the rehabilitation department the patients that addressed the service had a mean age in the first year of 58.8 years old, in 2014 – 58.1, in 2015

55.1 and in 2016 - 61.1 years old. In the space of four years from 2013 until 2016 the gender difference of population that accessed the services of neurology and neurosurgery was in majority females in proportion of 58.31% and males in proportion of 41.69%.

In neurosurgery department from Clinical Emergency Hospital "N. Oblu" in 2013 the incidence was 31.55% from the total patients, versus 19.60% in the County Emergency Hospital from Vaslui. The percentage of incidence vary for the next years also: in 2014 was 28.60% versus 51.80%, in 2015 was 27.01% versus 67.64% and in 2016 was 23.43% versus 78.72%. In the rehabilitation department the incidence was as follows: in 2013 – 34.26%, 2014 – 36.69%, 2015 – 24.48% and in 2016 – 24.51%.

The number of days spent in the hospitalization regimen during the discectomy varies in concordance with the difficulty of the cases. In general we have found to be between 6.84 days and 10.78 days. In the rehabilitation service the patients generally stay longer because of the specific of the therapy that needs more time to take its effects on the functionality of musculo-skeletal system.

A very important part of the social reinsertion of the different categories of pathology patients are the costs that the social security services pay. These costs depend of the type of pathology, comorbidities, predisposition to chronic development of the pathology, and days of hospitalization. We have divided and compared the costs between the two groups. First group is comprised of patients undertaking surgery treatment and the second group the patients that choose the rehabilitation and physiotherapy treatment. We have found in the neurosurgery department from the first center in 2013 for a mean number of 7.29 days a cost of 2700.2 lei, in 2014 for 7.37 days a cost of 2800.59 lei, in 2015 for 7.26 days a cost of 2758.8 lei and in 2016 for a mean number of 6.84 days a cost of 2800.59 lei. In the second center in the neurosurgery department we have found in 2013 for a mean number of 10.78 days a cost of 3202.55 lei, in 2014 for 8.40 days a cost of 2635.43 lei, in 2015 for 9.35 days a cost of 2415 and in 2016 for a mean number of 8.27 days a cost of 2070.20 lei.

From the trends seen on the evolution graphics it may seem that the general costs for the treatment of one patient suffering from LDH is higher for those who access the services of physiotherapy and rehabilitation centers. While the general cost may seem higher for the rehabilitation centers in fact this cost is influenced by the number of days. Because of the physiotherapy specific medical treatment the number of days may vary depending on the

functional outcome and residual capacity. The cost per day of treatment for one patient is summarized in the Fig. 10.

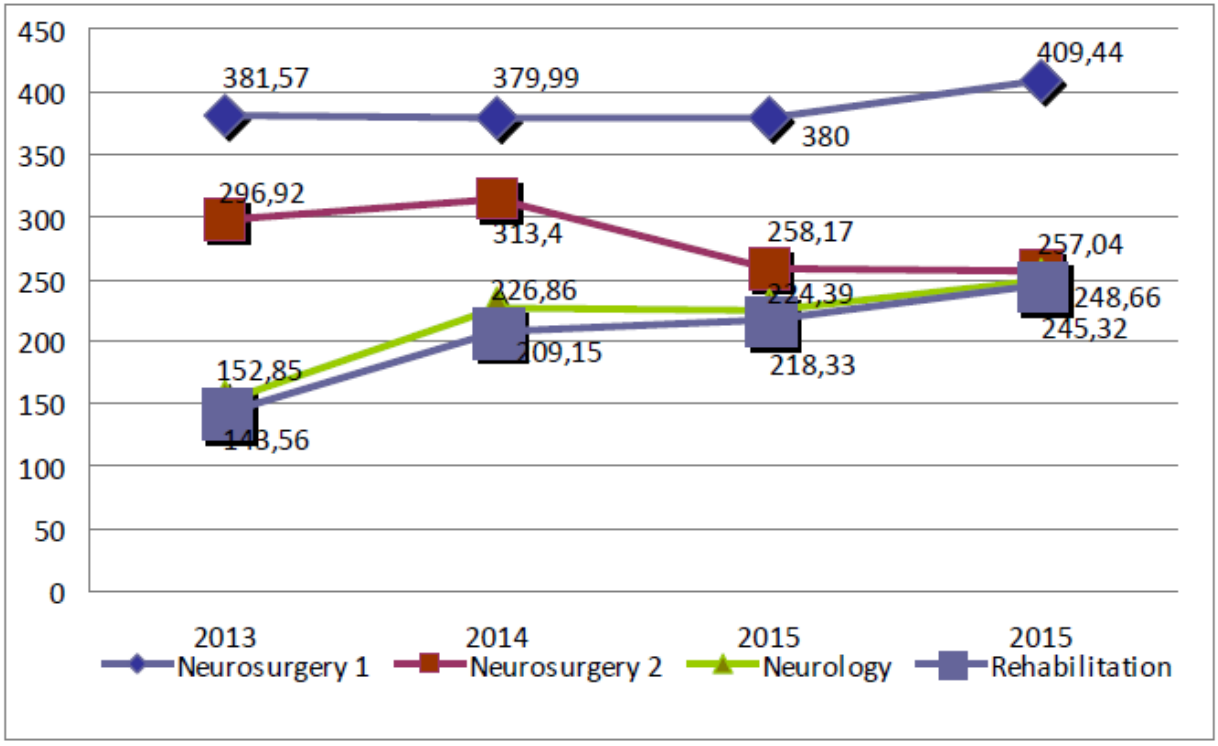

Fig. 10. Evolution of cost per patient per day in different treatment departments

2.4.5. Discussion

Study 1 - In this study, we presented two different techniques for functional electrical stimulation in peripheral nerve pathology, after having noticed the lack of published scientific evidence comparing Switch with EMG triggered FES, Also, we were interested in the uses and effects of functional electrical stimulation on peripheral nerve pathologies such as radicular nerve compression.

In order to regain central motor control, the peripheral nerve must first regain its integrity and conductibility. The problem with the utilization of FES on a peripheral nerve injury is that there is insufficient valid data to provide useful insights regarding the appropriate stimulation settings.

In our study, we enrolled fifty patients suffering from foot drop secondary to lumbar spinal disc herniation. They received 20 intensive physiotherapy sessions of FES training by means of two different modalities of triggering the electrical stimuli. We wanted to identify if

there is a difference on the peripheral nerve regeneration and muscle functional reintegration when using EMG as opposed to SWITCH triggering of the electrical stimuli. At the end of the treatment, the evaluation showed that the EMG-FES group had slightly better results than the SWITCH-FES group. The EMG-FES group showed improvements in CMAP ($p < 0.004$), which underlines an important process of axonal regeneration, also for dynamometry test ($p < 0.0001$) and similarly when analyzing the data from ODI index ($p < 0.0001$). Although the SWITCH-FES group had relatively poorer results we reported an increase in compound muscle action potential (CMAP) and a decrease in the ODI index score.

In our patients, lower limb electrical stimulation improved walking ability, increased mobility and increased voluntary EMG activity in EMG triggered FES. In the FES group we obtained increased isometric contractions of dorsiflexors and plantar flexors and improved walking ability. In the EMG triggered group we noticed an important correlation between the subjective overall functional status felt by the subjects (ODI score) in relation to the increased motor control of the dorsal ankle flexion (Dynamometry).

Study 2- We have collected data sensible to the costs and overall days of hospitalization from two hospital centers from the North-East region of Romania in order to try to understand the dynamic of social impact over a four year period. The data have a possible limitation due to the fact that we have not taken in consideration all the hospitals in the region with the neurosurgery departments because of the nonresponse rate to inquire.

The process of cost effectiveness evaluation for different categories of pathology should be taken in consideration by the health and social security systems. At this moment we did not find in the literature any scientific article to treat the subject of the patients suffering from LDH and its medical costs in correlation to work leave and its impact on social structure. From our study the data suggest that the costs per day of hospitalization for surgery treatment taking in account all the costs that come along with the hospitalization fees are higher than the cost alone of the physiotherapy treatment.

The minimum number of days that a person becomes social inactive it depends on whether he is treated only by surgical means in which case he leaves work between a mean number of 6,84 to 10,78 days. If after that he begins physiotherapy treatment than the number of days can rise up to 21,2 days. However the reality of the social cost is higher because a major

number of the patients suffering from LDH are developing a chronic state which means revisiting more than one a year the physiotherapy centers.

2.4.6. Conclusions

We found that the EMG triggered electrical stimulation technique produced better results in the quality of muscle action. Our recommendation for clinical practice is to start with the passively triggered technique if the patient does not have the possibility to tense the muscle and then, immediately after the recovery of motor control, to change to EMG triggered functional electrical stimulation.

The social impact for the patients suffering from lumbar disc herniation is related to the number of medical services that are provided, the indirect cost coming from work leave and hidden cost determined by social inactivity. This is why is not easy to correlate all these data in understanding the final and total costs. To ameliorate the negative influence over the social security system it is important for the medical specialists to make a good decision in differentiating the patients that must have surgical treatment versus patients that only need physiotherapy treatment in order to reduce the number of days of work leave and direct economic costs.

2.5. Vagus nerve stimulation in treating pharmacoresistant epilepsy

Epilepsy affects approximately 65 million people worldwide [140]. Although therapy has substantially developed, about a third of patients remain resistant to drug treatment [140]. This leads to high mortality and morbidity [141]. Prevention measures and recognition of modifiable risk factors may reduce epilepsy mortality.

Vagus nerve stimulation (VNS) represents an adjuvant treatment for medically refractory partial-onset seizures in adults and adolescents [142]. VNS consists of chronic intermittent electrical stimulation of the vagus nerve, delivered by a programmable pulse generator [142]. VNS may represent an earlier stage option in treating pharmacoresistant epilepsy, with positive long-term effects, reducing the frequency of seizures and ameliorating the quality of the interictal period [143].

This research direction has been materialized by publishing the following articles:

1. Constantinescu V, **Matei D**, Constantinescu I, Cuciureanu DI. Heart Rate Variability and Vagus Nerve Stimulation in Epilepsy. Transl Neurosci. 2019;10:223-232. doi: 10.1515/tnsci-2019-0036. eCollection 2019.
2. Constantinescu, V, **Matei D**, Constantinescu I; Cuciureanu, DI. Cardiac autonomic modulation in drug-resistant epilepsy patients after vagus nerve stimulation therapy. Neurologia I Neurochirurgia Polska, 2020, Volume: 54, Issue 4, pag. 329-336.

Since VNS was approved as a therapeutic approach for the treatment of refractory epilepsy, the search has been ongoing for nonpharmacological modulation of the ANS for different pathological conditions. The advantage of VNS therapy has also been evaluated for drug-resistant depression, heart failure, hypertension, and cardiac arrhythmias [144].

The precise mechanism of neuromodulation exerted by the VNS is still a matter of debate. 80% of the fibres of the vagus nerve are afferent pathways to the central nervous system. Only 20% are efferent pathways, some of them reaching the cardiovascular system [145]. The afferent pathways of the vagus nerve play an essential role in the neuromodulation process, influencing the interplay of various cortical networks probably involved in epileptogenic activity [146]. The activation of the vagal efferent pathways concerns the sinoatrial node and the cardiac conduction system [146]. Consequently, it may decrease the heart rate and reduce atrioventricular conduction and excitability of the His bundle.

Research dedicated to VNS's impact on cardiac rhythm has yielded contradictory results. A minor increase in sympathetic cardiovascular modulation without significant haemodynamic effects, probably related to the activation of sympathetic pathways from the brainstem, has been reported [145]. Cardiac Brady arrhythmia is a rare complication during ongoing VNS therapy [147]. An increase in cardiac vagal modulation appears to play a cardioprotective role against sudden death [148].

HRV analysis in epilepsy provides essential information about the risk of sudden death by cardiac arrhythmias in these patients [149].

2.5.2. Aims

study 1- Heart Rate Variability and Vagus Nerve Stimulation in Epilepsy- The aim of this research is to evaluate the impact of the VNS on cardiovascular autonomic function, through

sympathetic and parasympathetic activation tests, after three months of neurostimulation in drug-resistant epilepsy patients, using MTRS analysis of the HRV.

study 2- Cardiac autonomic modulation in drug-resistant epilepsy patients after vagus nerve stimulation therapy. The same group of patients was previously assessed using Multiple Trigonometric Regressive Spectral analysis. The actual evaluation based on Fast Fourier Transform provides, in addition to the spectral power analysis, the non-linear appraisal of the HRV.

2.5.3. Patients and methods

study 1 - ECG recordings of the first five drug-resistant epilepsy patients from our department who underwent left latero-cervical VNS procedures were analyzed using MTRS software. None of the five patients underwent epilepsy surgery and had no cardiovascular comorbidities, as well as no cardiovascular medication. No changes in the antiepileptic medication were performed three months prior to our first evaluation and between the two HRV tests.

ECG was registered before the VNS procedure and at least three months after the implantation of the electrode. ECG recordings were performed during ON and OFF time period of VNS stimulation.

We applied a standardized protocol including resting state and subsequently four autonomic activation tests, each test entailing a five minute ECG recording. Two sympathetic activation tests were performed: a maximal voluntary isometric contraction of the fist, using a dynamometer – "handgrip" test and a three-minute standing test. Two parasympathetic activation tests were considered: "deep breathing" test, consisting of six complete cycles of deep inhale and exhale over 60 seconds, with timing, 10 seconds for each cycle, and Valsalva maneuver.

BIOPAC acquisition system was used for data collection and analysis. Data processing was done using MTRS software version 7.3.2.0 (University Hospital, Center for Clinical Neuroscience, Dresden, Germany). This software assesses the HRV time-domain and frequency-domain parameters, based on the trigonometric regressive analysis

The analysis was performed using GraphPad Prism software version 8.1.0. For the statistical analysis of the data, having into consideration the small sample size, series normalization was very difficult. Wilcoxon matched-pairs tests were applied to compare the

parameters of the analyzed series. Spearman's rank correlation coefficient (rs) was used to assess the relationship between variables, and Student's t-test or non-parametric Mann–Whitney test were employed to determine differences. The significance level (p-value) was considered to be 0.05 (5%).

study 2- ECG recordings of the first five patients with drug-resistant epilepsy who underwent VNS procedure, in our department, were analyzed. In these patients, seizure control was not obtained within two years of multiple antiepileptic drug treatment.

Each patient had an ECG recording before VNS procedure and after three months of neurostimulation, during ON and OFF periods of the stimulation. All patients were monitored with prolonged EEG and ECG recordings before and after the autonomic tests (including night EEG), which excluded clinical or infraclinical seizures. A standardized protocol consisting of a resting state ECG recording followed by four autonomic activation tests, each lasting for five minutes, was applied. The four autonomic activation tests were performed in the same sequence in all patients, as follows: deep breathing, standing, hand-grip and Valsalva manoeuvre.

HRV analysis was performed using Kubios HRV software version 2.2 (Biosignal Analysis and Medical Imaging Group, University of Eastern Finland). HRV parameters were analyzed using Fast Fourier Transform. A minimum of 256 RR intervals on the ECG were analyzed for each recording. The HRV time/frequency-domain and non-linear analysis were done.

The output current of stimulation was 2 mA for the first, third and fourth patients, 1.5 mA for the second patient, and 1 mA for the fifth patient. The frequency of stimulation was set at 30Hz for all five patients, while pulse width (500 μsec), duty cycle (10%), ON time period (30 seconds) and OFF time period (5 minutes) was identical for all patients.

The first patient, a 33-year-old female, was diagnosed with focal epilepsy (left anterior temporal epilepsy) and secondarily generalized seizures. The clinical symptoms were rotatory vertigo, breathing difficulties, facial flush, dreamy state, and generalization. Brain MRI showed no abnormalities. The patient was being medicated with three antiepileptic's: lamotrigine, levetiracetam and oxcarbazepine.

The second patient, a 34-year-old female, presented focal epilepsy (left insular epilepsy) with secondarily generalized seizures. The clinical symptoms were nausea, dyspnea, abnormal

sensation of retrosternal pain, burning heat restricted to the perioral area. No epilepsy-related brain MRI abnormalities were found. The patient was under treatment with valproic acid and levetiracetam.

The third patient, a 34-year-old female, had been diagnosed with focal epilepsy (right insular epilepsy) and secondarily generalized seizures (rotatory vertigo, facial flush, sense of unreality) at the age of 22. No epilepsy-related brain MRI abnormalities. Patients under treatment with levetiracetam and oxcarbazepine.

The fourth patient, a 29-year-old female, presented multifocal epilepsy with secondarily generalized seizures (onset features: vertigo, sweating and motor unilateral symptoms, motor aphasia and generalization). Brain MRI showed parietal and occipital gyration abnormalities. Pharmacological treatment consisted of lamotrigine, levetiracetam and carbamazepine.

The fifth patient, a 31-year-old male, presented left insular focal epilepsy with secondarily generalized seizures (retrosternal ascending heat, hypersalivation and post-ictal psychomotor agitation with hetero-aggressive behaviour). Left insular atrophy with frontoparietal extension was revealed on cerebral MRI. Antiepileptic medication consisted of valproate and oxcarbazepine.

2.5.4. Results

study 1- Clinical symptoms, type of epilepsy, age of onset, cerebral MRI findings and current treatment are depicted in table 15. Patients 1 and 3 were seizure-free in last month before the second HRV evaluation, patient 2 had no seizure in the last three weeks before the second HRV evaluation and patient 4 had no change in seizure frequency after three months of neurostimulation. Seizure frequency decreased for patient 3 in the last month before the second HRV evaluation, during VNS therapy.

Patients Age/gender	Clinical symptoms	Age of onset	Type of epilepsy	Brain MRI	Current treatment
Patient 1 33/female	rotatory vertigo, breathing difficulties, dreamy state, facial rush, generalization	8	focal epilepsy (left anterior temporal), secondarily generalized seizures	no epilepsy-related abnormalities	Lamotrigine, Levetiracetam and Oxcarbazepine
Patient 2 34/female	abnormal sensation of retrosternal pain, nausea, dyspnea, burning "heat" restricted in the perioral area, anarthria	6	focal epilepsy (left insular epilepsy)"	no epilepsy-related abnormalities	Valproic acid and Levetiracetam
Patient 3 31/male	retrosternal ascending "heat", hypersalivation and post-ictal psychomotor agitation with hetero-aggressive behavior	4	focal epilepsy (left insular epilepsy) with secondarily generalized seizures	left insular atrophy with frontoparietal extension	Valproate and Oxcarbazepine
Patient 4 29/female	vertigo, sweating and motor unilateral symptoms, motor aphasia and generalization	8	multifocal epilepsy with secondarily generalized seizures	Parietal and occipital gyration abnormalities	Lamotrigine, Levetiracetam and Carbamazepine
Patient 5 34/female	rotatory vertigo, facial flush, sense of unreality and generalization	22	focal epilepsy (right insular epilepsy) with secondarily generalized seizures	no epilepsy-related abnormalities	Levetiracetam and Oxcarbazepine

Table 15. Patients description

MTRS analysis of the ECG recordings from the five patients during resting state and autonomic activation tests provided time-domain and frequency-domain parameters. To reduce the influence of HR on the HRV, the parameters that revealed a negative relationship with HR, as RMSSD, pNN50 and HF were divided by mRR squared to become HR independent. The parameter positively related to HRV (LF/HF ratio) was multiplied by mRR squared.

The response pattern to autonomic activation tests (deep breathing, hand-grip, standing, Valsalva maneuver) displayed by several HRV parameters was similar after normalization (results illustrated in figure 16). Appropriate responses to parasympathetic (deep breathing and Valsalva maneuver) and respectively sympathetic (hand-grip and standing) activation tests were observed for all patients, indicated by increases in RMSSD, pNN50 and HF values, respectively decreases of the values of the aforementioned parameters during challenge, in both evaluations.

The first patient presented a slight decrease of RMSSD values after three months of VNS, while the second, the third and the fifth patient displayed an increase of several parasympathetic specific parameters values in the second test. Time and frequency-domain parameters did not reveal a significant change in the cardiac autonomic state after three months of VNS for the fourth patient (figure 11).

The frequency-domain parameters for patient 3 during the second HRV evaluation in resting state are illustrated in figure 12. During the ECG recording, the patient presented the activation of the generator (ON period), being clinically symptomatic (voice alteration and

cough). In the interval between seconds 85 and 115 of the ECG recording we identified the ON period, which determined further on an increase of the RMSSD values (figure 13).

This could be an example of the response to left VNS at intensity sufficient to impose immediate changes in cardiovascular autonomic modulation. In the OFF period, the RMSSD values recovered rapidly to baseline values, with transient overshoot of baseline values (figure 13).

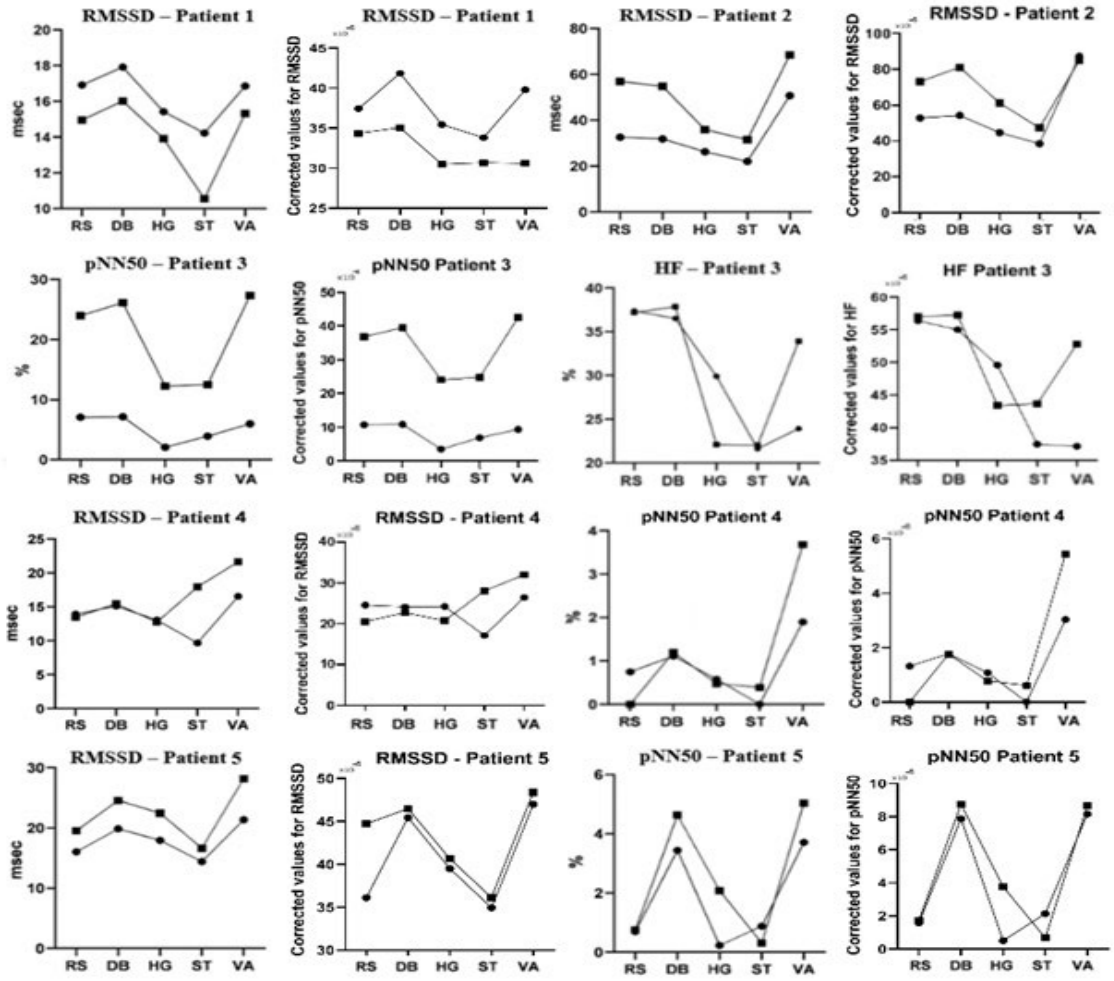

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Figure 11. HRV parameters of the five patients. RS=resting state, DB=deep breathing test, HG=hand-grip test, ST=standing test, VA=Valsalva maneuver, ●Test 1, ■Test 2

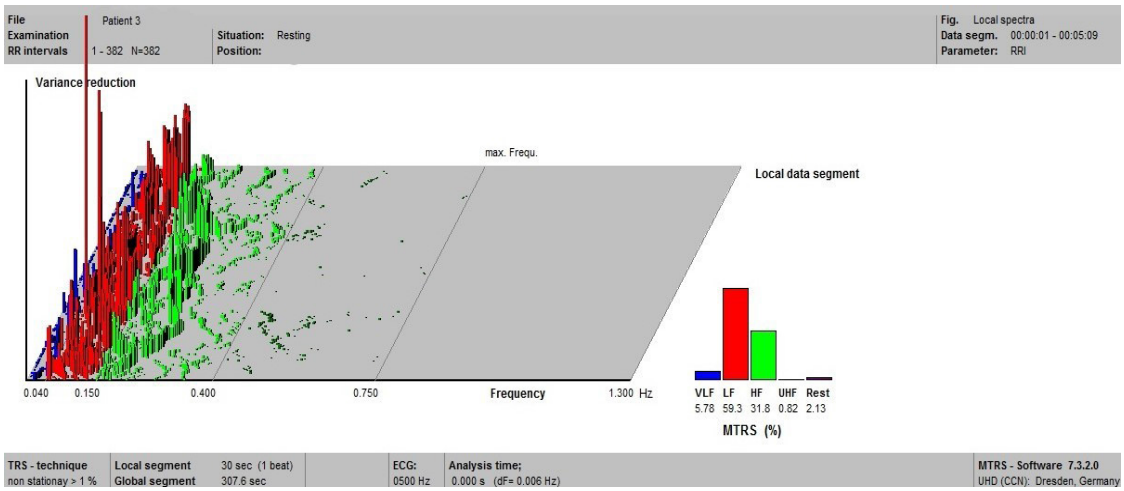

Figure 12. HRV parameters of the third patient in resting state during test 2

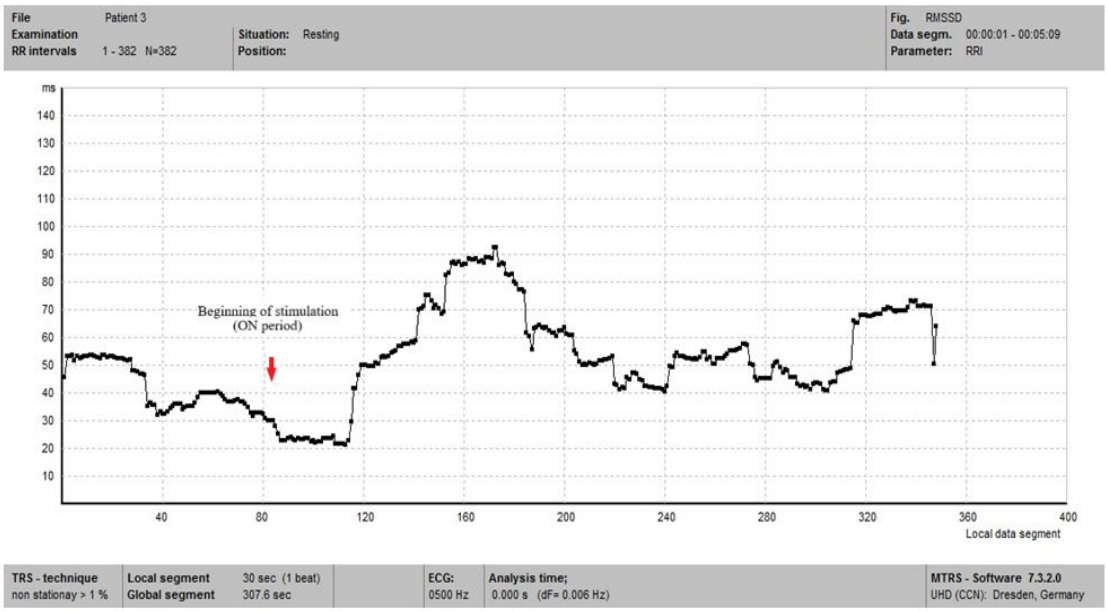

Figure 13. RMSSD values of the third patient in resting state during test 2

study 2- In first patient there was no significant difference concerning the dynamic of HFnu and LF/ HF parameters in response to activation tests in the two HRV evaluations (Fig. 14). After three months of neurostimulation, an improvement in HRV was noticed, as shown by an increase of ApEn and SampEn during standing test, hand-grip test and Valsalva manoeuvre (Fig. 14).

The second patient presented normal responses to parasympathetic and sympathetic activation tests, reflected in HFnu, LF/HF and RMSSD values during challenge, in both HRV evaluations. A decrease in the HRV after sympathetic activation tests in both evaluations was seen regarding DFA α1 values (Fig.14).

The third patient presented appropriate responses to parasympathetic and sympathetic activation tests mirrored in the dynamics of HFnu and LF/HF ratio in both HRV evaluations. DFAα1 presented similar values after three months of neurostimulation (Fig.14).

The fourth patient presented normal dynamic of the HFnu and LF/HF ratio in response to autonomic activation tests, similar in both HRV assessments. During sympathetic activation tests, there was a shift to sympathetic predominance, seen in LF/HF values, correlated with a low HRV after three months of neurostimulation, revealed by DFAα1 values (Fig.14).

For the fifth patient, during the first evaluation, the Valsalva manoeuvre determined an increase of vagal modulation and of the HRV, illustrated by the values of RMSSD, pNN50, HFnu, SD1 and DFAα1. During the second evaluation, deep breathing test induced an increase in the parasympathetic control over the heart rate (higher RMSSD, HFnu values, lower LF/HF ratio), compared to resting state, highlighting a normal response to the vagal activation test. ApEn and SampEn illustrated similar responses to the activation tests in both HRV evaluations (Fig. 14).

All five patients presented an increase of vagal modulation after parasympathetic activation tests, specifically at the Valsalva manoeuvre ($p < 0.05$), during the first HRV evaluation (T1) and after deep breathing test during the second HRV evaluation (T2), as shown by the RMSSD values (Fig. 15).

LF/ HF ratio decreased after deep breathing test ($p < 0.05$) and Valsalva manoeuvre during the first HRV evaluation. The same dynamic of the LF/HF values was observed during the second HRV evaluation, with increased values after standing and hand-grip tests ($p < 0.05$) (Fig. 20). HFnu presented increased values after deep breathing test ($p < 0.05$) during the first HRV evaluation. Similar features were observed at the second HRV evaluation, with an increment of the HFnu values after deep breathing test and a decrease after sympathetic activation tests ($p < 0.05$), reflecting a regular response of the heart rate to sympathetic and parasympathetic modulation (Fig. 15).

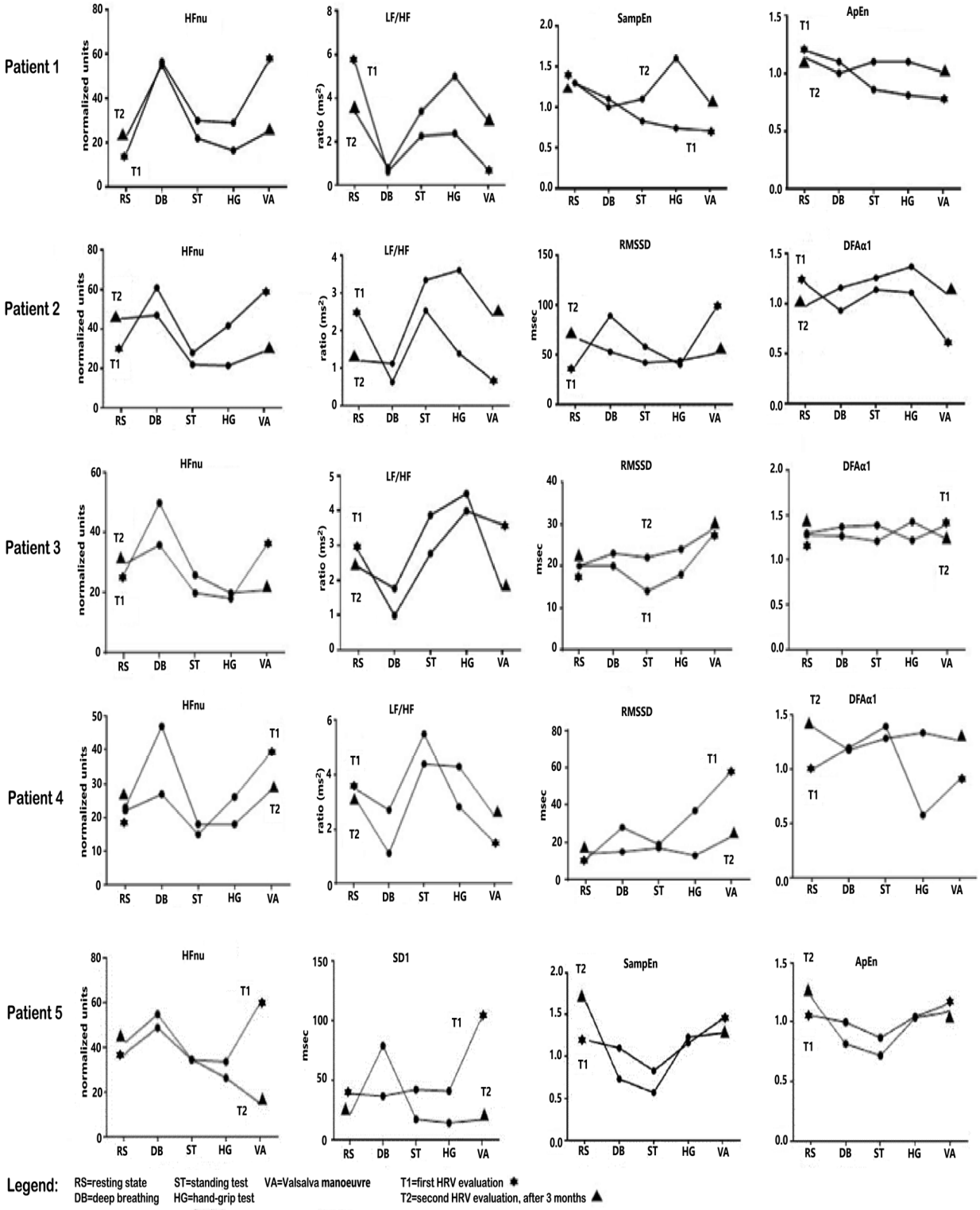

Figure 14. Heart rate variability (HRV) parameters for the five patients

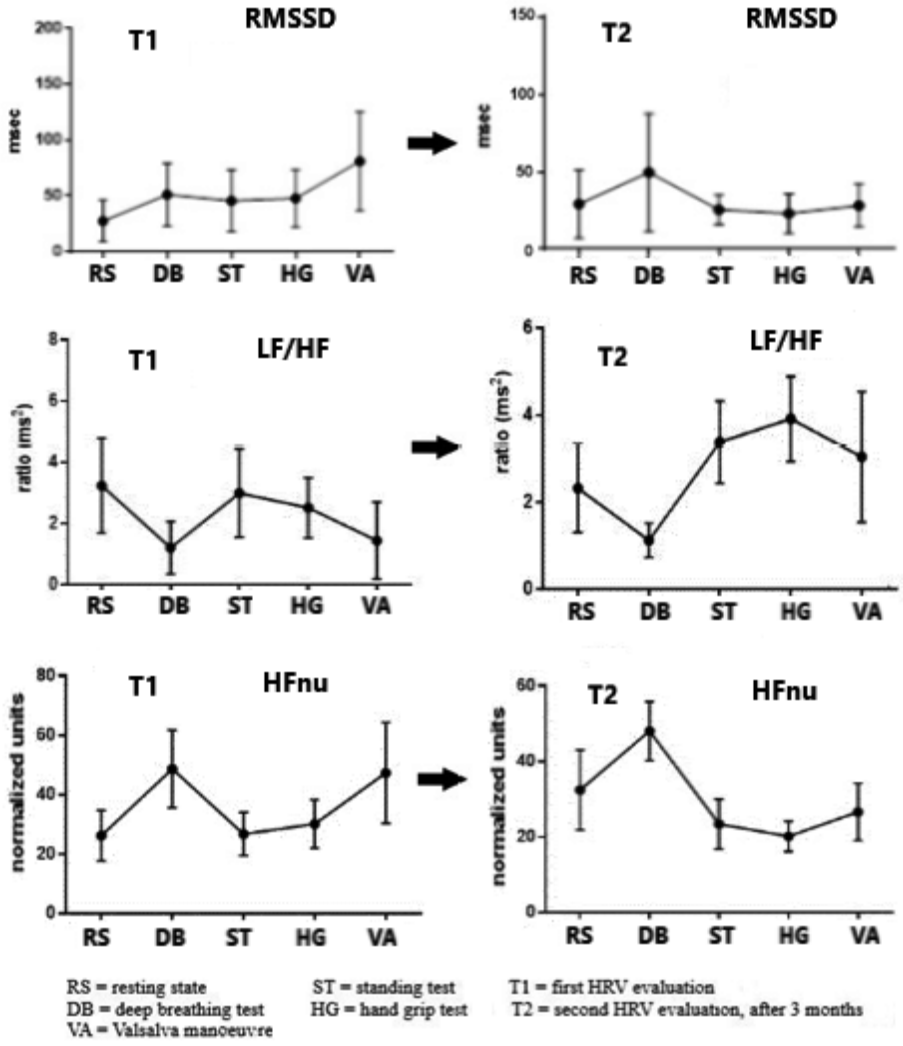

Figure 15. Root Mean Square of the Successive Differences (RMSSD), low frequency power (LF)/ high frequency power (HF) ratio and HFnu dynamics for the five patients

2.5.5. Discussion

Patients with refractory epilepsy may present decreased HRV, raising the concern that altered autonomic function might contribute to sudden unexpected death in epilepsy (SUDEP). Long-term recordings in these patients indicated that severe bradycardia or asystole may occur [150], probably related to increased vagal tone associated with sleep.

The positive effect of VNS in patients with drug-resistant epilepsy is considered to be mediated by the afferent pathways of the vagus nerve, modulating the activity of different

cerebral structures, probably involved as trigger-points of seizures [146]. Recent findings indicate that activated vagal afferents initiate centrally mediated reflexes that inhibit parasympathetic efferent outflows to the heart [151], without consequent bradycardia, a clinical feature not found in our five patients after VNS therapy. Efferent vagal fibres do not directly synapse with cardiomyocytes, but rather with the intrinsic cardiac nervous system, acting as a buffer in modulating the commands to the cardiomyocytes [152]. The intrinsic cardiac nervous system comprises a complex network of ganglia and its neurons that can independently operate or connect with its complement structures of the autonomic pathways in the spinal cord, brainstem or cortex, in order to balance the intracardiac reflexes [152]. Periodic VNS may effectively modulate heart rate dynamics.

The originality of our studies consists of using the autonomic activation tests (Ewing tests) and the analysis HRV parameters through 2 different methods: MTRS and time, frequency-domain, and non-linear HRV parameters for describing the cardiac autonomic response after sympathetic and parasympathetic challenge in patients with drug-resistant epilepsy, three months after vagal stimulation.

The non-linear parameters ApEn, SampEn, SD1 underlined an increase of HRV during the vagal activation tests compared to sympathetic activation tests. DFA α1 confirmed the increase of HRV, especially during the Valsalva test, in all five patients, in the first evaluation. Thus, the non-linear analysis of HRV validated the results from time- and frequency-domain analysis, reflecting the shift in the sympatho-vagal balance during the autonomic tests.

VNS appears not to disrupt the cardiac autonomic activity, with no significant alteration in HRV parameters during autonomic tests being registered during the ECG recordings. The first and fourth patients presented sympathetic predominance over the heart rate control. The first patient displayed an increase of HRV, while the fourth patient displayed a decrease of HRV requiring further cardiac monitoring. Also, the second patient presented a decrease of HRV after sympathetic activation tests in both evaluations, while the third and the fifth patients kept constant features regarding the non-linear parameters.

All five patients presented normal responses to sympathetic and parasympathetic activation tests in the first HRV evaluation. VNS did not alter HR modulation in response to autonomic activation tests after three months of vagal neuromodulation. This data may indicate

the minor contribution to cardiac control of the sympathetic efferent axons contained within the vagosympathetic complex in response to VNS. Four of our five patients had insular or temporal epilepsy. Patient 3, with left insular epilepsy, and patient 5, with right insular epilepsy, presented both appropriate responses to autonomic activation tests and a slight increase in several parasympathetic specific parameters after three months of VNS.

One of the limits of our report is the reduced number of patients while the other is using surface EEG study, because of the distant location of the insular cortex relative to scalp electrodes and the rapidly spreading activity.

We propose HRV analysis as a useful tool to assess sympathovagal balance and identify high-risk patients for cardiac arrhythmias. Moreover, HRV analysis could be a practical tool in identifying suitable patients for VNS therapy.

2.5.6. Conclusions

Our results revealed that VNS does not alter the cardiac autonomic responses to the sympathetic and parasympathetic activation tests, having no clinically relevant effects on cardiac autonomic activity at the analysed stimulation threshold.

Patients with decreased HRV should be periodically monitored. Further studies on larger groups of drug-resistant epilepsy patients, and longer follow-up periods, are needed in order to observe the cardiac autonomic response after neurostimulation.

2.6. **Monitoring the circadian rhythm of arterial blood pressure for dementia rehabilitation**

Hypertension is a risk factor for cardiovascular disease, stroke, and dementia. Increasing evidence shows that hypertension is involved in the pathogenesis of the most common forms of dementia, such as Alzheimer's disease (AD) and vascular dementia (VaD).

Matei Daniela, Carmen Grigoras, Dan Cuciureanu, Victor Constantinescu. The circadian rhythm of arterial blood pressure in Alzheimer's disease and vascular dementia. Acta Neurologica Belgica 2021. https://doi.org/10.1007/s13760-021-01664-8

2.6.1. Introduction

High systolic blood pressure (SBP) in midlife increases the risk of dementia in the elderly [153]. Not only high SBP values but also excessive fall in blood pressure (BP), including orthostatic and postprandial hypotension, contribute to cerebral damage which progresses to cognitive impairments. A reduction in BP is observed several years before the diagnosis of AD and is considered by some researchers as an early change of the dementing process, which can be attributed to impaired cerebral autoregulation and endothelial dysfunctions [154]. Recently, high pulse pressure or increased arterial stiffness was associated with damage to the endothelium and dementia [155]. Ambulatory blood pressure monitoring (ABPM) for 24 h has become a useful tool in the diagnosis and treatment of hypertension, thus preventing future cardiovascular events.

2.6.2. Aim

This study aimed to describe BP characteristics in AD and VaD patients compared to healthy controls, using ABPM. We also searched for a correlation between the clinical, psychological, biochemical profile, and BP parameters.

2.6.3. Patients and methods

Sixty patients with diagnosed dementia (30 patients with AD and 30 patients with VaD) and 30 age-related normal subjects were evaluated in our study. The inclusion criteria for the patients were the following: presence of cognitive impairment, neurological, imagistic, and psychiatric evaluation before the admittance in the study. The exclusion criteria were the presence of acute ischemic stroke or brain hemorrhage, white matter lesions due to non-vascular etiologies (e.g. multiple sclerosis), or major diagnosed psychiatric disorders.

Systolic and diastolic blood pressures (DBP) were measured in a supine position after ten minutes of rest, using an Omron MX blood pressure recorder, and every 60 s for 3 min in the upright position. Orthostatic hypotension was defined as a decrease in SBP of at least 20 mm Hg or in DBP of at least 10 mm Hg, within 3 min of standing. Blood tests including total cholesterol, high-density lipoprotein cholesterol (HDL), triglycerides, and fasting blood glucose were performed in all patients.

The assessment of the cognitive function was based on applying several scores such as Mini-Mental State Examination test (MMSE), Frontal Assessment Battery (FAB), Geriatric Depression Scale (GDS), Instrumental Activities of Daily Living (IADL) scales.

ABPM was used for the evaluation of the circadian BP. This was recorded every 15 min from 6 AM to 10 PM, and every 30 min from 10 PM to 6 AM with an ABPM Holter device. The mean values of daytime and nighttime BP, diurnal index ([daytime SBP–nighttime SBP]/daytime SBP × 100) were calculated automatically. Also, pulse pressure, measured as differences between maximal SBP and DBP, and mean arterial pressure (MAP) calculated as [SBP + 2×DBP]/3 were calculated. According to the diurnal index, circadian BP variation could be subdivided into: dipping 10–20%, non-dipping < 10%, extreme dipping ≥ 20%, reverse dipping < 0% or nocturnal BP elevation [156]. Hypertension was defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg or both during the day, and SBP ≥ 125 mmHg or DBP ≥ 75 mmHg or both during the night period [156].

2.6.4. Results

We included in our study 30 AD patients (18 women, 12 men; mean age 75.33 ± 4.71 years), 30 VaD patients (13 women, 17 men; mean age 73.70 ± 3.90 years), and 30 normal subjects (15 women and 15 men; mean age 75.10 ± 5.07 years). The incidence of hypertension was higher in VaD groups compared to the AD group [VaD—23 patients (76.6%) vs AD—16 patients (53.3%)]. Also, the diabetes mellitus was more common in the VaD group (12 patients—40%) compared to the AD group (9 patients—30%). The VaD group had higher levels of blood glucose, total cholesterol, triglycerides, and lower values of HDL-cholesterol compared to AD and controls (Table 16).

Cognitive tests revealed differences between the three groups. VaD and AD patients had lower MMSE, FAB, GDS, IADL scores compared to controls. The lowest FAB score was found in VaD patients and the lowest IADL score was found in the AD group (Table 16). VaD patients presented higher SBP values compared to AD patients and healthy controls. There was no significant difference between AD patients and the control group (Table 17). VaD patients presented higher values for daytime and nighttime SBP compared to the other two groups, while the AD patients presented the lowest diurnal SBP values (Table 17). DBP values in the AD group were the lowest, while VaD patients presented the highest DBP values, including day and

nighttime (Table 17). MAP values were also the highest in the VaD group, including daytime and nighttime values, while AD patients had similar values with the control group. The VaD patients presented the lowest systolic diurnal index compared to AD patients and controls (Table 17). The mean pulse pressure and nighttime pulse pressure values were higher in both groups of dementia patients when compared with the control group.

VaD patients presented the highest pulse pressure values in the three groups (Table 17). Mean heart rate (HR) and nighttime HR were increased in AD and VaD patients compared to controls, while diurnal HR displayed no significant differences between the three groups.

Parameter	VaD $N=30$	AD $N=30$	Control $N=30$
Age (years)	73.70 ± 3.90	75.33 ± 4.71	75.10 ± 5.07
Sex M/F	17/13	12/18	15/15
BMI (kg/m^2)	27.3 ± 2.4**	24.7 ± 2.5	25.7 ± 2.4•
MMSE score	22.06 ± 1.99**	18.6 ± 2.92††	28.52 ± 1.54••
FAB total score	9.5 ± 1.85*	12.6 ± 1.2†	17.4 ± 1.15••
GDS score (short)	4.3 ± 3.28	4.1 ± 2.2†	2.45 ± 2.1•
IADL score	8.83 ± 2.37**	7.1 ± 1.58††	26.25 ± 0.85••
Fasting blood sugar (mg/dl)	112.4 ± 14.4*	104.4 ± 17.8	102.3 ± 13.25•
Total cholesterol (mg/dl)	194.1 ± 44.7*	151.2 ± 49.7†	176.3 ± 22.3
HDL-cholesterol (mg/dl)	39.5 ± 2.84	43.5 ± 3.4	46.82 ± 2.65•
Triglyceride (mg/dl)	169.5 ± 54.1*	124 ± 42.8	128.5 ± 45.06
Uric acid (mg/dl)	5.4 ± 1.76	4.26 ± 1.436	3.21 ± 1.45•
Creatinine (mg/dl)	1.1 ± 0.45	0.96 ± 0.34	0.98 ± 0.46

Data: expressed as means ± standard deviation

BMI body mass index, *MMSE* mini-mental status examination, *GDS* geriatric depression scale, *IADL* instrumental activity of daily living

*$p<0.05$; **$p<0.01$ when comparing VaD with AD groups

† $p<0.05$; ††$p<0.01$ when comparing AD with C groups

• $p<0.05$; ••$p<0.01$ when comparing VaD with C groups

Table 16 Clinical and biochemical features of the groups

Adding a supplementary factor, sex, to the analysis, the volumes of the groups decrease and the power of the test is diminished accordingly. The results are shown in Figure 16 presents comparative box plots for each variable for the 3 disease categories: VaD, AD, and Control, which had shown to be discriminative for the disease, for each of the two sexes. Figure 17 presents the 3D interpolation of the points determined by the three coordinates, SBP mean, SBP day, and MAP mean, for the 2 sexes, F and M, for each disease condition: Control, VaD, and

AD. This thin plate spline interpolation can serve as a disease predictor for the population from which the samples were extracted, the spline regression surfaces being distinct for each condition.

Parameter	VaD patients $N=30$	AD patients $N=30$	Control group $N=30$	p value		
				VaD-AD	VaD-C	AD-C
SBP mean	131.60 ± 13.83	120.40 ± 16.82	123.20 ± 10.01	0.0061	0.0111	0.2402
SBP max	163.40 ± 25.30	147.0 ± 19.18	154.40 ± 12.39	0.0153	0.1736	0.1329
SBP min	108.70 ± 13.40	96.40 ± 16.05	97.70 ± 10.78	0.0025	0.0016	0.4242
SBP day	135.0 ± 14.81	121.80 ± 14.45	128.13 ± 10.89	0.0012	0.0616	0.0218
SBP night	128.06 ± 14.55	119.09 ± 20.84	118.25 ± 9.67	0.0442	0.0061	0.6464
S-DI	2.74 ± 9.87	5.0 ± 6.72	8.61 ± 4.02	0.4783	0.0213	0.0027
DBP mean	77.57 ± 11.06	70.18 ± 11.69	75.49 ± 9.53	0.0166	0.6783	0.0222
DBP max	104.40 ± 15.19	92.77 ± 15.38	96.87 ± 11.54	0.0025	0.0623	0.0551
DBP min	55.93 ± 10.45	48.78 ± 11.85	55.40 ± 9.35	0.0462	0.9093	0.0504
DBP day	81.23 ± 11.98	73.68 ± 15.02	79.32 ± 10.13	0.088	0.5642	0.0934
DBP night	73.91 ± 11.51	66.69 ± 10.91	71.66 ± 9.43	0.0465	0.7108	0.0692
MAP mean	96.72 ± 11.37	86.79 ± 10.54	91.12 ± 9.36	0.0039	0.0418	0.1098
MAP max	121.74 ± 15.65	108.23 ± 12.28	112.77 ± 12.53	0.0005	0.0126	0.1388
MAP min	74.97 ± 10.78	67.47 ± 10.46	71.50 ± 9.34	0.0113	0.1785	0.1273
MAP day	100.32 ± 12.19	90.39 ± 13.24	95.48 ± 10.05	0.0020	0.2037	0.0222
MAP night	93.11 ± 12.03	83.19 ± 14.70	86.76 ± 9.08	0.0163	0.1065	0.2647
PP mean	57.45 ± 12.58	54.73 ± 12.26	48.52 ± 8.07	0.3560	0.0056	0.0585
PP max	78.07 ± 16.16	73.93 ± 15.73	68.50 ± 12.74	0.2975	0.027	0.3186
PP min	34.27 ± 9.19	32.17 ± 7.80	31.27 ± 7.0	0.5935	0.3215	0.5938
PP day	53.82 ± 12.0	51.11 ± 10.90	48.76 ± 9.39	0.5109	0.1493	0.4875
PP night	54.14 ± 11.59	54.03 ± 14.65	46.49 ± 8.88	0.6623	0.0129	0.0790
HR mean	79.57 ± 8.98	78.98 ± 10.16	71.20 ± 8.20	0.7551	0.0002	0.0012
HR max	108.73 ± 27.76	107.60 ± 23.56	110.40 ± 30.18	0.8687	0.8283	0.9327
HR min	59.60 ± 9.52	60.70 ± 7.56	57.93 ± 9.05	0.5149	0.5391	0.1249
HR day	85.42 ± 9.43	85.45 ± 11.59	84.08 ± 11.67	0.8516	0.4295	0.6946
HR night	73.73 ± 9.70	72.64 ± 10.2	61.22 ± 10.14	0.5945	0.0001	0.0001

Table 17 Blood pressure and heart rate characteristics in the three groups

In terms of the BP pattern, 6 patients (20%) in VaD, 4 patients (13.3%) in the AD group were dippers vs 14 patients (46.6%) in the control group. Non-dipper profile was found in 16 patients (53.3%) in age-matched subjects, 18 patients (60%) in VaD and 15 patients (50%) in AD patients. An extreme dipping profile was found only in 6.6% VaD and AD patients. Reverse dipping was found in 4 (13.3%) patients with VaD and in 9 (30%) patients with AD. During the night period, DBP below 60 mmHg was found in 5 patients (16.6%) with VaD, in 7 patients (23.3%) with AD groups, and in 1 patient (3.3%) in the control group.

We found a positive correlation between the SBP and BMI ($r = 0.41$, $p < 0.05$), mean pulse pressure, and triglycerides ($r = 0.37$, $p < 0.05$). Mean pulse pressure presented a negative correlation with the mean HR ($r = -0.60$, $p < 0.001$).

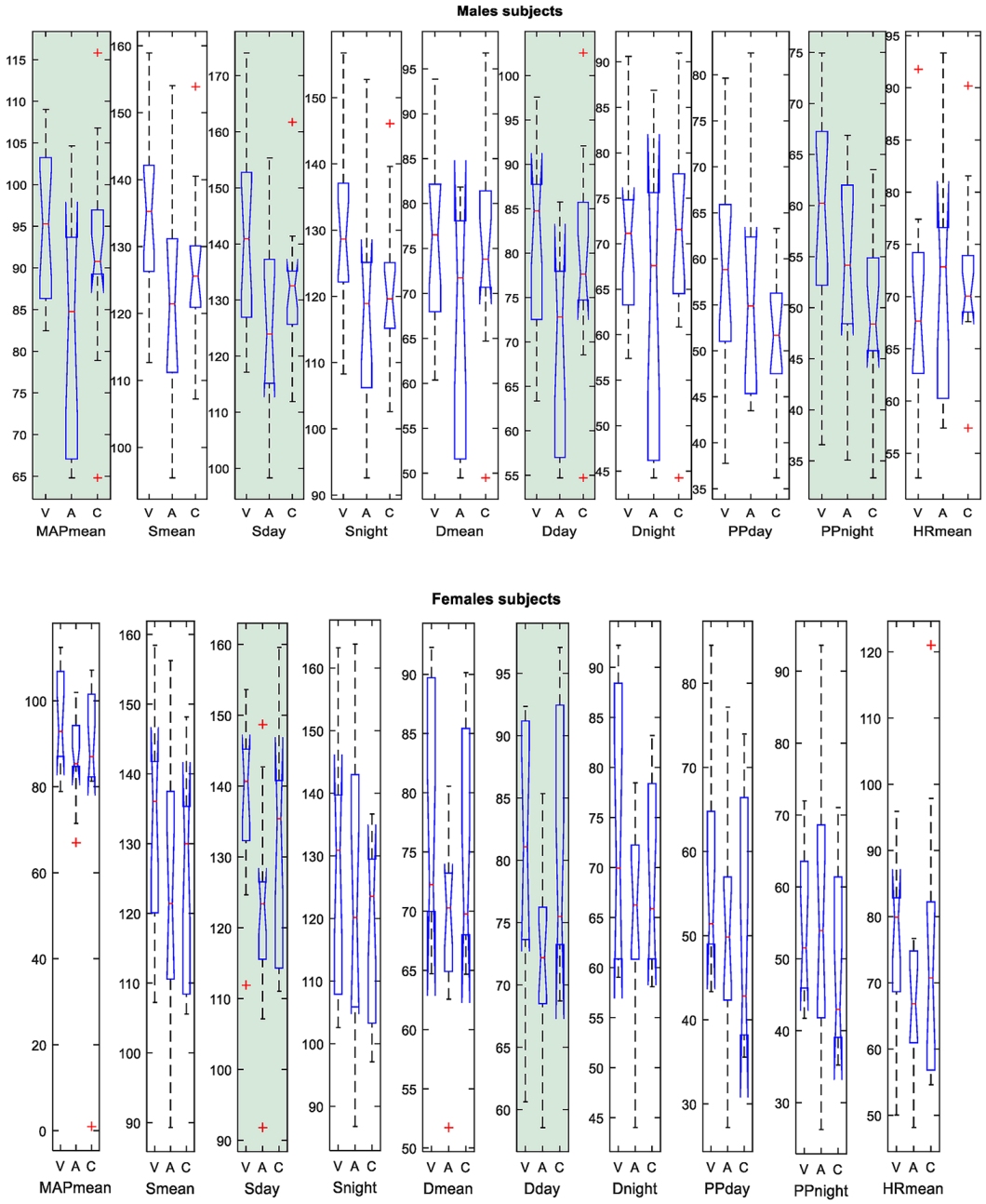

Fig. 16 Comparative boxplot representation of the most significant variables for the discrimination of VaD, AD and Control groups simultaneously. S = SBP: systolic blood pressure (mmHg), D = DBP: diastolic blood pressure (mmHg), MAP: mean arterial pressure (mmHg), PP: pulse pressure (mmHg), HR: heart rate, b/min = beats/ min, A = AD: Alzheimer's disease, V = VaD: vascular dementia, C = control group

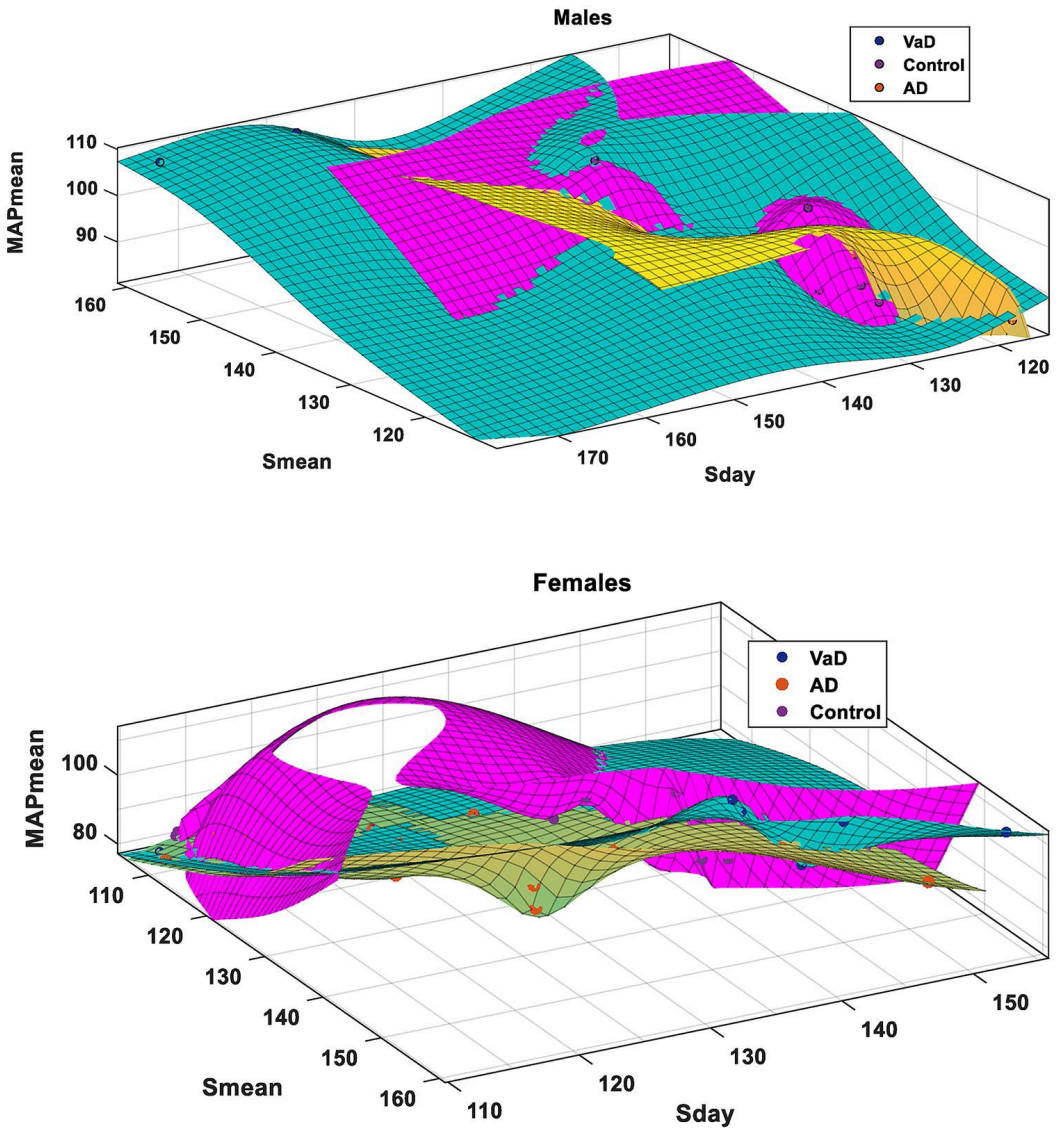

Fig. 17 Surface interpolation of 3D plot of measured points (Smean, Sday, MAPmean), for males and females. S = SBP: systolic blood pressure (mmHg), MAP: mean arterial pressure (mmHg)

2.6.5. Discussions

Aging promotes increases in SBP and decreases in DBP, leading to increased pulse pressure which is a risk factor for cardiovascular events and might be involved in the development of cognitive impairment [157]. Hypertension is the main factor that causes small vessel disease, responsible for leukoaraiosis, lacunar infarcts, and microinfarcts [158]. On the other hand, increased pulse pressure may affect the diastolic coronary perfusion and thus

contribute to ischemic heart disease and heart failure [159]. Low DBP in late life may contribute to cerebral hypoperfusion which can accelerate disease processes leading to AD and VaD [160].

Our results showed that VaD patients displayed higher SBP, MAP, and pulse pressure compared to controls and AD patients. Also, decreased values of DBP where found in AD patients especially during the night period. HR was increased in the groups of patients compared to healthy controls, especially during nighttime. Both groups of patients with dementia were associated with an alteration in the circadian pattern with the highest incidence of the non-dipper and reverse dipper pattern when compared with the healthy elderly. Hypertension is as common in people with dementia as in other populations, but early initiation of antihypertensive treatment can help prevent further cognitive decline [161].

Low values of DBP reflect higher degrees of arterial stiffness associated with cerebrovascular atherosclerosis. Also, DBP reduction during nighttime in patients with AD requires greater attention paid to monitoring BP using ABPM devices and also caution in administering antihypertensive therapy. In addition to the impaired cerebral autoregulation, excessive antihypertensive therapy may exacerbate the risk of cerebral hypoperfusion in patients with dementia [162].

The main limitation of our study was the relatively small sample size, which makes it difficult to draw more specific conclusions. Another drawback is the limited number of patients with advanced dementia because of the difficult cooperation for testing. ABPM monitoring device is ideal to wear at home and not under hospital conditions as in our case. Also, patients with dementia enrolled in the study were treated with various drugs and the effect of those treatments on BP could not be neglected.

2.6.6. Conclusions

Increased SBP, pulse pressure, and alteration in the circadian pattern with the highest incidence of the non-dipper and reverse dipper pattern were found in patients with dementia when compared with the healthy elderly. Also, decreased values of DBP where found in AD patients, especially during nighttime. Further studies describing the influence of BP pattern, specifically during nighttime on cognitive decline are needed. Personalized antihypertensive management according to ABPM parameters is essential in dementia patients, considering the disruption in the sleep–wake cycle often encountered in these patients.

Chapter 3

PREVENTION IS BETTER THAN CURE

The global burden of atherosclerotic cardiovascular disease is steadily increasing and it is considered the leading cause of mortality and morbidity worldwide. Unchangeable constitutional factors include age, sex and genetic factors, while modifiable risk factors such as hyperlipidemia, hypertension, smoking, diabetes and physical inactivity are the target of the main studies on preventing and combating the formation of atherosclerotic plaques.

ANS and endothelial dysfunctions, inflammation, and oxidative stress appear to be major contributing factors to the development and progression of atherosclerotic cardiovascular disease.

This research direction has been materialized by publishing the following articles:

1. Daniela Matei, Ioana Buculei, Catalina Luca, Calin-Petru Corciova, Doru Andritoi, Robert Fuior, Daniel-Andrei Iordan, and Ilie Onu. Impact of Non-Pharmacological Interventions on the Mechanisms of Atherosclerosis. Int. J. Mol. Sci. 2022, 23, 9097. https://doi.org/10.3390/10.3390/ijms23169097
2. Matei Daniela, Luca Catalina, Onu Ilie, Matei Paula, Iordan Daniel-Andrei and Buculei Ioana. Effects of Exercise Training on the Autonomic Nervous System with a Focus on Anti-Inflammatory and Antioxidants Effects. Antioxidants 2022, 11, 350. https://doi.org/ 10.3390/antiox11020350
3. Elvina Mihalaş, Lăcrămioara Ionela Şerban, Daniela Matei, Dan Caşcaval, Anca Irina Galaction. Changes of oxidative stress caused by physical activity. STUDIA UBB CHEMIA, LXIV, 2,Tom I, 2019 (p. 35-47)

3.1. Autonomic nervous system and atherosclerosis

Endothelium is of crucial importance for internal homeostasis, and it is considered a “first line” physiological defense against atherosclerosis [163]. Endothelium can regulate vascular tone through endothelium-derived contracting factors (endothelin, prostaglandin F2a and thromboxane A2) and endothelium-derived relaxing factor (prostaglandin I2 and nitric oxide).

These substances can directly act on vascular smooth muscle cells (VSMCs) or by affecting sympathetic activity [164].

In normal endothelium, there is a balance between vasoconstrictors and vasodilators factors. When this balance is altered, endothelial dysfunction occurs, which is a key initiating event in atherosclerosis. Endothelial dysfunction predisposes the vasculature to vasoconstriction; leukocyte adherence, platelet activation, mitogenesis, pro-oxidation, thrombosis, impaired coagulation, vascular inflammation, and all these processes lead to atherosclerosis.

Higher plasma lipid levels with smaller lipoproteins, such as low-density lipoproteins (LDL), small dense LDL and triglyceride, accumulate in the intima and activate the endothelium. Proatherogenic-modified low-density lipoproteins (mLDL) bind with proteoglycans of the extracellular matrix in the intima of blood vessels, causing the aggregation of lipoprotein particles, foam cell formation, endothelial destruction, leukocyte recruitment and inflammation [165].

During chronic inflammation, at the site of atherosclerosis-diseased adventitia, a tertiary lymphoid organ occurs, due to the accumulation of immune cells through the involvement of lymphoid tissue-organizer-like cells [166]. At the site of the inflammatory process, several adhesion molecules and chemokines promote monocyte recruitment, as well as the attraction of T cells. Moreover, plaque macrophages and T cells secrete pro-inflammatory cytokines, such as interferon, interleukin-2 (IL-2), tumor necrosis factor, as well as anti-inflammatory cytokines such as IL-10 and IL-4 [166].

In this way, the inflammatory process can stop or continue depending on the type of cytokine that predominates. The artery tertiary lymphoid organ (ATLOs) contains both pro- and anti-inflammatory immune cells, the inflammatory process in atherosclerosis can be stopped if interventions occur in time. ATLOs that form in the adventitia during atherosclerosis can regulate the immune response and may modulate reverse cholesterol transport (RCT) pathway [167]. The lymphatic vasculature, in addition to the drainage of inflammatory cells and cytokines, is important for the removal of cholesterol from macrophages in RCT, accounting for 50% of cholesterol delivery from cholesterol-loaded macrophages into the plasma compartment.

Vessels contain sympathetic nerves, which are distributed between smooth muscle and adventitia layers. At present, it is well established that the SNS contributes to the modulation of vascular function and this relationship is a key factor in the development of cardiovascular

diseases. The PNS does not directly act on the blood vessels, the necessary parasympathetic endings missing from this level.

In the adventitia layer, which is very close to ATLOs, expanded nerve ending networks were highlighted. Researchers have introduced a neurotropic virus into the adventitia of mice and tracked its path to the central nervous system [168]. They were able to establish a structural artery–brain circuit (ABC): nociceptive afferents from abdominal adventitia entered the central nervous system through spinal cord T6–T13 dorsal root ganglia and reached structures such as the parabrachial nucleus, central amygdala [168].

Sympathetic efferent fibers start from these structures, projected from medullary and hypothalamic neurons to the adventitia through spinal intermediolateral neurons and both coeliac and sympathetic chain ganglia [168]. The increased activity of sympathetic nerve fibers increases the growth of atheromas, whereas coeliac ganglionectomy reduced disease progression and enhanced plaque stability.

The SNS contributes to the differentiation, maturation, recruitment, and regulation of immune cells via lymphoid organs and also influences vascular and lymphatic flow. Cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor-alpha, ROS, RNS can stimulate SNS.

Even if there are no necessary terminations in the adventitia of the vessels, the PNS innervates the cells of the immune system. The efferent parasympathetic nerves directly release acetylcholine or instruct immune cells to produce acetylcholine at the site of inflammation, which inhibits pro-inflammatory cytokine release.

This inhibitory pathway mediated by parasympathetic vagus nerve is called the cholinergic anti-inflammatory pathway [169]. The components of this neuroimmune circuit include the vagus nerve, the splenic nerve, choline acetyltransferase expressing Tcells (TChAT), and the alfa 7 nicotinic acetylcholine receptor subunit (α7nAChR). The α7nAChR is reportedly expressed in human atherosclerotic lesions and their ablation increased aortic atherosclerosis [170]. Acetylcholine (ACh), a neurotransmitter of the vagus nerve, is biosynthesized by ChAT and is an agonist for α7nAChR. ACh can also be released to the extracellular space by TChAT, which relays neural signals to α7nAChR-expressing macrophages in spleen and attenuates the production of pro-inflammatory cytokines [171].

3.2. Autonomic Nervous System and the Immune System

Inflammation plays a key role in promoting most chronic diseases such as atherosclerosis, type II diabetes, neoplasia and cardiovascular, respiratory, digestive, neuroendocrine and neurodegenerative diseases.

The stimulation of the immune cells leads to the release of pro- and anti-inflammatory cytokines, the balance of which is ensured by the ANS, giving adequate host defense with minimal tissue damage.

The HPA axis, the SNS and more recently, the PNS have been shown to regulate the immune system. The HPA axis and the PNS have anti-inflammatory effects and the SNS has been shown to have both pro- and anti-inflammatory effects [172]. Signals from cytokines can result in the central and peripheral activation of the SNS.

Cytokines, important mediators of inflammation, are synthesized and secreted by different cells like macrophages, monocytes, lymphocytes, CNS neurons, microglia, astrocytes, oligodendroglia and endothelial cells. Macrophages are implicated in the production of interleukins (IL) 1, 6 and 8 and tumor necrosis factor alpha (TNF-α) and neutrophil produces IL-1b, IL-6, TNF-α and IL-8.

During inflammation, immune cells recruited at the site of inflammation or cells from affected tissues release chemical attractants (chemokine) that cause leucocytes to adhere to the endothelium and to migrate into the tissue spaces. In inflammation, after the release of chemokines from affected cells the attraction of immune cells to the site of injury generates a phase of destruction of the affected tissues mediated by the infiltrating cells. At the same time the body tries to promote tissue repair by producing anti-inflammatory cytokines (IL-4, IL-10, IL-13 and perhaps IL-6), which attenuates inflammation by restricting inflammatory cytokine production and suppressing inflammatory cell activity.

Both primary and secondary lymphoid organs are innervated by the SNS [173]. When the distribution and density of sympathetic nerves in lymphoid organs are not stable there are different changes during immune response [173]. This communication between the SNS and immune cells occurs via the release of NE and subsequent intercellular signaling via postsynaptic

adrenergic receptors (ARs) expressed by T-and B-cells, stromal cells, granulocytes, macrophages and mast cells.

During chronic inflammation, the SNS and HPA axis activity are increased, which leads to a local repulsion of SNS fibers from inflamed tissue, including lymphoid organs, to create zones of permitted inflammation. If this condition is prolonged, overactivation of the SNS can lead to dangerous effects such as toxic shock, tissue damage, immune deficiency and autoimmunity [173].

Stimulation of the β_2AR on human T-and B-cells increases cAMP levels and adenylate cyclase activity. Under catecholamine and cortisol action, TH2 immunity may be enhanced and the TH1 to TH2 shift will not allow adequate tumor cell surveillance by the immune system [174].

SNS fibers from the superior mesenteric celiac ganglion form the splenic nerve. Preganglionic cholinergic sympathetic neurons innervate postganglionic neurons and these nerves reach the spleen alongside blood vessels, mainly innervating the white pulp and slightly innervating the red pulp [175]. This pathway has been named the inflammatory reflex and is controlled by NE and cholinergic neuronal inputs, which result in the attenuated activation of splenic macrophages [175].

Despite the fact that there is no neuroanatomical evidence of PNS innervation of the immune organs, there is evidence that the spleen receives both sympathetic and parasympathetic signals. The vagal immune reflex system sends signals to the SNS and the HPA axis centrally, resulting in the peripheral release of anti-inflammatory glucocorticoids and NE; acetylcholine is also released from efferent vagal nerve fibers and results in the negative feedback control of inflammation [176]. The cholinergic anti-inflammatory pathway is mediated by the α7 subunit of the nicotinic receptors (nAChR), expressed on macrophages, monocytes and dendritic cells inhibiting the release of pro-inflammatory mediators such as Il-1, TNF-α and IL-6 without affecting anti-inflammatory cytokines such as IL-10.

In summary, both the afferent and efferent vagus nerves mediate anti-inflammatory effects. Afferent vagus pathways are involved in the activation of the HPA axis and adrenal gland corticosteroid release. By contrast, efferent vagus nerves mediate anti-inflammatory processes via a direct effect on immune cells or through the splenic sympathetic nerve. Cytokines released in peripheral tissues activate vagal afferents, resulting in an inflammatory

reflex in which efferent vagus nerves inhibit inflammation by suppressing cytokine production via the cholinergic anti-inflammatory pathway.

3.3. Autonomic Nervous System and Oxidative Stress

Oxidative stress (OS) is defined by the high production or low inactivation of reactive oxygen species and an imbalance between the levels of oxidants and anti-oxidants, with an increased level of oxidants having a destructive effect. Nitrogen species and reactive oxygen (RNS and ROS) in excessive amounts can be harmful because they can produce lipid peroxidation, proteins and ADN oxidation.

The brain is vulnerable to oxidative stress because at this level the concentrations of polyunsaturated fatty acids are high, the catalytic activity is reduced and the antioxidant capacity is minimal. The hippocampus, amygdala and prefrontal cortex are the most vulnerable structures to oxidative stress. Oxidative stress in the brain compromises biochemical integrity of the hippocampus, the amygdala and PFC affecting neuroplasticity and neurogenesis and disturbing normal synaptic neurotransmission as well as neurogenesis factors like brain-derived neurotropic factor.

NO released from the endothelium inhibits central and peripheral SNS activity and increases central and peripheral PNS activity [177]. This suggests that NO released from endothelial cells may play a role in the modulation of the balance between the SNS and the PNS branches of the ANS. Additionally, NO inhibits the oxidation of LDL-cholesterol, the proliferation and migration of smooth muscle cells, the adhesion and aggregation of platelets and the production of vasodilatation.

Besides NO and ROS, other factors influence the SNS. Some of these factors are implicated in the regulation of the vascular function: endothelin (ET) and the renin–angiotensin system. Normal endothelin levels may suppress SNS activity, whereas endothelin excess may enhance the central and peripheral SNS and influence hemodynamic regulation by the baroreflex, chemoreflexes and vascular tone. Additionally, SNS stimulation can increase endothelin release.

High levels of ROS can cause DNA damage and hence higher frequencies mutation (Figure 18).

Greater SNS activation and PNS withdrawal after exposure to physical and psychosocial has been shown to be associated with shorter telomere length in children [178] withdrawal was measured during HRV by measuring HF. A very interesting study showed that elderly people with shorter telomeres had lower vagally mediated compared to people of the same age group with longer telomeres [179]. Telomerase was related to lower vagal tone and greater sympathetic reactivity to an acute stressor [179]. HRV is also inversely related to IL-6 and other inflammatory markers, C-reactive protein [178]. These discovered aspects indicate that low vagal correlates with increased amounts of cytokine-induced activation of NFkB and, in with increased ROS production. Thus, the reduction in PNS activity stimulates the inflammatory process and the production of ROS, which lead to reduce telomeres length [178].

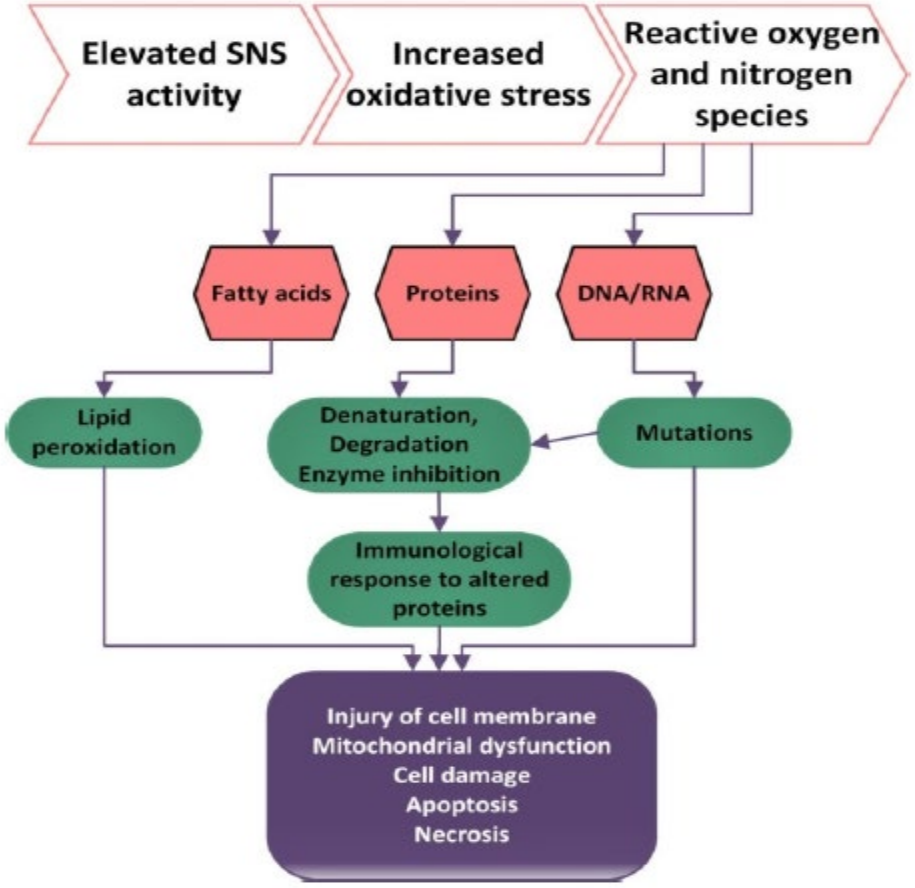

Figure 18. The pathophysiological mechanism by which oxidative stress contributes to the various diseases.

Antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and other antioxidant molecules are the defense used against ROS: ascorbic acid (vitamin C), tocopherol (vitamin E), vitamin A, flavonoid and ubiquinone. In response to ROS, cells increase their antioxidant defenses through the activation of nuclear factor erythroid 2–related factor (Nrf2), which increases the expression of several endogenous antioxidants [180].

3.4. Effects of Exercise Training with a Focus on Anti-Inflammatory and Antioxidants Effects

Physical exercise (PE) can be used as a primary non-pharmacological clinical tool because it can improve antioxidant capacity, reduce oxidative stress and inflammation and increase energy efficiency. During exercise, an increase in respiration and oxygen uptake directed to the body's vital organs take place. Increased oxygen consumption due to higher energy requirements result in increased levels of reactive oxygen and nitrogen species.

During exercise, the blood flow is increased to the vital organs and muscles but is lowered to the liver, and this has an impact on antioxidant levels. The intra and extracellular transportation of glutathione is affected and the synthesis and degradation of glutathione is also affected. This explains why the efficacy of antioxidant systems differs after acute exercise and exercise training

PE can impact the activity of antioxidants during effort, and this is one of the mechanisms considered to be implicated in lowering the risk of age-related diseases. High levels of ROS caused by effort induce the activation of antioxidant defenses and this causes a positive adaptation of the CNS.

Moderate physical activity has a positive impact on the body because it helps maintain the health of bones, muscles and joints; helps maintain normal levels of cholesterol and body weight; and also decreases levels of cholesterol and overweight. During this type of effort, the level of free radicals produced is moderate and the body can adapt. The body also tries to adapt during exhaustive physical activity but the levels of oxidants produced are much higher so this will cause an imbalance between oxidants and antioxidants resulting in oxidative damage (lipid

oxidation, protein oxidation and DNA oxidation). This makes body more vulnerable to fatigue, injury and disease.

In a study conducted by González-Bartholin et al., ten older healthy subjects were asked to perform 30 min work-outs that included different types of exercises (moderate intensity concentric and eccentric cycling and high-intensity eccentric cycling) in a randomized manner. These exercises included moderate-intensity concentric cycling with 50% maximum power output, moderate-intensity eccentric cycling with 50% maximum power output and high-intensity eccentric cycling with 100% maximum power output. After the exercises were conducted, the effects of different types of exercises were studied by measuring VO2 and HR and the results showed that high-intensity eccentric cycling had a greater impact on VO2 and HR. The next day, the subjects were examined again and the researchers looked at the muscle strength loss, peak soreness, creatine kinase activity, malondialdehyde levels and IL-6 levels. Muscle strength loss and peak soreness were greater in subjects that performed high-intensity eccentric cycling and the activity of creatine kinase was high in these subjects, along with IL-6 levels. MDA levels did not decrease after any type of exercise. This study, even though it was conducted on a small number of participants, shows a connection between the intensity of the effort and the impact of oxidative stress [181].

The adaptation of the ANS is one of the ways in which the positive impact of exercise is achieved. The recommendations regarding moderate-intensity exercises for most people are 30 min/day 5 days/week. For people with diseases such as autonomic disorders, training should be carried out under expert supervision.

Exercises conducted on a daily basis can cause the ANS to adapt to parasympathetic dominance, which translates to a lower HR at rest. Nitric oxide seems to be associated with bradycardia induced by exercise and studies have shown that the transfer of nitric oxide synthase into the atrial wall has the same effect as the exercise-induced vagal phenotype.

In a review, A.J. Hautala et al. showed that regular aerobic fitness training can cause an increased cardiac vagal modulation of the heart rate and they also showed that normal or pathological functioning of the ANS causes individual responses to aerobic training. Individuals with high vagal modulation at the start of the training obtain greater improvements in their VO2 peak. The use of methods to assess and monitor the ANS can help optimize the exercises chosen for aerobic fitness [182].

Because studies show that in patients with obesity the activity of the sympathetic nervous system and oxidative stress are high, Li et al. investigated the impact that exercise had on four groups of rats with different diets. One of the groups received a high-fat diet for 12 weeks. Rats from the group with a high-fat diet and the ones from the group that received a regular diet were trained on a treadmill 5 days/week 60 min/day for eight weeks. The activity of the sympathetic nervous system was assessed by measuring the plasmatic levels of norepinephrine and oxidative stress was assessed by measuring the plasmatic and muscular levels of malondialdehyde, superoxide anion and F2-isoprostanes. The results showed that in the group of rats who underwent exercise training the activity of the sympathetic nervous system and oxidative stress was lower compared to the activity in the three other groups of rats [183].

Because overload training (large volume or long-term exercise) causes oxidative distress, this will nullify the positive impact of the physical training on health outcomes. The kinds of physical exercises recommended due to the increased levels of antioxidant enzymes they generate are moderate exercises that can improve individuals' physiological and functional capabilities.

Fibroblasts, myoblasts, endothelial cells and smooth muscle cells have been shown to be capable of producing IL-6. Skeletal muscle cells are capable of producing IL-6 in response to reactive oxygen species that are produced as a result of the oxidation of fat and glucose. A small net release of IL-6 from the internal jugular vein has been reported, suggesting that the CNS may contribute to the IL-6 found in the circulation [184]. In the brain, IL-6 predominantly comes from activated astrocytes. IL-6 levels in the plasma increase rapidly during exercise, whereas the production of IL-6 in the brain increases more slowly.

Levels of other cytokines that are expressed in the skeletal muscle following exercise, such as TNF-α and IL-1b, increase, but the circulating concentration of these cytokines does not change (or only increases slightly) [185]. Conversely, the circulating concentrations of IL-1 receptor antagonist (IL-1ra) and IL-10 increase markedly, but these cytokines are not expressed in skeletal muscle after exercise [185].

There are studies that show that physical exercise can alter the inflammatory mode of microglial cells. Microglia, the primary immune cells in the CNS, can be activated by the M1 (pro-inflammatory subtype) and M2 (anti-inflammatory subtype) pathways. The M1 secretes

pro-inflammatory cytokines and free radicals that are toxic to the surrounding cells. The M2 secretes anti-inflammatory cytokines and promotes tissue healing by secreting trophic factors.

Exercise in mice can increase levels of the growth hormone insulin growth factor 1 (IGF-1) in the prefrontal cortex and hippocampus, which have an anti-inflammatory effect by stimulating the M2 macrophage phenotype [186].

Exercise can lead to increased levels of neurotrophic factors, especially nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and insulin-like growth factor (IGF-1). BDNF plays many important roles in neuroplasticity, neuronal growth and differentiation. Physical exercise has been found to normalize BDNF. It has been suggested that higher aerobic fitness levels are associated with larger hippocampal volume and improved neuronal health and that acute aerobic exercise can induce increased BDNF levels in the peripheral blood [187].

The phenomenon of "wellbeing" in which PE is involved is the consequence of three mechanisms by which endorphins are stimulated: the 'runner's high', addiction to exercise and pain tolerance [188]. The most serious limitation of "endorphin theory" is the measurement of endorphins from the bloodstream, as it cannot be considered an indicator of central effects due to the endorphins being too large to cross the blood–brain barrier.

Studies demonstrated that high-intensity, long-term PE (marathon running) suppressed immune function for a period of several hours to days, increasing the risk of infections, as was confirmed by Goh et al. in 2019 [189]. Pedersen and Ullum showed that high intensity PE had antagonistic effects compared to moderate PE. The study was conducted on six healthy individuals who did a 25%, 50% and 75% VO2max ergometric bike program for one hour. Blood samples were collected 2 h after the end of the PE. After moderate PE, no immunosuppression was recorded but prolonged high intensity PE caused the down-regulation of the immune function. They suggest that natural killer cells are highly influenced by PE and the mechanisms behind the changes induced by intense exertion are related to cytokines, adrenaline, noradrenaline, cortisol, stress and growth hormones, hyperthermia and beta-endorphins. This causes high-performance athletes to have high levels of natural killer cells at rest while after acute high intensity PE their levels drop dramatically, leading to immunosuppression and low resistance to pathogens [190].

Suzuki et al., documented the systemic kinetics of cytokines after PE, especially TNF-α and IL-1b, which induce cytokines in acute phase reactions. They found that the circulating

concentration of these cytokines remains almost unchanged after exertion. Plasma interferon (IFN)-alpha and IFN-gamma remain unchanged, while IL-2 decreases and IL-8 increases after endurance exercises. They concluded that long-duration high intensity PE suppresses the production of immune modulatory cytokines [191].

It is clear that the intensity, type and duration of exercise and the muscle mass involved in the exercise influence the secretion of cytokines into the circulation. Thus, high-intensity and long-duration PE can be dangerous from the point of view of the secreted inflammatory cytokines.

Health is a result of the harmonic interchange between the SNS and PNS branches of the ANS. Acute stress response with predominant SNS activity is important for survival, performance and achieving various goals. However, when this activation becomes chronic it can be detrimental to our health and wellbeing.

Chronic stress leads to the dysregulation of ANS, causing SNS predominance and the non-involvement of the PNS. This disorder is associated with neuroendocrine, cardiovascular, respiratory, digestive and psychiatric diseases (Figure 19).

Exercise training is protective against cardiovascular diseases, obesity, metabolic syndrome and type 2 diabetes mellitus and is also effective to improving the performance of the autonomic nervous system.

Exercise is associated with reduced resting heart and respiratory rate and blood pressure; improved baroreflex, cardiac and endothelial function; increased skeletal muscle blood flow; and more effective redistribution of blood flow during exercise.

SNS is activated during PE but repeated physical training can reduce SNS activity and improve autonomic balance. It is generally believed that reductions in sympathetic outflow represent a major adaptation of exercise training.

After exercise, slow breathing shifts the autonomic balance to parasympathetic dominance. The salutary effects of slow and deep breathing are mediated by an increase in tidal volume and the activation of the Hering–Breuer reflex, an inhibitory reflex triggered by lung stretch receptors and mediated by vagal afferents, which may increase baroreflex sensitivity.

In addition to stimulating the PNS, slow breathing also improves pulmonary ventilation, gas exchange and arterial oxygenation. Additionally, reduced SNS activity may be the result of a

decrease in chemoreflex activity due to the reciprocal influences of the baroreflex and chemoreflex [192].

During exercise, an increase in respiration and oxygen uptake take place with the purpose of directing a high quantity of O2 to the body's vital organs.

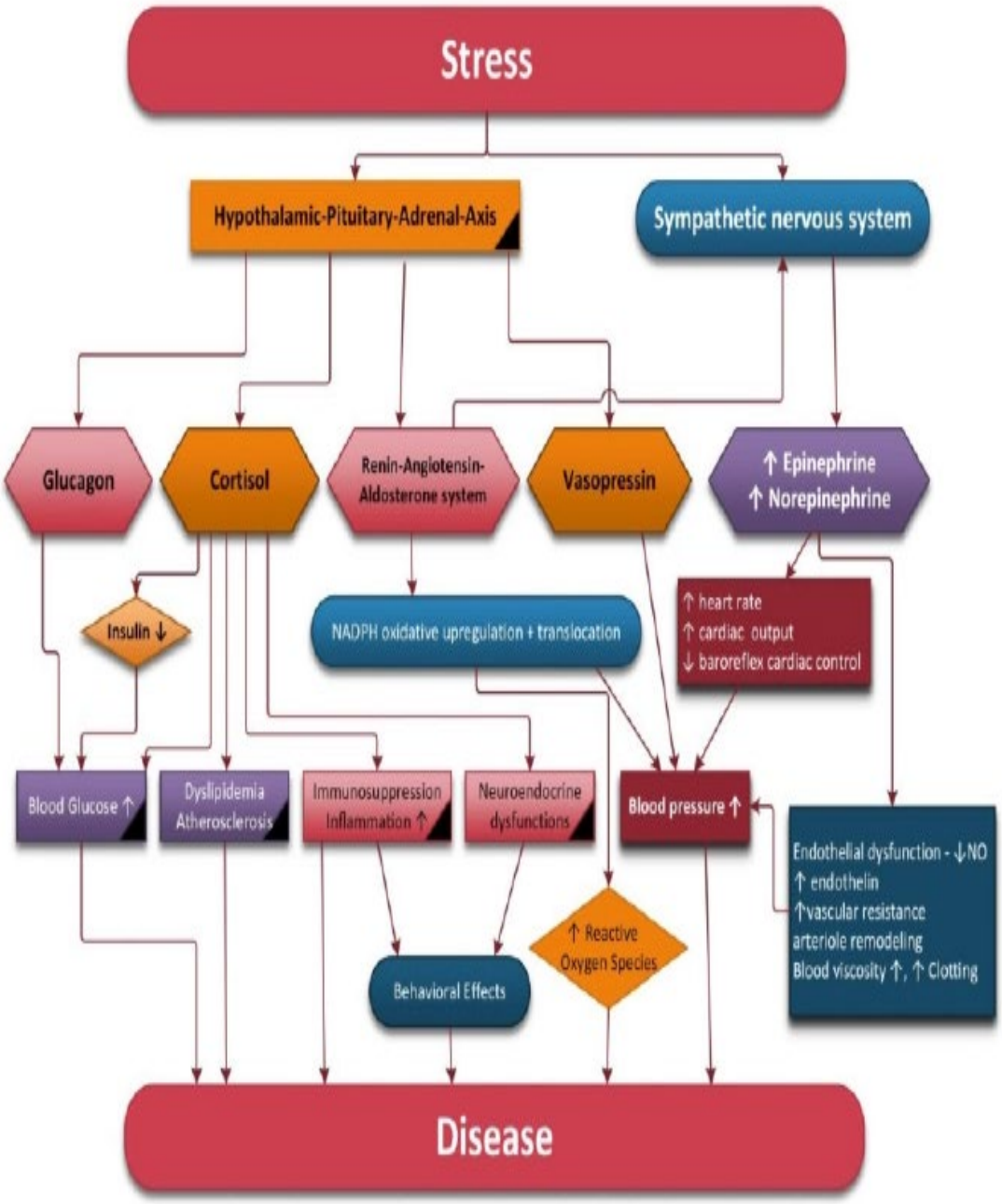

Figure 19. Physiological modifications under stimulation of the hypothalamic–pituitary–adrenal axis and sympathetic nervous system.

After the oxygen is used, a lot of ROS/NRS are produced. High levels of ROS induce the activation of antioxidant defenses and this will cause a positive adaptation of the nervous system.

Training can decrease systemic oxidative stress and it also has a positive impact on antioxidant defenses. A single session of overload training (in large volume or over a long time period) can cause oxidative distress, leading to the loss of beneficial health outcomes related to physical activity. However, if training continues the body can adapt to the exhaustive physical activity by increasing the expression of antioxidant enzymes. If oxidative stress is reduced or antioxidant capacity is increased with training then less inflammatory process will occur during training. Physical exercise is an efficient clinical tool that limits chronic inflammation using complex mechanisms which activate immune system, which increases the level of anti-inflammatory cytokines and limits pro-inflammatory cytokine levels in the blood plasma and serum.

Physical activity, by enhancing the parasympathetic tone and activating the cholinergic anti-inflammatory pathway, may be a therapeutic strategy for reducing chronic inflammation and preventing many chronic diseases. If PE can produce inflammation during and after its execution, regular physical exercise training may be considered an anti-inflammatory therapy. Moreover, pro-inflammatory processes that occur after exercise may be vital for long-term adaptive responses to exercise training.

PE causes lower HPA axis, SNS, oxidative stress and inflammatory activity and increased PNS activity. In addition, PE also contributes to greater cardiovascular and respiratory function, insulin sensitivity and neuroplasticity and higher levels of neurotrophic factors, which may all contribute to the beneficial effects of regular exercise (Figure 20).

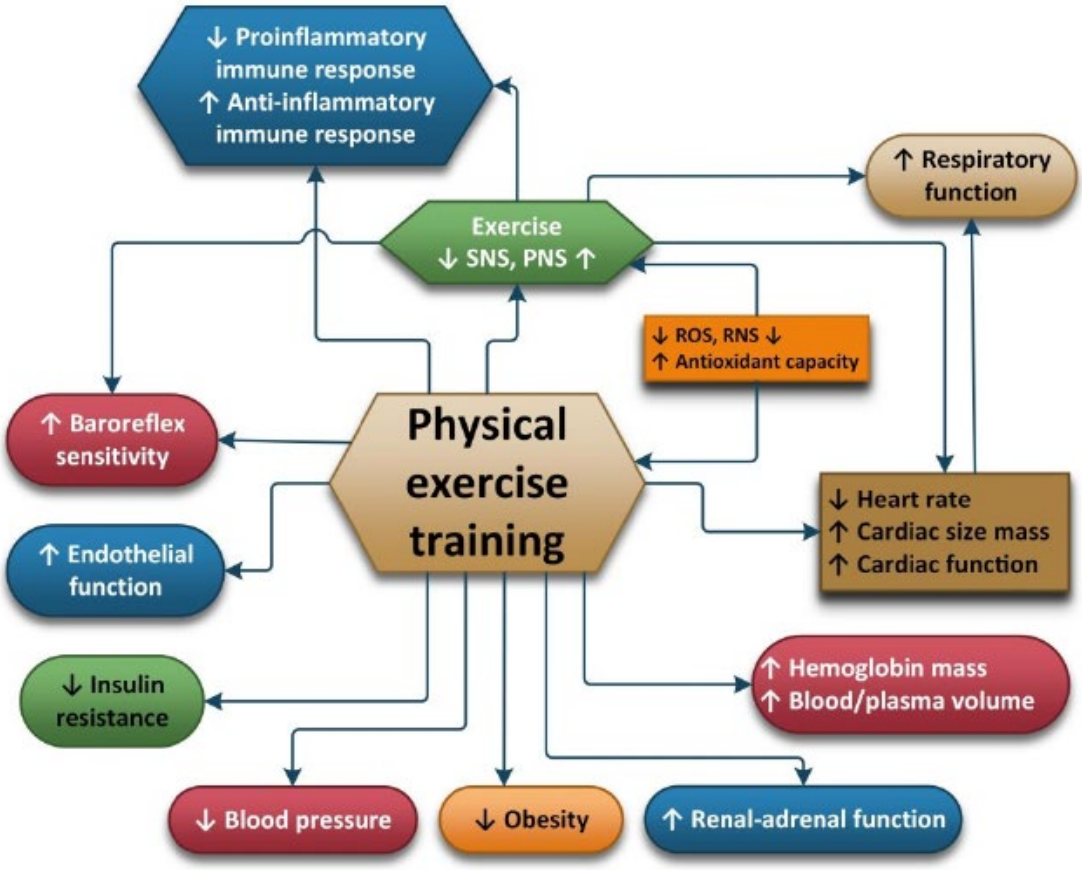

Figure 20. Pathophysiological changes under regular physical training.

The protective impact that physical exercise (PE) has on the on endothelial dysfunction are shows in Figure 21

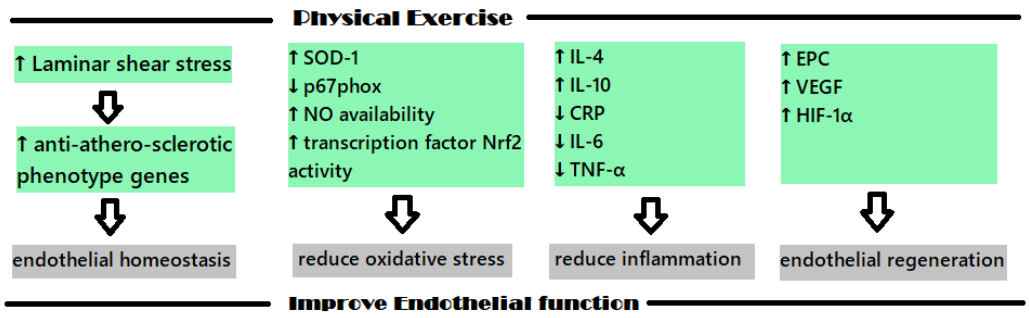

Figure 21. The main mechanisms involved in the protective cardiovascular effects of PE

PE can prevent and restore the endothelial dysfunction caused by aging by increases NO availability through its antioxidant and anti-inflammatory role [193]. It is known that sustained PE leads to an improvement in antioxidant levels, which can be explained by the increase in oxidative stress levels that occur during PE. The fact that ROS increase as a result of this effort causes the organism to adapt to this type of stress, which translates to a better response to the repair mechanisms for oxidative damage, an increased resistance to oxidative stress and a decrease in the levels of oxidative damage [193].

For example, the transcription factor "nuclear factor (erythroid-derived 2)-like 2 (Nrf2)" plays an important role in PE's positive effect on the endothelium dysfunction because it is linked to the organism's fight against oxidative stress [193]. Nrf2 regulates the expressions of some antioxidants, such as NQO-1, glutathione-S-transferase, glutathione peroxidase, and HO-1 when located in the nucleus; however, in the absence of oxidative free radicals, it usually remains dormant in the cytoplasm of the cells [193].

During PE, a short-term inflammatory response appears, which is followed by a long-term anti-inflammatory adaptive response. This short-term inflammatory response correlates with an increase in the number of leukocytes, oxidants and C-reactive protein (CRP) level. When exercise is methodically performed, a decrease in the pro-inflammatory molecules level is noticed, and an increase in anti-inflammatory molecules levels is also noticed; substances such as IL-4 and IL-10 are produced and CRP, IL-6 and TNF decrease [193].

Endothelial precursor cells (EPC) are important cells that are implicated in the regeneration of the endothelium and, depending on their number, they are positively associated with vascular function. These cells originate in the bone marrow and are circulating precursors of endothelial cells, but their circulating levels are usually small and, when injuries appear, the body has to mobilize them in higher levels with the aim of supporting endothelial repair. Studies conducted in this field have revealed that physical effort can act as an impulse for the mobilization of EPC from bone marrow [194].

Ribeiro et al. conducted a study with the aim of assessing the impact that different intensity resistance exercise, performed one time, has on EPC levels over 24 h. All participants in this study were females (n = 38). Along with the determination of EPC levels, the underlying mechanisms for EPC mobilization by effort was assessed using vascular endothelial growth factor (VEGF), the angiogenic factors stromal-cell-derived factor 1 (SDF-1α), erythropoietin

(EPO) and hypoxia-inducible factor 1-alpha (HIF-1α) [194]. After exercise, the number of EPCs increased, with the greatest increase occurring 6 h after effort was performed. This was also correlated with increasing levels of VEGF and HIF-1α. It was also observed that higher-intensity exercises were associated with a greater increase in EPC levels [194].

PE is considered a valuable non-pharmacological therapy, but it must be included in a lifestyle strategy designed to enhance overall wellbeing, in which diet also plays an important role. Caloric restriction (CR) is a concept involving dietary interventions with chronic or periodic reduced energy intake, without malnutrition. CR generates cellular and metabolic adaptations that delay aging processes, prolong the maximum lifespan and consistently improve insulin resistance. CR reduces body weight, waist circumference by decreasing visceral fat, insulin levels and improves insulin sensitivity, and serum lipids, which will induce a reduction in proinflammatory adipokines, IL-6, IL-12, IL-18, and TNF, etc., as well as an increase in anti-inflammatory adipokines, adiponectin and omentin, and lead to a significant reduction in oxidative stress [195]. Weight loss due to CR improves flow-mediated dilatation, which, in turn, significantly improves endothelial function in vitro in overweight adults [195]. Along with CR, fasting is proving to be an effective strategy to optimize health, reduce weight, and delay aging. Intermittent fasting has demonstrated its efficacy against the clinical progression of cardiovascular disease, inflammation, glucose intolerance, insulin resistance, obesity, and hypertension. The known mechanisms of intermittent fasting are the enhancement of cellular and molecular adaptive responses to stress, and here we mention endogenous antioxidant enzyme activity, mitochondrial function, proteostasis, and autophagy, as well as reductions in inflammation and oxidative stress [196].

It has been observed that the incidence of cardiovascular diseases is much lower in Mediterranean countries, and this has mainly been attributed to dietary habits. The Mediterranean diet is rich in fish, vegetables, whole grains, fruit, nuts, and extravirgin olive oil. In a systematic review on the effect of lifestyle and dietary changes in patients with coronary heart disease, Iestra JA et al. demonstrated that the mortality rate decreased in subjects who consumed extra-virgin olive oil compared to those who consumed other fats [197]. This could be explained by the improved endothelial function, reduced serum cholesterol and lower blood pressure found in individuals following the Mediterranean diet.

Fasting, the Mediterranean diet, and CR are positively associated with increased endothelial function, anti-inflammatory effect, and reductions in low oxidative stress, thus reducing the risk of cardiovascular disease (Figure 22).

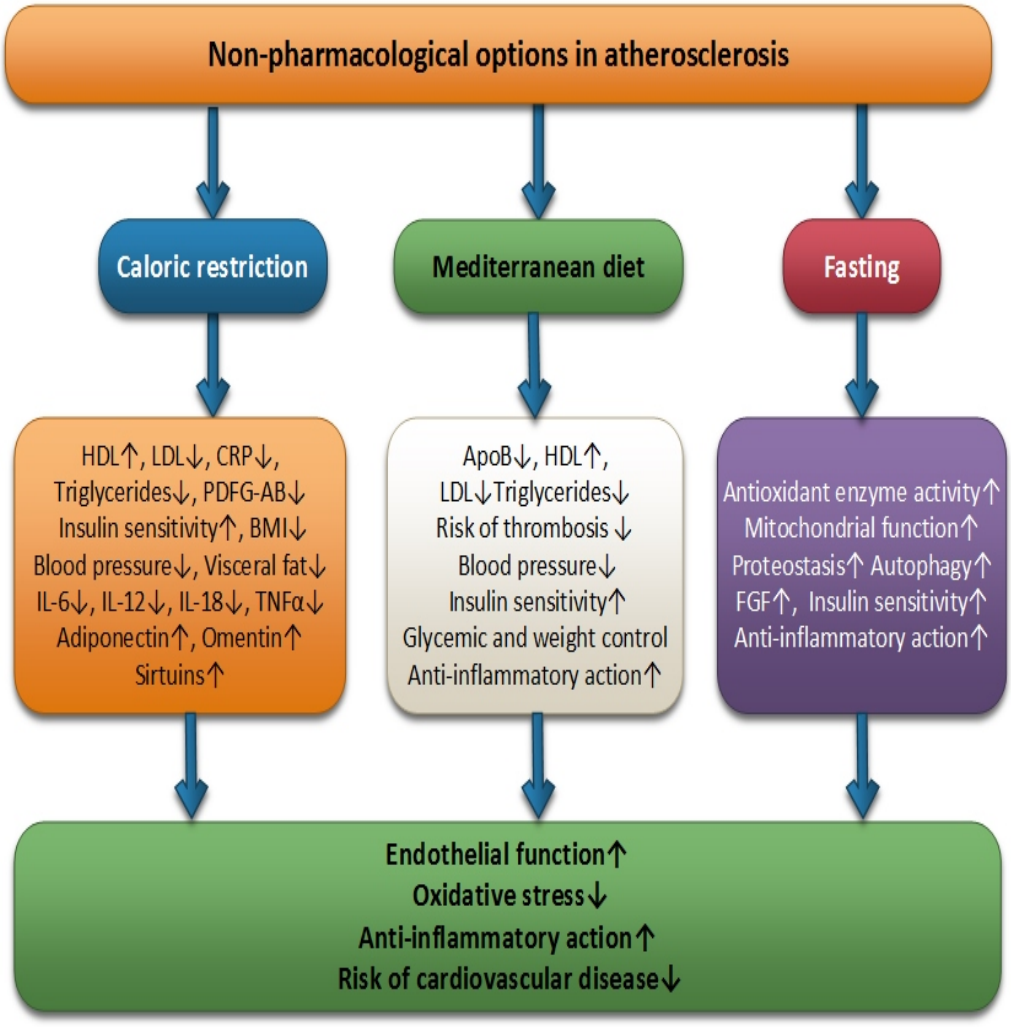

Figure 22. Non-pharmacological options in atherosclerosis: caloric restriction, Mediterranean diet and fasting

SECTION II

FUTURE PROJECTS IN THE ACADEMIC, PROFESSIONAL AND RESEARCH FIELD

1. IMPROVEMENTS IN THE ACADEMIC FIELD

Over the course of my academic career, I have always strived to continuously improve my teaching skills. Having the opportunity to teach students of both programs Medical Bioengineering and Physio-kinetotherapy and Rehabilitation together with my team, I am always preoccupied with development of solid skills in the field of paraclinical functional testing, neuromuscular exploration, and neuromuscular rehabilitation as well as in physiology, physiology of physical activities for better interdisciplinary. Updating textbooks and on-line resources available on the University's website it was an active concern for our groups.

Another concern will be to modernize the teaching process and making it compatible with the skills and competencies required by the labor market, stimulating the student's creativity and innovative spirit. The education law specifies that the student is an equal member of the academic community, both in relation to his educators and in relation to other students. Respecting the principles specified in the University Charta, for the promotion of student-centered education, paves the way for a teacher-student partnership as a defining element for obtaining the proposed performances. Interactive teaching and learning strategies are used, based on critical and reflective thinking, on group work, so that students will, first of all, develop professional skills and competences. Activities will be promoted to ensure a closer connection between the teaching staff and students: consultations, stimulating participation in congresses, student conferences.

I will encourage and coordinate students and master students to dedicate themselves and persevere in scientific activity and providing an information exchange at national and international level.

I'll try to increase the enthusiasm for postgraduate education, by involving as many master students as possible in practical and research activities. Creating a core working group, consisting of students with an interest for physiology research and practice; the outlined themes could be used later for the elaboration of graduation or doctoral theses. I'll intensify the collaboration with my colleagues from other specialties, and together we will hold interdisciplinary sessions for students.

I intend to be an example and in the same time a mentor for young PhD students, who must receive the best information and guidance for planning and organizing an original research.

I intend to attend as many national and international conferences as possible that would help me to obtain and update the necessary research skills in the future and to improve my professional and research performances. Therefore I intend to extend my collaboration with scientists from other Universities and research institutes and initiate and develop the necessary collaborative networks.

I also intend to contribute to expanding the prestige of the University by publishing the research results in prestigious ISI Thomson Reuters-indexed journals and to participate in competitions of funding for modernization of infrastructure and equipment necessary for a modern research activity.

I will continue to promote a performing academic environment, of mutual respect between teaching staff as well as in relations with students, which stimulates the achievement of the professional and personal potential of each member of the academic community. For the formation and development of professional skills, both the theoretical and informative activity is necessary, but especially the practical activity, carried out in a real professional context. That is why it is necessary to ensure the instructional process in the spirit of training valuable graduates, whose professional training meets the demands of the labor market, graduates prepared not only for the profession but also as a person and who can represent the Faculty of Medical Bioengineering successfully in the country and abroad.

2. **PERSPECTIVES IN SCIENTIFIC ACTIVITY**

In order to continue my research activity in the future, I will have to make more efficient and effective the professional interaction between the current and future collaborations, between my ability of coordinating original researches and the need to supervise young students and PhD students.

I will put the emphasis in the future activity on rehabilitation because in modern society, medical recovery is a field of great relevance, in continuous development. The increasing incidence of chronic diseases, as well as the numerical increase of the third-age population with different degrees of disability have brought into discussion the importance of the reintegration of

the individual, in the family and socio-professional environment, in the direction of promoting the patient's autonomy in everyday life, the final goal being the optimization of the quality of life, by increasing the degree of functional independence.

The rehabilitation process is a process that involves a multidisciplinary team in which, in addition to doctors of various specialties, physiotherapists and bioengineers cannot be missing. Medical recovery, being a complex field of activity, requires a close multidisciplinary collaboration between physiotherapist and bioengineer. The physiotherapist assists the patient in carrying out functional recovery, participates in the development of the rehabilitation project carries out the therapeutic program regarding infirmity and motor disability, according to the indications of the specialist doctor.

The role of the bioengineer in rehabilitation is complex. He creates fundamental concepts and knowledge from the molecular level to the systemic level and develops new biological products, materials, implants and IT products for the prevention, diagnosis and treatment of diseases and for the rehabilitation of the patients. Medical bioengineer develops strategies and provides equipment for communication, environmental control, home design (it is carried out according to the needs and specifics of the patient who uses that space, aiming to facilitate movement and self-service inside the home as well as to prevent the risk of accidents), making biocompatible prostheses, diagnostic and treatment medical devices that vary from clinical equipment to micro-implants with a role in re-education, etc. Neural engineering is a rapidly developing discipline that uses engineering techniques to repair, replace, or improve the neural system. Bioengineers working in this field are specially qualified to solve problems that arise at the neural tissue-artificial structures interface.

Rehabilitation bioengineering, through its neuromotor rehabilitation component, aims to train specialists (physiotherapists, bioengineers, doctors) to apply biomedical sciences to find medical and technical solutions that allow the correction or replacement of functions that affect motor function, vision, hearing, communication among people with disabilities to improve their quality of life and social reintegration. The bioengineer helps to adapt existing medical devices and builds new devices that are so necessary for recovery (making intelligent systems for diagnosis and monitoring the control of rehabilitation processes based on functional electrical stimulation; making software for monitoring parameters involved in rehabilitation processes; complex robotic recovery systems, virtual reality.

Assistive technology is a generic term that includes assistive, adaptive and rehabilitation devices for people with various types of disabilities. Assistive technology ensures greater independence for people with disabilities, allowing them to perform tasks otherwise impossible or very difficult to accomplish.

Functional electrical stimulation, a form of electrical stimulation applied in rehabilitation practices on a nerve pathway or motor point to produce a muscle contraction, is able to improve axonal conduction velocities, axonal growth, and the myelination of peripheral nerves [198]. It has been shown that FES device enhances peripheral nerve activity (efferent activation) and corresponding muscle and joint proprioceptive feedback (afferent activation). A major goal of rehabilitation is to make quantitative and qualitative improvements in daily activities in order to improve quality of independent living.

Functional electrical stimulation is a certified method of rehabilitating central motor neuron damage and less used in the recovery of peripheral motor neuron disorders. We want to continue studies on functional electrical stimulation using EMG biofeedback. Stimulated by the positive results of our study, we want to continue with FES as an efficient method in the functional re-education in foot drop and muscle re-innervation due to peripheral nerve injuries. The functional reeducation in a peripheral nervous system affliction differs consistently in its mechanisms from that of the central nervous system. In order to regain central motor control, the peripheral nerve must first regain its integrity and conductibility. The problem with the utilization of FES on a peripheral nerve injury is that there is insufficient valid data to provide useful insights regarding the appropriate stimulation settings.

The EMG triggered electrical stimulation training involved voluntary muscle contraction to trigger the FES. A prerequisite for this type of stimulation is the capacity of the paretic muscle to tens at a level that allows for an EMG signal to be measured by a surface electrode which makes this an active technique for the patient. In contrast to the foot-floor switch technique, where the patient follows a pre-established electrical pattern of movement, in the EMG triggered stimulation technique the movement pattern is activated and determined actively by the patient. EMG triggered technique therapy implies more motor control and cognitive abilities from the part of the patient. EMG triggered stimulation may be more effective than the simple or switch triggered techniques in improving motor control [199].

In addition to the mirror therapy, we want to continue the rehabilitation studies of the upper limb using a robotic system that was purchased through an institutional project. Robots appear for the first time in medicine in rehabilitation medicine with the scope to support the rehabilitation team in order to accomplish more results in less time. Robots are being developed for an extensive applications within rehabilitation domain, including use as exercise aids, activity of daily life, mobility aids, and remote some devices. Of these potential applications, robotic exercise devices have been best studied in clinical research and appear safe and also beneficial. Therapeutic robots collect data that can be used to quantitatively measure the patient's progress throughout the recovery process, enabling therapists to optimize treatment techniques. Robotic rehabilitation therapy is extremely attractive in post-stroke recovery as multifunctional pre-programmed devices can deliver individualized intensive training. Robot-assisted therapy for upper limb motor function training in sub-acute stroke patients showed comparable or superior results to conventional therapy [200].

We would also like to combine mirror therapy, the robotic arm with virtual therapy. This modern computer technology emulates learning process in the real world, while allowing the addition of extrinsic feedback and increasing the frequency, duration and even intensity of an exercise. Virtual environment enables the user to have the opportunity to interact with objects and situations produced by the hardware.

Virtual reality technologies allow creation of a simulated environment so that proper adjustment of exercise intensity and feedback would provide the patient with safe and effective training and rehabilitation [201]. Virtual reality therapy for stroke victims was proven to help improve motor impairment [202]. Up to date, there is too few data on cognitive functions rehabilitation with this approach.

Robotic devices and virtual reality have capabilities to continue as technological development and in the near future these methods are expected to become an integral part of rehabilitation like an important accessory to traditional rehabilitation approaches.

In recent years, physical disabilities have been greatly aided by technology. Starting from the post-stroke rehabilitation program to creating a safer and easier-to-use environment at home are another area of our research. Home medical equipment, intended for functional investigations and treatment, respectively monitoring at the home of the patient with disabilities is an attractive

research topic for our collective. This type of devices and equipment are useful for recovery at home and ensure the patient's independence (walking, dressing, enjoying hobbies, etc.).

Another area of interest would be analysis of biomedical signals that will help to create functional models, improve the performance of existing medical devices, design new techniques and methods of investigation and data analysis useful in diagnosis and monitoring.

Biomedical signal processing has recently one of the hottest topics in the research community due to E-health initiatives and widespread of mobile technology. Efficient acquisition, storing, processing and analysis of medical data have been subjects of many researches with both univariate time series such as ECG or PPG or multivariate ones such as EEG. Combining ECG or EEG with biomedical imaging it is a future research area for us.

An attractive topic for me was Alzheimer's disease, a disease carrying a huge negative effect upon both patients and their families, and also on society in terms of health services. AD is the most frequent form of dementia in old people, manifesting by a progressive damage in cognitive fields and emotional abilities, which interfere with the social and professional life of the individual.

Hallmark brain abnormalities in AD consist in accumulation of beta-amyloid plaques and tau tangles, also deterioration of the nerve cells, and eventually, death inside the brain. Beside this, new concepts about the pathogenesis of AD began to emerge, such as oxidative stress, mitochondrial dysfunction, neuron inflammation, endothelial dysfunction, impaired insulin signaling, synaptic dysfunction, and decrease of neurotropic factors.

A better control of vascular risk factors, especially in midlife decades is today considered possible to be associated to a decline of cognitive impairment due to dementia. Studying the biology and pathological biology of normal aging and AD, biomarker evidence and neuroimaging, all contribute to understand the basic mechanisms of this disease, possible identification of markers for early detection, establishing the risk of evolution towards dementia in at-risk populations, and for all that, a better management of the public health impact of AD.

The pathophysiological mechanisms involved in the formation of atherosclerosis, one of the major vascular risk factor, are excessive sympathetic stimulation, the inflammatory process, oxidative stress and endothelial dysfunction. Finding a way to simultaneously combat these factors would be ideal for the prevention of atherosclerosis and cognitive decline.

Vagus nerve stimulation could meet these criteria. Several non-pharmacological methods to activate the vagus nerve exist, such as the invasive and non-invasive electrical stimulation of the vagus nerve, and non-invasive stimulation of vagus nerve during different types of deep breathing or during heart rate variability biofeedback (HRVB) training.

Using the cholinergic anti-inflammatory pathway, VNS could reduce cytokines levels with attenuation of the inflammatory process. This inhibitory pathway may be of interest to control the neural reflex of inflammation, with great potential in combating inflammatory diseases [203]. Several clinical studies have used implanted nerve stimulators to activate the vagus nerve, which have reported encouraging results in relieving chronic inflammation.

The least invasive tVNS methods use superficial stimulation of the vagus nerve through the skin, which can be applied to the anterolateral surface of the neck (cervical tVNS) or to the cymba conchae on the ear (auricle tVNS) [204]. Studies on the reduction in inflammation caused by tVNS are just beginning, but some are promising [205, 206].

Given the novelty of VNS in humans for anti-inflammatory effects, there is no standardized method of treatment (administration methodology, duration, current levels) so the development of new guidelines for researchers and practitioners interested in vagus nerve modulation for inflammation control is needed.

Furthermore, tVNS contributes to reduced activity in limbic brain regions [207], and was more recently found to increase activity in the anterior cingulate and the left prefrontal cortex [208]. These studies may suggest that tVNS can increase executive control and emotional regulation. Higher executive function can modulate risk factors such as smoking, unhealthy diets and sedentary behaviors, and can moderate the intention–behavior link between physical activity and dietary behavior [209].

Expanding knowledge of the mechanisms of neural control of vascular inflammation, balance autonomic nervous system activities, reduce ROS/RNS will be important for the treatment of cardiovascular diseases such as atherosclerosis.

Another non-invasive method that stimulates the vagus nerve is paced vagal breathing, which is practiced during different types of physical activities, yoga, or during heart rate variability biofeedback (HRVB) training. HRVB is a non-invasive therapy training that aims to increase heart rate oscillations through real-time feedback and slow breathing training [210]. HRVB has a positive effect on psychological symptoms and increases wellbeing [211,212].

Lehrer et al. recently performed a systematic and meta-analytic review on the efficacy of HRVB and/or paced breathing (six breaths/min) and conclude that HRVB improves emotional and physical health and performance [213,214].

HRVB may have regulatory effects on the autonomic nervous system function. By enhancing vagal activity and reducing SNS activity, HRVB could represent a promising method for the management of a wide range of chronic diseases. HRVB significantly decreased systolic blood pressure and improved baroreflex sensitivity [215]. HRVB was associated with a reduced systolic blood pressure in response to exercise [216]. Thus, HRVB is a relatively simple, non-invasive technique that could be implemented in cardiac rehabilitation programs. Autonomic nervous system activity improvements represent one of the essential mechanisms through which HRV-biofeedback influences cardiovascular outcomes.

Psycho-emotional stress can cause dysfunction in both the autonomic and neuroendocrine nervous systems, which can lead to increased serum cortisol, catecholamine and an imbalance between SNS and PNS. Stress produces increased sympathetic activity, reduced vagal activity, and causes inflammation, oxidative stress and endothelial dysfunction. Psychosocial stress was associated with the upregulation of SNS and the increased proliferation of neutrophils and inflammatory monocytes in mice and humans [217]. Individuals with a higher tonic vagal output showed a lower expression of NF-_B transcription [218].

Resting respiratory sinus arrhythmia was inversely related to increases in inflammatory cytokines, such as: IL-6, IL-8, IL-10, and TNF_α [219]. The current understanding of the complex network that links the central and peripheral autonomic nervous system, hypothalamic–pituitary–adrenal axis, immune system, and the main biological systems provides a physiological explanation that links psycho-emotional stressors and social adversities to cardiovascular diseases [220].

In order to reduce inflammation and psychosocial stress, dosed physical effort is indicated. Physical exercise can be used as a primary non-pharmacological clinical tool because it can improve antioxidant capacity, reduce oxidative stress and inflammation and increase energy efficiency. Depending on the volume, the intensity and the frequency of exercise, acute or chronic biochemical and physiological responses are induced. PE intensity is usually expressed as a percentage of the individual's maximum oxygen uptake (VO2max), which is the maximum aerobic capacity during PE. Metabolic equivalent (MET) is defined as the amount of oxygen

consumed at rest, and it is an optimal method used to describe the functional capacity or tolerance of an individual to certain PEs [221].

The positive impact of physical activity is well known and has been studied by many researchers. But physical activity may also have negative impacts on the body, depending on the type of effort, the duration of the effort, and the individual characteristics of the person exerting the effort (age, gender, diseases, etc.). These negative impacts are less well studied and seem to be linked with the oxidative stress and inflammation induced by effort, mainly reflected in the increase in oxidants and decrease in antioxidants during physical activity. Due to the fact that the level of antioxidants in the body decreases with age, age is an important factor in the body's response to oxidative stress.

Because overload training (large volume or long-term exercise) causes oxidative distress, this will nullify the positive impact of the physical training on health outcomes. It showed that high-intensity PE could be dangerous and is associated with chronic musculoskeletal injury, anaphylaxis, and sudden death. A single session of overload training (in large volume or over a long time period) can cause oxidative distress, leading to the loss of beneficial health outcomes related to physical activity. However, if training continues the body can adapt to the exhaustive physical activity by increasing the expression of antioxidant enzymes. If oxidative stress is reduced or antioxidant capacity is increased with training then less inflammatory process will occur during training.

We know a lot about the benefits of exercise, but lack information about the correct mode, type, length and frequency of exercise necessary to gain such benefits. Even though there are many ways in which physical exercise can be structured, it is important that individualized training is prescribed that takes into account the characteristics of the person doing the training with the aim of achieving the optimal physiological outcomes. Stanley et al. suggested that a monitory system should be used in which the HRV and training logs are included in the daily routine of the person doing the exercise [222]. Their results show that the time required for complete cardiac autonomic recovery after a single aerobic-based training session is up to 24 h for low-intensity exercise, 24–48 h for threshold intensity exercise, and at least 48 h following high-intensity exercise [222].

That's why we propose that using wireless systems we can acquire ECG signals and monitor using HRV each stage of physical effort, in different types of physical activities. In this

way, we can contribute to the practice of physical effort in safe conditions and to prevent the occurrence of cardiac arrhythmias and sudden death associated with physical exercise.

To conclude, My entire research activity will focus on rehabilitation of physical, psychological, social, professional, occupational and educational potential of each patient. Rehabilitation will support patients by offering them the possibility of regaining maximum independence. For some patients, regaining some lost functions means a new beginning this allowing for their return to work and a decent quality of life. For some patients, even the smallest degree of recovery of a motor deficit can generate immeasurable satisfaction. With the help of rehabilitation programs some of the patients "can start living again".

SECTION III

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