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DECLASSIFYING THE MASTERMIND

- HABILITATION THESIS -

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CONTENTS

ABBREVIATIONS

ACE - angiotensin-converting enzyme in
ANS - autonomic nervous system
ApEn - approximate entropy
ARB - angiotensin receptor blocker
ASL - arterial spin labeling
CVD - cerebrovascular diseases
HF - high frequency
HRV - heart rate variability
HTPR - high treatment platelet reactivity
LF - low frequency
MCA - middle cerebral artery
PCA - posterior cerebral artery
RespRate - respiratory rate
RMSSD - root mean square of the successive differences
SampEn - sample entropy
STRE - epilepsy after ischemic stroke
TIS - transient ischemic stroke
TTPA - partially activated thromboplastin time
VLF - very low frequency
VNS - vagus nerve stimulation

ABSTRACT

The Habilitation Thesis synthesizes postdoctoral professional, academic and scientific activity and it is structured in three major sections, according to the The National Council for Attestation of University Titles, Diplomas and Certificates (CNATDCU) recommendations and criteria. The paper represents a synopsis of my accomplishments in the concern domains. The title "*Declassifying the mastermind*" suggests the struggle of my research and the dilemmas of the clinical and academic approach in the field of neurology.

An academic career rises complex challenges and entrusts on indefatigabilityand the awareness of the need for self-enhancement. Pedagogy and teaching are complex professional challenges, which relies on receptivity to new ideas and concepts, flexibility, dynamism and critical reflection. To apply professional standards in the academic career is essential to ensure the constant high-quality enrichment of the educational system. Our current national standards required for the academic stuff focus on the awareness of continuous training, the integration of modern methods in their teaching activities (like technologies of information and communication), thus increasing the quality of the educational process.

Any academic career has important distant echoes and impact on the entire academic community. It should mix harmoniously with many qualities, such as: substantial scientific knowledge, availability and gratification to communicate, ambition to be part of a team, capacity to create and coordinate functional teams, ability to identify and motivate human resources receptivity to new ideas and concepts, flexibility, dynamism and critical evaluation.

In accordance with the CNATDCU criteria I have structured the thesis into two main parts:

- Section I - Abstract/Rezumat
- Section II - Professional, scientific and academic achievements. This last section is subdivided into three subsections:
    - Chapter 1 - Compandiary overview of the academic carreer.
    - Chapter 2 - Scientific and professional achievements
    - Chapter 3 - Referrences.

Section II contains a summarised survey of my professional, academic and scientific activities, where I reassessed my researches and the main areas of interest which I have followed after my PhD thesis. There is the assessment and augmentation of my research, didactic and medical activities, since the doctoral thesis fullfilment in 1999. Detailed descriptions of the plans for future research and for the continuation of the projects already started are also defined. This main section is organised in three chapters as follows, as I mentioned above.

It starts with an analytical perspective of all my research domains and the projects which I have conducted, together with the results of my academic work I have. These are written under the Chapter 1.

In Chapter 2 there are affiliated within three main study directions the most important results of my main research domains:

1. Stroke - "Brainology of the "vital spirit"

2. Epilepsy - "Carpe de morbo sacro"
3. New perspectives in neurology.

These research domains came out from my main clinical practise fields. Stroke research results led to to deepen the knowledge about the morphology, functionality and particular practical features o the brain.

The direction of study regarding the epilepsy led to important datas which allow us to expand the research of this vast field. We have explored the morphofunctional features of different epileptic focals topographies, risk factors and new treatment protocols through innovative and modern techniques.

The third chapter blends and underlines our newest collected datas from different codomains, such as: Parkinson disease study, Myastenia gravis and diabetes neuropathy.

The third section contains the most representative bibliographic refferences of my currently knowledge and for future projects. Within this manuscript I described and highlighted all my research findings in the two main directions that I have followed. These are derived from the knowledge accumulated so far by me and the team I coordinate in this direction.

I personally believe that by continuing the research in the vast and complex field of neurology we could achieve the development of management protocols for these patients that will balance the clinical need and the individual opportunity for their treatment.

The starting point of our experience and expertise on this topics represents the current level of understanding for the areas of interest and the related ones, marked by the specialized bibliography. Thus, at the end of the thesis I have attached the list of the most important references that we have used for this purpose.

REZUMAT

Prezenta teză de abilitare sintetizează activitatea mea profesională, academică și științifică postdoctorală și este structurată în trei secțiuni majore, în conformitate cu recomandările și criteriile Consiliului Național pentru Atestarea titlurilor, diplomelor și certificatelor universitare (CNATDCU). Lucrarea reprezintă o sinteză a realizărilor mele în domeniile principale de interes. Titlul „Deconspirarea secretelor creierului" sugerează eforturile făcute pentru relizarea cercetărilor mele și dilemele abordării clinice și academice ale domeniului neurologiei.

O carieră academică ridică provocări complexe și încredințează indefatigabilitatea și conștientizarea nevoii de auto-depășire. Pedagogia și predarea sunt provocări profesionale complexe, care se bazează pe receptivitatea la idei și concepte noi, flexibilitate, dinamism și reflecție critică.

Aplicarea standardelor profesionale în cariera academică este esențială pentru a asigura îmbogățirea constantă și de înaltă calitate a sistemului educațional. Standardele naționale actuale necesare și solicitate în cariera academică se concentrează pe conștientizarea formării continue, pe integrarea metodelor moderne în activitățile de predare (cum ar fi tehnologiile informației și comunicării), crescând astfel calitatea procesului educațional.

Orice carieră academică are ecouri și un impact importante asupra întregii comunități academice. Aceasta ar trebui să combine armonios anumite calități, cum ar fi: cunoștințe științifice substanțiale, disponibilitate și dorința de a comunica, alături de ambiția de a face parte dintr-o echipă, capacitatea de a crea și coordona echipe funcționale, capacitatea de a identifica și motiva resursele umane, receptivitatea la idei și concepte noi, flexibilitate, dinamism și evaluare critică.

În conformitate cu criteriile CNATDCU am structurat teza în două părți principale:

- Secțiunea I - Rezumat / Rezumat
- Secțiunea II - Realizări profesionale, științifice și academice. Această ultimă secțiune este împărțită în trei subsecțiuni:
  - Capitolul 1 - Prezentare generală obligatorie a carierei academice.
  - Capitolul 2 - Realizări științifice și profesionale
  - Capitolul 3 - Referințe.

Secțiunea a II-a conține un rezumat al activităților mele profesionale, academice și științifice, unde am reevaluat cercetările mele și principalele domenii de interes pe care le-am urmat după teza de doctorat. Sunt prezentate, de asemenea, evaluarea și extinderea activităților mele de cercetare, didactice și medicale, de la completarea tezei de doctorat din 1999.

În cadrul tezei sunt definite descrise detaliat planurile de cercetare viitoare și pentru continuarea proiectelor deja începute. Această secțiune principală este organizată în trei capitole, după cum am menționat mai sus.

Secțiunea începe cu o perspectivă analitică asupra tuturor domeniilor de cercetare și a proiectelor pe care le-am desfășurat, împreună cu rezultatele activității mele academice. Acestea sunt scrise în capitolul 1.

Capitolului 2 îi sunt afiliate trei direcții de studiu principale cele mai importante domenii de interes:

1. Accidentul vascular cerebral - „Știința creierului și a spiritului vital ”
2. Epilepsia - „Cunoașterea bolii sacre”
3. Noi perspective în neurologie.

Aceste domenii de cercetare au reieșit din principalele mele domenii de practică medicală. Rezultatele cercetării accidentului vascular cerebral au condus la aprofundarea cunoștințelor despre morfologia, fiziologia și particularitățile clinice specifice ale sistemului nervos central.

Direcția de studiu privind epilepsia a dus la obținerea unor date importante care ne permit să extindem cercetarea asupra acestui vast domeniu. Am explorat caracteristicile morfofuncționale ale diferitelor topografii de focare epileptice, factorii de risc și protocoalele noi de tratament prin tehnici inovatoare și moderne.

Al treilea capitol îmbină și subliniază cele mai noi date colectate de la diferite codomenii de activitate, precum: studiul bolii Parkinson, al miasteniei gravis și al neuropatiei diabetice.

A treia secțiune conține cele mai reprezentative referințe bibliografice ale cunoștințelor mele de până în prezent și care stau la baza proiectelor viitoare. În cadrul acestui manuscris am descris și am evidențiat toate rezultatele cercetărilor mele în cele două direcții principale pe care le-am urmat. Acestea sunt derivate din cunoștințele acumulate până acum de mine și de echipa pe care o coordonez în această direcție.

Eu personal cred că mergând înainte în cercetările din domeniul vast și complex al neurologiei am putea realiza elaborarea de noi și mai eficiente protocoale de management pentru acești pacienți care să echilibreze nevoia clinică și oportunitatea individuală de tratament.

Punctul de plecare al experienței și expertizei noastre pe aceste teme reprezintă nivelul actual de înțelegere pentru domeniile de interes și pentru cele conexe, marcate de bibliografia de specialitate.

SECTION I - PROFESSIONAL, SCIENTIFIC AND ACADEMIC ACHIEVEMENTS

COMPANDIARY OVERVIEW OF THE ACADEMIC CARREER

Individual high-end education is compulsory for the development of an academic career and inherently for the benefit of the entire society. Nowadays we areemboldened to make use of the opportunities offered by lifelongeducation. Career advancement should focus on theinvolvement and accomplishments gainedwith didactic and professional activity, their aftereffects and future plans. Meanwhile, the outcomesof our research activity contribute in its specific field and manner to establish the future objectives.

Reaching an academic education level is about acquiring the necessary knowledge and skills for each individual line of work and impetus.

My academic career development and perspectives has both a professional and, especially, a human side, ascumulative, objective and appreciative outgrowth of my life enlightening experiences. The overlapped efforts performed for the achievement of a top academic recognition and performance represent the background of successful currently knowledge.

Some of the characteristics of our time are accelerated social transformation and an opened competitive market for academic services. In this context, universities need to adapt and to evolve to reach the needs and requirements of high educational management standards. Which of us is an active part of this academic mechanism which enables university to further development and recognition among the top-ranking higher education institutions.

Some of our competences and finesses can be inherented, such as oratory and scholastic skills, others need tobe tought. These to be improved by practice, steady continuous works, pending to the reference models we have.

For all of these there is the demand of highly qualified teachers, professional mentors who have to form competitive and adaptable specialists in the field.

A member on University stuff shall undertake a full-time commitment to academic activity andstudy. Each member with responsibilities in the area of academic activity shall be entitled and expected to engage in Academic Activity, which involves some or all of the creation of new knowledge, including understanding or concepts, the creative application of existing knowledge, the organization and synthesis of existing knowledge and that is relevant to librarianship or archival practice.

These activities oblige each individual to didactic endeavor and scientific research as well. In the same time European Union policy and management of academic research causes a inconsistency between the two directions of activity by the fact that institutional and individual assessment is determined by the achievement of performance parameters/indicators in research area. The didactic activity should reflect the research activity exponentially expanding.

Didactic activity

I have more than 25 years ofdidactic experience materialized in practical works and courses in the field of Neurology. Especially in the last time, the teaching methods we have used have diversified with the modernization of the infrastructure. Even so, the key instrument in the academic neurology teaching process is still working the patients. The main teaching method in my field and other clinical ones remains the clinical practice.

To qualify new physicians for this there isa high demand for personalized professional bibliographic resources, correlated with the syllabus.

I have achieved all my teaching levels by concours:

- Associate Professor from 2004 until now at the Chair of Neurology, Faculty of Medicine, University of Medicine and Pharmacy "Grigore T. Popa" Iasi
- Lecturer from 2000, at the Chair of Neurology, Faculty of Medicine, University of Medicine and Pharmacy "Grigore T. Popa" Iasi;
- Assistant professor between 1994-2000, at the Chair of Neurology, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy Iaşi
  I have finished the PhD study in 1999.

Since I joined in the Chair of Neurology to the present day, I have had as basic principles the permanent improvement of clinical practice knowledge, skills and didactic methods, the assimilation of theoretical matter into the direct benefit of the patients. There is the need for consolidating the education process and continuous qualitative improvement in accordance with the requirements of the national education system, but also the international requirements.

An academic career within a clinical study direction comes with the opportunity to fuse its three main directions: didactic, clinical and research.

All this time I was looking to accomplish my didactic appropriateness by:

- ✓ continue renewing of contents and presentation of well-documented lectures;
- ✓ managing up-to-date, systematized and stimulating practical classes;
- ✓ conceiving of course and practical textbooks for romanian, english and french study directorates;
- ✓ modernizing the teaching-learning-evaluation methods by using specific computer equipment;
- ✓ developing evaluation written and practical tests for for all study directions;
- ✓ creation of groups for the analysis of substance and content of the disciplines' records in order to eliminate overlaps and parallels;
- ✓ self evaluating and capitalizing the results of the students' assessment for the improvement of didactic activity.

Research activity

The teacher has an important wind of change goal, to promote self-transcendence through knowledge, understanding and tolerance. Therefore, the responsibility placed on his shoulders is enormous, because they are among those who participate in the formation of the characters of the new generation and are models for students. Under the current conditions, in

which the contemporary world is evolving at such an alert rate, the need to constantly update and improve the level of research activity and proficiency as well as the teaching techniques used is obvious.

Equally, however, the knowledge-based society involves particular scientific research efforts, their transmission through education and training, their dissemination using the means of information and communication technology, the use of learning through technological innovation.

Therefore, a proper academic career involves scientific research by continuation and completion of research projects undertaken in group and/or individually and involvement in new ones. Capitalizing on the research results by publishing studies and articles in ISI quoted journals and in journals from the main international scientific stream, indexed to BDI is compulsory for worldwide recognition.

Via my entire research activity I have developed the opening towards interdisciplinary initiatives and extending the collaboration in order to elaborate research projects. I have stimulated the interdisciplinary research activity within the chair of neurology and increased the visibility at national and international level of the members of the department for our didactic and scientific activity carried out;

Attracting an increasing number of students and masters in the scientific research activity is another current personal engagement.

In 1999 I have finished my PhD Thesis, entitled "Neurological manifestations in acromegaly", which conferred me the title of Doctor of Medical Sciences, Medicine domain. I chose an ambitous theme because the study of the acromegaly raises great challenges. The most difficult aspect was the need for multidisciplinary approach of these patients patients.

The original character of the thesis is supported by the complex research directions, starting from the endocrinological study, continuing with the pure neurological one and including radiological and clinical trials.

The objectives proposed and achieved throughout my PhD research project have astrong practical and scientific impact with primarily regards on thegeneral and, particularly neurological statusof the acromegalic population.

The highlighted risk factors involved in the processes neurological disorders in acromegaly and the obtained results emphasize that the study hasstrong implications in the management strategy of these patients.

The theme that I approached gives new research perspectives andopens new opportunities for early identification and monitoring of acromegaly neurological comorbidities. All the efforts should be made towards continuous awareness of this disease among primary care physicians (including those in training), specialists, and other health professionals. The presence of neurological comorbid conditions contributes significantly to patient morbidity/mortality and impaired quality of life, and their appropriate management has the potential to improve long-term outcomes.

Hormonal control of acromegaly (e.g., with surgery and/or somatostatin analogues) may contribute to the management of some comorbid conditions. However, specific neurological therapies/treatments may be also be required to control these conditions (e.g., antihyperglycemic drugs, antihypertensives, various surgical interventions, psychological therapy). Thus, better awareness and a more aggressive approach to treat acromegaly

neurological comorbidities may contribute to improving quality of life and decreasing disease mortality.

I continued my research work, constantly improving myself by attending three new scientific domains: stroke, epilepsy and PArkinson disease. They allowed me to acquire the necessary skills in performing clinical and cohort studies, imagistic explorations, morphological research and statistical analyzes.

Together with the results of my research activityin another side domains - myastenia gravis, diabetes polineuropathy etc - I have disseminated our outcomes by scientific papers presented at congresses, symposiums, national and international conferences and scientific papers published in ISI-rated journals.

My scientific portfolio includes:

- ✓ 20 ISI articles
- ✓ *H-index* 7
- ✓ scientific papers published in extenso in BDI-rated journals;
- ✓ abstracts published in the volumes of national and international scientific events.

I was permanently looking for improving of personal research skills by advocating competitive research by finishing research into ongoing topics and accessing new national or international research founding resources. By identifyingnew research opportuneties and directions we shall benefit of information and training courses on the scientific research domains.

I intend to keep on stimulating the multidisciplinary approach in research by encouraging human resourses exchange.

Our research activity performance is mandatory in scientific and professional recognition, throughout the quality of the published articles which also empowers us to reach for founded research projects.

All of my personal and professional achievements warrants my individual academic performance recognition, but also they are increassing my international visibility, as memberof the Academic Corpus of the University of Medicine and Pharmacy"Grigore T. Popa" Iasi.

The international visibility must be constantly increased and I attend to do that by the sustanence the dissemination of the results of my researches by elaborating and publishing articles in top international journals. Also I promoteour research results through active participation in national and international scientific events, interdisciplinary collaboration with other universities and research centers. In the same order we must make usage of hosting services such as SlideShare to disseminate research results and ORCID platform that provides a persistent identity for humans.

- ***Professional achievements***

After an experience of 26 years in the fiels of neurology, I can say that the results of the academic career integrate into the complexity of the medical requirements and, above all, of the research. My academic career has been built, first and foremost on hard work, on the

awareness of the need for research and the opportunities offered by training sessions in the country and abroad.

I have work under the co-ordination by the elites of Romanian and international neurologists, coupled with access to cutting-edge information and technology, has allowed me to develop this side of my career.

Equally important was and is, in this respect, the opportunity that I am offered in correlating the three major aspects of my career - didactic, professional and research - around the main themes of interest.

25 of my professional experience of over 36 years has been complementary to my academic career and is integrated in the complexity of the current requirements and, first of all, qualifies me for the research career. In all this time I have enriched my experience in managing the series of students, the activity with the residents but also with regard to the main topics of interest - stroke and epilepsy.

The perspective of researching these fields has stimulated me throughout my university careers and focused on the medical activity, in order to ensure the highest professional quality. These topics have a major socio-economic impact and the research directions we have carried out so far are mostly aimed at prevention, early diagnosis and treatment, reducing the incidence of complications and a more adequate socio-professional reintegration of these. patients.

CHAPTER 1. BRAINOLOGY OF THE "VITAL SPIRIT"

1.1. State of the Art

What we call nowadays stroke was first noted from 460 to 370 bc by Hippocrates. At that time, the symptoms such as convulsions and paralysis were referred to as apoplexy. It was considered they were caused by different types of problems of the blood flow.

Blood flow was considered to assure the distribution of the "Vital Spirit" throughout the body (Darwin,1868).

Over the next several centuries, physicians focused on clinical symptoms and potential etiology, which led to patients to be treated with enemas and bloodletting.

Within the recent times, pathophysiological changes, noted by physicians such as Thomas Willis and Jakob Wepfer led to surgical interventions. Meanwhile, the magnitude of research has grown exponentially and the term stroke has replaced the apoplexia (Garrison, 1929; Pearce, 1997; Rocca,1998).

Medical science continued to study the cause, symptoms, and treatment of apoplexy and, finally, in 1928, apoplexy was divided into categories based on the cause of the blood vessel problem. This led to the terms stroke or "cerebral vascular accident (CVA)". Stroke is now often referred to as a "brain attack" to denote the fact that it is caused by a lack of blood supply to the brain, very much like a heart attack is caused by a lack of blood supply to the heart. The term brain attack also conveys a more urgent call for immediate action and emergency treatment by the general public (Barnett,2014).

Today, there is a wealth of information available on the cause, prevention, risk, and treatment of stroke. Although there is no cure, most stroke victims now have a good chance for survival and recovery. Immediate treatment, supportive care, and rehabilitation can all improve the quality of life for stroke victims (Wolf et al., 1991).

Medical students and researches in the 1940s observed that stroke victims were regarded as in a hopeless situation. Severely disabled patients by stroke and also those in coma from intracerebral clots and haemorrhage were confined to specific Observation Units. So, Stroke Prevention and Treatment Units could be finally formed. This mean formation and selection of neurologists, nurses, and imaging technicians specialized in stroke, to give expert care in these new units.

This was the first light of hope in stroke patients care. Meanwhile mandatory differences have resulted from epidemiological observations, dramatic scientific breakthroughs, including microbiology, treating of the infectious disease, pharmacology, and also technology. These have been coupled with the advent of rigid clinical trials.

One of the 100 Most Important People of the 20th century, Alexander Fleming discovered the penicillin and eliminated brain abscess and much pneumonia and strokes because of pneumococcal and syphilitic meningo-vascular changes (The Nobel Foundation 1945; Cruickshank, 1955; Hugh and Howard, 2002).

Streptomycin, discovered by Albert Schatz could cure tuberculous meningitis (Rawlins, 2012).

This is the explanation for how endocarditis with embolic brain infarction was becoming uncommon and since than there were counted home discharged and well recovered individuals, even from coma.

All these pioneering events that before those days were fatal but today we take the cure for granted. Confirmation of the risk factors emerged from a combination of research strategies was one of the keys to affirmation of many.

The collecting of life-time records of populations as in the Framingham Study created a wealth of data about physical and mental health, especially about cardiovascular disease (Kannel, 1976; Levy and Wang, 2013).

Major findings from the study are the background of the Framingham Risk Score of future coronary heart disease:

- ↗ smoking increases risk of heart disease;
- ↗ increased cholesterol and elevated blood pressure increase risk of heart disease; exercise decreases risk of heart disease, and obesity increases it;
- ↗ elevated blood pressure increases risk of stroke;
- ↗ in women who are postmenopausal, risk of heart disease is increased, compared with women who are premenopausal;
- ↗ psychosocial factors affect risk of heart disease;
- ↗ high levels of HDL cholesterol reduce risk of heart disease
- ↗ having an enlarged left ventricle of the heart (left ventricular hypertrophy) increases risk of stroke.
- ↗ obesity is a risk factor for heart failure;
- ↗ serum aldosterone levels predict risk of elevated blood pressure (Christakis and Fowler, 2008).

The Oxfordshire Stroke Project help clinicians predict the safety and efficacy of thrombolysis by highlighting "The Four Horsemen of the Stroke Apocalypse" as: hypertension, tobacco, diabetes mellitus, and cholesterol excess (Yang et al., 2016).

The management of all of these has been shown clearly to reduce stroke occurrence. Some important factors cannot be changed (eg, old age, male sex, and heredity) but with these in the patient's background assiduous management of the risk profile is imperative needed. All the clinical trials have been of particular consequence in proving the necessity to regulate blood pressure in all age groups and to maintain normal levels of the low-density lipoprotein fraction of cholesterol (Wahlgren et al., 2007; Wahlgren et al., 2008; Mazya et al., 2012; Kent et al., 2006; Cucchiara et al., 2008; Lou et al., 2008; Saposnik et al., 2012; Saposnik et al., 2013; Pagola et al., 2011; Sarikaya et al., 2011; Breuer et al., 2011; Bamford et al., 1991).

Another breakthrough was when Moniz introduced cerebral angiography in 1928. He triggered subsequent leaps forward in the ease, safety, and exactitudes of clinical diagnoses. This leaded us to nowadays 3-dimensional imaging of the cerebral arteries from the neck up to the small branches of the Circle of Willis. His technique followed the pneumo-encephalography, a method developed by the American neurosurgeon Dandy which consists in the injection of air into the brain ventricles (Doby, 1992).

Collateral supply can be also identified but not quantified and there is hope that we will routinely be able to visualize the condition of the penetrating arteries of the basal ganglia/thalamic area and cerebellum and have understanding of and more accurate diagnosis of lacunar stroke (Antunes,1974).

He indirectly demonstrated for the first time the importance of the extracranial arteries in stroke, their importance later being confirmed pathologically by Hultquist in Norway and 10 years later by Fisher. Magnetic resonance imaging (MRI) and computed tomography angiography have become standard in seeking obstructive lesions (Gross and Schäfer, 2011).

Diffusion weighted MRI of the brain has become essential in deciding on the extent of the early infarction in developing stroke making the early use of thrombolytic agents safer and their use more precise when there has already been infarction (Baliyan,2016).

The goal of the imaging procedures is generation of an image contrast with a good spatial resolution. Initial evolution of diagnostic imaging focussed on tissue density function for signal contrast generation. In 1970s, Lauterbur, Mansfield and Ernst have brought into common medical practise the modern MRI technique (Geva, 2006).

MRI provides an excellent contrast resolution not only from tissue (proton) density, but also from tissue relaxation properties. After initial focus on T1 and T2 relaxation properties researchers explored other methods to generate contrast exploiting other properties of water molecules. Diffusion weighted imaging (DWI) was the next step as a result of such efforts by researchers like Stejskal, Tanner and Le Bihan (Le Bihan,2014).

Denis Le Bihan tried to differentiate liver tumors from angiomas, before MRI contrast became available. He hypothesized that a molecular diffusion measurement would result in low values for solid tumors, because of restriction of molecular movement. Based on the pioneering work of Stejskal and Tanner in the 1960s, he thought that diffusion encoding could be accomplished using specific magnetic gradient pulses. It was a challenging task to integrate the diffusion encoding gradients in to the conventional sequences and initial experience in the liver with a 0.5T scanner was very disappointing. Firstly diffusion MRI was a very slow method and it was very sensitive to motion artifacts due to respiration (Stejskal and Tanner, 1965).

It was not that DWI could become a reality in the field of clinical imaging until the availability of Echo-Planar Imaging (EPI) in the early 1990s. This technique is based diffusion sequences is fast and solves the problems of motion artifacts. By the early work by Moseley et al and Warach et al it established DWI as a cornerstone for early detection of acute stroke (Carr and Purcell, 1954; Torrey, 1956).

The sequence diffusion sensitization gradients are applied on either side of the 180° refocusing pulse in DWI and the parameter “b value” decides the diffusion weighting and is expressed in s/mm2. That is proportional to the square of the amplitude and duration of the gradient applied. The diffusion is qualitatively evaluated on trace images and quantitatively by the parameter called apparent diffusion coefficient (ADC). Thus, tissues with restricted diffusion are bright on the trace image and hypointense on the ADC map (Nicholson and Phillips, 1981; Hrabe et al., 2004; Kingsley, 2006).

The white matter contrast on diffusion images changes according to the spatial direction of the diffusion encoding gradients habe been noticed for the first time by Moseley et al (Bernstein and Quach, 2003).

In addition to these, Douek et al suggested that this was due to the fact that water diffusion in white matter fibres was faster in the direction of the fibers and slower perpendicular to them, i.e., anisotropic. The initial attempts were not very impressive, because diffusion measurements were done only along two directions but, with the use of a tensor formalism by Basser et al and development of 3D representation algorithms for fibre bundle depiction modern DTI came into existence. Initial clinical applications of DTI were limited to the central nervous system. The imaging artefacts and the small calibre of peripheral nerves hampered its use in the peripheral nervous system. However recent advances in MRI technology have extended its application to the peripheral nervous system (Lim and Helpern, 2002; Callaghan, 1991; Haacke et al., 1999).

Most of the early trials do not distinguish between causes of the strokes in the subjects entered nor do they identify the causes of the outcome events. As because modern treatment depends on the cause, this has become a sine qua non of a credible trial.

The Harvard Stroke registry plus as well as the Oxfordshire Stroke Project pioneered the quest for answers here. Many of the findings in this study were comparable to those in previous registries based on postmortem data. New observations include the high incidence of lacunes and cerebral emboli, the absence of an identifiable cardiac origin in 37 % of all emboli, a nonsudden onset in 21 % of emboli, and the occurrence of vomiting at onset in 51 % and the absence of headache at onset in 67 % of hematomas (Mohr et al., 1978).

Later came new data emerging from other two clinical trials (Trial of Org 10172 in Acute Stroke Treatment - TOAST - and North American Symptomatic Carotid Endarterectomy Trial - NASCET. The main causes for ischemic stroke are cardioembolism, large artery arteriosclerotic disease, spontaneous arterial dissections and small vessel disease - lacunes (Ferguson et al., 1999).

The advancing in stroke research was only possible due to the combination of the disciplines of neuroepidemiology, biostatistics, and clinical trial methodology as major factors. The multidisciplinary approach introduced in the mid-1940s has become mandatory in the evaluation of new therapy or even for proof of the value of therapy accepted by custom and traditional knowledge coming from individual datas. Without proper trials regulatory agencies should not authorize the introduction of new therapeutic agents or surgical strategies (Lansberg et al., 2012).

Clinicians and patients need to consider the risk of bleeding when making treatment decisions, specifically for interventions for which the recommendation is weak. In situations of uncertain benefit of a treatment and an appreciable probability of harm, a “primum non nocere” approach must be taken into consideration and recommended against such treatment.

Their recommendations are:

- ✓ In patients with acute ischemic stroke in whom treatment can be initiated within 3 h of symptom onset, we recommend IV r-tPA over no IV r-tPA (Grade 1A).
- ✓ In patients with acute ischemic stroke in whom treatment can be initiated within 4.5 h but not within 3 h of symptom onset, we suggest IV r-tPA over no IV r-tPA (Grade 2C).

- ✓ In patients with acute ischemic stroke in whom treatment cannot be initiated within 4.5 h of symptom onset, we recommend against IV r-tPA (Grade 1B).
- ✓ Intraarterial (IA) thrombolytic therapy is delivered by local infusion adjacent or into the thrombus. This approach has the potential to increase recanalization rates and enhance safety due to targeted administration of a lower dose of thrombolytic. Disadvantages include the need for specialized facilities and personnel, delays in drug administration related to the logistics of assembling an appropriate team and performing an angiogram, the risks inherent in performing an invasive procedure within the cerebral vasculature, and the risk of general anesthesia that may be used for the procedure.
- ✓ IA Thrombolysis Compared With No Thrombolytic Therapy in Patients With Ischemic Stroke and Contraindication for IV r-tPA. There is moderate-quality evidence that in patients with an ischemic stroke with a demonstrable cerebral artery occlusion, IA thrombolysis is associated with an increased chance of good functional outcome, whereas results failed to show or exclude a beneficial or detrimental effect on mortality (Guyatt et al., 2011; Vandvik et al., 2012; Alonso-Coello et al., 2012; You et al., 2012; Whitlock et al., 2012; Guyatt et al., 2012; MacLean et al., 2012).

An increased understanding of the morphofunctional processes underlying poststroke recovery has led to the development of targeted approaches to improve motor deficits. One of the most successful of such targeted strategy uses brief bursts of Vagus Nerve Stimulation (VNS) paired with rehabilitation to enhance plasticity and support recovery of upper limb function after chronic stroke. The stimulation of the vagus nerve triggers release of plasticity promoting neuromodulators, such as acetylcholine and norepinephrine, throughout the cortex (Engineer et al., 2019).

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

1. Constantinescu V, Arsenescu-Georgescu C, Daniela M, Moscalu M, Corciova C, **Cuciureanu D**. Heart rate variability analysis and cardiac dysautonomia in ischemic stroke patients. Clinical neurology and neurosurgery, 2019; 186, Article Number: UNSP 105528.
2. Constantinescu V, Matei D, Ignat B, Hodorog D, **Cuciureanu DI**. Heart rate variability analysis a useful tool to assess poststroke cardiac dysautonomia. The neurologist, 2020; 25(3): 49-54.
3. **Cuciureanu DI**, Constantinescu VA, Statescu C, Sascau RA, Hodorog DN, Preda C, Hinganu D, Hinganu MV, Cuciureanu T. Antithrombotic therapy in patients with stroke An observational study. Revista de chimie, 2019; 70(4): 1283-1287.
4. **Cuciureanu ID**, Hinganu MV, Statescu C, Sava A, Hinganu D, Turliuc MD, Cuciureanu T, Sascau RA. Morphopathological particularities of cerebrovascular diseases for patients in the northeastern area of Romania. Romanian Journal Of Morphology And Embryology, 2019; 60(1): 227-232.

1.2. Apoplexia and the wandering ticker

1.2.1. 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 (Lopez et al., 2006; Ingall, 2004). As we assist to a growth in life expectancy and global aging of population, cerebrovascular pathology becomes an important aspect. 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 (Brainin et al., 2000).

Cardiac disease is a risk factor for ischemic stroke (Kamel and Healey, 2017) and cardiovascular complications represent the second leading cause of poststroke death (Yoshimura et al., 2008). The bidirectional relationship, however, has been less explored. Recent evidence shows that brain ischemia can conversely alter cardiovascular and autonomic function (Samuels, 2007; Kumar et al., 2010; Soros and Hachinski, 2012).

Although investigations and treatment possibilities in the acute phase of ischemic stroke have evolved during the last decades, primary and secondary prevention strategies play an essential role in reducing long-term stroke-related mortality. Monitoring cardiovascular parameters in patients with acute ischemic stroke is important because of the increased risk of secondary vascular events, such as cardiac arrhythmias, myocardial infarction, uncontrolled arterial hypertension, and cardiogenic shock (Chen et al., 2017). Less obvious are the delayed consequences of ischemic stroke on cardiovascular function. The aforementioned cardiovascular complications are related to disturbances of the autonomic nervous system, arisen secondary to the cerebral lesion. In this context, understanding the underlying mechanisms responsible for autonomic dysregulation together with a quantification of autonomic cardiac dysfunction represents a necessary step in managing patients who had a stroke, bringing new perspectives on the therapeutic approach of these patients.

There is considerable evidence regarding the role of forebrain lateralization in cardiovascular autonomic regulation in patients with ischemic stroke (Ozdemir and Hachinski, 2008). Certain specialized cerebral structures, such as the insular cortex, are actively involved in this bidirectional brain-heart axis mediation with an immediate impact on the cardiocirculatory parameters, thus influencing the patients' clinical outcome. Damage to the insular cortex, a complex structure supplied by the middle cerebral artery (MCA), is associated with a more pronounced autonomic imbalance leading to life-threatening arrhythmias and sudden death (Ozdemir and Hachinski, 2008).

Approximately, 40 % of stroke patients present a risk of recurrence in the first 5 years (Amarenco et al., 2006) and a higher risk for myocardial infarction, uncontrolled hypertension, cardiac arrhythmias and cardiogenic shock (Dhamoon et al., 2007; Tokgozoglu et al., 1999). 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 (ANS) structures and catecholamine storm (Makikallio et al., 2004; Myers et al., 1982). Increased sympathetic nervous system and reduced

parasympathetic nervous system activity is common in acute stroke patients (Dorrance and Fink 2015; Korpelainen et al., 1994). These disturbances of autonomic cardiac function, and also, the preexisting cardiac disease, may be responsible for 2–6 % of the total mortality 3 months after acute ischemic stroke (Prosser et al., 2007).

Cardiac dysautonomia is a common complication of stroke (Orlandi et al., 2000; Bassi et al., 2007; Sykora et al., 2009). Post stroke autonomic nervous system dysregulation has attracted significant interest in the last couple of decades. Many facts involved in short term evolution and prognostic still need to be clarified.

Cardiovascular system presents cortical modulation. 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 (Graig, 2003; Critchley and Harrison, 2013; LeDoux, 2007). 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 (Daniele et al., 2002).

Studies about heart–brain connections proposed the concept of neurogenic cardiac disease, clinically and pathogenically different from the actual cardiac disease (Oppenheimer and Hachinski, 1992; Davis et al., 1993; Samuels, 1997; Oppenheimer, 1994).

Identifying highrisk 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 (Ozdemir and Hachinski, 2008) has been illustrated using modern neuroimaging data, including positron emission tomography and functional magnetic resonance imaging data (Critchley et al., 2000).

Autonomic nervous system dysfunction, caused by an acute cerebral lesion, predisposes to cardiovascular complications, such as uncontrolled arterial hypertension, cardiac arrhythmias, myocardial infarction, or cardiogenic shock (Chen et al., 2017). The assessment of cardiovascular autonomic activity is a pivotal step to prevent these vital risk complications.

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 (Rincon et al., 2008). Insular cortex, a complex structure supplied by the middle cerebral artery (MCA), was often used as a model to illustrate the possible lateralization impact on sympatho-vagal balance, depending on the hemispheric localization of the stroke. It has been reported that cardiosympathetic centers are located in the anterior, medial and superior parts of the insula, while posterior insula and inferior parietal lobe are responsible for inhibiting and modulating the cardiosympathetic outflow of the other parts of the insula (Al-Qudah et al., 2014). Autonomic imbalance associating increased sympathetic activity may be reflected in cardiovascular impairment post insular stroke (Orlandi et al., 2000).

Quantification of heart rate variability (HRV) represents a non-invasive method of sympathovagal balance evaluation (Perkiömäki, 2011). It reflects the cardiac ability to comply with hemodynamic fluctuations and external environment variations, a relevant

parameter for the appraisal of the autonomic modulation on the cardiovascular activity (Task Force of the European Society of Cardiology, 1996).

Among different variables assessing the autonomic response, heart rate variability (HRV) quantifies sympatho-vagal modulation at sinoatrial level. It has been shown that a reduction of HRV may be an indicator of general illness, including acute stroke, and it correlates with enhanced sympathetic or reduced vagal tone, which may predispose to higher risk of arrhythmia (Stein et al., 1999) and increased risk of sudden cardiac death. HRV might provide prognostic information in ischemic stroke. Graff and collaborators underlined the contribution of HRV in-depth analysis to stroke prognosis and stated that while HRV assessed by linear methods may provide long-term prognostic value, complex, non-linear measures of HRV may rather assess the impact of the neurological state on temporary patterns of heart rate post stroke (Graff et al., 2013). In the same line of evidence, it has been recently shown that acute ischemic stroke patients had a significant reduced complexity of HRV. Early assessment of HRV by non-linear methods can be a potential predictor of stroke-in- evolution in newly admitted non- atrial fibrillation ischemic stroke patients (Chen et al., 2015).

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 post-stroke heart rate (Task Force of the European Society of Cardiology, 1996; Kleiger et al., 1987; Orlandi et al., 2000; Bigger et al., 1992; Nolan et al., 1998), highlighting qualitative properties of the heart rate fluctuation (Perkiömäki 2011; Pincus and Goldberger 1994).

Considerable evidence regarding the role of cortical lateralization in cardiovascular autonomic dysregulation exists in patients with ischemic and hemorrhagic stroke (Oppenheimer et al., 1992). 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 (Hachinski et al., 1992; Cechetto 1993). Clinical observations also suggest an association between right hemispheric lesions and supraventricular tachycardia (Lane et al., 1992).

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 (Woo et al., 1992) by fitting an ellipse to the shape of the Poincaré plot in order to calculate HRV indices (Tulppo et al., 1998). This geometrical technique can be used to assess the dynamics of HRV by a representation of the values of each pair of R–R intervals into a simplified phase space, describing the dynamics of a phenomenon that can recognize the hidden correlation patterns of a time series signal (Karmakar et al., 2010; Hoshi et al., 2013). Each pair of successive elements in a time series (tachogram) is pictured into a simplified Cartesian plane (Brennan et al., 2001; Lerma et al., 2003). 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 R–R intervals without a requirement for a stationary quality of the data (Karmakar et al., 2010; Brennan et al., 2001; Tulppo et al., 1996; Huikuri et al., 2003).

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 (Hoshi et al., 2013; Porta et al., 2007), which quantifies signal complexity robustly within short time segments (Lewis and Short 2007). The name refers to the applicability to time series data sampled from a continuous process and the algorithm suggests ways to employ sample statistics to evaluate the results (Richman and Moorman 2000). It measures system complexity and unpredictability (Javorka et al., 2002).

The power spectral analysis of heart rate variability (HRV) is a simple method to assess ANS activity, especially the modulation of the sympathetic and parasympathetic tone upon heart rate under experimental and clinical conditions (Pomeranz et al., 1985). Power spectral analysis of heart rate has shown a pronounced reduction of spectral power in the field of sinus arrhythmia after right-sided lesions when compared with left-sided lesions (Baron et al., 1994).

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 (Task Force of the European Society of Cardiology, 1996; Stein et al., 1999), being a mortality predictor (Bruyne et al., 1999). The decrease in HRV was observed not only in the acute phase but also up to six months after the acute cerebrovascular event (Makikallio et al., 2004; Montano et al., 1996; Lanfranchi and Somers 2002).

Specific cerebral structures, such as the insula, are involved in the bidirectional heart-brain mediation, having an immediate impact on the cardio-circulatory parameters (Cereda et al., 2002). Insular involvement in the ischemic stroke generates autonomic imbalance (Cereda et al., 2002). There is still controversy about hemispheric lateralization and the involvement of specific cortical structures in central autonomic nervous system control that determine post-stroke prognosis (Al-Qudah et al., 2015; Constantinescu 2018).

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 (Tokgozoglu et al., 1999; Naver et al., 1996). 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 (Sander and Klingelhofer 1994; Li and Dong 1999).

Complex systems present a temporal variation of biological processes, characterizing the biological rhythms (Ziemssen et al., 2013). The analysis of biological rhythms may serve as an important tool for unraveling the pathophysiology of different diseases, including cerebrovascular pathology. Heart rate variability (HRV) illustrates the ability of the heart to adapt to different hemodynamic and external environmental changes, or in response to certain

pathologic conditions, outlining the autonomic cardiac control. There is a close relationship between sympathetic hyperactivation and reduced cardiac vagal modulation associated with low HRV, involving a higher risk of cardiac arrhythmia and sudden death. Some studies reported a decrease in HRV in patients who had a stroke, not only in the acute phase but also present up to 6 months after the acute cerebrovascular event (Montano et al., 1995; Lanfranchi and Somers, 2002; Mäkikallio et al., 2004).

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 (Rüdiger et al., 1999). 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, as the local data segments required for MTRS analysis can be as short as 20 seconds (Li et al., 2018).

The sympathetic hyperactivity represents an independent risk factor for long-term cardio and cerebrovascular events (Sander and Klingelhofer 1994; Sander et al., 2001). 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 co- morbidities, like coronary artery disease (Lowattana et al., 2006).

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. We also evaluated 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.

1.2.2. Material and methods

⇒ **Study 1 on heart rate variability analysis and cardiac dysautonomia in ischemic stroke patients**

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 or hemodynamic decompensation upon admission, renal insufficiency, oncologic pathology, dementia, diagnosed diabetes mellitus with polyneuropathy, current beta-blocker medication. A study population diagram is presented in **Figure 1.1**.

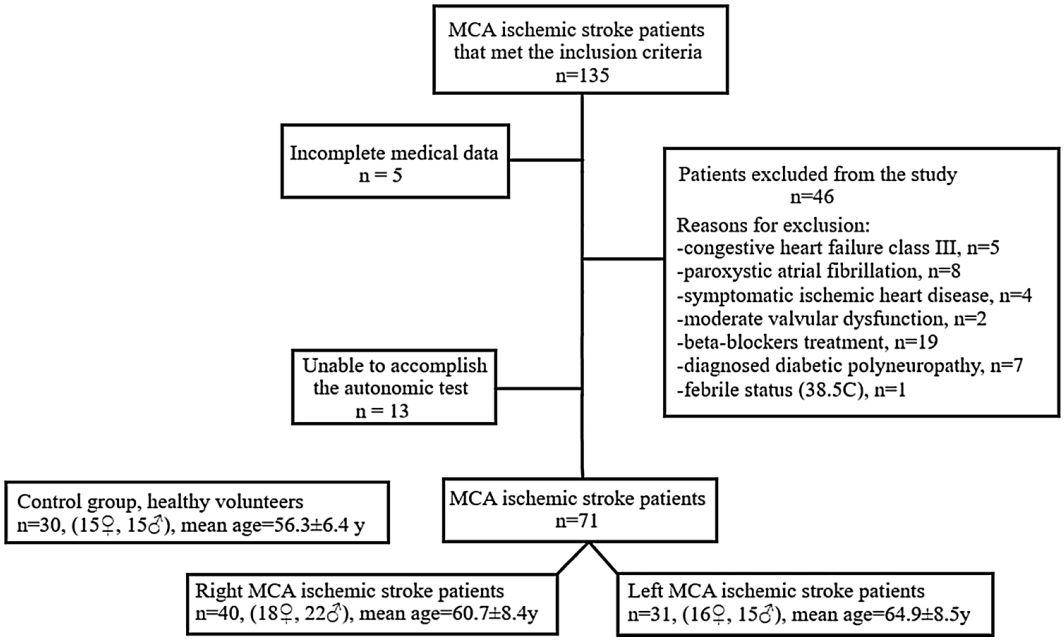

Figure 1.1. *Study population diagram.*

Other specific medications for associated pathologies were allowed: statins or fenofibrate, antiplatelet agents, antihypertensive medication in various combinations, including diuretics, Angiotensin-converting enzyme inhibitors (ACEI), calcium channel blockers or Angiotensin Receptor Blocker (ARB). The control group was not under 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. AcqKnowledge software version 3.9.1.6 was used for refining the recorded data. Data processing was done using Kubios HRV software version 2.2 (Biosignal Analysis and Medical Imaging Group, University of Eastern Finland). A standardized protocol was applied for each HRV 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. A minimum of 256 RR intervals was analyzed for each evaluation.

HRV time-domain parameters RMSSD ("Root Mean Square of the Successive Differences") and pNN50 describe the vagal influence on the heart rate. pNN50 represents

the ratio of successive "NN" with differences higher than 50 ms between them (NN50) and the total number of NN intervals.

The frequency-domain analysis concerned the following parameters: HF ("High Frequency"), LF ("Low Frequency"), VLF ("Very Low Frequency") and the LF/HF ratio. The HF parameter (expressed in ms2) illustrates the vagal control on the heart rate. The LF and HF values may also be calculated in normalized units ("LFnu", "HFnu"), defining the relative values of each frequency spectrum reported to the total spectral power, from which the VLF ("Very Low Frequency") component was excluded from the calculation.

These parameters were analyzed from the ECG recordings together with non-linear parameters SD1 and DFAα1, that reflect the variability of the heart rate.

We applied a standardized protocol for the ECG recording: assessment at the same time range (4–6 PM), after 20 min of clinostatism rest, in a quiet room, constant temperature (22OC), in the absence of prior physical effort or ingestion of caffeinated or alcoholic beverages 24 h before the test.

Two sympathetic activation tests were performed: a 5 min standing test and the "handgrip" test corresponding to the maximal voluntary isometric contraction of the fist, using a dynamometer, and two parasympathetic activation tests: "deep breathing" test, consisting of 6 complete cycles of deep inhale and exhale over 60 s, with timing, 10 s for each cycle, and Valsalva maneuver.

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. (IBM Statistical Package for the Social Sciences, Chicago, Ilinois). 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 method, respectively Kruskal – Wallis test were applied. The One Way Anova test (95% CI) was applied for the comparative analysis. The "t Student", Pearson-$\chi 2$, Fisher, ANOVA, linear regression, logistic regression, and multivariate analysis test were also applied, using generalized linear models. The descriptive statistics indicators (mean, standard deviation, standard error, minimum, maximum and quartile intervals) were calculated for the continuous variables. In the case of the categorical or ordinal variables, the nonparametric analysis based on comparative tests founded on a "Chi-squared" distribution was tracked. Pearson's Chi-square test was the most used $\chi 2$ significance test. 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 2 on heart rate variability analysis in order to assess poststroke cardiac dysautonomia**

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. The study protocol was approved by the local ethics committee and all subjects provided written informed consent before inclusion. The study was carried out in accordance with the Helsinki Declaration.

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, cardiologic assessment before being included in the study.

Patients presenting the following comorbidities were excluded: moderate or severe valvular dysfunction, heart failure, cardiac arrhythmia present upon the current admission, history of myocardial infarction, febrile status, hypoxic status, impaired consciousness or hemodynamic decompensation during admission, dementia, severe renal insufficiency, diabetes mellitus, or other already diagnosed metabolic pathology with present polyneuropathy.

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 control group was not under medical treatment. The autonomic control over heart rate in patients who had a stroke and healthy volunteers was evaluated under standardized conditions. The ECG recordings were performed according to the following criteria: in the afternoon (4 to 6 PM), after 30 minutes of the supine position, in a quiet room, at a constant temperature of 221C, in the absence of prior physical effort or ingestion of caffeinated or alcoholic beverages 24 hours before the test. The evaluation sequence was similar for all the patients and the healthy subjects, consisting 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, converting biologic parameters to numeric data. AcqKnowledge software, version 3.9.1.6, permitted to refine the data, detecting, measuring, and analyzing the recorded signal.
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: $\sum (RRI(t(i))-Reg(t(i)))2=>$minimum, with RRI(t(i)) being the original RR intervals and $Reg(t(i))=A^* \sin(\omega t(i)+\varphi(i))$ being a trigonometric function of the parameters A (amplitude), ω (frequency), and ϕ (phase shift).[14] 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).

Time-domain RMSSD parameter (root mean square of the successive differences) describes the vagal influence on the heart rate. pNN50 represents another time domain parameter, reflecting the ratio between NN50 (number of RR intervals considered normal-successive "NN" with differences >50 ms between them) and the total number of RR intervals, being expressed in percentages. It characterizes the vagal control on the heart rate.
Heart rhythm oscillations may be categorized into 4 primary frequency bands: ultra-low frequency, very low (LFnu, HFnu), defining the relative values of each frequency spectrum reported to the total spectral power, from which the very low frequency component was excluded from the calculation as it is considered to be influenced by thermoregulation mechanisms and renin-angiotensin system activity. LF/HF ratio describes the sympathovagal balance.

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 con- sideration 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 $P < 0.05$.

1.2.3. Results

⇒ **Study 1 on heart rate variability analysis and cardiac dysautonomia in ischemic stroke patients**

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 sub-cortical strokes ($p < 0.05$), while for the left MCA infarctions there was no significant difference.

Regarding the corticosubcortical localization, there was no difference concerning stroke severity (reflected by NIHSS) between the left ($n = 11$) and right ($n = 12$) hemisphere infarcts ($p = 0.182$).

For the subcortical ischemic stroke group, there were no significant differences between right ($n = 28$) and left ($n = 20$) subgroups ($p = 0.332$).

Patients with right MCA infarctions presented a decreased vagal modulation, reflected by lower values of RMSSD, HFnu, SD1 (**Figure 1.2**) during the autonomic activation tests in both corticosubcortical and subcortical stroke groups.

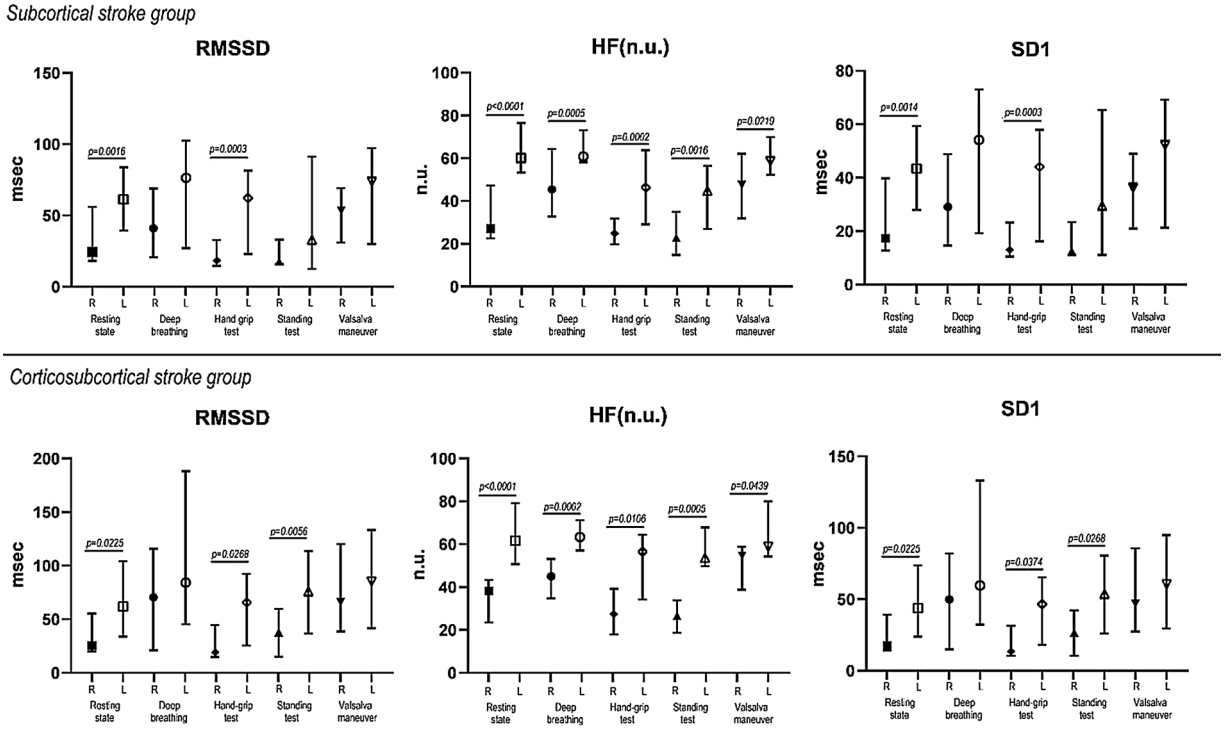

Figure 1.2. *Comparison between stroke localization: subcortical vs. corticosubcortical.*

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 (**Table 1.1**). This has also been noticed during parasympathetic and sympathetic activation tests (**Table 1.1**). 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 1.3**) and de- creased LF/HF. After the vagal activation maneuvers, we noticed a tendency towards re-balancing the sympathovagal activity (**Figure 1.3**).

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 1.3**).

HFnu (expressed in normalized units) is unanimously considered a marker of the parasympathetic activity. This parameter showed the same differentiation in the two groups of stroke patients according to the involved cerebral hemisphere, as shown by other parameters also (vagal predominance in patients with left MCA ischemic stroke and sympathetic predominance in patients with right MCA ischemic stroke). The reference value of the control group (45.24 nu) confirms the tendency of polarisation of the sympathovagal balance depending on the hemispheric lateralization of the stroke again (**Figure 1.4**).

We evaluated HRV non-linear parameters (SD1, DFAα1) in resting state and during the Ewing tests, correspondingly to the linear parameters ahead discussed (**Figure 1.4**). 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

Table 1.1. *Groups differences reflected in HRV parameters during autonomic tests.*

HRV Parameters		Right vs left MCA stroke	Right MCA stroke vs control	Left MCA stroke vs control
RMSSD (msec)	RS	36.68/71.63**	36.68/57.32*	71.63/57.32
	DB	57.26/87/29*	57.26/44.35	87.29/44.35**
	HG	30.75/65.17**	30.75/31.96**	65.17/31.96**
	ST	31.31/62.55**	31.31/40.81	62.55/40.81*
	VA	65.61/82.61	65.61/89.77	82.61/89.77
pNN50 (%)	RS	3.06/10.21*	3.06/14.50**	10.21/14.50
	DB	6.33/9.77*	6.33/14.84*	9.77/14.84
	HG	3.25/8.40*	3.25/6.07	8.40/6.07
	ST	3.38/7.44*	3.38/9.52**	7.44/9.52
	VA	5.55/9.55*	5.55/11.35*	9.55/11.35
HF (ms^2)	RS	430.60/884*	430.60/758.10	884/758.10
	DB	1405.4/1761.2*	1405.4/1656.11	1761.21/1656.11
		*		
	HG	425.27/1072.8*	425.27/409.13	1072.80/409.13*
	ST	467.23/111.6.7*	467.23/284.97	1116.70/284.97*
	VA	1383.30/1644	1383.3/933.53	1644/933.53*
LF (ms^2)	RS	668.40/424.71	668.40/897.33	424.71/897.33*
	DB	562.01/399.30	562.01/557.80	399.30/557.80
	HG	600.35/920.48	600.35/882.27	920.48/882.27
	ST	801.70/864.77	801.70/700.60	864.77/700.60
	VA	1290.9/1059.3	1290.9/675.07*	1059.3/675.07
LF/HF	RS	2.56/0.62**	2.56/1.24**	0.62/1.24*
	DB	1.55/0.57**	1.55/0.62*	0.57/0.62
	HG	3.32/1.46**	3.31/2.89	1.46/2.89**
	ST	3.91/1.45**	3.91/2.57*	1.45/2.57*
	VA	1.59/0.66**	1.59/0.64*	0.66/0.64
HF (n.u.)	RS	33.35/64.52**	33.35/45.24**	64.52/45.24**
	DB	45.84/64.85**	45.84.60.54**	64.85/60.54
	HG	28.15/47.75**	28.15/27.92	47.75/27.92**
	ST	26.67/47.68**	26.67/35.03*	47.68/35.03*
	VA	48.61/61.86**	48.61/61.26**	61.86/61.26
LF (n.u.)	RS	66.50/35.09**	66.50/54.20**	35.09/54.20**
	DB	53.97/34.77**	53.97/37.79**	34.77/37.79
	HG	71.72/51.53**	71.72/71.97	51.53/71.97**
	ST	73.18/52.04**	73.18/64.84*	52.04/64.84*
	VA	51.72/37.86**	51.72/38.35**	37.86/38.35
SD1 (msec)	RS	25.99/50.81**	25.99/39.18*	50.81/39.18
	DB	40.57/61.91*	40.57/34.44	61.91/34.44
	HG	21.79/46.21**	21.79/22.67	46.21/22.67**
	ST	22.18/45.88**	22.18/30.51	45.88/30.51*
	VA	45.52/58.55	45.52/63.64*	58.55/63.64
DFAα1	RS	1.14/0.70**	1.14/0.91*	0.70/0.91*
	DB	1.02/0.73**	1.02/0.73	0.73/0.97*
	HG	1.24/0.86**	1.24/1.25	0.86/1.25**
	ST	1.30/0.89**	1.30/1.14	0.89/1.14*
	VA	0.98/0.78*	0.98/0.67**	0.78/0.67

*RS=restaing state, DB=deep breathing, HG=handgrip test, ST=standing test, VA=Valsalva maneuver, (*)=p<0.05, (**)=<0.001; * HRV values are expressed by mean.*

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.

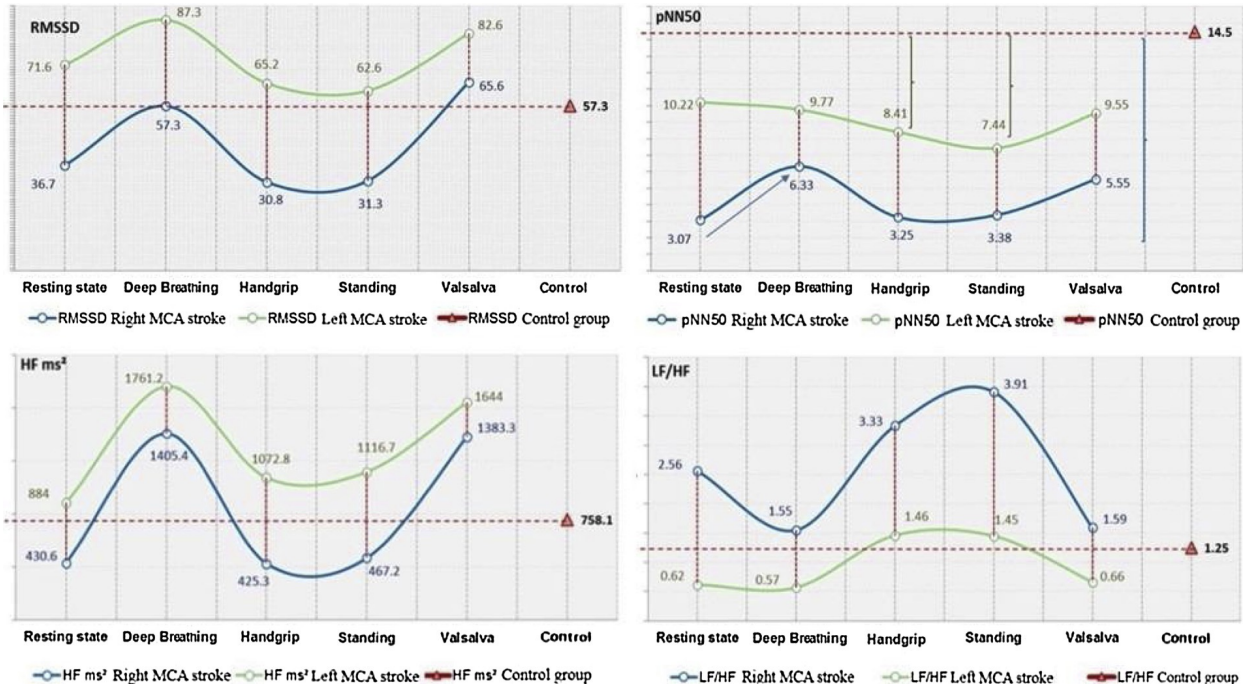

Figure 1.3. *Comparative assessment of RMSSD, pNN50, HF(ms2) and LF/HF values in dynamics.*

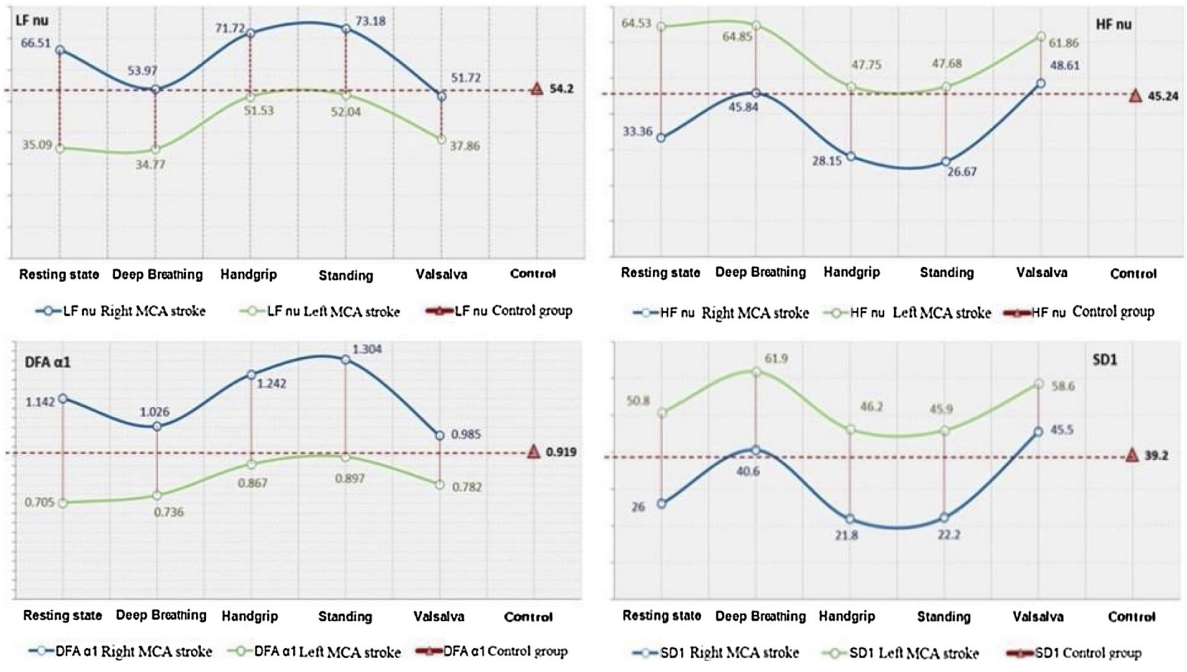

Figure 1.4. *Comparative assessment of LF(nu), HF(nu), DFA α1 and SD1 values in dynamics.*

We estimated reference values (cut-offs), depending on the hemispheric lateralization

(**Table 1.2**). We determined the optimal cut- offs for each of the parameters, successively comparing the three groups, having into consideration the values of the area under the ROC curve (AUC) (**Table 1.2**).

Table 1.2. *Cut-off values and Area Under the ROC Curve (AUC) estimated for several HRV parameters in ischemic stroke patients*.

Cut-off values / AUC for differenr linear and non-linear parameters in ischemic stroke patients					
Parameters	Resting state	Deep breathing	Handgrip	Standing	Valsalva
RMSSD (ms)	60.15/0.771	73/0.651	63.40/0.783	63.40/0.677	-
pNN50 (%)	2/0.691	11.65/0.650	-	1.50/0.798	7.80/0.596
LF/HF	1.20/0.937	0.94/0.834	1.66/0.829	2.18/0.838	1.06/0.753
LFnu (n.u.)	54.50/0.936	48.57/0.833	56.30/0.834	55.70/0.814	51.45/0.731
HFnu (n.u.)	48.80/0.939	51.29/0.835	28.45/0.831	44.25/0.812	48.25/0.717
SD1 (ms)	40.45/0.773	51.70/0.687	39.95/0.783	44.95/0.708	-
DFA α1	0.96/0.844	0.93/0.747	0.86/0.810	1.01/0.80	1.10/0.760

Table 1.3. *Multiple linear regression HRV parameters vs. treatment*

Autonomic Tests		RMSSD		HF		LF/HF		SD1	
Deep breathing	(Constant)	4.607	.000	2.826	.006	5.191	.000	4.609	.000
	Diuretic	.024	.981	-.913	.364	.465	.643	.023	.982
	ARB	-.240	.811	.674	.503	-.978	.332	-.244	.808
	Ca-blocker	-.424	.673	-.278	.782	.509	.612	-.428	.670
	ACEI	.053	.958	-.255	.799	-1.895	.033*	.051	.960
	Statin	1.054	.296	1.080	.284	-.221	.826	1.055	.295
Handgrip	(Constant)	4.129	.000	1.527	.131	6.453	.000	4.132	.000
	Diuretic	.684	.496	.808	.422	-1.755	.044*	.681	.498
	ARB	-1.130	.262	-.777	.440	.380	.705	-1.130	.263
	Ca-blocker	-.200	.842	-.652	.517	-.251	.802	-.203	.839
	ACEI	-2.174	.033*	-2.339	.022*	.500	.619	-2.173	.023*
	Statin	2.825	.043*	2.266	.027*	-1.183	.241	1.825	.033*
Resting state	(Constant)	4.724	.000	2.392	.020	4.667	.000	4.729	.000
	Diuretic	.936	.353	-.159	.875	-.179	.8.58	.933	.354
	ARB	-.522	.603	.203	.839	-.568	.572	-.526	.601
	Ca-blocker	-1.267	.210	-1.058	.294	.864	.391	-1.269	.209
	ACEI	-.282	.779	-.621	.537	-.865	.390	-.288	.774
	Statin	.252	.802	1.265	.210	-.088	.930	.258	.797
Standing	(Constant)	4.080	.000	1.851	.069	6.436	.000	4.013	.000
	Diuretic	.779	.439	1.782	.039*	.085	.932	1.085	.282
	ARB	-1.552	.126	-1.505	.137	-.087	.931	-1.575	.120
	Ca-blocker	-.508	.613	-.722	.473	1.235	.221	-.686	.495
	ACEI	-2.084	.031*	-1.777	.030*	-.040	.968	-1.948	.036*
	Statin	1.849	.039*	1.516	.134	-2.786	.007*	1.978	.032*
Valsalva	(Constant)	4.399	.000	3.343	.001	4.258	.000	4.119	.000
	Diuretic	.412	.681	-.910	.366	-.578	.565	.466	.643
	ARB	1.272	.208	2.373	.021*	-.823	.413	1.278	.206
	Ca-blocker	-.927	.357	-1.972	.043*	1.299	.199	-.880	.382
	ACEI	-.737	.464	.771	.443	.763	.448	-.667	.507
	Statin	1.414	.012*	.855	.396	-1.806	.0.35*	1.569	.121

To analyze the treatment effect on HRV parameters in ischemic stroke patients, we used multivariate linear regression analysis. This regression model was chosen taking into account that the dependent variable is a continuous one (HRV parameters). The patients enrolled were under treatment for secondary stroke prevention, having different vascular risk factors. Fifty-five patients were diagnosed with arterial hypertension, being treated with following drugs: Angiotensin-converting enzyme inhibitors - ACEI (31 patients), diuretics (21 patients), ARBs (10 patients), Calcium channel blockers (20 patients). The beta- blocker medication was excluded. Fifty patients were under treatment with statins. Data analysis regarding medication is detailed in **Table 1.3**.

In resting state, ACEI did not alter the sympathovagal balance after analyzing the following parameters: RMSSD, HF, SD1, LF/HF ratio. No consistent changes in sympathovagal balance were noticed in patients under the other antihypertensive drugs, as shown by the analyzed HRV parameters (**Table 1.3**).

⇒ **Study 2 on heart rate variability analysis in order to assess poststroke cardiac dysautonomia**

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 1.4, 1.5**).

Table 1.4. *HRV Parameters for the 3 Groups*

	Right MCA Ischemic Stroke			Left MCA Ischemic Stroke			Control Group		
Parameter	RS	DB	ST	RS	DB	ST	RS	DB	ST
RMSSD (ms)Mean	15.26	17.97	11.98	26.40	31.77	23.79	32.26	38.99	27.82
SD	6.06	9.75	4.49	17.95	19.29	16.61	16.65	22.09	17.31
HF (rel.values) Mean	23.83	21.59	16.48	32.13	37.35	22.85	32.28	31.54	25.71
SD	10.97	10.04	7.37	14.00	13.49	13.74	12.86	10.42	8.74
LF (rel. values) Mean	51.94	52.59	58.12	41.92	38.25	50.39	48.13	50.66	56.81
SD	12.91	10.79	8.98	13.82	14.40	14.36	12.71	11.39	10.95
VLF Mean	14.37	17.57	18.54	12.93	11.23	15.47	9.73	9.15	11.22
SD	6.87	7.96	6.94	7.63	4.88	8.71	3.57	3.54	4.31
LF/HF Mean	4.47	4.63	7.62	2.72	2.41	4.44	2.41	2.44	3.83
SD	3.20	4.02	6.08	2.29	2.27	2.71	1.35	1.38	2.28
HFnu Mean	27.38	25.28	19.73	36.23	41.41	26.28	35.55	34.84	28.54
SD	11.90	10.83	8.67	14.14	14.36	14.44	13.89	11.51	8.84

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 (**Figsure 1.5, 1.6**). The same characteristics of the sympathovagal balance were observed after the activation tests when analyzing the LF/HF ratio, indicating increased sympathetic activity in the patients who had a right MCA ischemic stroke. There was no significant difference between the left MCA ischemic stroke group and the control group (**Table 1.5**).

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 1.6**). The parasympathetic activation test did not induce a significant change in the sympathovagal balance in this group of patients (**Figure 1.7**).

Table 1.5. *Differences in HRV Parameters Between the 3 groups*

	Mann-Whitney		Test (P-value)	All Groups
HRV Parameter	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

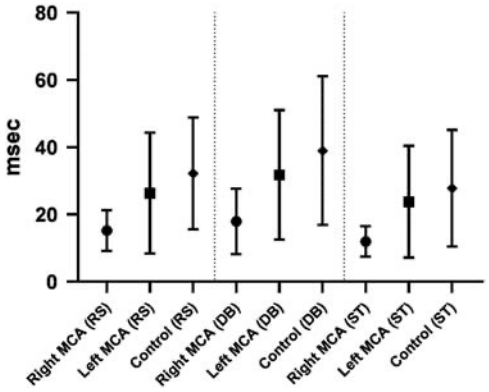

Figure 1.5. *RMSSD values for stroke patients and controls during tests. Values are depicted as mean with standard deviation. DB indicates deep breathing test; RMSSD, root mean square of the successive differences; RS, resting state; ST, standing test.*

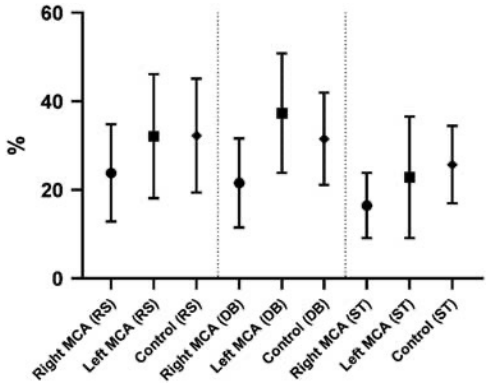

Figure 1.6. *HF values for stroke patients and controls during tests. Values are depicted as mean with standard deviation. DB indicates deep breathing test; HF, high frequency; RS, resting state; ST, standing test*.

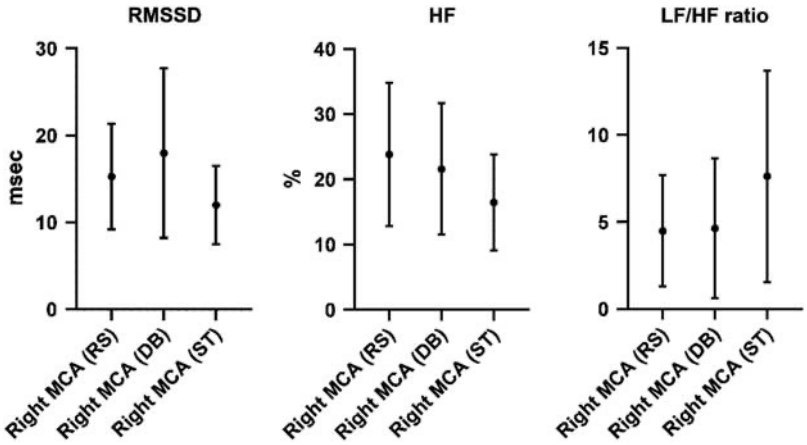

Figure 1.7. *RMSSD, HF, and LF/HF values during tests for patients who had a right MCA stroke. Values are depicted as mean with standard deviation. DB indicates deep breathing test; HF, high frequency; LF, low frequency; MCA, middle cerebral artery; RMSSD, root mean square of the successive differences; RS, resting state; ST, standing test.*

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 (**Figure 1.8**), indicating a sympathetic activation in accordance to the autonomic test (**Table 1.6**). Higher values of RMSSD and pNN50 compared with resting state indicate a regular vagal activation response after deep breathing test.

Table 1.6. *Differences in HRV parameters between tests for stroke patients*

	Wilcoxon Matched-Pairs Signed-Rank Test (P-value)		
HRV Parameter	Autonomic Activation Tests	Right MCA Ischemic Stroke	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*

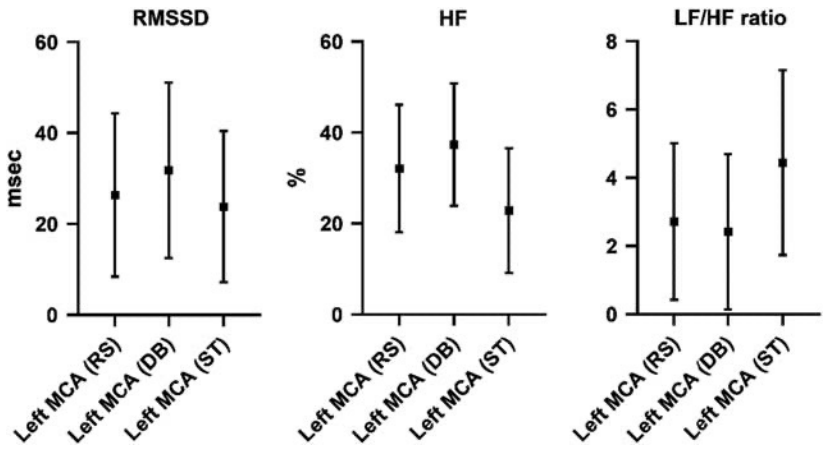

Figure 1.8. *RMSSD, HF and LF/HF values during tests for patients who had a left MCA stroke. Values are depicted as mean with standard deviation. DB indicates deep breathing test; HF, high frequency; LF, low frequency; MCA, middle cerebral artery; RMSSD, root mean square of the successive differences; RS, resting state; ST, standing test.*

1.2.4. Discussions

In the past decades, post stroke autonomic nervous system dysregulation has attracted vivid interest and there are facts that still need to be clarified, with possible implication in short term evolution and prognostic. 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 (Van Bree et al., 2010; Baranchuk et al., 2009). Moreover, insular cortex involvement is associated with non-dipper profile of blood pressure (Sander et al., 1994), myocardial injury and sleep- related disordered breathing (Ozdemir and Hachinski 2008). Additionally, patients with right insular cortex ischemia had increased incidence of ECG abnormalities (atrial fibrillation, A–V block, ectopic beats and inverted T wave) (Christensen et al., 2005), higher blood pressure values compared to left insular cortex ischemia (Meyer et al., 2004). High norepinephrine levels were correlated to right insular infarction and QTc prolongation (Sander et al., 2001). However, it is not clear whether cardiac injury and ECG abnormalities result from elevated serum catecholamine levels or from direct neural intervention (Koppikar et al., 2013).

HRV represents a simple, non-invasive method to assess and monitor the sympathovagal balance in dynamics in healthy and pathological condition. In 1987, Kleiger demonstrated a possible role of HRV in predicting mortality after acute myocardial infarction (Kleiger et al., 1987). Since then, HRV has been investigated as a risk marker in cardiology, intensive care, neurology and many other fields (Ernst et al., 2017; Ernst 2014). It was established that reduced HRV is a predictor for general mortality and anticipated the development of a number of risk factors, such as hypertension or obesity, and that lowering risk profiles is associated with increased HRV (Thayer et al., 2010).

It is known that healthy physiological systems have several parallel regulatory mechanisms that increase stability, and that stability is associated with complex patterns in time series like heart beats (Goldberger 1997). These systems are organized in such a way that they display scale invariance (Goldberger 1996) and long-range order (West and Goldberger 1987). Pathologic states are defined by a breakdown of these two related fractal attributes. A consequence of such reorganization is often a loss of fractal, multi- scale complexity and the emergence of highly periodic (single-scale) behavior. This approach may serve important implications for new approaches to early disease detection and prognostic assessment (Goldberger 1997; Goldberger 1996; West and Goldberger 1987; Goldberger et al., 1990; Glass et al., 1988).

There are currently no normative data universally assumed for short-term measures of HRV (O'Neal et al., 2016). Several recent studies on HRV data (Lee et al., 2017; Nunan et al., 2010) proposed such normative for short-term recordings of HRV obtained in normally healthy individuals, but discrepant values were identified. Age, ethnicity, habitual physical activity and cardio-metabolic condition contributed importantly to the variability of the results, even in large cohorts (O'Neal et al., 2016). Given the spectrum of physiopathological conditions that generate approximate values for the HRV, its role may principally serve to signal a general pattern of the autonomic nervous system response in a given, temporary

physiopathological context. As comprehensive investigations of HRV indices in large diseased populations are still lacking, it is difficult to formulate pertinent clinical measures based on HRV evaluation in neurovascular patients, and further research needs to establish precise sets of values reflecting autonomic response, pragmatically applicable in clinical setups.

Stroke can induce cardiac autonomic imbalance, therefore causing secondary cardiovascular complications. As suggested researchers (Ozdemir and Hachinski 2008), awareness of a ‘‘neurogenic heart syndrome’’ and recognition of right hemispheric involvement, especially the insular cortex, is important (Colivicchi et al., 2004). The risk of developing cardiac arrhythmia (atrial fibrillation, ventricular tachyarrhythmia) or other ECG modifications (prolonged QT, AV block, inversed T wave) 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 (Naver et al., 1996; Korpelainen et al., 1996; Mäkikallio et al., 2005; Lakusic et al., 2003). Amplified by catecholaminergic storm, it may induce cardiac ventricular arrhythmias or myocardial detriment, which is often associated with sudden death (Baranchuk et al., 2009; Van Bree et al., 2010). Early cardiac monitoring may change long-term prognosis, since sympa- thetic 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 (Proser et al., 2007).

Our results indicated a decreased vagal modulation of the heart rate in patients who had a right MCA ischemic stroke compared with healthy controls. The standing test determined sympathetic activation in both groups of patients and in healthy controls. During the deep breathing test, we observed an attenuated parasympathetic activation response in patients who had a right MCA ischemic stroke. Reductions in time and frequency domain HRV parameter values, as observed in our study for patients who had a right MCA ischemic stroke, are consistently associated with increased risk of stroke (Lees et al., 2018). *These data strongly indicate that ischemic stroke has an important influence on cardiac function and this is likely mediated through alterations of sympathovagal balance.*

Cerebral lesions involving the central network of the autonomic control may induce downstream effects on spinal cord preganglionic autonomic nerves, determining HRV changes (Korpelainen et al., 1996). Previous research has already shown a neural source of HRV changes in patients who had a ischemic stroke (Korpelainen et al., 1999).

The central autonomic control of the heart rate is reflected by the values of the linear and non-linear parameters of the HRV analysis. Changes in the sympathovagal balance associated with HRV reduction were described up to six months after stroke (Mäkikallio et al., 2004; Naver et al., 1996; Korpelainen et al., 1996; Lakusic et al., 2003).

Our results showed different values of the HRV parameters during resting state and the four autonomic activation tests, depending on the cortical lateralization of the ischemic stroke. A vagal predominance in the control of the heart rate in left MCA ischemic stroke patients was observed, while right MCA ischemic stroke patients presented a predominant sympathetic control on the heart rate.

Currently, there is no normative data for the HRV assessment over short periods for patients with neurological pathology (O'Neal et al., 2016*). Establishing specific cut-off values for the most frequently used parameters in the ECG analysis can be useful in the rapid analysis of the sympatho-vagal balance in stroke patients, in resting state or during autonomic activation tests. The identification of a sympathetic hyperactivity (low RMSSD or pNN50 values for time-domain parameters and low HF or HFnu, elevated LF/HF values for frequency-domain parameters), associated to a decrease of variability of the heart rate (high DFA α1 values, low SD1 values) may announce a potential risk of cardiac arrhythmia, having also therapeutic impact.*

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 (Soros and Hachinski 2012; Frangiskakis et al., 2009). Actually, many of these arrhythmias such as atrial fibrillation could be the cause and not the result of the acute cerebral event (Goldstein 1979).

The total spectral power of RR intervals from the HRV analysis is reduced in patients who had a stroke compared with age-/sex-matched controls (Barron et al., 1994). Cardiac parasympathetic neural activity is particularly affected after stroke, determining a shift in the sympathovagal balance. In patients with sympathetic hyperactivity, the risk for cardiac complications is higher in the period immediately after cerebral infarction (Colivicchi et al., 2005).

Some studies reported that cardiac sympathetic control on the heart rate was enhanced within 5 days of stroke symptoms onset, but similar findings were described by other authors for longer periods (Korpelainen et al., 1997; Giubilei et al., 1998; Strittmatter et al., 2003). Decreased vagal modulation and sympathetic hyperactivity can be underlined both during the resting condition and after exercises specific to the motor recovery, including sympathetic and parasympathetic activation activities, as a potential source of cardiovascular complications.

The originality of our study consists mainly of applying the MTRS analysis of the HRV in patients who had an ischemic stroke, whereas, most commonly, a power spectral analysis of HRV was performed in previous studies through the Fast Fourier Transform. Unlike the Fast Fourier Transform, MTRS analysis does not need interpolation on nonequidistant RR intervals (Rüdiger et al., 1999) and can be successfully applied to analyze shorter local data segments.

The influence of cerebral structures on the autonomic nervous system activity relies on several factors: the topography of the brain lesion, the hemispheric lateralization, and the functional remodeling of the cerebral structures, described as brain plasticity, depending on the type of aggression.

There is still controversy about hemispheric lateralization and involvement of specific cortical structures in central autonomic control, mainly attributed to the interhemispheric functional and anatomic asymmetries of the central nervous system (Oppenheimer, 2006; Reich et al., 2019).

Specific functional lateralization concerning the sympathetic and parasympathetic central control involving the right, respectively, the left insular lobe was described (Constantinescu et al., 2018; Oppenheimer et al., 1992; Oppenheimer et al., 1996). Our results support this

hypothesis of an inter-hemispheric functional differentiation of the central autonomic control, in accordance with other clinical trials (Colivicchi et al., 2004). Moreover, we performed four autonomic activation tests.

The results maintained differentiated characteristics between the two groups, depending on the hemispheric laterality. Patients with a right hemisphere infarction showed, after the vagal activation tests, both a significant increase in the total variability of the heart rate, as well as an increase of certain linear parameters specific to vagal activity (e.g., RMSSD, HF), highlighting the potentiation of the parasympathetic influence on the heart rate. This appears to be cardioprotective in elderly patients (McLachlan et al., 2010). Depending on the particularities of the autonomic nervous system activity, new therapeutic perspectives may emerge.

Furthermore, in order to clarify the contradictory results on the specific role and attributions of the central nervous system structures, functional cerebral imaging studies are needed. This would allow a thorough understanding of the cortical functional lateralization and a more accurate description of the brain structures involved in cardiac dysautonomic phenomena in cerebrovascular pathology.

In right MCA infarction with insular cortex involvement, a reduction of poststroke total spectral power of the HRV has been noticed (Naver et al., 1996; Tokgozoglu et al., 1999). Moreover, increased levels of serum noradrenaline, reduced circadian variability in blood pressure, prolongation of QTc, and recurrent cardiac arrhythmias were identified following right MCA ischemic stroke (Sander, 1994).

Other studies revealed a higher risk of cardiac complications and increased long-term mortality in left hemisphere brain infarction, particularly in the left insular ischemic stroke (Laowattana et al., 1996; Algra et al., 2003). The sympathetic hyperactivity was described as an independent risk factor of long-term unfavorable cardio and cerebrovascular outcome (Sander et al., 2001).

In our study, we found a decreased vagal modulation of the heart rate and enhanced sympathetic tone in patients who had a right MCA ischemic stroke compared with left MCA ischemic stroke. These findings were in line with previous results from our studies when HRV was analyzed using the Fourier Transform (Constantinescu et al., 2016; Constantinescu et al.,2018).

Our study is limited by the low number of participants which may decrease the statistical power. Further studies on larger groups are needed to confirm and strengthen these results.

The autonomic nervous system is becoming an attractive target for the therapeutic approach. Current options that may amplify the vagal component of the nervous system have already been exploited in patients with heart failure or epilepsy (Englot et al., 2011; Premchand et al., 2014). The widening of the spectrum of indications of cerebrovascular diseases can be analyzed.

Evaluating autonomic modulation may improve the outcome of patients who had an ischemic stroke by implementing early personalized pharmacological or nonpharmacological interventions for autonomic restoration in these patients.

Less used in the general neurological practice, HRV analysis may be added to current investigations in patients who had an ischemic stroke to identify possible dysfunction of the central modulation of the autonomic cardiovascular activity.

Autonomic dysfunction can predict poor outcome in patients who had an ischemic stroke. HRV analysis together with clinical evaluation (eg, NIHSS score and blood pressure measurements), other cardiovascular biomarkers such as troponin, proBNP, QT interval, hormonal markers (catecholamines, other steroids), and imagistic data depicting the right insular involvement, can contribute, through interdisciplinary consensus, to the development of a prognostic score relevant in current practice. Such a score would help identify patients who had a stroke at risk of cardiac complications.

Regarding the involvement of medication on autonomic control, the effects of several antihypertensive drugs are still debated. While some studies support the benefits of ACEI in patients with cardiac disease, data on cerebrovascular disease is scarce (Kontopoulos et al., 1997).

In patients receiving ACEI treatment within the first 30 days after myocardial infarction, an improvement in vagal tonus expressed by time-domain parameters has been shown, while others did not report any significant changes in sympathovagal balance (Kontopoulos et al., 1997; Kontopoulos et al., 1996). Studies performed in patients with heart failure sustain the recovery of parasympathetic tonus under ACEI treatment (Binkley et al., 1993; Menezes et al., 2004).

Our results support this observation. During sympathetic activation tests (handgrip and standing) we observed a decrease of RMSSD, HF and SD1 in patients with ACEI treatment. The influence of ACEI on autonomic function may be related not to sympathetic suppression, but to other mechanisms, as described in heart failure patients (Inoko et al., 2001).

Other classes of antihypertensive medication did not influence HRV parameters at rest and did not provide conclusive results on sympathovagal balance changes during autonomic activation tests. Further studies on larger groups are needed to understand the impact of med- ication on sympathovagal balance better.

Statin-treated patients had higher values of parasympathetic specific parameters during autonomic activation tests. Studies are demonstrating the beneficial role of statins in modulating the autonomic nervous system activity in patients with heart failure (Horwich and Middlekauff 2008; Millar and Floras 2014), but there is scarce data related to cerebrovascular pathology.

This research attempts to establish a practical utility for the use of linear and non-linear analysis of HRV in neurological patients, offering a practical contribution to the current clinical activity in the neurovascular units. This supports the inclusion of HRV parameters reflecting independent cardiovascular activity in the assessment of ischemic stroke patients.

Recognizing the "neurogenic cardiac syndrome" (Ozdemir and Hachinski 2008) 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 (Colivicchi et al., 2005).

1.2.4. Final remarks

Our study reported 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).

Based on the results obtained in this study, prognostic scores could be developed for stroke patients at risk of neurogenic cardiac syndrome. This could complete the current treatment guidelines for patients with acute stroke in preventing fatal cardiac events in the short and long term.

Our research brings new perspectives on personalized therapeutic approaches and integrated management of patients who had a stroke presenting an alteration of the sympathovagal balance.

We propose MTRS analysis of the HRV as a useful tool to assess the autonomic control on the heart rate using short local data segments to prevent fatal cardiac events in patients who had an ischemic stroke.

1.3. The saga of disambiguation

1.3.1. Introduction

Strokes have an increasing percentage among young population, are of extreme gravity and have serious psychological and social consequences. For these reasons, we considered it useful to evaluate the risk factors, epidemiology data, onset disease data, evolution and treatment methods in order to complete the diagnosis and prevention methods of this disease.

The increased incidence of this pathology is correlated with the decreasing in onset age of the disease.

Cerebrovascular disease is the second leading cause of death in developed countries after cardiovascular, with a prevalence of 794 percent of hundred thousand of people. Mortality is 70% and loss of workforce patients as well as extension of hospitalization creates an important economic impact (Piffer et al., 2018).

The incidence of IS is higher in economically developed countries, increases with age, and predominates in males at a rate of 1.3 compared to women. Unfortunately, the possibilities of treatment in IS are not commensurated with the advances made in its pathophysiological knowledge.

Cerebrovascular diseases has as a starting point a cumulus of risk factors such as hypertension, dyslipidemic syndrome, diabetes, obesity. In many patients with atrial fibrillation a stroke occurs as the first clinical manifestation. Thus, in these patients, pulse rate variability parameters can predict cerebrovascular events (Sluyter et al., 2019; Davison et al., 2019; Boangher et al., 2018).

Atrial fibrillation is a serious health problem because it can lead to important complications for stroke. Management of this arrhythmia should include not only its

treatment but also antithrombotic therapy. All these aspects are more serious as there is a large proportion of cases of undiagnosed atrial fibrillation and untreated with oral anticoagulants (Afabe study 2013; The Task Force of the European Society of Cardiology, 2010).

Stroke generates about 10% of new cases of epilepsy and 55% of newly diagnosed cases in elderly pacients. In the literature there is a consecutive increase in the prevalence of epilepsy related to stroke. Epilepsy may begin early (in the first 7 days from stroke onset) or may appear later convulsions (after more than 7 days).

Early onset of epilepsy after IS (STRE) is considered to be the consequence of local metabolic disorders without alterations of other neural networks, and the latter onset of epilepsy occurs when the brain has a predisposition for convulsions.

The occurrence of IS after epilepsy may lead to increased morbidity, longer hospitalization and greater disability. Further controlled trials are needed to explore the primary and secondary STRE prevention as well as to provide new proofs on the efficacy and tolerability of anti-epileptic drugs in order to guide STRE treatment. Preclinical and clinical STRE predictive models are also required to develop treatments in order to prevent transformation of the devascularized tissue into an epileptic source.

The diagnosis of stroke and/or epilepsy after stroke is confirmedby MRI. This will show a series of abnormalities that can be missed by CT examination, such as cortical malformations, hippocampal sclerosis and cavernomas (Roberts 2003).

The treatment of IS consists of that of the underlying disease but also of its secondary effects. After CT and MRI exclusion of non-thromboembolic haemorrhage, anticoagulation therapy is practiced. Thus, in acute transient cerebral ischaemia (TIS) it has prophylactic value (the risk of stroke after an TIS is 24-29%). Anticoagulants are also recommended in: case of significant arterial stenosis, especially in vertebro-basilar territory, in patients with transient cerebral ischemia of cardioembolic origin, in progressive stroke as an expression of atheromatic plaque instability, but only if the lesion area detected by CT is small.

Randomized clinical trials (Shahpouri et al., 2012; Shah et al., 2018; Hakma et al., 2014; Goldstein 2014) highlights the benefits of anticoagulant therapy in stroke patients, meaning that anticoagulant drugs prophylaxise the progression of neurological deficit. This is a different approach to the protocols used at the moment. Anticoagulant indications in stroke with a defined embolic cause is to be administered after 48 hours. It only applies in the case of cardiac metal valves, due to the high risk of clogging (Shahpouri et al., 2012; Shah et al., 2018; Hakma et al., 2014; Goldstein 2014).

In this study we have updated informations on etiopathogenesis, epidemiology, pathological anatomy, clinical, laboratory and imaging investigations, differential diagnosis, evolution, complications, prophylaxis with antiplatelet treatment of patients with ischemic strokes (IS). IS remains an important public health issue of great importance in terms of its reality and the morbidity it implies, although the tendency is to reduce epidemiological indicators.

1.3.2. Material and methods

The study is conducted on a group of 165 patients aged between 25 and 50 years admitted to the I Neurology Clinic of ”Prof. Dr. Nicolae Oblu” Emergency Clinical Hospital in Iasi, berween 2014-2018 period, diagnosed with different forms of IS.

The 165 cases were analyzed taking into account all aspects of anamnesis and etiopathogenesis, suggested by the clinical examination and revealed by the laboratory exams.

The examination was performed according to the clinic protocol and special attention was paid to the ophthalmologic, cardiological, clinical, ECG, IRM and ECHO Doppler vascular examinations. Depending on the particularity of the clinically explored cases, it were performed:

- research of current biological samples regarding the proteic, lipidic, carbohydrate, hydroelectrolytic and acido-basic balance;
- research of immunological samples, CIC, Latex, Waller-Rose, immunoglobulins;
- research of bacteriological, haemocultures, urocultures samples in infectious complication;
- cerebrospinal fluid (CSF) examination - cytology, biochemistry, serology;
- EEG, ECG, CT, Angiography, Echo Dopler, as appropriate.

The results obtained were discussed and compared with those in the literature.

1.3.3. Results

All patients were aged between 25 and 50 years. The distribution on age and gender criteria is found in **Tables 1.7 and 1.8**.

The treatment objectives in these patients are restoration of cerebral circulation and provision of a cerebral vascular flow adequate to metabolic and cellular needs. This has been done in patients with thromboembolic etiopathogenesis at the macrovascular level in order to reopen the blood vessel, prevent the propagation and prophylaxis of recurrences.

The restoration of hemodynamic homeostasis aimed correction of systemic hypotension and restoration of cerebral perfusion pressure**.**

Table 1.7. *Study on affected group age*

Age	Ischemic stroke cases	Percent
25- 30	7	4%
31- 35	10	6%
36- 40	23	14%
41- 45	49	30%
46- 50	76	46%

Table 1.8. *Gender distribution of the 165 studied cases*

Gender	Number	Percent
Male	95	58%
Female	70	42%
Total	165	100%

The classification of patients according to their demographic origin shows that 54% are from the urban area and the rest from rural areas.

It can be observed an increased frequency of IS in the Carotid territory than in the vertebrobasilar one.

TIS had a more significant percent in the total IS, the frequency being approximately equal in both carotid and vertebrobasilar level (**Table 1.9**).

Table 1.9. *The clinical evolutive forms of IS and the affected vascular territory. RIS – recurrent ischemic stroke; CIS – constituted ischemic stroke; PIS- Progressive ischemic stroke.*

	Carotidid level	Sylvian level	Vertebrobasilar level	Total
TIS	22	14	18	66
RIS	14	26	5	56
CIS+PIS	11	21	9	55
Total	47	61	32	165

As a clinical manifestation, hemiparesis is the dominant syndrome of an IS. Located on the right in one quarter of the cases, it is accompanied by aphasia (**Table 1.10**).

There is a higher frequency of IS in carotid territory than in the vertebrobasilar. Thromboembolic occlusions are more frequent in the carotid and vertebral arteries: thrombotic occlusions are more common in the carotid artery while embolic occlusions are more common in the vertebral artery (**Table 1.11**).

In order to achieve these goals, treatment has been established with thrombolysis, surgical revascularization, anticoagulants, platelet antiaggregant, colloidal plasma substitutes.

Most commonly, the field on which IS is produced is the hypertensive dyslipidemic one. Cardiac disorders (valvular, chronic ischemic cardiopathy) are favorable conditions for IS.

Table 1.10. *Clinical symtoms*

Hemiparesis	84	84%
Aphazia	41	41%
Seizures	9	9%
Affecting the consciousness	10	10%
Hemianopsia	2	2%
Bulbare syndrom+ pontin syndrom	6	6%
Wallenberg syndrom	6	6%
Weber syndrom	1	1%
Millard- gubler syndrom	1	1%

Table 1.11. *Etiologic mechanism (thrombosis, embolism) and IS location*

	Thromboembolic (arterio-arterial embolism)	thrombosis	embolism
Carotid artery	25	19	11
Medial cerebral artery	12	9	7
Anterior cerebral artery	6	5	4
Posterior cerebral artery	16	10	11
Vertebral artery+ basilar trunk	22	12	16

Specification of an associated diagnosis (risk factor) is less common compared to literature data (**Table 1.11**).

Table 1.12. *Etiology - Frequency of etiological factors*

Arterial hypertension	58	32.58%
Atrial Fibrillation	27	15.17%
Valvular disorders	23	12.9%
Chronic ischemic cardiopathy	26	14.6%
Dyslipidemia	36	20.22%
Obesity	34	19.10%
Cerebral atherosclerosis	30	16.85%
Obliterant arteriopathy of lower limbs	13	7.30%
Diabetes	17	9.55%

Under anti-coagulant and platelet antiaggregant treatment, most patients left the hospital in improved condition, with a relatively low number of deaths (**Table 1.13**).

Table 1.13. *Patients condition at discharge*

Improved	141	86%
Stationary or aggravated	19	12%
Deceased	5	2%

At the microcirculation level, it was considered to eliminate vasoconstriction and cell edema by administering calcium antagonists, magnesium, 5 HT receptor blockers and hemodilution.

All patients with embolic sourse received after 48 hours heparin therapy which acts as antithrombin, antiprotrombin, antithromboplastin inhibition of platelet adhesion, prolonging coagulation time but without modifying bleeding time parameter. It was administered as follows:

- ✓ initial intravenous bolus of 5000-10000 u followed by continuous heparin perfusion at a rate of 100-1500-2000 u/h with automatic syringe. It is recommended when there is evidence that the thrombus has formed.
- ✓ subcutaneous in small but sufficient doses for activation of AT III and for prevention of coagulation at doses of 500-800 u heparin followed by repeated intravenous bolus of 5000-10000 u heparin at 4 hours. Intravenous continue perfusion were preferred instead of intermittent administrations.

The Intravenous heparin bolus that preceed perfusion is necessary, so the hypocoagulability is only achieved approximately 6 hours after the perfusion. In the case of continuous heparin perfusions, test blood control is performed once a day, with the recommendation that blood coagulability test to be done as close as possible to blood collection, in order to prevent neutralization of the anticoagulant effect by platelet factor 4 mobilized from endothelial deposits by heparin.

Blood testing involves the determination of partially activated thromboplastin time (TTPA), which under heparin therapy should reach 1.5-2.5 higher.

If continuous heparin perfusion is used, the coagulation tests will be necessary approximately 4 hours after the intravenous bolus injection.

Coumarin agents inhibit vitamin K-dependent coagulation factors resulting in inactive biological forms. They are orally administered and indicated for long-term treatment.

There are two types of coumarin agents, depending on the plasmatic half-life and the duration of action:

- ✓ Biohydroxycoumarin - a half-life of 2 to 3 hours and a duration of action of 24 to 48 hours. The average dose is 75 mg per day.
- ✓ Warfarin has a half-life of approximately 45 hours and a duration of action of 90 to 120 hours. The average maintenance dose is 2-15 mg per day.

In medical practice, Phenprocumon is used more frequently, the duration of action can be 240 hours. The thrombostop (2 mg / tablet) with a half-life of 8 hours and a duration of action ranging from 48 to 96 hours is also part of the acenocumarol group.

Nowadays we use for long time prophylaxy NOAC (non vit K anticoagulant compound), direct factor Xa inhibitors (apixaban, rivaroxaban), direct thrombin inhibitors (dabigatran) after 3 -14 days depending o the severity of stroke.

1.3.4. Discussions

The evolution of this pathology is related to the type of the ischemic stroke:

- ✓ *The TIS is short-lived from a few seconds and minutes in hours (not exceeding 1 hours), it is characteristic that it can usually relapse in the same territory and it ends with a major stroke (infarction or haemorrhage) beeing a signal alarm;*
- ✓ *Progressive stroke regresses after 2-3 weeks, sometimes the patient is completely recovered.*

It should be noted that 17 of these patients developed epilepsy seizures in the next two years.

Complete ischemic stroke can lead to exitus within the first 4 weeks, depending on evolution (13-42%) with more in the first 3-10 days.

In order to establish a prognosis, it should be considered the existence of associated risk factors: hypertension, cardiac disease, myocardial infarction, lung infections, leading to exitus after cerebral ischemic stroke.

Recurrence are possible in the first 5 years (25%). During this period it occurs some disturbances as well as the high degree of physical instability which may also favor the establishment of the exitus. Strokes of the brainstem and those with bilateral lesions have a more limited prognosis.

Of the patients who survived the stroke, most of them have a hemiplegic motor deficit that passes after 3-8 weeks, from the flaccid phase to the spastic phase. At first movements reappear in larger joints, especially the lower limbs, which gives to patient possibility to move.

In relation to the remaining sequelae which gives the patient a reduction or a total loss of self-care, social reintegration and work capacity, the sickness can fall into four categories (scales- Barthel scale):

1. *total independence, total resumption of social-professional life;*
2. *a reducing of professional activity, but patient can serve themselves;*
3. *pronounced dependence with considerable reduction of professional activity;*
4. *total dependence on all activities.*

The risk of cerebral infarction after the TIS is between 6-9% in the first months and 5% per year over the next 3 years (Goldstein 2014).

The main risk of heparin treatment is systemic and intracerebral haemorrhage. This side effect can be neutralized in minutes by intravenous administering of protamine sulphate 2mg per ml solution, slowly, not more than 50mg in 10 minutes.

Other major side effects are: cephalalgia, circulatory disturbances, allergic reactions, bone pain, severe thrombocytopenia.

Other frequently used antiaggregants are aspirin, clopidogrel and triflusal.

In the usual medical practice, measurement of platelet reactivity in patients with stroke treated with platelet antiaggregants is not a routine maneuver. Through our study we evaluated the response to the mentioned patients' therapies and compared them with the literature.

The response to aspirin was significantly better in combination with one of the two drugs used in this study and vice versa. Also, the combination of anticoagulant medication with a clopidogrel-type anti-aggregate potentiates the patient's response to treatment and improves the post-stroke hematoencephalic barrier activity in patient with severe heart disease (Steering Comm Investigators, 2018; Rosafio et al., 2017).

The occurrence of a recurrent stroke during treatment with antiplatelet agents has a therapeutic dilemma. One of the main causes is platelet resistance more commonly known as high treatment platelet reactivity (HTPR). Previous studies have established that proteinuria is associated with HTPR after myocardial infarction. Thus, proteinuria is an independent predictor of HTPR, but after risk factors such as older age, smoking, diabetes, hypertension. Platelet resistance is a genetic clinical entity that compromises the effectiveness of treatment with antityrombotics and, especially with platelet antiaggregants (George et al., 2018).

In order to prevent and treat in due course such situations, it is necessary to perform radiological monitoring of patients, especially MRI and/or angiography, and a complete

clinical balance. The discovery of an associated pathology can radically change the therapeutic protocol (Van der Graag et al., 2018).

Specialty literature shows that more than a third of patients with ischemic stroke caused by occlusion of large intracranial vessels do not recover their functional independence, despite of rapid and successful recanalization by acute mechanical thrombectomy. *Platelet antiaggregants and heparin may restore microvascular reperfusion but may also increase the risk of symptomatic intracranial haemorrhage*. Some studies indicate a slight increase in the risk of symptomatic intracranial haemorrhage, acceptable in the context of a beneficial effect on functional outcomes (Nesselroth et al., 2018).

1.3.5. Final remarks

Platelet function testing may potentially be useful in monitoring the biological effect of platelet antiaggregant medication. Aggregometry could provide personalized prognostic information and may thus become a useful tool in designing strategies for prevention and management of ischemic stroke. Thorough studies are needed to answer the question whether the potentially greater risk of symptomatic intracranial haemorrhage that is given by this type of medication, might be compensated by improved functional results.

1.4. FAST dissection

1.4.1. Introduction

Stroke is still a public health problem, with a high incidence in the working age population. Approximately one-third of ischemic strokes in young people remain undetermined, making secondary prevention unsuitable. The consumption of toxic substances and occult cardiac arrhythmia could be the cause of these ischemic strokes.

A mnemonic to remember the warning signs of stroke is *FAST* - facial droop, arm weakness, speech difficulty, and time to call emergency services.

An ischemic stroke is a prolonged interruption or insufficiency of the arterial supply in a cerebral zone, clinically translated by focal neurological signs. These signs are respecting the territory of the concerned artery, with sudden installation or by successive strokes, the most common of which is hemiplegia.

Cerebrovascular diseases are pathologies caused by the primary or secondary damage of one or more arteries supplyng the brain (extra-/intracranial). This can occur through ischemic, hemorrhagic or mixed lesions of the cerebral tissue. In acute form, a stroke represents a severe neurological pathology with a reserved or lethal prognosis, determining a clinical syndrome through the ischemic or hemorrhagic lesions of the cerebral tissue (Brown et al., 1996).

Cerebrovascular diseases (CVD) represent the first cause of acquired disability, the second cause of dementia and the third cause of mortality in industrialised countries. Prevalence in Romania is 13,9% in the case of over 70-year-old persons, according to a paper written by a research group from Bucharest (Cinteza et al., 2007). The Global prognosis is

reserved: 20% mortality rate after one month and 40% mortality at the one-year mark. CVD also represent a cause for increased mobidity.

There are two kinds of stroke: ischemic (80%) divided constituted infarctions and transient ischemic attacks and hemorrhagic strokes (20%) represented by spontaneous cerebral hemorrhage or hematomas.

Hemorrhagic transformation is a multifactorial phenomenon and involves the transformation of ischemic cerebral tissue into a hemorrhagic lesion through the rupture of blood vessels, the extravasation of liquids and consequent cerebral lesions. The rate of hemorrhagic transformation of ischemic strokes is estimated to be in the 30-40% range. This process can happen independently in embolic strokes or after thrombolytic therapy. In current times, with the increase in the use of anti-thrombotic therapy, the prevalence of hemorrhagic transformation is increasing and it occupies a central role in the discussion about the complications of cerebral infarctions (Zhang et al., 2014).

The incidence of symptomatic spontaneous hemorrhagic transformation is between 0.6% and 20%. The incidence depends on factors such as age, serum glucose levels, and in some cases on the thrombolytic agent used, the way in which it was administered and the time lapsed between the debut of the stroke and the administration of the treatment (Tan et al., 2014).

A haemorrhagic stroke could also have consequences of a similar severity to ischemic strokes. Each type of stroke has a specific treatment that requires an early differential diagnosis.

Classification of cerebrovascular diseases are made in relation with its topography, onset, pathophysiological mechanism or is considering the timing cryteria.

Another classification divides strokes into:asymptomatic - includes patients with no symptoms of cerebral or retinal vascular disease, but paraclinical investigations (CT, MRI) reveal signs of cerebrovascular disease;focal cerebral dysfunction which can be:

1. transient ischemic attack in the carotid or vertebrobasilar system.
2. constituted (cerebral infarction) by one of the mechanisms: thrombotic - is achieved by overlapping the thrombus on the atheroma plate; embolic-distal embolization and occlusion of an artery (Goldstein et al., 2001; Mohr et al., 1978).

Hemodinamic explorationmechanism are compulsory in patients with severe stenosis or proximal arterial occlusions with inadequate collateral circulation, with a critical decrease in global cerebral infusion. The anatomopathological mechanisms of different types of strokes occurs in conditions of severe hypotension or in paroxysmal rhythm disorders.Confirming the diagnosis of a stroke is usually easy if the anamnesis is known. Any sudden neurological deficit that involve an arterial territory must be considered a stroke. The differentiation between ischemic stroke (IS) and transient ischemic stroke (TIS) has no practical interest since the patient should be seen and treated in a similar manner in the first 24 hours and there is nothing to affect the clinical deficit. Usually, TIS announces a future constituted stroke.

MRI investigation is one of the most sensitive paraclinical diagnostic methods in the case of stroke. It is more sensitive than tomography, highlighting recent ischemic strokes, with signal abnormalities occurring a few hours after the ischemic process. It is also more sensitive in highlighting old post-haemorrhagic sequelae.

For high accuracy, contrast-injected MRI technique is used. There are numerous studies in specialised literature that assume that this radiological investigation technique is overstated by other emerging techniques and, in some situations, it may even be dangerous (Kilinc et al., 2003; Ing et al., 1989).

The symptomatology is caused by the ischemia in a cerebral territory, phenomena which can be transitory, seconds to minutes, or can persist for longer periods of time. After such a lesion permanent damage can occur because the cerebral matter is irreversibly damaged and an infarction takes place.

Neurological symptoms are not, sadly, pathognomonic for the certain diagnosis of cerebral infarction and their succession does not indicate the cause of the ischemia(Caplan 1989; Hegele and Dichgans 2010). All of these factors can delay a certain diagnosis and the correct and efficient implementation of an etiopathogenic treatment.

This pathology is multifactorial, among the causes being: age, smoker status, arterial hypertension, diabetes mellitus, obesity, chronic alcohol consumption and hypercholesterolemia. Of the modifiable risk factors we count: hypertension and other cardiovascular diseases, diabetes, dyslipidemia. These are conditioned by geographical location and socio-economic status. Among the unmodifiable risk factors we can count general status correlated with age, gender and ethnic background, Approximately 80% of strokes can be prevented only by lifestyle changes (Allen and Bayraktutan 2008).

Our study aims to create a link between the modifiable risk factors, the affected vascular territory and the clinical manifestation of CVD in patients from our geographical area. The purpose of our study is also to show the usefulness of the radiological technique of MRI with contrast substance and to propose a protocol for the investigation of clinically diagnosed patients with stroke by this method.

1.4.2. Materials and methods

1.4.2.1. Drain to lifelessness

Our study proposes the retrospective analysis of a lot of 70 patients diagnosed with ischemic stroke with spontaneous hemorrhagic transformation over a period of 5 years between the 1st of April 2015 and the 1st of April 2018 admitted in the 1st Neurology Clinic of the Clinical Emergency Hospital “Prof. Dr. N. Oblu”, Iasi.

Average age of the patients in the study group was 70.57 years old with a mean deviation of 13.7 years, a minimum age of 20 years old and a maximum age of 91 years old. For the female patients, the average age was 72.61 years old with mean deviation of 14.67 years and for the male patients of 68.78 years old with a mean deviation of 12.87 years. In the analysed group the number of male patients was 37 and the number of female patients was 33. 58.28% of patients were from rural areas and 45.72% were from urban areas.

The clinical study was done by collecting the data from patient files: demographic data (age, gender, area of provenance), personal pathologic history, comorbidities and risk factors (arterial hypertension, atrial fibrillation, diabetes, dyslipidemia, renal pathology history, neoplasms, and thrombotic risk quantified by chronic venous disease and mutations

of factor 5 Lyden). The behaviour regarding smoking and chronic alcohol consumption was also noted.

Usual laboratory investigations from which glycemia at admission and the presence or absence of thrombocytopenia were noted.

From the clinical exam at admission we noted the systolic and diastolic blood pressure.

The patients were examined using CT in emergency, and during the evolution we followed the topography and dimensions of the infracted area, the presence of sequellary infarctions, the mass effect of the lesion and the particular signs for hemorrhagic transformation.

Also we noted the evolution of the neurological state of the patient: stationary, favourable, worsening, the appearance of complications or death. For the patients where hemorrhagic transformation occurred while being hospitalised the neurological symptoms that accompanied the event were noted.

The criteria of admission in the study were the presence in the patient file of a main diagnosis of CVD confirmed by CT. In the case of ischemic strokes with hemorrhagic transformation this was documented either at admission or during the period of hospitalisation.

1.4.2.1. Built in apoplexy

Our study was conducted on a group of 165 selected patients diagnosed with different forms of IS, aged between 25 and 50 years, admitted to the I-st Neurology Clinic of the ”Prof. Dr. Nicolae Oblu” Clinical Hospital in the period 2014-2018.

The 165 cases were analyzed taking into account all aspects of the epidemiological and etiopathogenic data suggested by the clinical examination and revealed by the laboratory examinations.

The examination was performed following the clinic protocol, with particular attention to clinical, ophthalmic, cardiological, ECG, and ECHO Doppler vascular examinations.

All the selected patients were examined by MRI contrast technique after initial native scan. This was performed after entire routine examination protocol.

We must emphasize that patients or their owners have previously signed the informed consent forms on the methods of investigation and treatment.

The contrast agent routinely used in MRI examinations is based on iodine in various combinations and concentrations, with lately developing isosomolar and hypoallergenic compounds in order to reduce the frequency of possible side effects.

The radiologist will decide upon the need to use the contrast agent in the course of the investigation, depending on the pathology of the patient and of course the changes encountered during the initial native scan and taking into account the benefit/risk ratio of each case.

Routine contrast agents are used in MRI examinations to increase the natural contrast of blood vessels and certain portions of the encephalus, resulting in a diagnosis of certainty.

In the case of highlighted injuries, their particular vascular criteria give additional information for diagnosis.

For this we used the following exam protocol:

1. weight ratio in sagittal section (number of slots 30, slice thickness 4mm, matrix 218x256, TA 3:20)
2. weighted t2 sagittal iso_1.0 (3D sequence, sagittal acquisition and axial and coronal reconstruction, 1mm acquisition and reconstruction slice, matrix 202x256, TA 5:17)
3. ep2d_diff_3scan_trace_p2 (diffusion, slice number 30, slice thickness 4mm, matrix 192x192, TA 2:18)
4. t2_swi3d_tra (slice number 30, slice thickness 2mm, matrix 186x256, TA 3:38)
5. spc_ir_cor_p2_iso (oblique, perpendicular to the hippocampus) (3D sequence, coronal acquisition and axial reconstruction, 1mm acquisition and reconstruction slice, 202x256 array, TA 4:26)
6. t2_tse_cor (oblique, perpendicular to the hippocampus) (slice number 50, slice thickness 2mm, TA 4:26, matrix 307x256)
7. t1_mprage_tra (3D sequence, axial acquisition and sagiatal and coronal reconstruction, 1mm reconstruction acquisition slice, 246x256 array, TA 5:34) Contrast agent injected
8. t1_mprage_tra (3D sequence, axial acquisition and sagittal and coronal reconstruction, 1mm acquisition and reconstruction slice, 246x256 matrix, TA 5:34)
9. t1_se_tra_320_MTC (slice number 30, slice thickness 4mm, matrix 256x320, TA 3:25)

Device Type: Siemens Essenza Tim-Dot, 1.5T

Abbreviations list: TA = acquisition time; Total TA = 45min; FOV = field of view = 230-250mm.

1.4.3. Results

1.4.3.1. The hazard of the emptiness

The identification of the risk factors for hemorrhagic stroke, ischemic stroke or ischemic stroke with hemorrhagic transformation at admission or during hospitalisation represents a basic requirement in the diagnosis and secondary prevention of this disease (**Figure 1.5**).

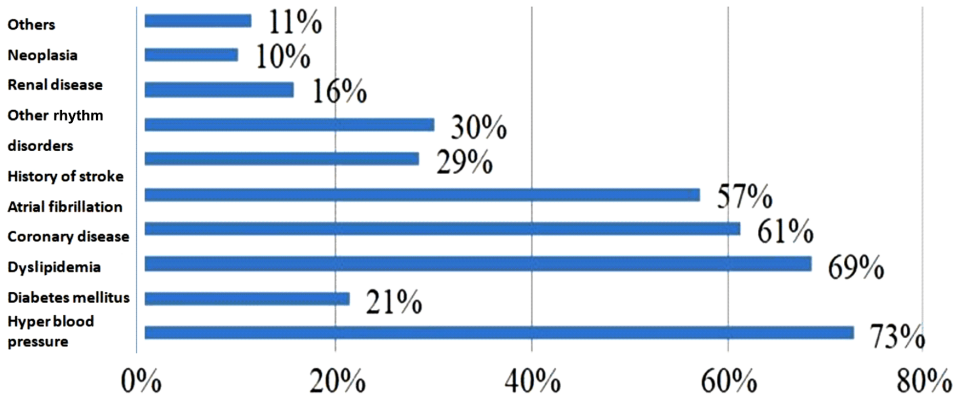

Figure 1.5. *Risk factor distribution in sample group.*

Myocardial infarctions, pectoral angina and ischemic cardiomyopathy of different causes were classified as coronary disease.

Dyslipidemia was documented by serum cholesterol values higher than 200 mg/dl and LDL-col higher than 100 mg/dl during admittance or in the patient's history.

For the diagnosis of arterial hypertension a history of hypertension, chronic hypertension treatment or persistent systolic blood pressure over 140 mmHg during hospitalisation were taken into account. Other risk factors considered were an interatrial septal aneurysm, a left subclavicular theft syndrome and venous thrombosis of the lower members.

The majority of the patients included in the study had associated cardiovascular risk factors which can frequently be found in the case of ischemic strokes. Of these well represented in this group were: arterial hypertension (72.85%), dyslipidemia (68.57%), atrial fibrillation (57.14%), history of stroke (28.57%), diabetes mellitus (21.42%). Of all these predisposing conditions for stroke the risk factors with the highest predictive value for the hemorrhagic transformation of an ischemic stroke were: atrial fibrillation, hyperglycemia associated or not to a poorly compensated diabetes, dyslipidemia

Many of these risk factors were associated at the same patient (**Table 1.14.**). In a large part of the cases (48.57%) there are 3 or 4 associated risk factors and more than 5 simultaneous risk factors are present in 32.8% of patients.

Table 1.14. *Distribution of simultaneous risk factors*

Risk Factors	Number of Patients
1-2	10
3-4	34
> 5	23

In the studied group the time period lapsed between the first symptoms and presentation at the emergency room was noted. Of the total of 70 patients studied, 51 presented with ischemic stroke and the rest with hemorrhagic stroke. Hemorrhagic transformation took place in 10 patient of which in 4 it was present at admission and in 6 cases it happened during hospitalisation (**Figure 1.9**).

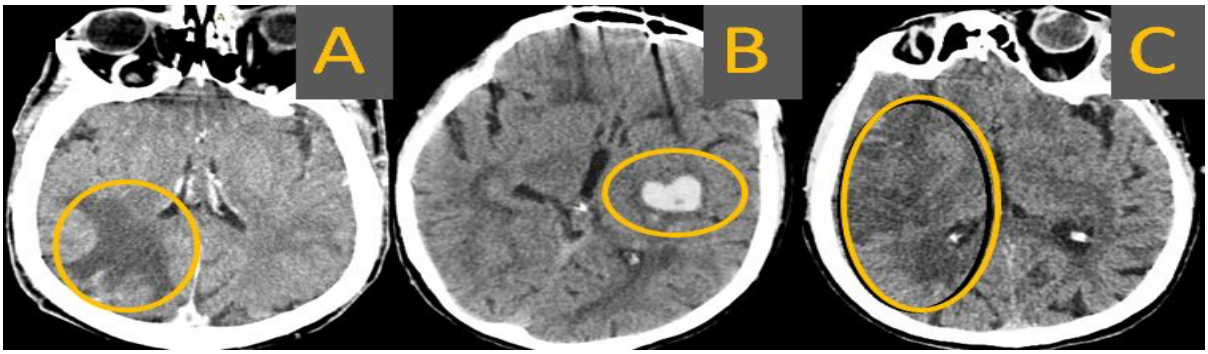

Figure 1.9. *CT examination for AVCI, AVCH and AVCI with hemorrhagic transformation*

In the analysed group motor symptoms were most common (94.28%). These can be represented by hemiparesis or hemiplegia. the diagnosis of motor deficits was done through the evaluation of active segmentary movements using the MRC scale for motor deficits (0 - no contraction, 1 - minimal voluntary contraction, 2 - movement possible when cancelling the effect of gravity, 3 - movement possible against gravity, 4 - movement possible against resistance, 5 - normal movement). Any result between 0 and 3 was considered a motor deficit.

The precocious identification of the complications of strokes and their treatment is defining as this influences the mortality and the short and long term functional prognosis. We note a high rate of correlation between hemorrhagic transformation and other possible precocious complications of an ischemic stroke. All of these manifestations were present at admission or appeared during the first week (**Tables 1.15 and 1.16**).

Table 1.15. *Frequency of complications*

Stroke complications			**Simultaneous complications**	**No. of patients**
Variable	***No. of patients***	***Percent***		
Respiratory tract infection	17	24.28%	*0*	21
Urinary infection	12	17.14%	*1–2*	39
Swallowing disorders	13	18.57%	*3–4*	10
Electrolyte disturbance	22	31.42%		
Epilepsy	2	2.85%		
Urinary incontinence	7	10%		
Depression	7	10%		
Cerebral edema	12	17.14%		

Table 1.16. *Main and associated neurological signs at admission*

Clinical neurological picture at admission			**Associated neurological signs**		
Variable	***No. of patients***	***Percent***	***Variable***	***No. of patients***	***Percent***
Glasgow Coma Scale (GCS)	14–52	74.28%	*Dysarthria*	6	8.57%
	15	21.42%	*Hemianopsia*	24	34.28%
	8–13	4.28%	*Hemihypoesthesia*	15	21.42%
	0–7	3%	*Vertigo*	7	10%
Aphasia	35	50%	*Cephalia*	16	22.85%
Motor deficiency	66	94.28%	*VIth nerve paresis*	51	72.85%
Visual field disorders	2	2.85%	*Conjugate deflection of the eyeball*	3	4.28%
			Signs of atherosclerosis	36	51.42%
			Others	20	28.57%

In the studied group only in 2 cases the ischemic stroke resulted in deep vein thrombosis and only one case presented hematemesis as a complication, in the absence of known digestive disease.

At admission, in the studied group we noted e mean systolic blood pressure of 144.17 ± 27,9 mmHg with a maximum of 230 mmHg and a minimum of 80 mmHg. The mean diastolic blood pressure was 82,8 ± 14,8 mmHg with a maximum value of 130 mmHg and a minimum of 50 mmHg. Of the 40 patients with hyperglycemia (57.14%) only 27.5% were previously diagnosed with diabetes, the rest of 72.5% presenting with hyperglycemia at admission without other determining factors. The glucose levels of these patients normalised in 1-2 days after admission.

According to the classification of blood pressure values in the ESH-ESC 2013 guide, the mean value of the study group's blood pressures is qualified as hypertension (systolic blood pressure over 140 mmHg and diastolic blood pressure over 90 mmHg).

The laboratory tests run at admission included serum glucose levels. Thus, the mean value for 70 cases was 132,97 ± 52,68 mg/dl, with a maximum of 368 mg/dl and a minimum of 72 mg/dl. Normal glycemia values are between 65 and 110 mg/dl. To sum up, the mean serum glucose levels show a tendency towards hyperglycemia.

Imagistic studies using CT without contrast allows for the differential diagnosis between ischemic and hemorrhagic strokes, but the role of CT as a predictive parameter for hemorrhagic transformation is still debated.

In this paper we examined the CT images obtained early after admission, in the first few hours (**Table 1.17**).

In the case of patients presenting with uncomplicated ischemic stroke possible candidates for thrombolytic couldn´t be evaluated because the time lapsed from the debut of the symptoms was impossible to quantify (**Figure 1.10**).

The first imagistic feature studied was whether the patient had older cerebral infarctions.

Table 1.17. *Distribution of patients by closed arterial branch*

Area of stroke	No. of patients	Percent
Carotid territory	2	2.85%
Anterior cerebral artery	2	2.85%
Right medial cerebral artery	32	45.71%
Left medial cerebral artery	24	34.28%
Posterior cerebral artery	16	22.85%
Vertebro-basilar system	1	1.42%
Cerebellar	3	4.28%
Anterior carotid artery	3	4.28%

From the 70 cases included, 30% had sequellary lesions visible on CT. These results reflect the recurrence of embolic events. The results of the statistic analysis show a large percent of medium-sized infarction areas: 70% (the volume of the infarction area was bigger than 1.5 cm^2 and involved the area supplied by one main artery). Massive ischemic

infarctions (he volume of the infarction area was bigger than 1.5 cm^2 and involved the area supplied by two or more main arteries) happened in only 28.57% of cases.

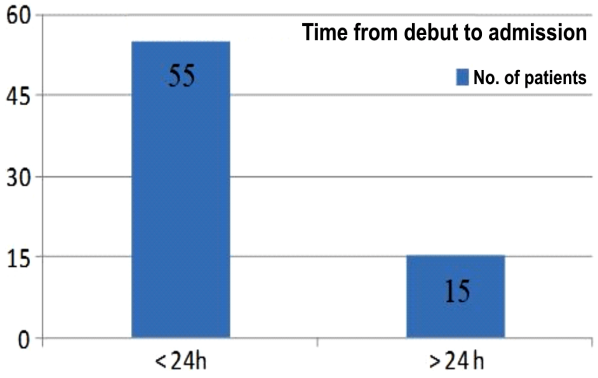

Figure 1.10. *From this graphic, we observe that 78.57% of patients with the debut of symptoms less than 24 hours before admission, allowing for acceptable clinical and imagistic diagnosis and appropriate therapy for ischemic strokes.*

Out of 70 cases only in 5 particular aspects were described at CT. Noted were the bilateral disappearance of the ambiens nucleus and of the right interpeduncular nucelus, a spontaneous hyperdensity in the right MCA (suggestive for a thrombus), the disappearance of the intergyral sulcus, the loss of differentiation between the white and grey matter and spontaneous hyperdensity in the M1 Sylvian segment (suggestive for a thrombus). All of these are signs of hyper-acute ischemic stroke. All strokes that resulted in death were embolic and had an aspect of liquefactive necrosis on HE staining (**Figures 1.11-1.13**).

The mass effect of the infarction are was present in 35.71% of cases. Of this group where mass effect complicated the stroke, 28% had massive infarction and 68% had a medium area of infarction. 56% of the patients in this group presented hematomas.

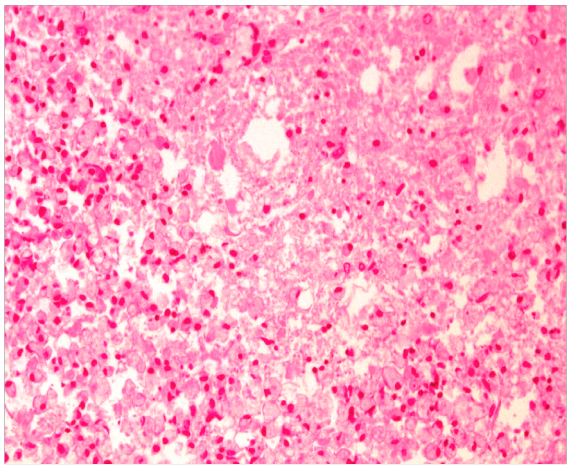

Figure 1.11. *Liquefactive necrosis surrounded by lipid-laden macro-phages that ingested the products of degradation of dead neurons and myelin (HE staining, ×200).*

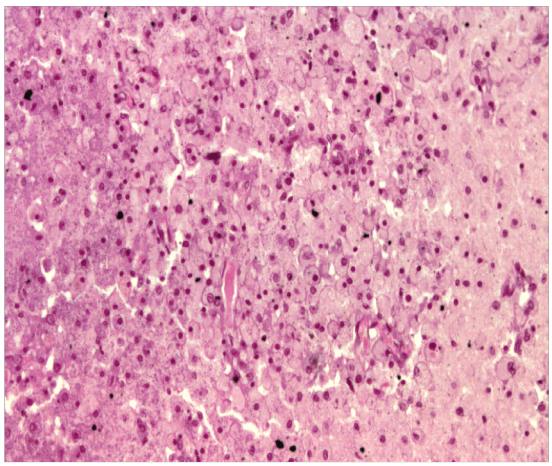

Figure 1.12. *Foamy macrophages that cleaned up the lipid debris from the liquefactive necrosis and newly formed capillary vessels (HE staining, ×200).*

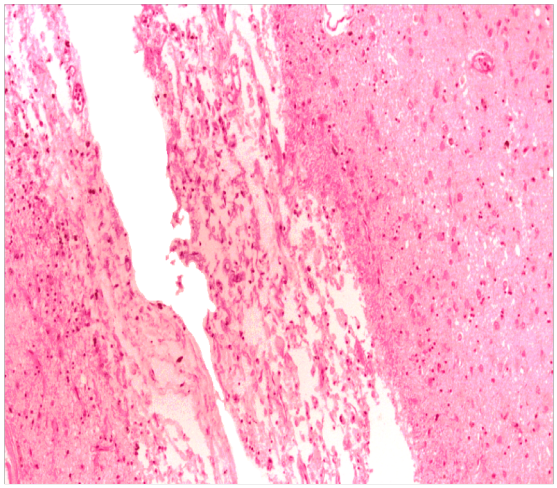

Figure 1.13. *Resolution of the lique-factive necrosis led to a cystic area surrounded by foamy macrophages, rare fibroblasts, rare lymphocytes, and few new capillary vessels. The nervous tissue around the cavity expressed reactive astrogliosis (HE staining, ×100).*

1.4.3.2. Watch me!

The cases investigated by the mentioned protocol were diagnosed as follows:

- 82 patients with atherosclerotic stroke (49.70%);
- 78 with embolic stroke (47.27%);
- 5 with haemorrhagic stroke (3.03%).

Five of them died shortly after paraclinical investigation due to the severity of stroke and associated comorbidities. 58% of the patients were male and 42% females.

The obtained images emphasizes the utility of contrast agents in MRI examination and the particularly high degree of acuity of this technique.

In patients with atherosclerotic disease, 92.13% of the strokes occurred in the territory of the internal carotid artery (**Figure 1.14**).

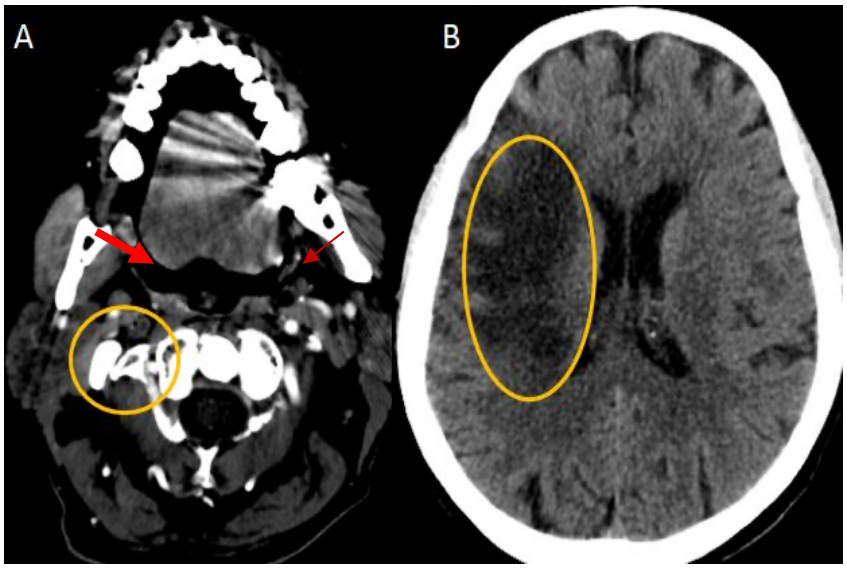

Figure 1.14. *Stroke caused by plaque occlusion of the right common carotid artery; A = MRI with contrast substance; B = native MRI that highlights the affected brain territory*

In the case of patients with embolic strokes, the territory of the internal carotid artery was also predominantly affected but with lesser extent - 53.85% (**Figure 1.15**).

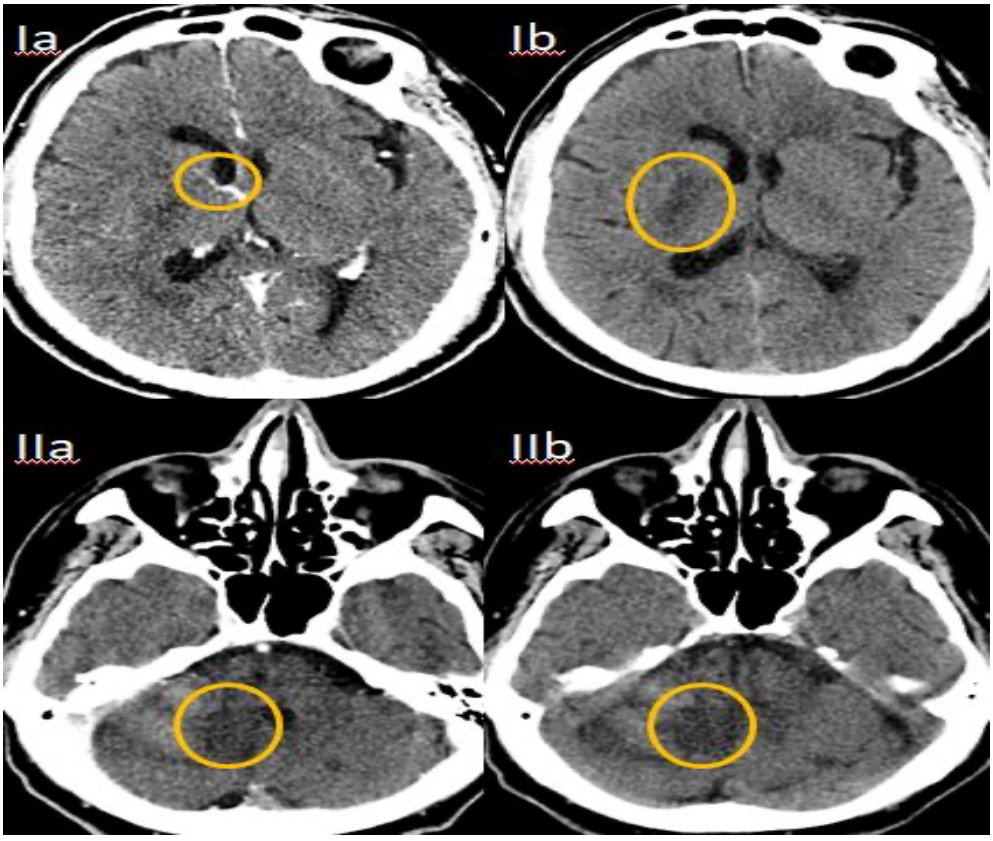

Figure 1.15. *Thrombotic stroke; I = on the middle right brain artery; II = on the right cerebellar artery; a = MRI with contrast substance; b = native IRM*

Most of the haemorrhagic strokes (80%) occurred in the territory of the internal carotid artery (**Figure 1.16**).

In the case of vascular accidents due to atherosclerosis and embolic causes, the MRI contrast technique shows with high acuity the affected blood vessel and its segment.

In the case of haemorrhagic vascular accidents, besides the objection of the affected blood vessel, the MRI with the contrast substance shows the degree of extravasation of the blood flow.

This is performed in T1-weighted images, left field without and right field with contrast medium administration. We have used Omniscan which is a gadodiamide and Magnevist which is a gadopentetate substance.

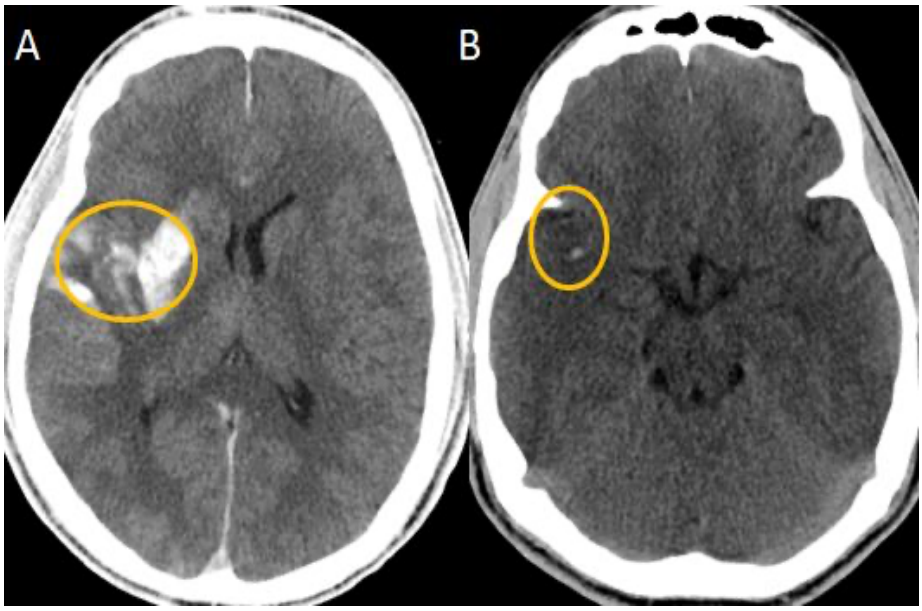

Figure 1.16. *MRI in patients with haemorrhagic vascular accident; A = with contrast substance; B = native*

T2-based arterial spin labeling (ASL) method with 3D readout could be used to measure the water transfer processes between arterial blood and brain tissue based on a 3D-GRASE (gradient and spin echo) pulsed ASL sequence with multiecho readout. Also, these agents are used for highlighting the blood vessels in MRI angiography and/or brain tumor associated with the degradation of the blood–brain barrier. In case of large blood vessels gadolinium dose could be lowered to 0.1 mmol/kg body mass and higher concentrations are used for thinner vasculature. This great utility of the paramagnetic agents is given by the chelates they contain which do not pass the intact blood–brain barrier because they are hydrophilic. Thus, these are useful in enhancing lesions and tumors where blood-brain barrier is compromised and the Gd leaks out. After a while the contrast agent will distribute into the interstitial space and will be eliminated by the kidneys.

1.4.4. Discussions

Of the risk factors for stroke some cannot contribute individually to the formation of the stroke, but can determine it if they are associated with other attributes (Parkinson et al., 2016). Some cases of stroke do not have risk factors as currently recognised in medical literature but some of these parameters can indicate a higher chance of stroke (Wulandari et al., 2019).

This studies aim to discover unusual, rare associations of risk factors in the population of our geographic area which lead to a high stroke potential.

In the case of our test group we observe that the predominant localisation of strokes with bleeding potential is the right or left medial cerebral artery (MCA). In this case the deeper territories represented by the basal nuclei and the thalamus were very rarely affected.

We note the fact that there were no bilateral ischemic strokes in the area of the MCA. In the case of multiple infarctions, these were common in the border area between the MCA and the posterior cerebral artery (PCA). The evaluation of the infarction area is of utmost importance because a wide infarction area is recognised as a risk factor for hemorrhagic transformation and as negative prognosis factor for subsequent evolution of the patient.

The physiologic reason why a larger volume of the ischemic infarction predisposes the patient to hemorrhagic transformation is that more of the hematoencephalic barrier, where ruptures occur, is involved.

Hyperglycemia is considered damaging for cerebral metabolism in the infarcted area and can constitute a predictive factor for the hemorrhagic transformation of an ischemic stroke. Chronic hyperglycemia in the case of uncontrolled diabetes has the same risks as acute hyperglycemia, rising the risk of hemorrhagic transformation and unfavourable evolution. Acute hyperglycemia can occur because of the stress through the activation of the hypothalamic-hipophyseal-suprarenal axis or can be the expression of an undiagnosed case of diabetes or low tolerance to glucose.

The ratio of complications of ischemic strokes through hemorrhagic transformation is 1.18 times grater in patients from rural areas than in those from urban areas.

With ageing, changes occur in the small vessels of cerebral circulation and more cardiovascular risk factors appear (Awad et al., 1986). In particular, it is demonstrated that amyloid angiopathy predisposes to hemorrhagic intracerebral bleeding (Arles et al., 2010). At the same time, patients that have leucoaraiosis (the asymptomatic rarefication of the white cerebral matter) as a mark of ageing increases the risk of hemorrhagic remaniation of ischemic strokes (Biffi A, Greenberg et al., 2011).

The clinical evaluation of patients with neurological symptoms has to account for the understanding of CVD classification and has to realise a rapid initial evaluation in order to stabilise vital signs and to determine whether intracranial bleeding is present, justifying reperfusion therapy in the case of patients with ischemic stroke. Forming a etiological hypothesis based on history, physical exam, and initial imaging study (usually a CT scan without contrast) is an integral part of the exploration protocol for these patients.

In speciality literature other rare or very rare risk factors are described. Tyrosin kinase inhibitor therapy for chronic myeloid leukemia was associated with progressive

periferic arterial disease, and more recently there were reported cases showing rare intracranial vascular stenosis.

Another rare cause is fibromuscular dysplasia which represents a non ateromatous vasculopathy, non-inflamatory of unknown origin which mostly affects small and medium sized arteries. It affects mostly female patients and the renal, carotid and extracranial portion of the vertebral vessels (Lummus et al., 2014 ; Sur et al., 2019).

Thus, the patient having a high risk for stroke has at least 2 elevated risk factors together with a small or medium risk factor. Patients having an association of 3-4 major risk factors already had sequellary lesions in over 30% of cases.

The association characterising the study group is between arterial hypertension, dyslipidemia and atrial fibrillation causing ischemic stroke in the right or left MCA territory. The clinical signs common to these cases are motor deficits and facial paralysis.

Adding smoking and/or chronic alcohol consumption, to diabetes increses the risk for ischemic stroke by 47% and of hemorrhagic stroke by 155 in the test group.

Stroke risk higher than 30% is present in paraneoplastic syndrome and/or in patients who take anticoagulat therapy for a long time or who have chronic kidney disease.

The data in our study clearly shows a morphopathologic mechanism mostly based on right MCA ischemia caused by aterosclerosis and dyslipidemia.

Apart from the already known advantages of the IRM radiological investigation technique, particularly in the case of brain exploration, it has some great advantages. These include the high acuity and sensitivity of this technique, the possibility of functional explorations and 3D reconstruction. Native exploration is important in highlighting the affected brain territory (Dickie et al., 2019; Armitage et al., 2011).

Applying these notions to patients diagnosed with stroke, MRI is of particular interest.

In MRI, the very recent hematoma is iso-intensive in T1 and hypointense in T2. Gadolinium (Gd/paramagnetic) are the most common agents used to detect the efect upon the blood–brain barrier (Abbott et al., 2010) of a stroke shown in MRI. These signals change differently to become hyperintense on T1 and T2 from day 5. From day 15, a hyposignal ring appears at T1 and T2, corresponding to hemosiderin deposits.

Exploration includes blood and tissue compartment, T1 and T2 relaxation, and a blood-to-tissue transfer sequency (Liu et al., 2011). T1 sequences are extremely useful in detecting transient ischemic strokes and their differentiation from epilepsy attacks or other causes of loss of consciousness.

Even if gadolinium-based contrast agents appear to be safe in pregnancy, none of the female patients in our group was confirmed so (Lentschig et al., 1998; Garcia-Bournissen et al., 2006).

In case of contrast-extravasation following haemorrhagic stroke, the images obtained by MRI technique are also iso-intensive in T1 and hypointense in T2.

In the case of thrombotic cerebral accidents, T1 weighs an iso-intensive signal on both sides of the lesion and hypointense in T2.

Certain studies in the literature show that iodinated contrast agents used in CT and MRI techniques could disrupt the effect of thrombolytic medication. This would mean that the

method is contraindicated in patients diagnosed with embolicstroke. This theory has not been proven, and is thus denied.

MRI with contrast substance is more useful for patients diagnosed with embolic stroke as long as blood-brain barrier permeability changes can be tracked, using dynamic contrast-enhanced magnetic resonance imaging.

Iron oxide (IO) nanoparticles such as Ferumoxytol are being increasingly used to image cellular contribution to neuroinflammation using MRI. They provide additional information on neuroinflammatory phenomena, as compared to conventional Gadolinium-enhanced MRI. This is posible because these particles are allowing to assess cell trafficking from the blood to neuroinflammation sites by phagocytic activity. They have a widespread availability and relatively low costs together with great accuracy (Aghighi et al., 2015; Bulte et al., 2004).

In this way, vascular intracerebral phenomena occurring after reperfusion of affected area can be monitored by native MRI also (Kouichi et al., 2017).

Knowing the functional anatomy of the blood-brain barrier is of utmost importance for the understanding of ischemia-reperfusion injuries that are directly related to haemorrhagic transformation. rtPA (the recombinant human plasminogen activator) increases the risk of symptomatic post-reperfusion intracranial haemorrhage.

Modern radiological techniques allow understanding of the pathological disorders of blood-brain barrier, and MRI can be a rigorous method for assessing the risk of bleeding. In this regard, a micellar polymeric MRI contrast agent is used (Krueger et al., 2015; Whiteley et al., 2014).

1.4.5. Final remarks

The results of this study must be interpreted carefully as the study was undertaken in one single hospital and thus does not reflect the situation in the general population. Patients with hemorrhagic or ischemic with hemorrhagic transformation stroke were evaluated only using CT scans, without benefitting from MRI scans. MRI has a much higher sensibility for the detection of hemorrhagic transformation risk in a patient, thus there was a chance that eligible patients were not included.

Furthermore some important information for determining the risk of hemorrhagic transformation in a patient with ischemic stroke were not included in patient files such as: ferritin levels, urinary albumin, a complete lipid profile including LDL levels, NIHSS score at admission and dismissal and angiographic data.

The morphopathology of strokes is determined by risk factors and comorbidities, which have a clear demographic pattern. The existance of a correlation between demographic risk factors of CVD and the clinical manifestation allows for the individualisation of a clinical examination protocol leading to a rapid diagnosis.

MRI with contrast substance is a basic technique in diagnosing and evaluating the prognosis of patients diagnosed with stroke. The use of special contrast agents opens the way for their MRI functional exploration and monitoring.

2. CARPE DE MORBO SACRO

2.1. State of the Art

The oldest detailed notification of epilepsy is on a Babylonian tablet in the British Museum. It represents a chapter from a Babylonian textbook of medicine comprising 40 tablets dating as far back as at least 2000 BC and accurately records many of the different seizure types we recognize today. The tablet also emphasizes the supernatural nature of epilepsy, with each seizure type associated with the name of a spirit or god - usually evil. In this context, treatment was, therefore, largely a spiritual and divine matter (Magiorkinis et al. 2010).

The Babylonian's view was the preliminary of the Greek concept of "The Sacred Disease", as there was described in the famous treatise of that title by Hippocrates. However, Hippocrates believed that epilepsy was not sacred, but a disorder of the brain - a revolutionary view. He did not believe "that a human could be invaded by a god, the basest by the most pure". He recommended physical treatments and stated that if the disease became chronic, it was incurable (Francis, 1891).

It is thus with regard to the disease called Sacred: to Hippocrates it appeared to be nowise more divine nor more sacred than other diseases, but having a natural cause from the originates like other affections. "Men regard its nature and cause as divine from ignorance and wonder, because it is not at all like to other diseases. And this notion of its divinity is kept up by their inability to comprehend it, and the simplicity of the mode by which it is cured, for men are freed from it by purifications and incantations" (Fielding, 1966).

Those who first referred this malady to the gods appear to me to have been just such persons as the conjurors, purificators, mountebanks, and charlatans now are, who give themselves out for being excessively religious, and as knowing more than other people. They were using the divinity as a pretext and screen of their own inability to of their own inability to afford any assistance, have given out that the disease is sacred, adding suitable reasons for this opinion, they have instituted a mode of treatment which is safe for themselves, namely, by applying purifications and incantations, and enforcing abstinence from baths and many articles of food which are unwholesome to men in diseases (Fielding, 1966).

The history of chemical substances known to cause or treat epilepsy is as old as well. Initially, of sea substances, the surmullet, the blacktail, the mullet, and the eel are the fishes most to be guarded against. And of fleshes, those of the goat, the stag, the sow, and the dog are the kinds of flesh which are aptest to disorder the bowels. Of fowls, the cock, the turtle, and the bustard, and such others as are reckoned to be particularly strong. Also, potherbs, mint, garlic, and onions were considered to be digested only by strong persons (Fielding,1966).

These people forbid to have a black robe, because black is expressive of death; and to sleep on a goat's skin, or to wear it, and to put one foot upon another, or one hand upon another; for all these things are held to be hindrances to the cure. All these they enjoin with reference to its divinity, as if possessed of more knowledge, and announcing beforehand other causes so that if the person should recover, theirs would be the honor and credit. If they

should die, they would have a certain defence, as if the gods, and not they, were to blame, seeing they had administered nothing either to eat or drink as medicines, nor had overheated him with baths, so as to prove the cause of what had happened (Fishchenko and Khimich, 1986).

The word "epilepsy" is derived from the Greek "epilepsia" which means "to take hold of" or "to seize."

Hippocrates' view of epilepsy as a brain disorder did not begin to take root until the 18th and 19th centuries. The intervening 2000 years were dominated by the earlier supernatural views. This was reinforced, for example, in the account of Christ casting out a devil from a young man with epilepsy (Miller, 1953).

Throughout this time, people with epilepsy were viewed with fear, suspicion and misunderstanding, and were subjected to enormous social stigma. They were treated as outcasts and punished. However, some of them succeeded and, in fact, became famous the world over. Outstanding personalities were among them: Julius Caesar, Czar Peter the Great of Russia, Pope Pius IX, the writer Fyodor Dostoevsky, the poet Lord Byron and others. Even today, people with epilepsy continue to suffer discrimination in the family, marriage, employment, law, education and society (Margotta, 1968).

Epilepsy affects social and psychological well-being by reffering to social isolation, stigmatization, or disability. These effects may result in lower educational achievement and worse employment outcomes. Learning disabilities are common in those with the condition, and especially among children with epilepsy. This stigma affects also the families of those with the disorder (Engel, 2008).

Since medieval times, St. Valentine has been the patron saint of people with epilepsy and sites of pilgrimages included Rome and Terni in Italy, Ruffach in France, Poppel in Belgium, and Passau in Germany (Cooper,2013).

Only since the 19th century, as neurology emerged as a new discipline, distinct from psychiatry, the concept of epilepsy as a brain disorder became more widely accepted, especially in Europe and North America. Bromine, discovered by Carl Jacob Löwig and Antoine Balard, as the world's first effective anti-epileptic drug, became widely used in Europe and North America during the second half of the last century and helped to reduce the stigma associated with the disorder (Löwig,1829; Balard, 1826; Balard, 1826).

First hospital for the "paralyzed and epileptic" was established in London in 1857 and many other establishments of epilepsy "colonies" for care and employment have appeared worldwide. Examples include Bielefeld-Bethel in Germany, Heemstede in Holland, Chalfont in England, Zurich in Switzerland, Dianalund in Denmark, and Sandvikain in Norway (Buzard,1870).

The foundation of our modern understanding of the etiopathogeny and pathophysiology of function seen in epilepsy was also laid in the 19th century with the proposal by John Hughlings Jackson, an english neurologist, that seizures were the result of sudden brief electro-chemical discharges of energy in the brain - the character of the seizures depending on the location and function of the seat of the discharges (Critchley and Critchley, 2013).

It proceded the electrical excitability of the cortex of the brain in animals and man discovery made by David Ferrier in London and Gustav Theodor Fritsch and Eduard Hitzig in Germany (Hubert, 1961).

Hans Berger, a psychiatrist, developed the human electroencephalograph (EEG "brainwaves") with so important applicability in the field of epilepsy. The EEG revealed the presence of the electrical discharges in the brain and also showed different patterns of brainwave discharges associated with different seizure types (Haas and Berger, 2003).

The technique helped to locate the site of seizure discharges and expanded the possibilities of neurosurgical treatments, which became much more widely available from the 1950s onwards in London, Montreal and Paris.

The main primary drugs for the treatment of epilepsy were phenobarbitone (1912) and phenytoin (1938) but since the 1960s there has been an accelerating process of drug discovery. It was based on a much greater understanding of the electrochemical activities of the brain, especially the excitatory and inhibitory neurotransmitters. Nowadays, seizures could be controlled in approximately three-quarters of newly-diagnosed children and adults (Brodie and Kwan, 2012).

The development of the medical imaging devices, especially computer tomography (CT) scanning, magnetic resonance imaging (MRI) and MRI spectroscopy and positron emission tomography is another victory in the understanding and treatment of epilepsy. The first neuroimaging technique ever is the so-called 'human circulation balance' and was invented by Angelo Mosso in the 1880s. It was able to non-invasively measure the redistribution of blood during emotional and intellectual activity. These techniques have revealed many of the more subtle brain lesions responsible for epilepsy. Any type of brain lesion (e.g. trauma, congenital, developmental, infection, vascular, tumour, degenerative) can lead to epilepsy in some patients (Sandrone et al., 2012).

As in any other disorders' management, during the last decades greater attention has been paid to the psychological and social needs and quality of life issues of people with epilepsy.

In modern days 80% of people with epilepsy live in developing countries and for most of them the older supernatural views, social stigma and discrimination still prevail. Even in the developed world, the disorder is still shrouded in secrecy, and people prefer not to reveal or discuss their illness (Newton and Garcia, 2012).

The International League Against Epilepsy, a world-wide professional organization, was founded in 1909 and is growing rapidly, with chapters in 60 countries. Also, the International Bureau for Epilepsy, the equivalent lay organization, was founded in 1962 and is also rapidly expanding, with 50 national chapters. Since 1997, they joined forces with the World Health Organization in the Global Anti-Epilepsy Campaign aimed at improving prevention, treatment, care and services for those with epilepsy and raising public awareness of the disorder and its acceptability (Schachter, 2008; WHO, 2013).

World Health Organisation admitted types of seizures are:

- Focal (or partial) seizures - occur when seizure activity is limited to a part of one brain hemisphere. There is a site, or a focus, in the brain where the seizure begins. There are two types of focal seizures: with retained awareness or its loss.

- Generalized Seizures - occur when there is widespread seizure activity in the left and right hemispheres of the brain. The different types of generalized seizures are: absence seizures (formerly known as petit mal); tonic-clonic or convulsive seizures (formerly known as grand mal); atonic seizures (also known as drop attacks); clonic seizures; tonic seizures; myoclonic seizures
- Additional Seizure are infantile spasms and psychogenic non-epileptic seizures (PNES) (Stafstrom and Carmant, 2015).

Risk factors for epilepsy are still under scientific disputing. The importance of inheritance in the etiology of epilepsy is well established, but the underlying genetic mechanisms remain poorly understood. One important question is the degree to which genetic susceptibility contributes to epilepsy following an identified environmental insult (Ottman et al. 1996).

Approximately 25% of prevalent epilepsy is associated with an antecedent central nervous system (CNS) injury and accordingly is classified as “symptomatic”. The remainder without identified cause is assigned by the International League Against Epilepsy (ILAE) classification of epilepsy syndromes into two broad classes, “idiopathic,” reserved for syndromes of presumed genetic origin, and “cryptogenic,” for syndromes presumed to be nongenetic but with insufficient evidence to assign a specific etiology (Hauser et al., 1991; ILAE, 1989).

A history of febrile seizures, family history of seizures, birth difficulties and neonatal insults is significantly associated with the development of lifetime epilepsy (Mung'ala-Odera et al., 2008).

The unique anatomy of the vagus nerve provides a convenient peripheral medium in which to influence the brain without the invasiveness of intracranial surgery. While clinical efficacy is often modest, vagus nerve stimulation (VNS) has been successful, due in large part to patient acceptability, safety, and a low incidence of side effects. Stimulation of the right-sided cervical vagus nerve appears to be feasible, both from a safety and efficacy perspective. Second, the laterality of VNS effects may be important for the ultimate goal of any epilepsy therapy: complete remission of seizures (Krahl, 2012).

The epileptic seizures along with mental deterioration may compromise the oral and dental care resulting in numerous decayed teeth. This condition rises particular endodontic, surgical, and prosthodontic issues of the management of epileptic mentally challenged patient in the dental office (Joshi,2013).

Epileptic patients have a variety of unique medical and dental needs. A good health history to fully understand the patient's disease, and the medications they are taking is essential. To minimize the seizures due to stress and anxiety, appointments were scheduled during a time of the day when seizures activity is less likely to occur. A seizure triggering factor like operating light on the eyes was prevented by using dark glasses. A proper oral examination to uncover any dental problems and possible oral effects of anti-epileptic drugs is necessary. Some simple and straightforward treatment planning considerations will ensure the patient's oral health is properly maintained (Aragon and Burneo, 2007; Fiske and Boyle, 2002; Jacobsen and Eden, 2008; Károlyházy et al., 2003).

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 patients. Translational neuroscience, 2019; 10(1): 223-232.
2. Constantinescu V, Matei M, Constantinescu I, **Cuciureanu DI**. Cardiac autonomic modulation in drug-resistant epilepsy patients after vagus nerve stimulation therapy. Neurologia i Neurochirurgia Polska; 2020; DOI:10.5603/PJNNS.a2020.0044.
3. Mihai C, Budacu CC, Timofte A, **Cuciureanu DI**, Forna NC. Epilepsy, a neurological emergency in oro-maxi-facial surgery-neuro-psychic field. Revista de chimie, 2019; 70(7): 3041-3045.

2.2. The excited paroxysm

2.2.1. Introduction

Epilepsy affects approximately 65 million people worldwide (Moshé et al., 2015). Although therapy has substantially developed, about a third of patients remain resistant to drug treatment (Moshé et al., 2015). This leads to high mortality and morbidity (Devinsky et al., 2016; Harden et al., 2017). Prevention measures and recognition of modifiable risk factors may reduce epilepsy mortality.

Patients with drug-resistant epilepsy, defined as seizure occurrence despite judicious association of two or more antiepileptic drugs over a minimum of 12 months period (Kwan et al., 2010), may benefit from epilepsy surgery. Currently, this is performed in a small subset of drug-resistant patients. Vagus nerve stimulation (VNS) represents an adjuvant treatment for medically refractory partial-onset seizures in adults and adolescents (Panebianco et al., 2016). VNS consists of chronic intermittent electrical stimulation of the vagus nerve, delivered by a programmable pulse generator (Panebianco et al., 2016). VNS may represent an earlier stage option in treating pharmacoresistant epilepsy, with positive long-term effects (Morris et al., 2013; Ryvlin et al., 2014), reducing the frequency of seizures and ameliorating the quality of the interictal period (Ryvlin et al., 2014).

Since VNS was approved as a therapeutic approach for the treatment of refractory epilepsy, the search has been ongoing for nonpharmacological modulation of the autonomic nervous system (ANS) for different pathological conditions. The advantage of VNS therapy has also been evaluated for drug-resistant depression, heart failure, hypertension, and cardiac arrhythmias (Premchand et al., 2016; Lee et al., 2018).

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 (Garamend et al., 2017). 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 (Schachter and Saper, 1998; Henry, 2002). The activation of the vagal efferent pathways concerns the sinoatrial node and the cardiac conduction system (Henry, 2002; Premchand et al., 2016). 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 (Garamendi et al., 2017). Cardiac bradyarrhythmia is a rare complication during ongoing VNS therapy (Amark et al., 2007). An increase in cardiac vagal modulation appears to play a cardioprotective role against sudden death (Schomer et al., 2014).

Heart rate variability (HRV) describes the variations between consecutive heartbeats, known as RR intervals, on ECG recordings. Frequency-domain analysis allows the assessment of the global variation of a biologic signal, divided into its different spectral components (Bernardi et al., 1995). The presence of different frequency spectra can be attributed to the modulation of the ANS on cardiovascular activity (Saul, 1991; Hilz, 2002). Time-domain indices of HRV evaluate the amount of variability in measurements of the interbeat interval. Mechanisms involved in cardiovascular regulation interact with each other in a non-linear manner (Voss et al., 1996; Huikuri et al., 2003). Non-linear dynamics can be evaluated with the help of chaos theory, offering a more detailed perspective of the HRV. Fractal methods assess the scaling exponent of the signal which indicates the presence of fractal properties, or self-similarity of beat-to-beat intervals — the RR intervals on the ECG recordings (Golińska, 2012). Entropy measures have been widely used in HRV analysis, assessing the irregularity and complexity of HRV (Chen et al., 2017).

The rationale for implementing the analysis of the non-linear dynamics of HRV is thus to better understand the mechanisms of cardiac autonomic control. It has been demonstrated that alteration of fractal properties precedes the onset of fatal cardiac arrhythmias (Mäkikallio et al., 1999), as the increased regularity and the loss of complexity in the heart rate signal is related to the dysregulation of cardiac autonomic control (Mäkikallio et al., 1996; Varadhan et al., 2009). A decreased HRV was initially shown to be predictive of mortality in the elderly population (Tsuji et al., 1994). Moreover, in epilepsy patients, it appears to be associated with an increased risk of sudden unexpected death (SUDEP) (Myers et al., 2018). Therefore, HRV analysis may identify patients with autonomic dysregulation at risk of fatal cardiac arrhythmias (Myers et al., 2018).

The same group of patients was previously assessed using Multiple Trigonometric Regressive Spectral analysis, using a different analytical approach (Constantinescu, 2019). The actual evaluation based on Fast Fourier Transform provides, in addition to the spectral power analysis, the non-linear appraisal of the HRV. Since this has not been previously described (Constantinescu, 2019), we considered it worthy of further analysis.

VNS exerts through its diffuse projection via the nucleus of the solitary tract, or the reticular system, a cortical modulating effect, especially involving cerebral structures related to autonomic regulation, such as thalamus, amygdala or prefrontal region (Fornai et al., 2011). The impact of VNS on cardiovascular autonomic function in drug-resistant epilepsy patients remains a controversial subject and in need of further studies (Garamendi et al., 2017).

Heart rate variability (HRV) represents a simple and non-invasive method to evaluate the sympathovagal balance, outlining the cardiac ability to adapt to hemodynamic and pathological conditions. Sympathetic hyperactivation and reduced cardiac vagal modulation associated with low HRV determines higher risk of cardiac arrhythmia and sudden death (Task Force of the European Society of Cardiology 1996).

Rüdiger et al. proposed a novel algorithm to detect physiological oscillations of the heart rate (HR) based on RR intervals measurements – multiple trigonometric regressive spectral (MTRS) analysis (Rüdiger et al., 1999). HRV analysis in epilepsy provides essential information about the risk of sudden death by cardiac arrhythmias in these patients (Myers et al., 2018).

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. The clinical rationale of this study was to assess the effects of VNS on cardiovascular autonomic function in different physiological conditions in drug-resistant epilepsy patients, over three months of neurostimulation. The results of the study may be clinically useful for detecting a cardiac activity adjustment in the analysed epilepsy patients, and may contribute to better understanding of the effect of VNS on the autonomic cardiac activity.

2.2.2. Material and methods

⇒ **Study 1 - heart rate variability and vagus nerve stimulation in epilepsy patients**

ECG recordings of the first five drug-resistant epilepsy patients from our department who underwent left laterocervical 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.

Blood tests including electrolytes, hepatic and renal function were within the normal range for all patients in both evaluations.

All patients were informed about the study protocol and gave written consent in accordance with ethical principles. The study was carried out in accordance with the Helsinki Declaration.

We applied a standardized protocol including resting state and subsequently four autonomic activation tests, each test entailing a five minute ECG recording. There was a HR stabilization before starting the evaluation. Tests were performed at the same time range (4-6 PM), after 30 minutes of resting position in clinostatism, at a constant temperature of 22OC, in a quiet room, in the absence of sounds or human voices, without prior physical effort or ingestion of caffeinated or alcoholic beverages 24 hours before the evaluation. All patients were asked to empty the bladder before starting the evaluation. Moreover, the tests were performed at least three hours after lunch in order to avoid gastric distension. Two sympathetic activation tests were performed: a maximal voluntary isometric contraction of the fist, using a dynamometer – "hand- grip" 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. The test sequence was standardized: resting state, deep breathing test, hand-grip test, standing test and Valsalva maneuver for all patients.

BIOPAC® acquisition system was used for data collection and analysis. BIOPAC® represents an integrated hardware and software system that converts biologic parameters like HR to numeric data. AcqKnowledge software version 3.9.1.6 was used for refining the recorded data. Data processing was done using MTRS software version 7.3.2.0 (University Hospital, Center for Clinical Neuroscience, Dresden, Germany). Before each analysis, a manual data correction of ECG artifacts was carried out.

The dynamic assessment of HRV by MTRS analysis allows a precise evaluation of cardiovascular modulation under different conditions. This software assesses the HRV time-domain and frequency-domain parameters, based on the trigonometric regressive analysis (Li et al., 2011). 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 (Rüdiger et al., 1999).

MTRS works with two data segments, a global and a local data segment. The global data segment considered was the length of the ECG recording (5 minutes) and the local data segment was set - 30 seconds. The analysis is performed in all localdatasegments, until the end of the global data segment. All oscillations of the biosignals (RR intervals) are analyzed using a trigonometric function of the parameters A (amplitude), ω (frequency), and φ (phase shift) (Li et al., 2011). For each recording, the same settings of the local data segment were applied: a minimum variance reduction of 1%, a shift of the local data segment of 1 RR interval and Delta frequency 0.006 Hz. These settings remained unchanged for all patients. Considering non-stationary signals analysis, a trend correction over every local data segment was performed automatically in each local time window before the final analysis.

Heart rhythm oscillations may be categorized into four primary frequency bands: Ultra Low Frequency (ULF), Very Low Frequency (VLF), Low Frequency (LF) and High Frequency (HF). Respiration modulates vagal activity and contributes to the HF component of the spectra, ranging from 0.15Hz to 0.4Hz. Recent clinical and animal studies concluded that the LF component (0,04-0,15 Hz) of the HRV probably reveals a complex and not easily discernible mix of sympathetic, parasympathetic, and other factors interaction, and does not accurately reflect changes in the sympathetic activity (Billman 2013), as initially presumed. Other studies suggested that LF component reflects the baroreflex-mediated, phasic changes

in cardiovagal and sympathetic noradrenergic outflows (Moak et al., 2007). As a consequence, the physiological basis for LF/HF is difficult to discern (Billman 2013). The LF and HF values may also be calculated in normalized units (LFnu, HFnu) defining the relative values of each frequency spectrum reported to the total spectral power, from which the VLF component was excluded from the calculation.

The following time-domain parameters were considered: pNN50 (the proportion of pairs of successive RR intervals that differ by more than 50 ms to the total number of NN intervals) and RMSSD (the square root of the mean squared differences of successive NN intervals). These two parameters reflect the parasympathetic influence on the cardiac rhythm (Task Force of the European Society of Cardiology 1996).

HRV is significantly correlated with an average HR, dependent on the influence of autonomic nervous system activity (Task Force of the European Society of Cardiology 1996) and also mathematically determined (i.e., the inverse non-linear relationships between HR and RR interval) (Gasior et al., 2018). Therefore, it is needed to distinguish if the clinical significance of HRV comes from the variability or from HR (Sacha and Pluta2008). If the variability of a slow HR is compared with a fast HR (based on the fluctuations of RR intervals), greater HRV in patients with former than with the latter can be obtained (Sacha and Pluta 2005). To reduce this influence, we corrected the HRV for the average HR. The correction consisted of dividing or multiplying standard HRV indices by the power of two of their corresponding mean RR interval (mRR) (Gasior et al., 2018).

This correction procedure does not remove any information about the signal's oscillations but only makes the oscillations relative to the signal's average value (Sacha and Pluta 2005). This allows the comparison of HRV among patients with different average HRs (Sacha et al., 2013; Monfredi et al., 2014).

HRV is also influenced by the respiratory rate (RespRate). Therefore, alterations in these parameters may impose changes in HRV (Gasior et al., 2018; Gasior et al., 2016). A decrease in respiratory frequency generally corresponds with a lengthening of the heart period (Bruce 1996; Quintana and Heathers 2014). RespRate may be calculated from ECG recordings according to reaserchers (Sinnecker et al. 2014).

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 - cardiac autonomic modulation in drug-resistant epilepsy patients after vagus nerve stimulation therapy

ECG recordings of the first five patients with drug-resistant epilepsy who underwent VNS procedure, in our department, were analysed. In these patients, seizure control was not obtained within two years of multiple antiepileptic drug treatment. Epilepsy surgery was not a viable option in any of the five patients. 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 standardised protocol consisting of a resting state ECG recording followed by four autonomic activation tests, each lasting for five minutes, was applied. Standardised conditions imposed the following criteria: ECG recording at the same time range after 30 minutes of clinostatism rest, in the absence of noise, at a constant temperature of 22°C, without previous physical activity or the ingestion of beverages containing caffeine. The four autonomic activation tests were performed in the same sequence in all patients, as follows: deep breathing, standing, hand-grip and Valsalva manoeuvre. To remove the respiratory influence on the heart rate, the patients followed a paced breathing pattern at 15 cycles per minute, as described in other studies (Budrejko et al., 2018). During the deep breathing test, a complete deep inhale and exhale lasted 10 seconds, six complete cycles per minute, to emphasise the vagal activation. The following sympathetic activation tests were performed: the standing test and the hand-grip test, consisting of a three-minute isometric contraction of the fist, using a dynamometer. BIOPAC® acquisition system was used for the ECG recording. AcqKnowledge software version 3.9.1.6 eliminated artifacts of the recorded signal. HRV analysis was performed using Kubios HRV software version 2.2 (Biosignal Analysis and Medical Imaging Group, University of Eastern Finland). HRV parameters were analysed using Fast Fourier Transform. A minimum of 256 RR intervals on the ECG were analysed for each recording.

The following HRV time-domain parameters were analysed: Root Mean Square of the Successive Differences (RMSSD), the proportion of pairs of successive RR intervals that differ by more than 50 ms to the total number of NN intervals (pNN50), the standard deviation of the so-called normal-to-normal NN interval that reflects all the cyclic components responsible for variability in the period of recording (SDNN), the mean RR interval, and the heart rate [9]. The frequency-domain analysis referred to the following spectral components: VLF (very low frequency power, 0.02–0.04 Hz), LF (low frequency power, 0.04–0.15 Hz) and HF (high frequency power, 0.15–0.4 Hz). These parameters were expressed in absolute values (ms^2), and in relative values calculated as a percentage (%) to total power. Given the complex mechanisms that seem to influence the values of the VLF spectrum (i.e. thermoregulatory mechanisms, activity of the renin-angiotensin-aldosterone system), we analysed the normalised units (nu) for the LF and HF spectrum, which excluded from calculation VLF values, and the LF/HF ratio (the ratio of absolute LF power to HF power). RMSSD, pNN50, HF and HFnu parameters are considered to be markers of parasympathetic autonomic control on the heart rate (Hilz, 2002).

The following HRV parameters were considered for the non-linear analysis: SD1, SD2, SampEn (Sample Entropy), ApEn (Approximate Entropy), DFA α1 and DFA α2 (Detrended Fluctuation Analysis α1 and α2). When analysing the Poincaré graph, SD1 shows the short-term variability of a chronological series, while SD2 is the second component of the ellipse formed from the point cloud (which has as abscissa and ordinate two consecutive R-R values from the ECG recording) and it illustrates the long-term variability of the biological signals (Shaffer and Ginsberg, 2017). SD1 represents the standard deviation of the Poincaré plot and

is graphically perpendicular to the line-of-identity, while SD2 is along the line-of-identity. SD1 is considered a parameter that reflects the influence of parasympathetic tone on the control of the sinus node, being an expression of the rapid changes of the RR interval, since the vagal effects on the sinus node manifest faster than those mediated by the sympathetic nervous system (Mourot et al., 2004). ApEn measures the 'disorder' in the heart rate signal and quantifies the regularity and complexity of the chronological series (Peng et al., 1995). SampEn is a more constant measure derived from ApEn (Corino et al., 2006), which quantifies the complexity of the signal in short time segments (Yeh et al., 2009), low values of SampEn indicating a greater similarity between successive RR in chronological series. Detrended Fluctuation Analysis (DFA) is an evaluation method of the statistical 'self-affinity', assessing the regularity and complexity of the biosignals (Absil et al., 1999; Acharya et al., 2002) when the RR interval is analysed. Values of the scalar exponent α that are higher than 1 illustrate an increase in regularity and a decrease in signal complexity in the chronological series, with an increased self-correlation power, constantly associated with pathological conditions (Pikkujämsä et al., 1999; Germán-Salló et al., 2016). α1 represents the short-range scaling exponent, while α2 represents the long-range scaling exponent.

We referred mainly to the SD1, SampEn, ApEn and DFA α1 parameters, as our ECG recordings lasted five minutes.

All five patients underwent left laterocervical stimulation of the vagus nerve and had no cardiovascular comorbidities or cardiovascular medication. 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.

Biological parameters (blood pressure, oxygen saturation, renal and hepatic function and blood electrolytes) were within the normal range for all five patients. The antiepileptic medication was unchanged either in the three months before the first ECG recordings or between the two HRV tests. Blood pressure was measured in supine and orthostatic position for each patient after both evaluations.

Patients were recruited from the neurological department, and all patients were duly informed according to the study protocol and consented to the assessment in accordance with ethical principles. This study was carried out in accordance with the Helsinki Declaration.

For the statistical analysis of data, taking into consideration the small sample size, series normalisation was very difficult. Applied comparative tests were specific to the characteristics of the analysed parameters. A value of $p < 0.05$ was considered significant. GraphPad Prism software version 6.07 was used for the analysis and graphical presentation of the data.

Description of patients

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

The second patient, a 34-year-old female, presented focal epilepsy (left insular epilepsy) with secondarily generalised seizures. The clinical symptoms were nausea, dyspnoea, abnormal sensation of retrosternal pain, burning heat restricted to the perioral area, and anarthria. 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 generalised seizures (rotatory vertigo, facial flush, sense of unreality, and subsequent generalisation) at the age of 22. No epilepsy-related brain MRI abnormalities. Patient under treatment with levetiracetam and oxcarbazepine.

The fourth patient, a 29-year-old female, presented multifocal epilepsy with secondarily generalised seizures (onset features: vertigo, sweating and motor unilateral symptoms, motor aphasia and generalisation). 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 generalised 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.

2.2.3. Results

⇒ **Study 1 - heart rate variability and vagus nerve stimulation in epilepsy patients**

Clinical symptoms, type of epilepsy, age of onset, cerebral MRI findings and current treatment are depicted in **table 2.1**. 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. VNS parameters of the five patients are summarized in **table 2.2.**

Our ECG recordings did not reveal bradycardia, tachycardia or other cardiac arrhythmias in resting state or during challenge for all five patients. MTRS analysis of the ECG recordings from the five patients during resting state and autonomic activation tests provided time-domain (**table 3**) 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.

In order to analyze the relationship before and after correction of the parameters, we performed Wilcoxon matched-pairs signed rank test. Spearman's rank correlation coefficient "rs", calculated comparing values before and after normalization, is displayed in **table 2.4** for each patient.

Table 2.1. *Patients description*

Patient age/gender	Clinical Symptoms	Age of onset	Type of epilepsy	Brain MRI	Current Treatment
Patient 1 33/female	Rotatory vertigo, breaathing 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 senzation of retrosternal pain, nausea, dyspnea, burning "heat" restricted in the pectoral area, anarthria	6	Focal epilepsy (left insular epilepsy)	No epilepsy-related abnormalities	Valproic acid and livetiracetam
Patient 3 31/male	Retrosternal ascending "heat", hypersalivation and post-ictal psyhomotor agitation with heteroaggressive 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 2.2. *VNS parameters of the five patients*

VNS parameters	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Normal mode - Output current	2 mA	1.5 mA	1 mA	2 mA	2 mA
Normal mode - Frequency	30Hz	30Hz	30Hz	30Hz	30Hz
Normal mode - Pulse width	500 µsec	500 µsec	500 µsec	500 µsec	500 µsec
Normal mode - Duty Cycle	10%	10%	10%	10%	10%
Normal mode - ON time	30 sec	30 sec	30 sec	30 sec	30 sec
Normal mode - OFF time	5 min	5 min	5 min	5 min	5 min

The HR and RespRate correlation with the same above mentioned parameters is displayed in **table 2.5**. Similar to HR correlation, RMSSD, pNN50 and HF presented a negative correlation with RespRate, respectively LF/HF presented a positive correlation with RespRate.

Table 2.3. *Time-domain parameters RMSSD and pNN50 provided by MTRS analysis*

RMSSD (ms) / mRR (ms)					
Criteria	RS	DB	HG	ST	VA
Patient 1	16.92/672/04	17.92/654.24	15.43/659.62	14.23/648.60	16.86/650.82
	14.96/659.72	16.02/675.86	13.92/675.74	10.55/586.11	15.33/707.65
Patient 2	32.60/784.96	31.92/766.67	26.32/767.27	22.13/757.84	50.74/762.54
	56.97/882.40	54.84/822.0	35.99/766.96	31.57/816.03	68.51/897.17
Patient 3	30.60/814.14	30.66/814.73	19.62/766.57	21.78/760.08	25.01/802.67
	52.72/807.33	51.86/813.28	49.29/713.58	50.15/710.23	51.86/801.46
Patient 4	13.95/753.19	15.11/790.86	13.07/734.40	9.70/752.70	16.55/790.91
	13.47/809.55	15.47/825.38	12.77/784.77	17.94/799.35	21.64/822.14
Patient 5	16.04/666.29	19.85/660.71	17.96/674.05	14.40/641.75	21.37/674.19
	19.53/660.71	24.59/727.13	22.47/743.25	16.64/678.53	28.18/763.04
pNN50 (%) / mRR (ms)					
Patient 1	0/672.04	0/654.24	0/659.62	0/648.60	0.650.82
	0.50/659.72	0.42/675.86	0.675.74	0/586.11	0.65/707.65
Patient 2	10/784.96	8.02/766.86	4.5/767.27	3.14/757.84	11.11/762.54
	28.03/882.40	15.31/822.0	10.95/766.96	12.59/816.03	28.07/897.17
Patient 3	7.08/814.14	7.17/814.73	2.08/776.57	3.94/760.08	5.99/802.67
	23.99/807.33	26.14/813.28	12.26/713.58	12.50/710.23	27.33/801.46
Patient 4	0.75/753.19	1.11/790.86	0.58/734.40	0/752.70	1.90/790.91
	0/908.55	1.20/825.38	0.48/784.77	0.39/799.35	3.68/822.14
Patient 5	0.70/666.29	3.44/660.71	0.23/674.05	0.88/641.75	3.71/674.19
	0.75/660.71	4.63/727.13	2.08/743.25	0.31/678.53	5.04/763.04

Test 1 (HRV parameter/mRR), Test 2 (HRV parameter/mRR); RS=resting state; DB=deep breathing test; HG=handgrip test; ST=standing test; VA=Valsalva maneuver; mRR=mean RR interval

Table 2.4. *Wilcoxon matched-pairs signed rank test before and after correction for HRV parameters*

Petient	**RMSSD**		**pNN50**		**HF**		**LH/HF**	
Patient 1	rs=0.87	p=0.0008	rs=0.99	p=0.0014	rs=0.92	p=0.0002	rs=0.96	p<0.0001
Patient 2	rs=0.90	p=0.0004	rs=0.97	p=0.0001	rs=0.93	p=0.0001	rs=0.97	p<0.0001
Patient 3	rs=0.81	p=0.0030	rs=0.99	p=0.0001	rs=0.90	p=0.0004	rs=0.95	p<0.0001
Patient 4	rs=0.83	p=0.0024	rs=0.98	p=0.0001	rs=0.93	p=0.0001	rs=0.96	p<0.0001
Patient 5	rs=0.90	p=0.0004	rs=0.98	p=0.0001	rs=0.45	p=0.0956	rs=0.83	p<0.0019

Table 2.5. *Correlation of HR (bpm) and Respiration Rate (breaths/min) with standard HRV parameters*

Heart rate (bpm)	RMSSD		pNN50		HF		LF/HF	
Patient 1	rs=0.63	p<0.05	rs=0.50	p<0.05	rs=0.63	p<0.05	rs=0.70	p<0.05
Patient 2	rs=0.78		rs=0.94		rs=0.65		rs=0.69	
Patient 3	rs=0.13		rs=0.21		rs=0.80		rs=0.80	
Patient 4	rs=0.64		rs=0.45		rs=0.46		rs=0.23	
Patient 5	rs=0.81		rs=0.44		rs=0.66		rs=0.63	
Resp Rate(breaths/min)								
Patient 1	rs=0.34	p<0.05	rs=0.71	p<0.05	rs=0.04	p<0.05	rs=0.32	p<0.05
Patient 2	rs=0.90		rs=0.80		rs=0.58		rs=0.42	
Patient 3	rs=0.72		rs=0.86		rs=0.60		rs=0.41	
Patient 4	rs=0.70		rs=0.61		rs=0.51		rs=0.16	
Patient 5	rs=0.97		rs=0.80		rs=0.64		rs=0.45	

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 2.1**).

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 2.1**).

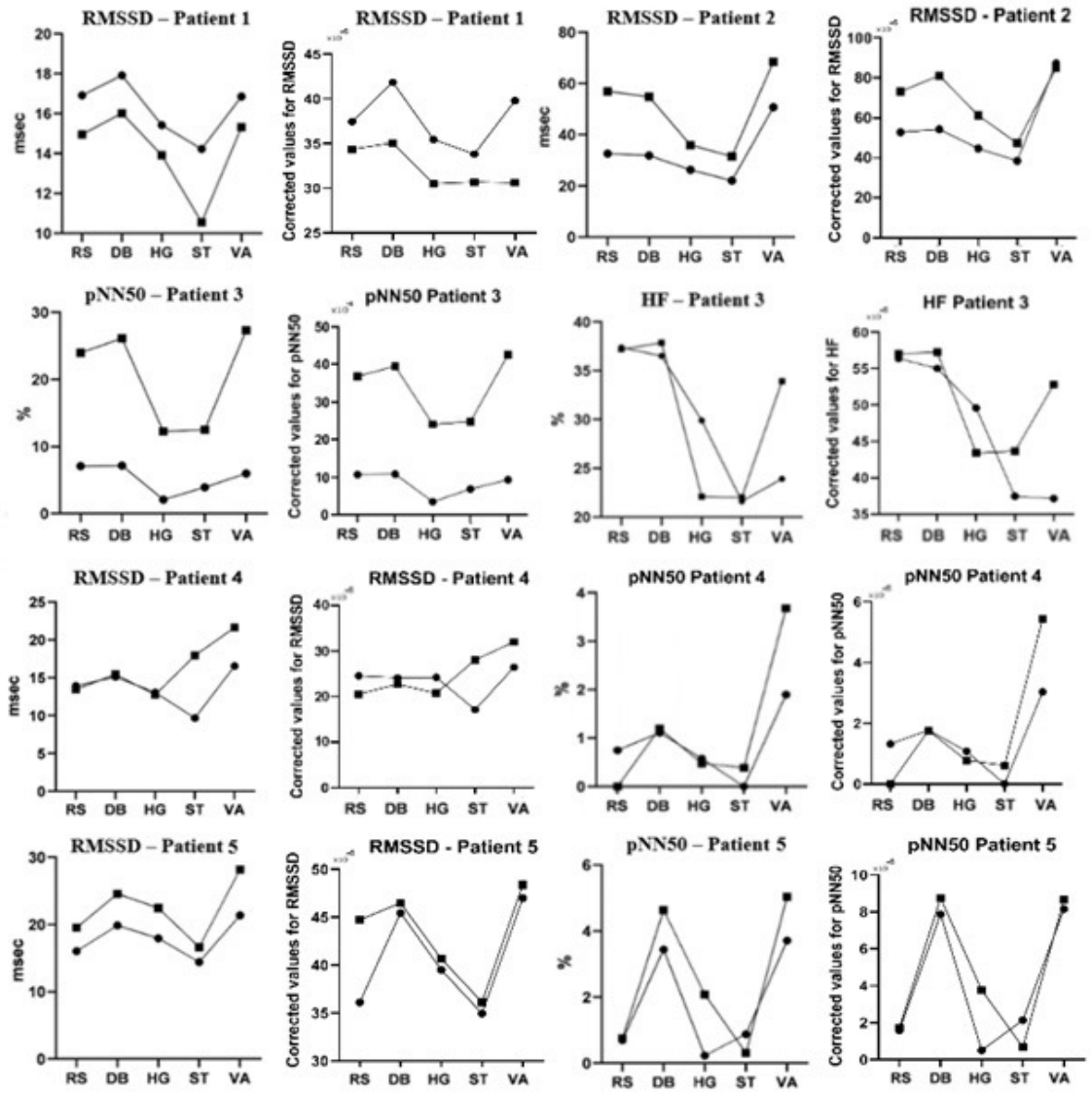

Figure 2.1. *HRV parameters of the five patients. RS=resting state, DB=deep breathing test, HG=hand-grip test, ST=standing test, VA=Valsalva maneuver, Test 1, Test 2*

The frequency-domain parameters for patient 3 during the second HRV evaluation in resting state are illustrated in **figure 2.2**.

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 of RMSSD values (**figure 2.3**).

This could be an example of the response to left VNS at an 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 2.3**).

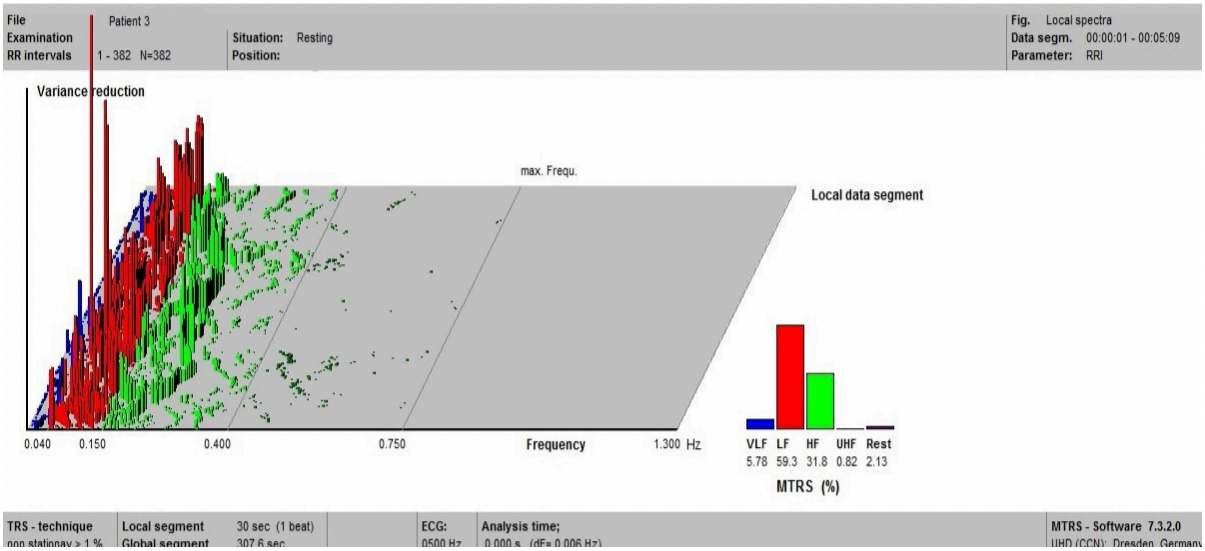

Figure 2.2. *HRV parameters of the third patient in resting state during test 2*

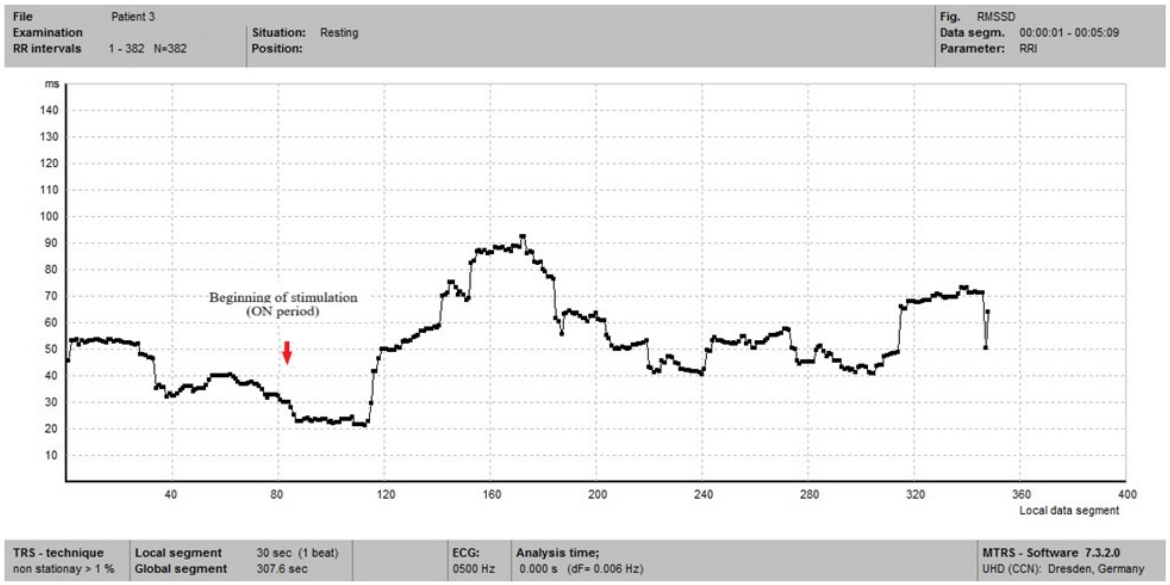

Figure 2.3. *RMSSD values of the third patient in resting state during test 2*

⇒ **Study 2 - cardiac autonomic modulation in drug-resistant epilepsy patients after vagus nerve stimulation therapy**

The first patient presented a sympathetic predominance on the heart rate control, as indicated by RMSSD, pNN50, HFnu and LF/HF values in resting state (**Tabel 2.6**). After the autonomic activation tests, the patient displayed an appropriate response of the cardiac autonomic regulation, as marked by the values of HFnu and LF/HF. Parasympathetic activation tests increased the HRV, illustrated by DFAα1 values (**Tabel 2.6**). There was no significant difference concerning the dynamic of HFnu and LF/ HF parameters in response to activation tests in the two HRV evaluations (**Figure 2.4**). 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 (**Figure 2.4**).

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 (**Figure 2.4**).

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 (**Figure 2.4).**

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 (**Figure 2.4**).

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 (**Tabel 2.6**). 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 (**Figure 2.4**).

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 (Figure 2.5). 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 ($p < 0.05$) (**Figure 2.5**). 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 (**Figure 2.5**). SDNN values presented a similar dynamic as RMSSD in response to the autonomic tests. Mean RR values were correlated to the heart rate values during the four autonomic tests (**Tabel 2.6**).

Table 2.6. *Heart rate variability (HRV) parameters for the five patients*

Test 1/ /Test 2	HR	Mean RR	SDNN	RMSSD	pNN50	LF (nu)	HF (nu)	LF/HF	SD1	ApEn	SampEn	DFAα1
RS Patient 1	90/91	671/660	39/31	13/15	0.6/0.5	84.8/77.4	14.7/22.5	5.74/3.4	16/10	1.2/1.1	1.3/1.3	1.1/1.4
DB Patient 1	92/87	653/685	48/36	66/45	1.7/0.5	36.5/44.6	56.4/55	0.64/0.8	46.8/32	1.1/1	1.1/1	0.6/1.2
ST Patient 1	92/96	655/632	62/25	77/22	2.1/2.8	69/77	30/22	2.27/3.4	54.5/15	0.8/1.1	0.8/1.1	0.8/1.3
HG Patient 1	92/87	653/683	72/22	86/14	2.7/0.3	70/83.5	29/16.5	2.39/5	60/10.4	0.8/1.1	0.7/1.6	0.7/1.5
VA Patient 1	92/93	659/642	69/35	77/14	2.6/1	42.7/74.7	57.3/25.2	0.74/2.9	50/10.4	0.7/1	0.7/1	0.6/1.3
RS Patient 2	76/67	793/903	49/67	39/67	12/36	68/54.7	31/45.3	2.4/1.2	28/47	1.1/1	1.6/1.6	1.2/0.9
DB Patient 2	78/73	777/824	72/54	89/53	9.2/14.5	38.7/52.9	60.9/47	0.63/1.1	63/38	1/1	1/1.3	0.9/1.1
ST Patient 2	85/75	711/802	80/58	58/42	4.5/14.5	71/77	28/22	2.53/3.3	41/29	0.7/1	0.6/1.3	1.1/1.2
HG Patient 2	76/76	787/797	40/58	40/44	12.3/15.8	58/78.4	41.7/21.5	1.39/3.6	29/31	1.2/1.1	1.6/1.4	1.1/1.3
VA Patient 2	81/70	740/863	75/57	96/51	6.3/20	41/71	58.7/28.6	0.69/2.4	68/36	0.8/1	0.8/1.4	0.6/1.1
RS Patient 3	89/81	670/735	41/24	20/20	2.4/1.7	73.9/70	26/29.5	2.84/2.3	14/14	1/1.2	1.1/1.7	1.3/1.2
DB Patient 3	90/81	665/740	30/29	20/23	3.6/2.7	50/63.9	50/36	0.99/1.7	14/16	1/1.1	1.1/1.6	1.3/1.2
ST Patient 3	93/86	640/700	19/29	14/22	0.9/2.8	73/78	26/20	2.77/3.8	10/16	1.2/1.1	1.7/1.6	1.3/1.2
HG Patient 3	89/83	673/724	22/32	18/24	0.2/4.4	80/81.8	20/18.2	3.99/4.4	12.7/17	1.2/1	1.7/1.4	1.2/1.4
VA Patient 3	89/78	672/766	39/36	28/29	4.4/7	78/63	21/36	3.6/1.72	20/21	1/1	1.2/1.6	1.4/1.2
RS Patient 4	79/74	755/811	9/16	11/13.9	0.4/0.3	77/76	22/23	3.5/3.2	35/9	0.8/1.1	0.8/1.6	1/1.4
DB Patient 4	76/72	790/823	31/18	28/15	4/1.2	73/52	26.9/47	2.7/1.1	20/11	1/1.1	1.1/1.6	1.2/1.1
ST Patient 4	82/81	737/743	27/25	19/17	2.5/0.9	84/81	15/18	5.5/4.4	14/12	0.9/0.9	1.2/0.6	1.4/1.2
HG Patient 4	81/76	743/787	40/17	37/13	2.5/0.5	73.7/81	26.1/18	2.82/4.3	26/9	0.9/1.2	0.9/1.5	0.5/1.3
VA Patient 4	76/72	792/826	46/32	58/23	2.3/4.9	60.4/71	39.3/28	1.52/2.5	41/16	0.9/1	0.9/1.3	0.9/1.2
RS Patient 5	75/73	805/814	85/43	54/29.9	25/6.9	62/58.3	37/41.7	1.68/1.3	38.8/21	1/1.2	1.2/1.6	1.1/1
DB Patient 5	74/76	811/791	87/88	52/111.5	27/6.8	50.9/43.7	48.9/55	1.04/0.7	36.8/78	1/0.8	1.1/0.7	1.2/0.9
ST Patient 5	81/77	754/782	104/93	59.8/24.6	20.1/5.1	65.2/65.2	34.6/34.8	1.88/1.8	42.3/17	0.8/0.7	0.8/0.5	1.1/1.4
HG Patient 5	75/77	805/776	93/42	57.8/20.2	27.8/2.5	66.1/73.4	33.8/26.5	1.95/2.7	40.9/14	1/1	1.1/1.2	1.1/1.2
VA Patient 5	69/75	883/795	132/46	146/24.2	52.4/4.7	39.2/84.9	60.2/15.1	0.65/5.6	104/17	1.1/1	1.4/1.2	0.8/1.4

HR — heart rate; RR interval — variations between consecutive heartbeats; SDNN — standard deviation of Normal-to-Normal intervals; RMSSD — Root Mean Square of Successive Differences; pNN50 — the proportion of NN50, representing the number of pairs of successive NNs that differ by more than 50 ms, divided by total number of NNs; LF — low frequency power; HF — high frequency power; ApEn — Approximate Entropy; SampEn — Sample Entropy; SD1 – standard deviation of instantaneous beat-to-beat interval variability; DFA – detrended fluctuation analysi

No patient presented orthostatic hypotension, defined as a decrease of at least 20 mmHg in systolic blood pressure or of at least 10mmHg in diastolic blood pressure, after a three-minutes standing test, performed after ECG recording, incurred after the two HRV evaluations. During autonomic evaluations and the prolonged ECG recordings, no cardiac arrhythmias were identified for the five patients. Furthermore, the patients did not recall seizure-related symptoms during the current hospitalisation. During the autonomic tests performed, including the deep breathing test, the patients were not monitored by EEG, but no seizure was observed by the examiner or reported by the patient.

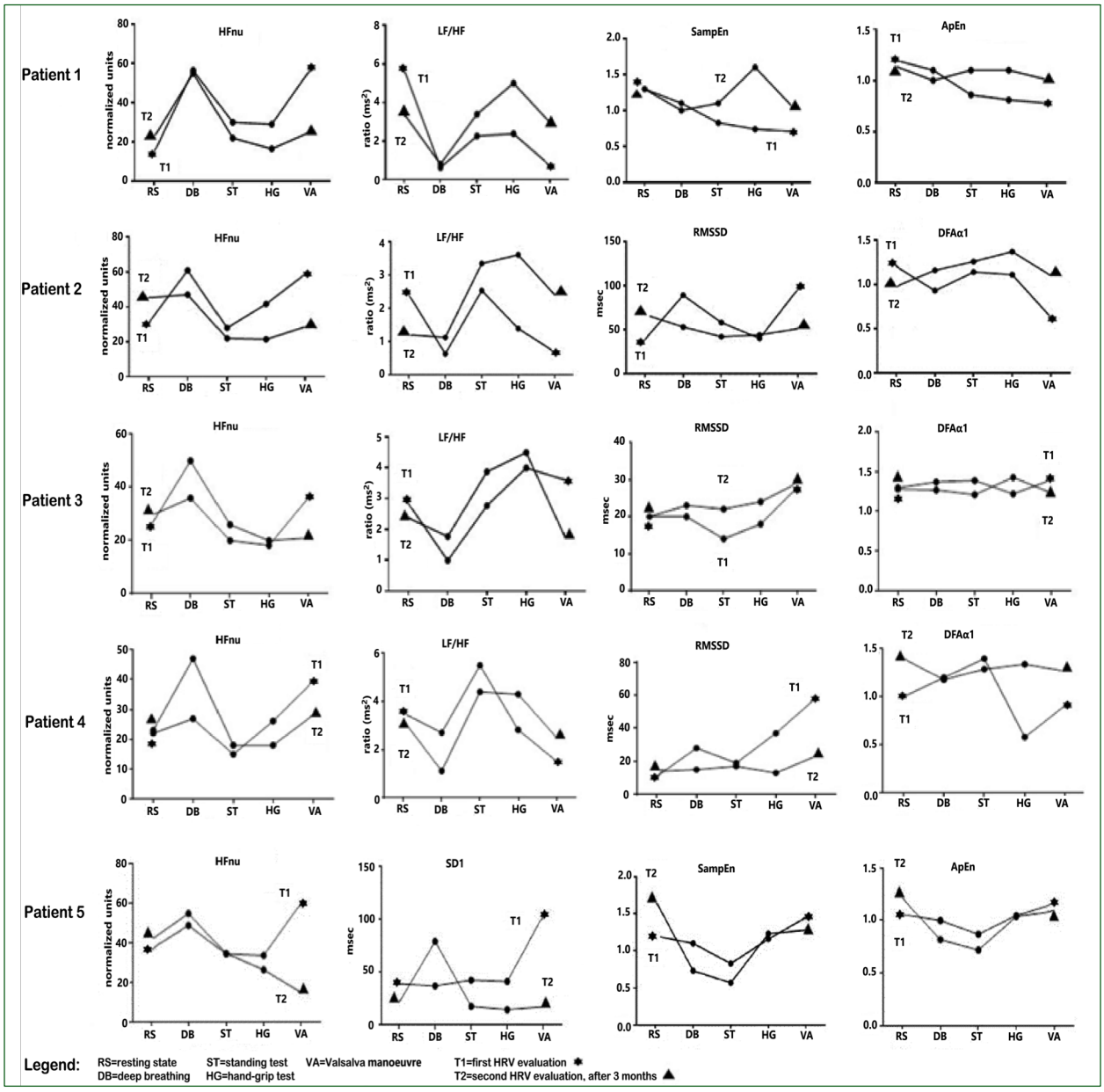

Figure 2.4. *Heart rate variability (HRV) parameters for the five patients*

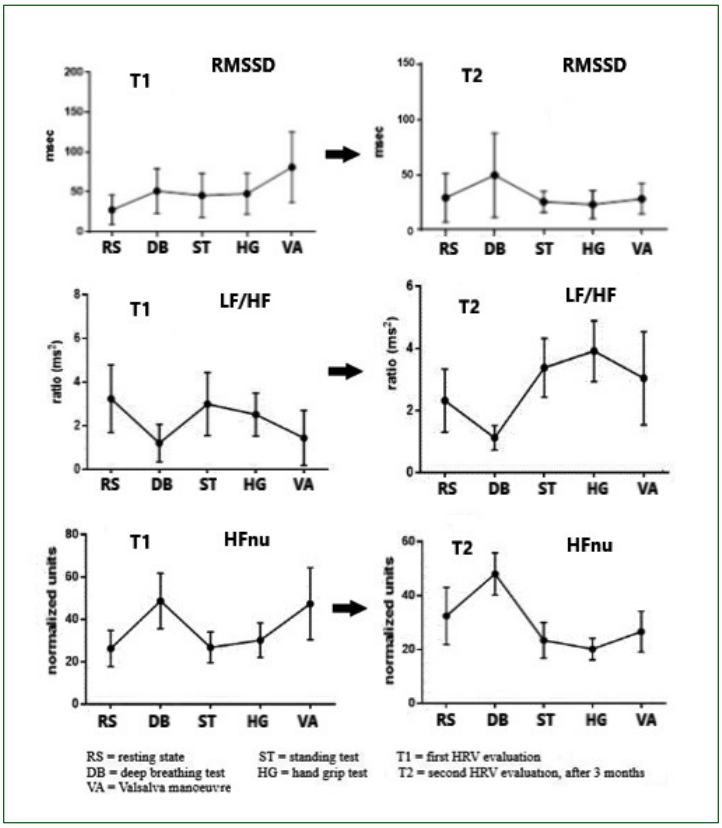

Figure 2.5 *Root Mean Square of the Successive Differences (RMSSD), low frequency power (LF)/ high frequency power (HF) ratio and HFnu dynamics for the five patients*

2.2.4. Discussions

Patients with refractory epilepsy may present decreased HRV, raising the concern that altered autonomic function might contribute to sudden unexpected death in epilepsy (SUDEP) (Massetani et al., 1997; Ansakorpi et al., 2002). Long-term recordings in these patients indicated that severe bradycardia or asystole may occur (Nei 2009; Rugg-Gunn et al., 2004), probably related to increased vagal tone associated with sleep. Clinical significance and potential association with the sympathovagal alteration still needs to be clarified. Epileptic seizures involving temporal or insular lobe are susceptible to such complications (Rugg-Gunn et al., 2004; Lacuey et al., 2016), highlighting the importance of interictal cardiac evaluation in these patients.

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 (Henry 2002). Activation of the vagal efferent pathways may alter the cardiovascular activity, through the sinoatrial node and the cardiac conduction system (Henry 2002; Premchand et al., 2016).

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. Cervical vagus nerve is a

mixed nerve containing both afferent and efferent axons, belonging to the sympathetic and parasympathetic nervous system (Bonaz et al., 2013; Hoover et al., 2008). Cardiac-related efferent projections contained within each cervical vagosympathetic trunk are predominantly parasympathetic preganglionic axons (Randall et al., 1985). Parasympathetic preganglionic projections arising bilaterally from nucleus amibiguus innervate multiple intrinsic cardiac ganglionic plexuses located within atrial and ventricular tissues, making direct contact with parasympathetic postganglionic neurons (Ardell et al., 2015).

There is anatomic and functional evidence indicating that the vagosympathetic trunk also contains a small number of sympathetic efferent fibers (Ardell et al., 2015; Onkka et al., 2013), that modulate cardiac function via the intrinsic cardiac nervous system (Randall et al., 2003), contributing to the beat-to-beat regulation. Left cervical VNS is believed to minimize potential bradycardia or asystole, primarily mediated by the right vagus nerve (Howland 2014). While left cervical VNS is approved for refractory epilepsy and resistant depression, right cervical VNS was clinically tested for heart failure (Howland 2014).

The cortical neuromodulation exerted by VNS therapy involves brain structures related to autonomic regulation, such as the prefrontal region, thalamus and amygdala (Fornai et al., 2011). Recent findings indicate that activated vagal afferents initiate cen- trally mediated reflexes that inhibit parasympathetic efferent outflows to the heart (Ardell et al., 2015), 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 (Ardell et al., 2015). 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 (Ardell et al., 2015).

Periodic VNS may effectively modulate heart rate dynamics. A phenomenon of pharmacological tolerance has been described, in which both vagus nerve and the autonomic nervous network adapt to periodic stimulation.

The originality of our study consists of using the autonomic activation tests (Ewing tests) and the analysis of non-linear HRV parameters besides time- and frequency-domain parameters for describing the cardiac autonomic response after sympathetic and parasympathetic challenge in patients with drug-resistant epilepsy, three months after vagal stimulation. Non-linear parameters have been used to analyse and predict the behaviour of biological phenomena. These parameters have proved to be good predictors of morbidity and mortality in clinics (Vanderlei et al., 2009).

Although it is known that drug-resistant epilepsy is associated with significant inhibition of vagal modulation of heart rate and lower HRV (Ronkainen et al., 2006; Liu et al., 2019), 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 sympathovagal balance during the autonomic tests.

Epilepsy and seizures can have dramatic effects on car- diac function, through the ANS. While some authors have concluded that VNS seems not to change decreased HRV in drug-resistant epilepsy patients (Ardell et al., 2015), others have reported that VNS improves HRV shortly after implantation via the extensive innervation of the vagus nerve into the sinoatrial and atrioventricular nodes (Galbarriatu et al., 2015). However, cardiac autonomic dysfunction related to VNS in epilepsy patients is rare (0.1%) (Ben-Menachem et al., 2001; Stemper et al., 2008; Ryvlin et al., 2014).

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 se- cond 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. This observation underlines the importance of non-linear analysis, which may provide useful information about the cardiac autonomic state.

Our results reveal that all patients presented adequate autonomic responses after sympathetic and parasympathetic activation tests, before VNS therapy, and three months after neurostimulation.

Although not necessarily reflecting a novel mechanism, the dynamic autonomic tests provide useful information regarding cardiac regulation. An impaired response to the autonomic challenge reflected by HRV parameters is not only a potential biomarker for monitoring progressive decline of the ANS system regulation, but is also a probable risk factor for sudden unexplained death determined by cardiac arrhythmias in patients with epilepsy (Baysal-Kirac et al., 2017).

Further studies are needed to assess whether a perpetuation of the sympathetic control and a decreased HRV, observed in some of our patients during sympathetic activation tests, is confirmed in larger populations of drug-resistant epilepsy patients during VNS therapy, and if it might pre- dispose to negative outcomes in patients with concurrent diseases. Currently, there is insufficient data to show how VNS influences different cardiac autonomic activity parameters within 24 hours, implying sleep-wake alternation, including physiological vulnerability periods of cardio-circulatory or respiratory control.

HRV analysis may be included in the current drugresistant epilepsy patient evaluation. Drug-resistant epilepsy patients who are non-responders to VNS therapy, defined as the lack of an at least 50% seizure reduction after one year of treatment, had significantly lower RMSSD, pNN50, HF, and SD1 than the responders. Thus, presurgical HRV evaluation measurements representing parasympathetic control on heart rate were significantly associated with the responsiveness to VNS (Liu et al., 2017) and may serve as a marker for the effectiveness of this therapeutic option, although further studies are needed to evaluate this hypothesis.

VNS has been shown to exert antiarrhythmic effects, improve left ventricular function, and reduce mortality in pa- tients with heart failure (Rousselet et al., 2014). The optimum VNS parameters define a stabilised state in which both afferent and efferent fibres are activated in a balanced manner, called ‘neural ful- crum’. VNS performed near this neural

fulcrum ensures an adequate response to stressors involving both central and peripheral components. It would be of interest to analyse whether reaching this cardiac autonomic balanced state would reduce the risk of fatal cardiac arrhythmia in epilepsy patients.

HRV study could, therefore, provide essential data about the neural fulcrum and could guide the adjustments of VNS parameters for the neurological target as well.

One limit of our study, besides the limited number of patients, is the interfering antiepileptic medication, especially sodium or potassium channel blockers that may alter the depolarisation-repolarisation potentials of the cardiac cells. It is therefore difficult to distinguish the medication-mediated effects from the cardiac dysautonomia present in epilepsy patients.

Chronic VNS influences both sympathetic and parasympathetic modulation but does not negatively influence autonomic cardiovascular regulation (Stemper et al., 2008; Ronkainen et al., 2006). Increasing parasympathetic activity seems to have cardioprotective effects against SUDEP (Schomer et al., 2014). Cardiac bradyarrhythmia represents a rare complication, few isolated cases during ongoing VNS therapy being reported (Asconape et al., 1999; Tatum et al., 1999; Ali et al., 2004; Iriarte et al., 2009; Amark et al., 2007). VNS may also enhance sympathetic output, raising hippocampal noradrenaline levels (Raedt et al., 2011). Locus coeruleus, the principal noradrenergic nucleus of the brain, is involved in the circuitry necessary for the anticonvulsant effectiveness of VNS (Krahl et al., 1998). Seizure suppression by VNS in drug-resistant epilepsy patients may, therefore, depend on the release of noradrenaline, a neuromodulator that has anticonvulsant effects.

Another cortical structure involved in autonomic regulation is insula. Insular lesions are associated with mortality through autonomic dysfunction (Rugg-Gunn et al., 2004). Electrical stimulation of the human insula produced cardiac chronotropic and blood pressure responses in epilepsy patients (Oppenheimer et al., 1992). Right insula seems to regulate the sympathetic tone, while left insula is associated with prevalent parasympathetic control (Oppenheimer et al., 1992). There is contradicting evidence supporting cortical lateralization of autonomic control (Di Gennaro et al., 2004).

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.

Antiepileptic drugs can also influence autonomic activity. Carbamazepine has been shown to affect the autonomic modulation among patients with temporal lobe epilepsy (Isojarvi et al., 1998; Ansakorpi et al., 2000), and withdrawal can increase cardiac sympathetic activity during sleep (Hennessy et al., 2001).

Patient 4 was under Carbamazepine and HRV analysis indicated a predominance of the sympathetic tonus in both evaluations, requiring further cardiac monitoring. Lamotrigine is supposed to have cardiac arrhythmogenic potential in certain metabolic conditions. Further research is needed to analyze the clinical impact.

The strengths of our report include MTRS analysis of the HRV on short ECG recordings (5 minutes) after autonomic activation tests. The advantage of MTRS analysis, which does not need interpolation on non-equidistant RR intervals contrary to Fast Fourier Transformation, is the assessment of shorter local data segments (Li et al., 2018). An

adequate correction designed to remove the HR influence on HRV should always be performed, due to physiological but also mathematical reasons (Sacha and Pluta 2008; Daniellson et al., 2005).

2.2.5. Final remarks

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.

Autonomic nervous system should be evaluated in epilepsy patients because of the risk of cardiac arrhythmias and SUDEP, particularly in the drug-resistant ones. We propose HRV analysis as a useful tool to assesssympathovagal balance and identify high-risk patients for cardiac arrhythmias. Moreover, HRV analysis could be a practical tool in identifying suitable patients for VNS therapy.

The involvement of ANS in patients with epilepsy has been insufficiently explored, and has produced conflicting results. Epilepsy patients present a risk of sudden unexpected death, autonomic dysfunction being one of the causes. However, the exact mechanism remains unclear. HRV is a useful method to assess the influence of ANS at the cardiac level.

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.

2.3. Serrated tremor

2.3.1. Introduction

The term epilepsy comes from ancient Greek and means crisis, convulsion or attack. It is popularly called black disease, a group of long-lasting neurological disorders characterized by one or more epileptic seizures.

Crises have a tendency to repeat, with no underlying cause, while crises arising due to a particular cause are not necessarily considered epilepsy. Epileptic seizures are the result of an excessive or abnormal activity of nerve cells in the cerebral cortex; diagnosis involves invalidating other conditions that may cause similar symptoms (such as syncope) and identifying other immediate causes. The most common types of seizures are convulsive; the rest of the crises are of an unconvulsive nature.

Epileptic seizures are periodic disorders of the electrical activity of the brain, leading to temporary cerebral dysfunction. When the nerve impulses are abnormally low, a crisis may occur (Fisher et al., 2005; Luders and Soheyl 2000; Meierkord et al., 2006; Skodda et al., 2001).

Epilepsy is a disease that originates in the cerebral cortex, in the so-called gray matter, which is part of the central nervous system; is not a form of intellectual retardation or mental illness and it is not contagious. The appearance of epilepsy is characterized by the imbalance between inhibition and excitability, which leads to damage to the cortex that becomes thus hypexcitable and will have uncontrolled electrical activity (Eadie, 2012; Thurman, 2011; Brodie, 2009).

Epilepsy is often the result of other diseases such as: cranial traumas, brain tumors, cerebral infections, strokes. The most common are the Jacksonian seizures that consist of tonic contractions localized to a member segment, have a whole membership (Goldberg and Coulter 2013). They are gradually propagated in the oil slick, and then secondary, they can be generalized, and transformed into a major crisis (Holmes et al., 2010; Newton, 2012).

The epileptic crisis, the tonic form evolves with cerebral anoxia, the patient becomes cyanotic. It is unjustified and exaggerated the fear, the unusual panic that encompasses entourage at the sight of an epileptic seizure. A crisis is a sudden electrical discharge in the brain that usually affects the way a person feels or behaves for a short period of time. Crises are not a disease in themselves, but are a symptom of several factors that can affect the brain. Some crises can go almost unnoticed, while others are dramatic.

In fact, even the generalised tonico-clonic seizures or major crises, by far the most dramatic forms of paroxysmal epileptic manifestation, occur spontaneously, they develop and pass by themselves, some especially if they occur in sleep, remaining unaware of anyone (Wilden, 2012; Berg, 2008; Devlin, 2012; Duncan, 2006).

A number of people with epilepsy suffer from seizures that are often triggered by certain events, known as reflex epileptic seizures. Individuals with reflex epilepsy suffer from seizures that are only triggered by certain stimuli.

Epilepsy can have various effects on social and psychological well-being. These effects include social isolation, stigmatization or disability, they can lead to poor school results and poor workplace outcomes. Learning difficulties are common in people with this condition, especially among children with epilepsy. The stigma of epilepsy can also affect the families of the patients. People with epilepsy may experience certain disorders, depending partially on the current epileptic syndrome, including depression, anxiety, and migraines.

The underlying cause of epilepsy may be of a genetic nature or due to metabolic or structural problems, but some of the cases are unknown. Genetic, congenital and developmental problems are the most common among young people, while brain tumours and strokes are more likely to occur in the elderly (Gross 1992). Genetics is a factor involved in most cases, either directly or indirectly. Some cases of epilepsy are due to a defect of a single gene; themajority of cases are due to the interaction between multiple genes and environmental factors.

In case of identical twins, if one of them is affected, the odds are of 60% so the other one is affected; in the case of non-identified twins, the risk is 15%. These risks are higher in people with generalized rather than focal seizures. If both twins are affected, most of them have the same epileptic syndrome (80-90% of cases). The close relatives of a person suffering from epilepsy are at a five times higher risk than the general population.

Down syndrome is the most common genetic cause of mild and moderate mental retardation caused by the presence of an additional chromosome 21. Chromosomes are

microscopic chromatic structures present in almost every constituent cell of the human body tissues. They bear the plan of all the features we inherit. This plan is carried in the form of a coded message present in deoxyribonucleic acid (DNA). In case of man, there are 23 pairs of chromosomes in each cell, of which 22 pairs of autosomes and one pair of heterosomes. A set of 23 chromosomes is inherited from the father and the other set is received from the mother.

Epilepsy may occur as a result of a number of other diseases, such as: tumours, strokes, cranial trauma, previous central nervous system infections, genetic abnormalities, as well as brain injuries occurring around birth (WHO 2012).

Posttraumatic epilepsy in a causal relationship directly with trauma meets several conditions, of which the most important are: there was no form of epilepsy prior to trauma; there is no other epileptogenic brain lesion (tumour, angioma, tuberculoma, parasitic cyst, etc.); the incriminated trauma was intense enough for the lesional brain effect to be epileptogenic.

The impact-epilepsy interval may be very short, of a few minutes, or very long, of a few years, but basically the first epileptic seizure can occur in any post-traumatic moment.

The impact-epilepsy interval, with a particular prognostic and therapeutic significance, can be considered as a criterion for the classification of post-traumatic epilepsies, such a classification would be post-traumatic epilepsy: immediate, occurring within minutes after impact; recent occurrence at some hours, days or weeks after impact; late, occurring at least three months after impact (Fisher et al. 2005; Eadie 2012; Thurman 2011).

Clinically there may be almost any type of seizure.

The incidence of post-traumatic epilepsy cannot be globally assessed because it has a high variability depending on several factors. After closed cranio-cerebral trauma the incidence is 1-5%, whereas after incidence of open cranio-cerebral trauma the incidence increases up to 30% and if there was a cranio-cerebral plaque, the incidence is over 45%.

Age is of great importance. In children under the age of 5, recent post-traumatic epilepsy is more common than in other age groups, and late-onset epilepsy starts after a longer interval in children under 16 years of age. After the age of 30, post-traumatic epilepsy is rare, and after 50 years of age, exceptional.

Other factors, including pre-existing non-traumatic brain disorders (arteriosclerosis, meningoencephalitis), influence posttraumatic epilepsy. Immediate epilepsy has good prognosis, generally after a few months it yields. Early epilepsy is symptomatic, it is due to a posttraumatic complication (hematoma, abscess, sequalae), heals after suppressing the cause. Tardive epilepsy is rarely due to a complication (abscess), but rather due to a meningocerebral scar or a diffuse sclerosis of the brain. In the case of diffuse sclerosis of the brain, epileptic seizures usually become more common and neuropsychotic symptoms occur. Prognosis is reserved and treatment is conservative. In the case of a meningo-cerebral scar, epilepsy often takes a surgical aspect (Brodie 2009).

The epileptic crisis has a very short duration and usually ends before a treatment is introduced, in which case the precautionary measures for the recurrence of the crisis are mandatory.

In case of generalized crises (tonic, clonic, tonic-clonic) that are assisted by a sanitary framework it is obligatory to establish the following measures: ensuring the freedom of the

airways; preventing the occurrence of secondary trauma to the crisis. These measures can also be provided by the patient's family members after prior training

The prophylaxis of the recurrence of the crisis is achieved by: administering a fast-acting antiepileptic: diazepam, IV. diluted in 10 ml physiological serum or glucose: 0.15-0.25 mg/kg; by rectal route: 0.2mg/kgc. Venous administration may be repeated after min. 20 minutes, and rectal one after min. 4 hours (Holmes et al. 2008; Wyllie 2010; Newton 2012).

Combating precipitating factors are reffered to fever, hypoglycaemia etc.

The epileptic state is a severe complication in the evolution of known epilepsy, or it may even represent the onset of epilepsy or of acute epileptic seizures.

Based on the clinically-convulsive (tonic-clonic, clonic, tonic, myoclonic or partial motor) or non-convulsive (absent, focal) appearance - and patient age one may determine most likely the cause in the absence of a rapid diagnosis of certainty: insufficient or suddenly interrupted treatment, infectious, vascular, hypoxic, metabolic, toxic (alcohol) comorbidities,

The condition of generalized convulsive epileptic state is a neurological emergency due to the morbidity and mortality it causes.

To reduce the risk of seizures repetition, the administration of anticonvulsants may be needed. These medicines are not usually prescribed for people who had only one generalized crisis the cause of which has not been identified. However, treatment is needed in patients who have experienced more than one crisis (Wilden 2012).

Anticonvulsants can completely prevent convulsive seizures in over half of people receiving this treatment and significantly reduce the frequency of seizures in another third of patients. The efficacy of these drugs is slightly lower in patients with absence seizures.

There is no medicine the management of which can control all types of seizures. In most people, seizures can be controlled by taking a single medicine.

Anticonvulsants, although very effective, may have side effects. Many of them cause drowsiness, and sometimes - paradoxically - cause hyperactivity in children.

There are a number of epileptic syndromes, which are generally classified by age and onset of the disease: neonatal period, childhood, maturity, and cases with no correlation with age. There are also groups with specific constellations of symptoms, those due to specific metabolic or structural causes, or those due to an unknown cause. The ability to classify a cause of epilepsy in a specific syndrome is more common in children. Some of these types include: benign rolandic epilepsy (2.8 per 100,000), child absence epilepsy (0.8 per 100,000) and juvenile myoclonic epilepsy (0.7 per 100,000). Fever convulsions and benign neonatal convulsions are not epilepsy forms (Berg 2008).

2.3.2. Material and methods

Our study was conducted in the Oral and Maxillofacial Surgery Clinic on 187 patients studied between 2015 and 2018 and 27 cases with posttraumatic epilepsy and psychiatric problems studied in the 2014-2018 period.

The clinical examination highlights Hydantoin gingival hyperplasia, multiple scarring on the tongue due to stroke during the seizures, facial expression can sometimes express a more or less advanced mental debility (as a result of brain destruction), for appreciation the risk of epileptic seizures, the frequency of seizures, the pre-emptive conditions, the date of

the last crises must be specified and it is recommended that the patient stops the use of alchool but not interrupts the antiepileptic medication before the dental therapy.

With regard to patient interventions, it was observed that the most common neurological emergency in stomatology is epileptic seizure.

Emergency: place the patient on the floor, if possible, open the clothes, place the head on one side, place a rubber piece between the dental arches to avoid tussle, supervise the crisis; administration of O2; an injection of Fenobarbital 1-1.5mg/kg, Diazepam 10 mg intravenously orintramuscularly.

„It is considered that genetics is a factor involved in most cases, either directly or indirectly. Some cases of epilepsy are due to a single gene defect (1-2%); most cases are due to the interaction between multiple genes and environmental factors. Each of the single gene defects is rare, with a total of over 200 cases.

2.3.3. Results

Emergencies were installed as follows: syncope-27 cases (15.78%), allergic accidents - 19 cases (11.11%), cardiociculators - 21 cases (12.28%), other emergencies - 01%); cerebral emergencies-epilepsy-39 cases (22.80%) (**Figure 2.6**).

By gender there was revealed a higher number of women (February 2 patients-56.41%) than men (17 patients, 43.59%) (**Figure 2.7**).

Rural patients predominated over those in the urban environment, in a ratio of about 2/1.

Depending on the diagnosis, the oral surgery pathology for which surgery was performed was: acute apical periodontitis suppressed - 17 patients (43.59%), soft parts abcesses-8 patients (20.51%), radicular remains- 14 patients (35.89%) (**Figure 2.8**).

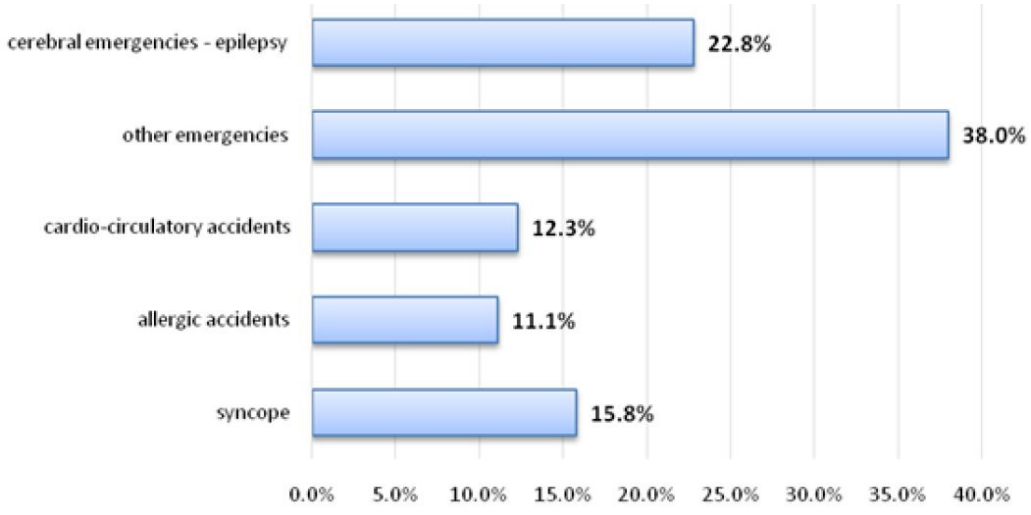

Figure 2.6. *Distribution of emergencies according with oral surgery procedures*

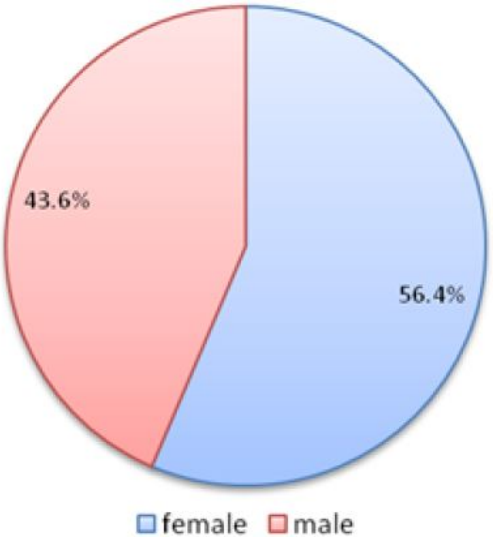

Figure 2.7. *Distribution by gender*

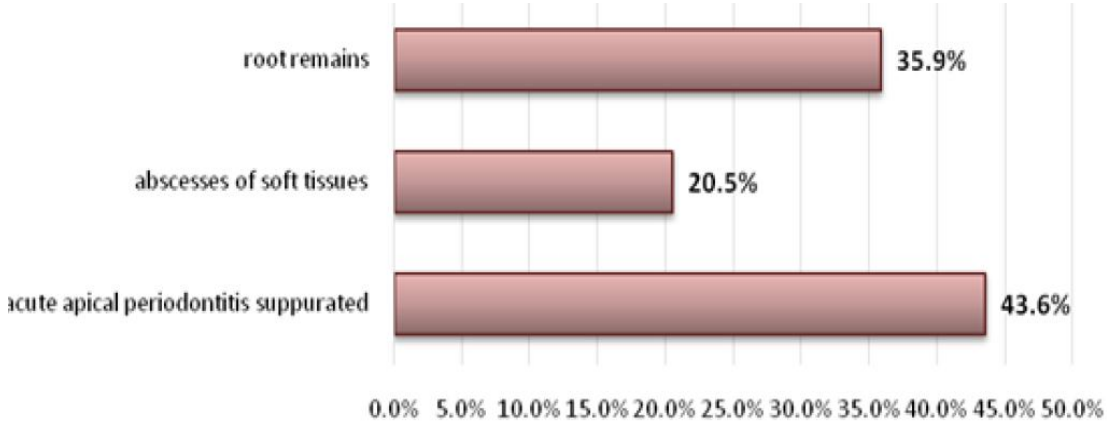

Figure 2.8. *Incidence of oral pathology solved by oral surgery intervention*

If the patient has not regained consciousness and the cure is repeated, Diazepam 10 mg intravenous will be administered, after which he will be hospitalized in an emergency hospital.

In the case of underlying seizures followed by a comatose state, the cardio-respiratory arrest can easily occur. In these situations the cardiac respiratory reanimation techniques are applied.

Establishing the diagnosis requires three important steps: knowing the patient, knowing the medical pathology and putting it in a precise nosological setting.

The moment of triggering the emergency-epilepsy crisis was performed during the intervention -63%; after performing anesthesia-12%; during anesthesia-22%; at the entrance in the surgery-8% (**Figure 2.9**).

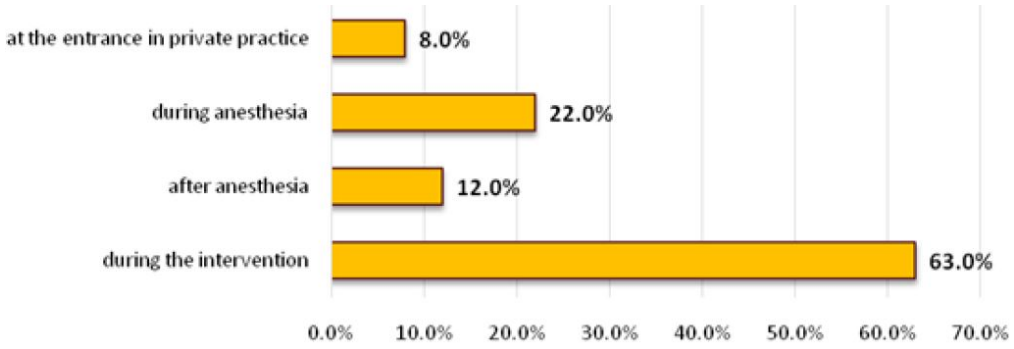

Figure 2.9. *Prevalence of moment of epilepsy crisis according with oral surgery procedures*

Epileptic seizures are the result of excessive or abnormal activity of nerve cells in cortex in the brain. Generally, diagnosis involves removing other conditions that may cause similar symptoms (such as syncope) as well as identifying other immediate causes. Epilepsy can often be confirmed with an electroencephalogram.

From many types of epileptic seizures, the grand mal is the most serious. Usually it is recognized without difficulty because it has a typical onset.

The preconvulsive and convulsive phase include selfbiting of the tongue, sputum, urinary incontinence, and faeces, are pathognomonic signs of the grand mal crisis.

The postconvulsive phase manifests with confusion, twilight or deep sleep, retrograde amnesia, characteristic EEG changes. There are six main types of generalized convulsions: tonic-clonic, tonic crises, clonic, myoclonic, epileptic absence and atonic convulsions. All of these involve loss of consciousness and generally occurs without any warning. Tonic-clonic convulsions are manifested by a contraction of the limbs, followed by their stretching and arched back, having a duration of 10-30s (the tonic phase). During the contraction of the chest muscles, a scream can be heard. This is often followed by a trembling of the limbs in unison (clonal phase).

Tonic convulsions produce constant contractions of the muscles. In most cases, the person's skin turns blue, because breathing is interrupted. In the case of clonic seizures, limb shaking occurs in unison. After the end of trembling, the time it takes for a person to return to a normal state is 10-30 min; this period is called the period of enrolment.

The epileptic seizure on the dental armchair may, during dental care, involve various foreign bodies (fingerprints, endodontic instrumentation, easily detachable fillings, dental fragments, etc.) in the respiratory tract, causing total or partial obstruction.

2.3.4. Discussions

The onset of epilepsy can be attributed to an internal neuronal stimulus, an external crash, medical injuries (such as cerebral vascular accidents, tumors) that hurt the brain or deprive it of oxygen intake, but in most cases epilepsy has no identifiable cause (idiopathic epilepsy).

Untreated epilepsy changes the personality of the patient, and it is serious that sometimes he can commit acts of aggression, even crimes which the patient does not know he

has done. Performing electroencephalography is absolutely essential in establishing the diagnosis and tracing the patient. This is done in shorter intervals at the onset of the disease, then less frequently, depending on the appearance and evolution of the disease clinics (NIHCE, 2012; Stephen, 2010; Hughes, 2009; Bradley and Walter, 2012).

Paroxysm appears as an expression of abnormal neuronal discharge of the brain. Very high frequency neuronal impulses are involved in this process, leading to an abnormal discharge of a large number of neurons. The two disorders hyperfrequency and hypersynchronization constitute the basic epileptic phenomenon. The paroxysmal diffusion of dysrhythmias leads to clinical focal access when diffusion is smaller or generalized when the diffusion is wide. The origin of the crisis can be cortical, subcortical, diencephalic, or centrocephalic (Engel, 2008; Simon et al., 2012; Steven, 2008; Xue, 2006).

In the mechanisms of the seizure discussing a trigger mechanism, a second mechanical way and a third stop mechanism of this crisis (Longo, 2012; Bergey, 2013).

The trigger mechanism imposes epileptogenic eunoxia, a predisposition to epilepsy (which involves increased reactivity, increased convulsion, or low convulsive threshold) and trigger factors (precipitants) (Malow, 2005; Tinuper, 2007; Holmes and Thomas, 2008).

The brutality of epileptic irradiation is performed synaptically but also extrasinaptically (efaptically), irradiation being facilitated by a series of spatial and temporal desumption phenomena, the diffusion of the intraparoxic excitation is self-sustained by the effects of entrainment and reverberation (Panayiotopoulos, 2010; James, 2009; Larner and Andrew, 2010; Stefan and Hermann, 2012).

Of the many factors and mechanisms that interfere directly or indirectly in the genesis of epileptic access, we quote: a neuronal epileptic with a subnormal bran potential, would be partially denerved because it brings repeated and frequent discharges to subliminal stimuli.

According to Ward (1969), the epileptogenic outbreak would be a partially disrupted aggregate of neurons in the cortex, in which the characteristic repetitive potentials appear and which either stimulate synaptically either by alterations in the local electric field or the ionic gradient bombarding by ortho or antidromo propagation neuronal cells, engaging them in epileptic activity (Plioplys et al., 2007; Reilly, 2011; Levisohn, 2007; Berg, 2010).

This may be done by various factors: intense affection, local metabolic, vascular or even small non-degraded subclinical lesions.

Epileptogenic responsiveness focuses on the anticonvulsant predisposition resulting from a functional disorder in the brain electromagnetism, whose substrate of neuronal hyperexcitability and which in relation to age is higher in the child (Neligan, 2012; Pandolfo, 2011; Dhavendra, 2008).

Epileptic access occurs in relation to epileptogenic noxes, which may be primarily cerebral, identifiable with the epileptic or extracerebral outbreak in the event that the paroxysmal manifestations are secondary to general processes, most commonly of a metabolic nature; the mechanism for the development of epileptic paroxysms is not fully elucidated, except for diffuse or partial seizures in which some Feed-Back mechanisms would occur; for the mechanism of stopping the epileptic crisis two factors are in discussion: progressive fatigue of neurons and an active inhibitory mechanism in which the talamo-caudal system would play a leading role (Bhalla et al., 2011; Simon, 2011; Sellner, 2012).

It is possible that this inhibitory role on certain nervous circuits is mediated by chemical mediators such as gamma -amino-beta-hydroxybutyric acid while excitement of excitatory systems (oxygen depletion, decrease of free acetylcholine) with consecutive changes in intra and extracellular distribution sodium, potassium, calcium, magnesium ions (Goldberg, 2013; Oby, 2006; Jerome et al., 2008; Panayiotopoulos, 2011).

The tonic-clonic convulsions manifest with a limb contraction, followed by their stretching and an arched back, with duration of 10-30 seconds (the tonic stage).

During the contraction of the thoracic muscles, a scream can be heard. This is often followed by a trembling of the limbs in unison (the clonic phase). Tonic convulsions produce constant contractions of the muscles (Martin et al., 2016).

Strong stimuli that irritate the brain, such as lesions, certain medications, sleep deprivation, infections, fever, lower blood oxygen levels, or lower blood glucose levels - can trigger a seizure regardless of whether or not a person is suffering from a convulsive disorder or not (Spatt et al, 2001; Zunker et al., 2014).

The major types of generalized seizures are: focal (or partial) seizures, with retained or lossed awareness and generalized.

The first type of focal seizure was previously known as a simple partial seizure. Focal seizures with a loss awareness may also be called a focal dyscognitive seizure (previously known as complex partial seizures)

Generalized seizures occur when there is widespread seizure activity in the left and right hemispheres of the brain. These are: absence seizures (formerly known as petit mal),tonic-clonic or convulsive seizures (formerly known as grand mal), atonic seizures (also known as drop attacks), clonic seizures, tonic seizures and myoclonic seizures. All of these involve the loss of consciousness and generally occur without any warning.

Myotonic seizures involve muscle spasms, either in restricted regions or at the level of the entire body. Epileptic absences may be subtle, with a slight head turning or blinking of the eyes. The person does not fall back and returns to a normal state immediately after the end of the crisis (Magiorkinis et al., 2010; Chang and Lowenstein, 2003).

When in children with crises beginning before the age of 2 years are usually caused by high fever or metabolic diseases such as abnormal blood glucose, calcium, magnesium, and vitamin B6 or sodium levels.

If seizures are recurrent, these are probably caused by a hereditary brain disease (as is the case with nocturnal frontal lobe epilepsy, which has an autosomal dominant transmission). Crises that begin after the age of 25 years old may be caused by structural brain injuries, such as those caused by cranial trauma, stroke or tumour. People with epileptic disease have an increased risk of having a crisis when they are under intense physical or emotional stress, or when they do not get enough sleep.

Strong stimuli that irritate the brain - such as injuries, certain medications, sleep deprivation, infections, fever, lowering blood oxygen levels, or lowering blood glucose levels - can trigger a seizure regardless of whether or not the person suffers from a convulsive disease or not. These seizures are known as cased seizures. Avoiding these stimuli can help prevent seizures.

Symptoms vary depending on the area affected by abnormal electrical discharge; depending on the magnitude of the seizure: partial (affecting only a cerebral area) or generalized (affects large areas of the brain located at the level of both cerebral hemispheres).

Partial seizures can be simple (the person is fully aware and perceives the environment) or complex (the state of consciousness is altered, but consciousness is not completely lost). Partial crises may be simple partial seizures, complex partial seizures, and partial continuous epilepsy. Generalized crises cause loss of consciousness and abnormal movements, which usually begin immediately. Loss of consciousness may be short or prolonged. Generalized seizures can be tonic-clonic seizures, primary generalized epilepsy, absence seizures, atone seizures, myoclonic seizures, and epileptic status.

To reduce the risk of seizures repeating, anticonvulsants may be needed. These drugs are not usually prescribed for people who have had only one generalized seizure the cause of which has not been identified.

Convulsions specific to epilepsy in children are characterized by body stiffness, repetitive movements, unusual sensations such as a strange taste or smell / unusual sensation in the stomach. It can take few seconds to a few minutes. The child may sometimes be conscious or, on the contrary, lose consciousness and will not remember anything during the seizure later. For some children, the seizures are carried out according to a pattern and follow at a certain amount of time (called the epileptic syndrome); they are likely to disappear by itself after the child celebrates a certain age (benign) or, on the contrary, aggravate and interfere with the development of the child, while associated disabilities may also arise.

During seizures dental problems could also occur. General emergencies in the dental office do not have a very high frequency but can occur at any time and the correct and prompt reaction of the physician may and should allow their professional management and therefore a proper dentist training and an appropriate patient evaluation.

The responsibility for decisions and consequences in these cases lies with the doctor, who has the professional, moral and forensic obligation to prevent urgency, to recognize it and to treat it properly, to know what he can do and not to do in these cases.

The risk assessment for the patient with dental conditions is of the highest importance in the usual practice in the cabinet.

Idiopathic epilepsy is one that poses dental problems either. It can evolve as the crisis: small to short-term absences, high to major epileptic crisis.

Triggering factors in the dental surgery may be diverse: odontogenic infections, dental neuralgia, alcohol abuse, work fatigue, reduction of anti epileptic medication, overdose of local anesthetics.

Epilepsy is a chronic cerebral disease characterized by recurrent seizures that are motor, sensorial, behavioral, with a change in consciousness, includes partial and focal seizures (simple, motor,sensitive, sensorial, vegetative, psychic) (Magiorkinis et al., 2013; Chang et al., 2003; Fischer et al., 2005).

Epilepsy presents groups of nerve cells, or neurons, that transmit abnormal signals; normal neuronal activity is disturbed, causing sensations, emotions or strange behaviors and sometimes seizures, muscle spasms and loss of consciousness.

Chemical factors are of great importance in the emergence of nervous system disorders. Among these we can mention the actions of certain toxic substances (benzene, aniline, lead, arsenic, carbon oxide and others).

Narcotics (chloroform, ether, morphine, alcohol etc.) have selective action on the central nervous system. Nervous system trauma causes the development of endogenous pathological conditions. During vascular sclerosis, a decrease or a strong contusion can cause cerebral haemorrhage. Such role has the traumatism which occurs in the course of epilepsy.

Generally, nervous system reactions to toxic action usually manifest through disorders of internal inhibition processes. Initially, there is an increase in the cortical excitability, and during the state of intoxication, phenomena of diffuse cortical inhibition are observed, which has a supraliminal protection inhibitory nature.

The normal correlations between cortical and subcortical activity are altered.

Symptoms are different depending on the child's age and other preexisting conditions (Devlin, 2012). The causes that give rise to epilepsy in children are: trauma to the brain (like a serious blow to the head), problems at birth (lack of oxygen to the brain or birth defects), metabolic diseases (chemical imbalances of the brain) brain tumours, blood vessel malformations, stroke, or an infection that affects the brain (meningitis orencephalitis).

Epilepsy having a known structural cause is designated as symptomatic epilepsy. Also, in some cases epilepsy has a genetic cause, namely, it is inherited from a parent or a change at the genetic level may occur (chromosomal disorders). Epilepsy with a hereditary probable cause is called idiopathic epilepsy (Duncan, 2006; NIHCE, 2012; Stephen, 2010).

The most common reasons for seizures and epilepsy in children are:

- ✓ premature delivery presents the risk of bleeding within the brain, which may cause seizure attacks and intracranial haemorrhage;
- ✓ children who at the time of their birth suffer from lack of oxygen in the brain are at risk of prenatal
- ✓ hypoxia; this can cause brain damage and may lead to epilepsy, low levels of glucose, sodium or calcium in the blood can cause epilepsy, infections such as encephalitis and meningitis are causes of seizures and epilepsy;
- ✓ children borned with abnormal or poorly functioning brain have a high chance of epilepsy; epilepsy is often inherited from parents.

Although children of all ages may tend to zone out, this may be a symptom of epilepsy in an easier form (Hughes, 2009; Bradley, 2012; Engel, 2008).

Absence crises last about 10 s, ending abruptly and consist of moments when the child may be unconscious and unresponsive. In many cases, the child can resume normal activity immediately after the crisis is over, but because it is not aware that it has occurred, diagnosis can be difficult. Absence crises can occur with a variable frequency from one person to another, between 1 and 100 times a day. Undiagnosed, these may affect school performance and may trigger tonic-clonic seizures later in life (Krishnan et al., 2011).

Anticonvulsants can completely prevent convulsive seizures in over half of people receiving this treatment and significantly reduce the frequency of seizures in another third of patients. The efficacy of these medicines is slightly lower in patients with absence seizures. Half of the patients responding to anticonvulsants may eventually discontinue treatment

without other seizures (Malkan and Beran, 2014). But in 10-20% of people with epileptic disease the administration of anticonvulsants does not help prevent seizures.

Anticonvulsants, although very effective, may have side effects. Many of them cause drowsiness, and sometimes - paradoxically - hyperactivity in children. Periodic blood tests are performed to determine whether the anticonvulsant affects kidneys, liver or blood cells (Simon et al., 2012; Steven, 2008).

Any anticonvulsant medication has side effects: decreased memory capacity, difficulty in concentrating, or lethargy. It is important to find the dose that prevents the onset of the seizure and causes the smallest side effects, a situation which can be determined after a few weeks of treatment. Regular tests are required to determine the concentration of the medicine in the blood. Neurological manifestations primarily involve the precipitation of convulsive seizures in epileptic people, by increasing the excitability of adrenergic receptors.

Administration of corticosteroids in epileptic patients requires the increase of anticonvulsant medication doses and clinical surveillance and ECG (Xue, 2006; Tinuper, 2007). Seizures are preceded by a premonitory sensation, such as noises, followed by loss of consciousness. Most anticonvulsants affect the degradation of other substances by the liver, influencing the action of other medicines.

Lately, a new form of epilepsy has emerged - the Dravet syndrome; this rare and incurable epilepsy manifests by seizures difficult to control even with a cocktail of multiple anti-epileptic medicines. Various other related events add to the seizures: developmental disorders, language requirement delays, walking instability, and in some cases autism (Van Klink et al., 2016).

Most side effects of the seizures are reffering to dental disorders. In patients with epilepsy, general anesthesia with intravenous barbiturates is anesthesia of selection. If there are no conditions of general anesthesia or there is a disproportion between the importance of narcosis and the dental act, it is possible to perform loco-regional anesthesia that includes compulsory in premedication a pituitarypreferred parenteral (Fenobarbital) batch, in a dose that provides the hypnotic and anticonvulsant effect.

In the dental surgery, during specialized care, a series of consciousness disorders may occur, ranging from a confusing minor state to coma. These disturbances of the consciousness may take from a few seconds to a permanent status, translating severe brain injury.

Disturbances in the state of consciousness have a clinical polymorphic picture, whether they precede, accompany or follow the disturbances of respiratory function and/or cardio-circulatory function.

Tagging epilepsy diagnosis and determining what type of crises a patient may have is difficult. The epilepsy diagnosis cannot and should not be considered a priori a drama. There are many cases of epileptic patients that did not prevent them from becoming prominent, and the research shows that 10% of the residents can get a better education and training. In addition, a properly administered and conscientiously administered treatment leads, in most cases, to the disappearance of crises, giving them the opportunity to embrace a very wide range of professions, of course avoiding activities involving risks of injury.

Prophylactic treatment: correct anamnesis, which notes that the patient is treated with antiepilectic preparations (Gabapentin, Pegabalin, Sodium valproate etc.) (Somjen, 2004).

Schultze's statement that the epileptic suffers more from the attitude of the entourage than from his illness, it should be stressed that the atmosphere of calm and understanding that the entourage must manifest, the elimination of reserves and susceptibilities that oppose even the most intimate and natural desires of any common, not to be regarded as an unusual patient, are indispensable conditions for socioprofessional recovery to the maximum of its physical and mental resources

Success in treating and improving the quality of life the patient depends heavily on his precocity the accuracy of the clinical and electroencephalographic diagnosis of the disease.

2.3.5. Final remarks

Epilepsy is a neurological disorder (it affects the brain and the nervous system) during which a person has a tendency for seizures with onset in the brain; this organ consists of millions of nerve cells that use electrical signals to control functions, senses and thoughts, and if the signals are interrupted, the epileptic seizure or convulsion occurs. Epilepsy located in temporal lobes affects cognitive functions, including feelings, emotions, thoughts, and experiences of each individual. Epileptic patients with behavioural disorders associate abundant paroxysmal activity and low sleep efficacy.

In dental surgery, during the specialized care, a number of disturbances of consciousness can occur, ranging from minor to coma. This state of consciousness disturbance can last from a few seconds to a permanent status, they translating severe brain injury. The epilepsy crisis (comitial seizures) occurs most frequently during the intervention, then during anesthesia.

3. FROM KAMPAVATA TO AVANT - GUARD NEUROLOGY

State of the art

The social and scientific interest on neurology and neurosciences is high since early ages of the mankind. Even so its recent datas recently, starting from 17th century. The *Journal of the History of the Neurosciences* and sections in general journals such as *Archives of Neurology*, *Neurology (Minneapolis)*, *The Lancet*, the *Journal of The Royal Society of Medicine* and others have conveyed the lessons of history to a wider readership (Gardner-Thorpe, 2000).

Probably Thomas Willis (1621–1675) was the father of the neurology, or so Sherrington thought, and the arterial circle at the base of the brain is one of Willis' eponymous claims to fame (Hughes, 1991).

He removed the whole brain from the body instead of dissecting from above and the cerebral body thus removed was seen to contain important solid portions. This maneuver liberated the theory of the humours of coldness, moisture, dryness and heat. The cerebral body was part of the whole of man's body, a body which was beginning to be anatomized. The primacy of the cerebral cortex in memory, imagination, passion and appetite was yet to

be described. Earlier workers had concentrated on the ventricles, perhaps echoing William Harvey's emphasis on the solid portions of the heart rather than its cavities—the empty areas (Molnár, 2004).

In the early days of the 19th century anatomy and pathology converged, parts of the body were portrayed by artists including Charles Bell, student of the painter David Allan. Bell was inspired by Alexander Monro Secundus, the middle of the three Monros (Kellie, 1824).

Robert Whytt, Professor of Medicine at Edinburgh was the first who described the nervous fluid (Eadie, 2000).

Jean-Martin Charcot is known as "the founder of modern neurology" and is "associated with at least 15 medical eponyms", including Charcot-Marie-Tooth disease and amyotrophic lateral sclerosis (Lou Gehrig's disease). He has also been referred to as "the father of French neurology and one of the world's pioneers of neurology" and named also "the Napoleon of the neuroses" (Siegel, 2000).

Santiago Ramón y Cajal was a Spanish histologist, psycologist, and Nobel laureate for his pioneering investigations of the microscopic invention of the brain were so original that he was considered by many to be the greatest neuroscientists of all time. He discovered the axonal growth cone, and demonstrated experimentally that the relationship between nerve cells was not continuous, but contiguous (Stanley, 2000).

Then, was the turn of Aloysius "Alois" Alzheimer, a German psychiatrist and neuropathologist and a colleague of Emil Kraepelin. He identified andt published the first case of "presenile dementia", which Kraepelin would later identify as Alzheimer's disease. In 1901, Alzheimer observed a patient at the Frankfurt Asylum named Auguste Deter. The 51-year-old patient had strange behavioral symptoms, including a loss of short-term memory and he would become his obsession over the coming years. Later, in April 1906, the patient died and Alzheimer had her records and the brain brought to Munich where he was working at Kraepelin's lab. Together with two Italian physicians, he would use the staining techniques to identify amyloid plaques and neurofibrillary tangles (Zilka and Novakuj, 2006).

Over the years, neurology became more and more complex and extended on many side domains. Neuromuscular disorders, Parkinson disease and diabetic neuropathy are just some of them.

Myasthenia gravis was first recognised as a distinct clinical entity by Thomas Willis. The first modern description was made in 1877 by Samuel Wilks, a London physician and only towards the close of the 19th century, primary muscle diseases and diseases due to denervation of muscle were studied by English, French, and German physicians. The first detailed and complete descriptions of myasthenia gravis were by Wilhelm Erb, of Heidelberg, and Samuel Goldflam of Warsaw (Hughes, 2005).

Simpson and Nastuck detailed the autoimmune nature of the condition, than Patrick and Lindstrom used rabbits to show that immunization with purified muscle-like acetylcholine receptors caused the development of MG-like symptoms in 1973 (Kahan, 2005).

The clinical signs of parkinsonism were identified in India, arround 600 bc. Evidences prove that as early as 300 bc, Charaka proposed a coherent picture of parkinsonism by describing tremor, rigidity, bradykinesia, and gait disturbances as its components. The 15th-

century classic "Bhasava rajyam" introduced the term kampavata, which may be regarded as an ayurvedic analogue of parkinsonism. The pathogenesis of kampavata centered on the concept of imbalance in the vata factor, which controls psychomotor activities. The therapy was essentially consisting of the administration of powdered seed of Mucuna pruriens, or atmagupta, which as per reports, which contains 4%-6% of levodopa (Ovallath and Deepa 2013).

In 1912 Frederic Lewy described microscopic particles in affected brains, later named "Lewy bodies" and than Konstantin Tretiakoff reported that the substantia nigra was the main cerebral structure affected, but this finding was not widely accepted until it was confirmed by further studies published by Rolf Hassler in 1938 (Lees, 2007).

This clinical condition is named after the English doctor James Parkinson, who published the first detailed description in An Essay on the Shaking Palsy, in 1817 (Parkinson 1817).

The history of diabetic complications, including neuropathies, cannot be separated fromthe one of diabetes itself. Ancient records of diabetes generally contain no reference to its complications involving the nervous system but some rare exceptions describing autonomic and painful neuropathies are allcoming from the Orient. It was not until the 18th century that Western physicians started studying diabetes and its complications. Eventually, the works of de Calvi and Pavyclearly established the link between diabetes mellitus and diabetic neuropathies (Skljarevski, 2007).

The clinical signs of this disease may be: balance disorders, numbness and tingling of extremities, sysesthesia, diarrhea, erectile dysfunction, urinary incontinence, facial, mouth and eyelid drooping, vision changes, dizziness, muscle weakness, difficulty swallowing, speech impairment, fasciculation, anorgasmia, retrograde ejaculation, burning or electric pain (Pop-Busui et al., 2016).

The epochaldiscovery of insulin in 1921 triggered a wide interest and more systematic approach to researchof diabetic complications, leading to S. Fagerberger's conclusion that many of them share theunderlying microvascular pathology (Fagerberg, 1959).

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

1. **Cuciureanu DI**, Croitoru CG, Toma C, Cuciureanu T. Effects of environmental and weather conditions on myasthenia gravis - in search of the missing link. Environmental engineering and management journal, 2019; 18(5): 1145-1152.
2. Firescu D, Sascau RA, Raftu G, Statescu C, Cuciureanu T, **Cuciureanu DI**. Thermography as method of paraclinic diagnosis in diabetic polyneuropathy. Revista de chimie, 2019; 70(4): 1449-1454.
3. **Cuciureanu DI**, Popescu RM, Cuciureanu T, Constantinescu VA, Hodorog DN, Szalontay AS. Dopaminergic centers neurodegeneration biochemical and radiologic approach. Revista de chimie, 2019; 70(5): 1835-1838.

3.2. Myastenia gravis up to date

3.2.1. Introduction

The environment plays an important role in the normal functioning of the human organism. Both chemical and physical factors influence human organs and systems at their most basic level such as homeostatic equilibrium and neurotransmission. Any imbalance in these factors causes or contributes to systemic diseases. A classic example is soil and water iodine deficiency enhanced by deforestation and the appearance of thyroid enlargement and other iodine deficiency disorders (Preda et al., 2013).

Other examples address allergic asthma secondary to exposure to environmental vegetal dust which can appear as an occupational or related profession disease (Constantin et al., 2015), health risks for patients with hepatitis A virus (Vata et al., 2018) environment for Mycobacterium tuberculosis growth (Cislariu et al., 2018), particulate matter air pollution effects on vulnerable and non-vulnerable people (Noor et al., 2015; Oprea et al., 2017).

This autoimmune disease is characterized by muscle weakness that fluctuates, worsening with exertion, and improving with rest. In about two-thirds of the patients, the involvement of extrinsic ocular muscles presents as the initial symptom, usually progressing to involve other bulbar muscles and limb musculature, resulting in generalized myasthenia gravis. In about 10% of myasthenia gravis patients, symptoms are limited to EOMs, with the resultant condition called ocular MG. In vivo, peripheral nerves form a functional unit with striated muscle fibres using the neuromuscular junction as an interface between the presynaptic and postsynaptic component. At the synaptic site the endplate potential is converted into muscle action potential (Verschuuren et al., 2016). Therefore, the effects of ambient temperature on the peripheric nerves are evident also in the muscle function.

Several electrophysiological studies have demonstrated influence of temperature on neuromuscular transmission in MG. Overall, local cooling improves muscular transmission (Borenstein and Desmedt, 1975) whereas local heating reduces the amplitude of the action potential (Racinais and Oksa, 2010). These facts support the much older clinical observation that myasthenic symptoms, especially muscle weakness and palpebral ptosis may improve with cold and may worsen with heat (Simpson, 1960). Both clinical observation and electrophysiological proof suggest that ambient temperature may influence the symptomatology of MG and even weather can have clinical implications (Borenstein and Desmedt, 1974). There is cited an increase in acute exacerbation in late summer and late winter (Melamed et al., 2014).

In spite of these correlations few data are available regarding seasonal patterns of MG worldwide, even fewer are reported from a specific region in Romania. Overall there are also few data regarding occurrence of MG in Eastern European countries (Ziedaa et al., 2018). Iasi is major city in north-eastern Romania and the existence of university neurology clinics explains a high addressability of the myasthenic patients. Also, the city possess an important meteorological center – The Moldova Regional Meteorological Centre.

The purpose of this study was to determine if there is in fact a correlation between environmental temperature fluctuations and MG exacerbations among myasthenic patients

which were hospitalized in I Neurology Clinic of "Prof. Dr. N. Oblu" Emergency Clinical Hospital Iasi. In addition, finding candidate variables for validation in a future prospective study was also a priority.

3.2.2. Material and methods

3.2.2.1. Data acquisition

Patient data were collected from patient files of I Neurology Clinic from "Prof. Dr. N. Oblu" Emergency Clinical Hospital Iasi, hospitalized between 1.01.2013 and 31.12 2017. Both prevalent cases and incident cases with a positive diagnosis of MG were included in the study. As stipulated in the medical literature, a positive case was defined by suggestive clinical aspect along with elevated specific autoantibodies titers and/or positive repetitive nerve stimulation test. The patients which had no significant decrement in spite of characteristic symptoms underwent a single fiber electromyography test or jitter test.

According to standardized methodology published in medical literature, low frequency repetitive nerve stimulation test consists of applying electrical current on a motor nerve with a frequency of two to five Hertz. Five cycles of ten stimuli each are applied. At this low frequency, presynaptic depression is greater than acetylcholine release facilitation. After stimulation the difference between negative waves' amplitude of the first and the forth stimuli is calculated. A decrement at least equal to 10% is considered highly suggestive for MG.

Environmental data were obtained from The Moldova Regional Meteorological Centre, Iasi and included daily values of maximum and minimum temperatures from 1.01.2013 and 31.12 2017 for Iasi city. A time interval of two weeks before an aggravation was taken into consideration. Six parameters were calculated: standard deviation for temperatures in the week prior to hospitalization (DST1) and the week before that (DST2), weekly temperature range defined as the difference between the highest and the lowest temperature from the week prior to hospitalization (RT1) and the week before that (RT2) and the average of the temperatures within the week prior to hospitalization (MT1) and the week before that (MT2).

3.2.2.2. Statistical analysis

One sided Fisher's exact test was used for comparison of categorical data. Mantel-Haenszel test was used for comparison of stratified categorical data. In order to verify the potential influence of the six weather parameters taken into consideration on myasthenic aggravations we used conditional forward binary logistic regression without intercept.

The reason of admission was considered the dependent variable and was given 1 for aggravation and 0 for assessment (stable disease). None of the independent variables were separately analyzed before they were introduced in the model, nor they were divided by category. P values<0.05 were considered significant. Excel version 1811 was used for data management and statistical analysis was performed using SPSS 20.

3.2.3. Results

3.2.3.1. Clinical data

In the period included in the study, a total of 122 myasthenic patients (83 females and 39 males) were registered from which 79 were incident cases: 22 in 2013, 17 in 2014, 14 in 2015, 14 in 2016 and 12 in 2017. The patients lived in Iasi city, Iasi county or neighbor counties. The median age at first presentation was 51 (range 19-83; mean 49) for women and 62 (range 31-78; mean 61) for men.

Regarding age at onset and gender, 51 of the cases had an age at onset under 50 years, from which 45 were women. From the 71 patients that were over 50 years old at the time of onset, 33 were men and 38 were women (**Figure3.1**).

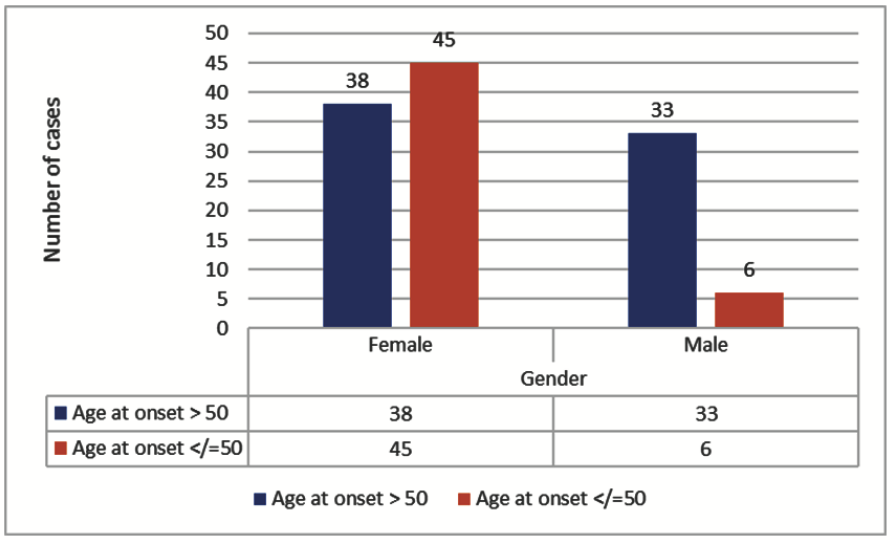

Figure3.1. *122 MG patients: gender and age at onset ($p < 0.001$, one sided Fisher's exact test)*

Table 3.1. *MGFA modified Osserman classification*

Osserman	*Female*		*Male*		*Total*
	Count	*% of total*	*Count*	*% of total*	
Osserman I	22	75.9	7	24.1	9
Osserman IIA	30	69.8	13	30.2	3
Osserman IIB	26	61.9	16	38.1	2
Osserman IIIA	2	100	0	0	
Osserman IIIB	1		2		
Osserman IVA	1	50	1	50	
Osserman IVB	1	100	0	0	
Osserman V	0	0	0	0	

Table 3.2. *Breakdown of admissions*

			Y ear					Total
			2013	2014	2015	2016	2017	
Worsening disease	Month	January	4	1	2	3	5	15
		February	3	2	3	6	7	21
		March	4	4	1	3	3	15
		April	7	6	5	3	2	23
		May	2	1	0	4	3	10
		June	3	1	1	1	2	8
		July	2	0	3	1	2	8
		August	3	1	3	2	3	12
		September	5	4	2	1	2	14
		October	3	3	2	3	2	13
		November	2	2	1	1	2	8
		December	1	6	0	1	0	8
	Total		**39**	**31**	**23**	**29**	**33**	**155**
			Y ear					Total
			2013	2013	2013	2013	2013	
Assessment	Month	January	0	0	0	0	0	0
		February	0	0	0	1	1	2
		March	0	0	1	3	3	7
		April	0	0	0	4	2	6
		May	0	1	0	1	2	4
		June	1	2	0	0	1	4
		July	0	0	0	2	3	5
		August	0	1	0	0	1	2
		September	0	1	1	0	0	2
		October	1	0	1	0	0	2
		November	0	1	1	0	3	5
		December	0	0	0	1	0	1
	Total		**2**	**6**	**4**	**12**	**16**	**40**

The clinical characteristics of the subjects are presented in **Table 3.1** using MGFA modified Osserman classification.

During the specific time interval taken into consideration 105 patients were admitted for worsening of symptoms. Overall, a total of 195 admissions with 155 aggravations were noted (**Table 3.2**).

From these, 144 admissions were from Iasi County. **Figures 3.2 and 3.3** reveal gender and age distribution among the 195 admissions. Female aggravations represented 83.58% of total female admissions while male aggravations represented 70.49% of total male hospitalizations. When age was used as stratifying factor for gender and admission reason distributions no statistically significant difference was found when using Mantel-Haenszel Mantel-Haenszel test.

3.2.3.2. Subgroup analysis

Given the fact that from the entire lot of 195 admissions 144 were from Iasi County, a subgroup analysis was performed. When only 144 admissions from Iasi county were taken

into account and all six temperature variables were used (without gender), again RT1 has correlated statistically significant with exacerbation of symptoms with $p < 0.001$ and exp(B) of 1.112 (95%CI: 1.068 – 1.158) (**Table 3.3 - 3.6**).

After adding gender, female gender was the only variable to corelate with exacerbation of symptoms with $p<0.001$ and exp(B) of 3.947 (95%CI: 2.386-6.53) (**Table 3.7**).

Remarks towards the statistical analysis performed are mandatory. Even though the type of analysis performed has an increased overfitting risk, the authors consider the results useful for generating hypothesis to be tested in a prospective study.

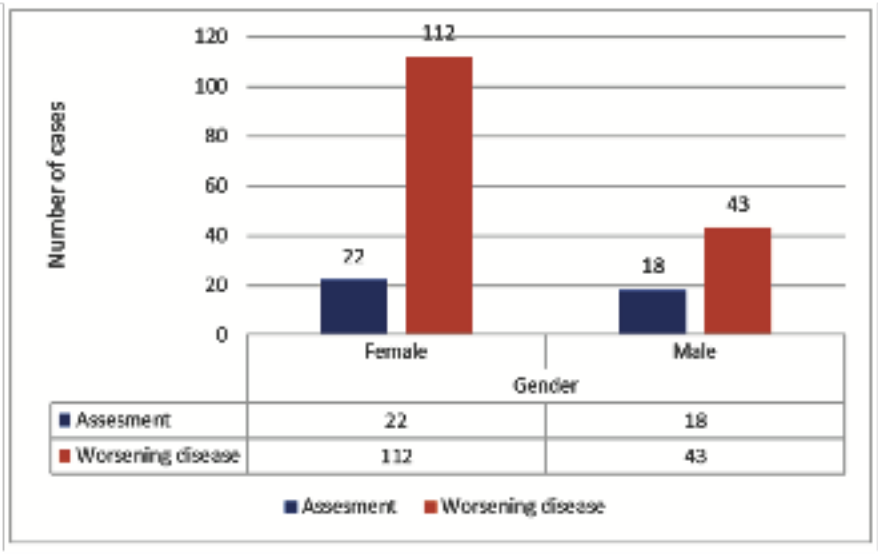

Figure 3.2. *Distribution of events according to gender and admission reason (One sided Fisher's exact test p = 0.03)*

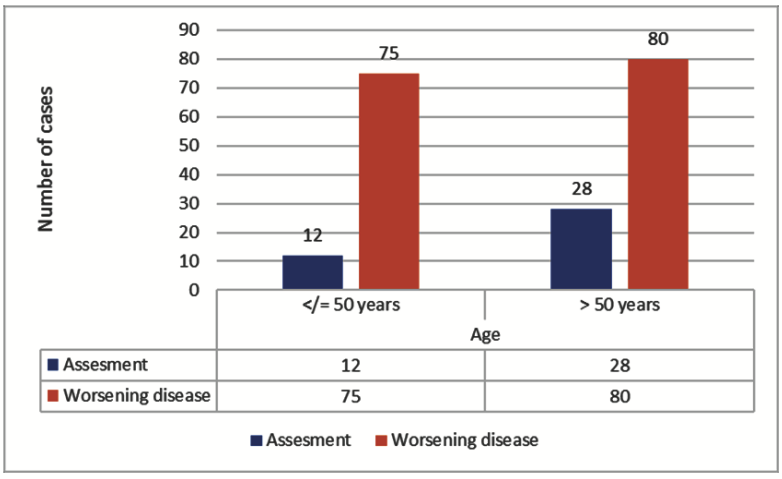

Figure3.3. *Distribution of events according to age and admission reason (One sided Fisher's exact test p=0.027)*

Table 3.3. *Influence of RT1 on symptom exacerbations*

		B	S.E.	Wald	Df	Sig.	EXP(B)	95% C.I.for EXP(B)	
Step 1	RRT1							*Lower*	Upper
		.133	.019	47.763	1	.000	1.143	1.100	1.187

Table 3.4. *Variables not introduced in the model*

			Score	*df*	*Sig.*
Step 1	Variables	DST1	.747	1	.387
		MT1	.449	1	.503
		DST2	.707	1	.400
		RT2	1.163	1	.281
		MT2	.576	1	.448
	Overall Statistics		2.910	5	.714

Table 3.5. *Influence of gender and temperature variability on symptom exacerbations*

		B	***S.E.***	***Wald***	***df***	***Sig.***	***Exp(B)***	95% C.I.for EXP(B)	
								Lower	***Upper***
Step1[a]	Gender(1)	1.627	.233	48.703	1	.000	5.091	3.223	8.041
	DST(1)	.195	.072	7.226	1	.007	1.215	1.054	1.401
Step 2[b]	Sec(1)	1.046	.310	11.379	1	.001	2.846	1.550	5.225
a. Variable(s) entered on step 1: Sex.									
b. Variable(s) entered on step 2: DST1b.									

Table 3.6. *Events from Iasi County - influence of temperature variables*

		B	***S.E.***	***Wald***	***df***	***Sig.***	***Exp(B)***	95% C.I.for EXP(B)	
								Lower	***Lower***
Step 1[a]	RRT1	.107	.021	26.859	1	.000	1.112	1.068	1.156

Table 3.7. *Events from Iasi County. Influence of temperature variables and gender*

		B	***S.E.***	***Wald***	***df***	***Sig.***	***Exp(B)***	95% C.I.for EXP(B)	
								Lower	***Lower***
Step 1[a]	Gender(1)	1.373	.257	28.580	1	.000	3.947	2.386	6.530

3.2.4. Discussions

Neurotransmission is the basis of communication between neurons and their targets. Information to other neurons or effector cells is transferred through electrical and chemical synapsis, the last being the most frequent. Influence of environmental factors, especially ambient temperature on neurotransmission velocity in the peripheral nervous system has been a subject of interest since the experimental studies published in the early 1900 (Gasser, 1928). High temperature non-linearly increases conduction velocity in motor and sensory fibres (Rutkove et al., 1997), while cold environments have an opposite effect (Todnem et al., 1989).

The complex chemical synapse between peripheral nerve endings and striated muscle fibres can be the target of several neuromuscular junction diseases, the most studied being myasthenia gravis (MG) (Vincent et al., 2001) Also known as Erb Goldflam disease, non-hereditary MG has a worldwide incidence which varies from 0.25 to 2 per million persons (Carr et al., 2010). In the last decades its prevalence has increased due to both prolonged life expectancy and also advances in diagnosis methods (Melzer et al., 2016). MG is an autoimmune disorder in which auto-antibodies attack the motor end-plate resulting in an impaired transmission of the nerve impulses to the muscle fibers (Ramanujam et al., 2011).

In 85% of cases, the target of the auto-antibodies is the acetylcholine receptor (Lindstrom, 2000) followed by the muscle-specific tyrosine kinasereceptor (Hoch et al., 2001). In 7% of seronegative myasthenic patients another antibody is detected, against lipoprotein related protein 4 (Romi et al., 2017). MG associated with timus neoplasia occurs in 10-15% of myasthenic cases and it is asociated with other auto-antibodies (Evoli et al., 2002).

MG has multiple clinical and paraclinical subtypes that may differ regarding prognosis and therapeutical approach. MG has an ocular and a general form. The ocular form is more frequent among people of asian descend (Zhang et al., 2007). The general form involves all striated muscles. It can appear before or after the age of 50 (Somnier, 2005; Suzuki et al., 2011). From this point of view, MG can be early-onset (EOMG) and late-onset (LOMG). For therapeuticall and general outcome reasons the Task Force of the Medical Advisory Board of the Myasthenia Gravis Foundation of America (MGFA) created a five grade clinical scale using Osserman's original stadialization as a model (Jaretzki et al., 2000).

According to this classification MG can be divided into five main classes and several subclasses: class I defines the ocular form whereas classes II to V, generalized forms in various degrees of severity. Each of the latter is subdivided in type A defined as predominantly limb and/or axial muscles and type B defined as predominantly bulbar and/or respiratory muscles.

Usualy MG has a prolonged and fluctuating evolution: in the first years relapses alternate with remission periods while in much more advance stages, relapses tend to be more scarce and a moderate motor deficit may be present permanently. Relapses can be spontaneus or can be triggered by pregnancy, sistemic infections or certain medication which alteres the normal transmission at the neuromuscular junction (Oosterhuis, 1989).

The clinical aspect of a relapse can vary from exacerbation of double vision or of generalized fatigability to life-threatening myasthenic crisis. Differential diagnosis varies with the clinical pattern of an exacerbation and can include ischemic or hemorrhagic stroke, central nervous system neoplasia, myopathies and encephalitis. One of the most controversial pathologies from the latter category is Herpes Simplex encephalitis which can mimic a myasthenic aggravation. According to Boangher, particular forms of Herpes simplex encephalitis with fluctuating neurological deficits might be a consequence of a secondary autoimmune phenomenon related to the presence of NMDA-R antibodies (Boangher et al., 2018). Myasthenic crisis is characterized by acute respiratory insufficiency and hemodynamic instability. Therefore, it can be easily mistaken with other causes of acute respiratory insufficiency. Among these, even though rare, one must not ignore the possibility of an autonomic malfunction secondary to a lesion in the vegetative nervous system, either

central or peripheric. In right handed patients, for example, it has been shown that a middle cerebral artery ischemic stroke is associated with different hart rate responses depending on the side of the lesion: right hemisphere infarcts have an enhanced sympathetic control on the heart rate while left hemispheric ones have a dominant parasympathetic control (Constantinescu et al., 2016, 2018).

There is no unanimous opinion regarding a certain age limit wich divides MG in early onset and late onset: some authors consider the age of 40, whereas others raise the limit to 60 years. However, the current tendency is to establish the age of 50 as the treshold (Somnier, 2005). EOMG is tipically associated with females, timus hiperplasia and high level of acetylcholine receptor antibodies, whereas LOMG tends to be more frequent in men which do not have any timus modification (Berrih-Aknin et al., 2014; Meriggioli and Sanders, 2009). The lot taken into consideration, representing both prevalent and incident cases of MG admitted in a university hospital in a major north-eastern city of Romania during a five-year period tends to fit in the already well-established worldwide epidemiology.

More specifically, the EOMG cases were predominantly women. Even though in the LOMG subgroup women were also the majority, the fact that the total number of men was 39, from witch 33 had over 50 years at the time of MG onset suggests that men are more prone to develop the disease later in life.

Including as much data as possible in order to obtain promising working hypothesis was the reason why six independent variables were introduced in the model. The present study is one of the few that focuses on possible correlations between MG exacerbations and weather variables. In fact, most of the articles concerning this subject find lack correlation between weather, temperature and patient general muscle weakness. One of the explanations is the existence of other much more evident trigger factors like therapy, rest and exercise, administration of certain medication, infections. However, one of the first articles that pioneered the idea of weather influenced myasthenic symptoms reveals that extremes of heat and cold do make the fluctuation of symptoms more evident to the patient because he is stressed by thermoregulation demands (Borenstein and Desmedt, 1974).

Apparently, aggravations were influenced only by RT and DST from the last week prior to month of hospitalization. The results obtained are consistent with the ones from the medical literature. In both the lot from Iasi County and also the entire lot, myasthenic exacerbations tended to be more frequent as the difference between the highest and the lowest temperature from that week was higher. In other words, as the extremes of temperature are greater, the thermoregulation effort is increased and this has a negative impact on the transmission at an already impaired neuromuscular junction. The result is a higher number of exacerbations.

Regarding gender related exacerbations, in the entire lot females tended to have an increased probability of aggravation when ambient temperature fluctuations were higher. This could have been a bias do to the fact that women represented the majority of the lot. However, if female aggravations related to total female admission are compared to men aggravation related to total male admissions, a tendency for female aggravations predominance is still obvious.

When the same reasoning was applied in the Iasi county subgroup, again females appeared to have a much higher rate of exacerbation. DST 1 and RT1 variables were at the

limit of statistical significance. Even though this suggested that the higher the ambient temperature fluctuations were, the greater the risk of exacerbation in women was, unfortunately no such conclusion can be drawn due to the fact p value is above the threshold considered.

3.2.5. Final remarks

The present study consists in a valid appraisal of possible influence of temperature fluctuations on MG aggravations.

In the lot considered the risk of myasthenic aggravation appears to be higher when the extremes of temperature from the week before hospitalization were greater. This finding has a potential great impact in the general management of MG. To be more specific, along the general dietary and hygienic recommendations that neurologist give to their myasthenic patients, advices to avoid exposure to very low or very high environmental temperatures can also be formulated. These behavioural precautions may be highlighted especially in women suffering from MG. This is because, according to the current study, apparently females have a greater risk of exacerbation when ambient temperature fluctuations were higher.

These are promising remarks that represent a hypothesis for a prospective study. If a certain cause- effect relation is demonstrated between environmental temperature fluctuations and myasthenic aggravations in a much larger lot the implications would be extraordinary in terms of both prophylaxis and treatment of MG. In other words, myasthenic aggravations could be attenuated or ideally avoided if the patient would not expose himself to extreme environmental temperatures. Also, if an exposure to a low or elevated temperature is anticipated then a myasthenic exacerbation could be avoided by temporarily modifying the medication posology.

In addition, further studies are mandatory in order to establish a link between myasthenic aggravations and other environmental parameters such as atmospheric pressure and humidity. The medical implications are extensive: myasthenic patients could prevent an exacerbation by avoiding exposure to certain weather conditions which could have a positive impact on reducing medication and improving quality of life and overall outcome.

3.3. Shaking palsy

3.3.1. Introduction

Parkinson's disease, also called "shaking palsy", has been known since the 19th century. The first attempts to treat the disease date back to the late nineteenth century, when atropine or belladonna root extracts were started (Charcot and Sigerson 1879).

Modern treatment with L-dopa and dopamine agonists have superior efficacy but do not have curative action.

Patients diagnosed with Parkinson's suffer because of a neurological or idiopathic degenerative condition that primarily affects the motor system. Thus, nervous cells are not

destroyed by a virus, but by a process of degeneration, whose origin seem to have a neurotoxic and genetic component. Other causes associated with the etiopathy of Parkinson's disease are stroke and drugs.

The social impact of this disease is extremely important for both the patient himself and the fact that it is the second pathological neurological condition as a frequency (Olanow et al., 2011).

The number of centers dedicated to the treatment of Parkinson's disease has increased in recent years. An association of Parkinson's patients have also been created. These have greatly contributed to the development of a better knowledge of Parkinson's disease and the problems faced by patients among the general public.

The onset of the disease is unknown, studies estimate the place of the beginning of degeneration process in gut to parasympathetic neurons level, 10- 20 years before the most obvious clinical signs are motor movements, related to agitation, stiffness, slow motion, difficulty walking, thinking problems and behavioural disorders.

These are called "parkinsonism", or a "parkinsonian syndrome". In the advanced stages of the disease dementia, depression and anxiety can occur. Other symptoms include sensory, sleep and emotional problems “nonmotors symptoms” (Sveinbjornsdottir 2016; Kalia and Lang 2015).

The positive diagnosis is based on the clinical examination and on the medical history of the patients. Patients who develop similar clinical manifestations of Parkinson's disease following a stroke or, especially, drug use, fall into the diagnosis of Parkinson's syndrome plus (Poewe and Wenning 2002; Jankovic 2008).

Among the radiological methods, MRI has become more accurate in diagnosing the disease over time, especially through the T2 and SWI sequences, and both can demonstrate the characteristic aspect of the substance nigra (Schwarz et al., 2014). This refers to the disappearance of swallow tail aspect at this level (Mahlknecht et al., 2001) but the technique is also used to exclude other diseases that may be secondary causes of parkinsonism, such as encephalitis, chronic ischemic lesions, tumors and hydrocephalus (Brooks 2010).

PET-CT can measure the metabolic activity of dopamine carriers from basal ganglia, reducing their activity characterized by Parkinson's disease.

Currently, the drugs used to treat this disease are levodopa (always combined with a dopa decarboxylase inhibitor and sometimes with a COMT inhibitor), dopamine agonists and MAO-B inhibitors.

The purpose of this study is to evaluate the efficacy of modern current drug therapy for Parkinson's disease by correlating clinical and imaging data.

3.3.2. Material and methods

The study group included 99 de novo patients diagnosed with Parkinson's disease, out of a total of 283, in the Department of Neurology at the Emergency Hospital "Prof. Dr. N. Oblu" hospitalized during 01.01.2015 - 31.12.2018.

On this group of patients, we conducted their demographic and clinical analysis, clinical examination and psychiatric in order to highlight clinical, motor and non-motoric manifestations and of these, which are the most useful therapeutic measures. We have

assessed the evolution of Parkinson's disease in these patients for a period of 3 years: 2015-2018.

This was possible by a follow up at a 6-month interval, occasionally being clinically reviewed. The MRI investigation was repeated over 12 months.

We evaluated percentual distribution of newly diagnosed patents, age group distribution, main motor manifestations, disautonomous disorders, neuropsychic dysfunction, follow up after medication.

3.3.3. Results

Our clinic's hospitalization registry shows 99 patients diagnosed with Parkinson's disease in 2015-2018, out of a total of 283 who were hospitalized within that time (**Figure 3.4.**).

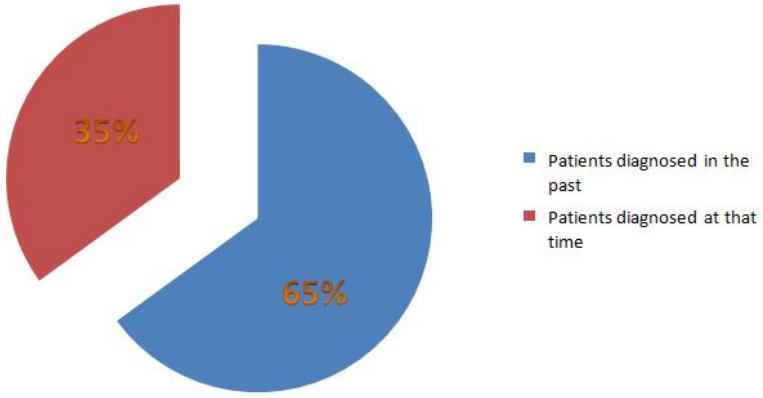

Figure 3.4. *Percentual distribution of newly diagnosed patents*

Age related distribution results in the group of patients studied is: 3 persons with an age belonging to the category of under 40 years; 34 people belong to the 40-60 age category and 246 people belong to the over 60 category. The incidence is therefore wider in the over 60 age group followed by the 40-60 age group.

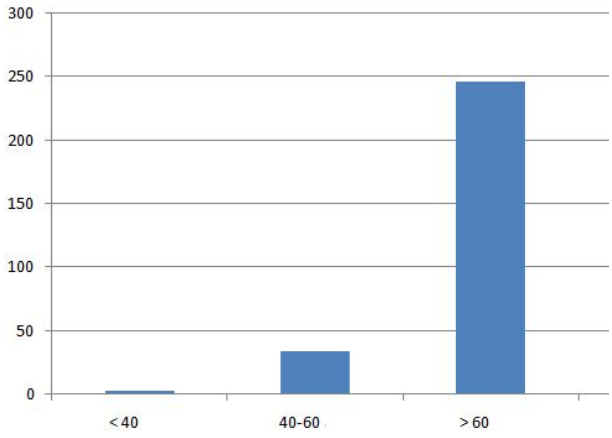

Figure 3.5. *Age group distribution*

The distribution by gender the group studied reveals 140 men (49%) and 142 women (51%). A slight predominance of females over males (**Figure 3.6**).

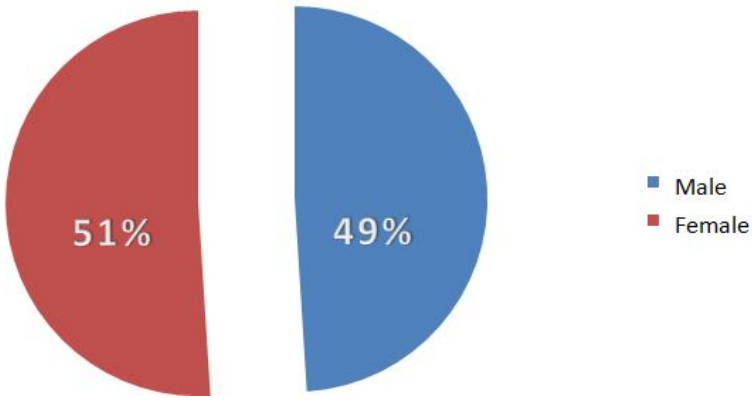

Figure 3.6. *Patients distribution by gender*

The patients studied showed motor clinical manifestations marked by tremor and muscle stiffness but also disautonomic (**Tables 3.8 and 3.9**).

Table 3.8. *The main motor manifestations*

Tremor	Muscular rigidity	Bradykinesia	Postural instability
269	258	256	199
95%	91%	90%	52%

Tabel 3.9. *Disautonomous disorders in non-motor symptomatology*

Digestive disorders	Olfactory disorders	Cardiac disorders	Urinary disorders	Sleepiness disorders	Sensory disorders	Thermoregulation
70	65	60	5	40	50	7
24%	23%	21%	1%	14%	17%	2%

The neuropsychiatric manifestations are present by: psycho-emotional lability, tendency to impulsiveness, depressive phenomenon in different grades of psychopathological intensity, psychotic disorders such as hallucination, confusion (**Tabel 3.10**).

Tabel 3.10. *Neuropsychic dysfunction in non-motor symptomatology*

Depression	Psychotic symptomatology (hallucination, delirium)	Cognitive dysfunction	Dementia
160	20	138	56
56%	7%	48%	19%

The drug treatment was administered according to the age of the patients. The age of 60 years is correlated with a late stage of illness in most cases (**Tabel 3.11**).

Tabel 3.11. *Medication of motor symptoms in Parkinson's disease*

	IMAOB (Rasagilina)	I-COMT (entacapone)	Dopaminergic agonist (Ropinirol, Pramipexol)	L-dopa and benserazida
40-60 years	3%	25%	53%	59%
> 60 years	7%	37%	81%	95%

In the radiological study we investigated the patients using MRI, after clinical diagnosis and then after one year. The images point indirect signs of basal ganglia disorders (**Figures 3.7, 3.8 and 3.9**).

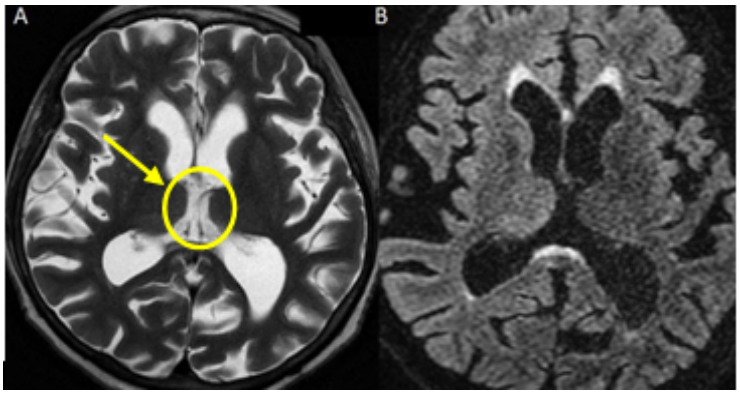

Figure 3.7. *Patient with Parkinson's disease in Hydrocephalus "ex vacuum"; A=Expansion of bilaterally symmetrical intergranular spaces. The arrow indicates a bilateral "swallow tail sign"; B= Chronic lesions, one year after.*

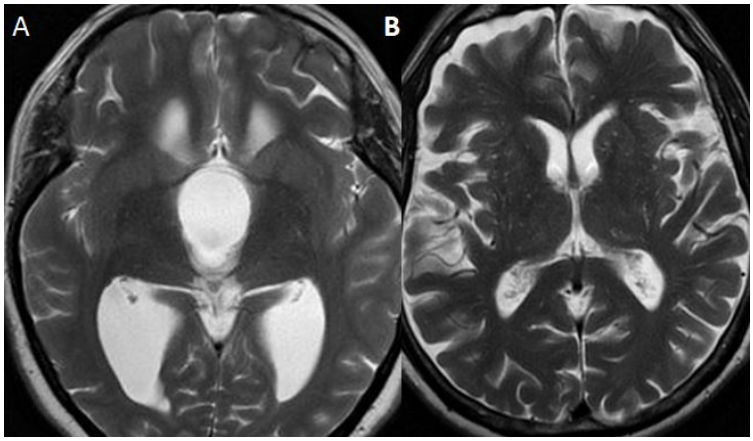

Figure 3.8. *A=Passive supratentorial hydrocephalus; B=Supra- and and subtentorial, symmetrical, central and peripheral cerebral atrophy that causes ventricular system expansion and exaggeration of intergiral sulci, aproximately one year after*.

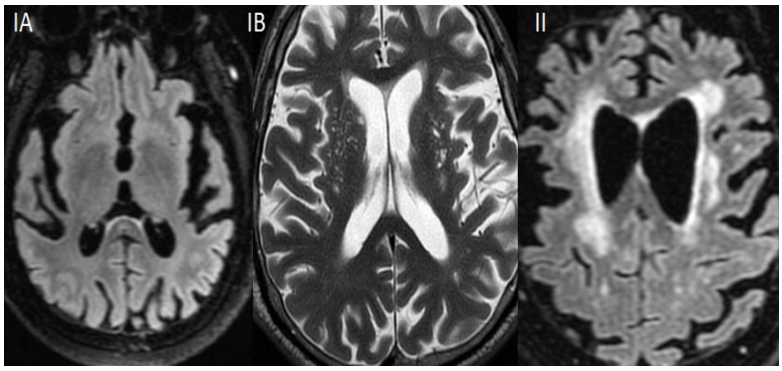

Figure 3.9. *71 year old patient with galloping neurodegenerative lesions; IA= Moderate cortico-subcortical cerebral atrophy associated with millimeter lesions in hypersensitive T2, FLAIR, discrete hyposemnal T1 located in the supratentorial white matter, with no tendency to confluence suggestive of non-specific degenerative vascular lesions (Fazekas 1 score); IB= Diffuse brain atrophy in combination with atrophy of the corpus callosum; II= Cortico-subcortical atrophy over and subtentortal associated with FLAIR, T2 confluent hyperstimulation lesions, located in diffuse white, periventricular and hemispheric bilayer, suggestive modifications of Fazekas III arteriosclerosis.*

3.3.4. Discussions

Since only 5-10% of levodopa crosses the blood-brain barrier, the rest is metabolised to dopamine elsewhere in the body, causing nausea, vomiting and orthostatic hypotension. Carbidopa and benserazide are dopa decarboxylase inhibitors, do not cross the blood-brain barrier and inhibit the conversion of levodopa to dopamine beyond the brain (Maria, 2017; Oertel, 2017).

This type of medication has as its effects the appearance of dyskinesia as well as fluctuations in its efficacy. Shutting down levodopa medication can have dangerous side effects such as neuroleptic malignant syndrome (Aquino et al., 2015).

Taking less doses of levodopa or using a controlled release version may reduce the risk and severity of these complications. Intestinal infusions of levodopa (Duodopa) can cause gastroparesis gastroparesis but are the latest efficient treatment for advances Parkinson diseases.

MAO-B inhibitors (safinamide, selegiline and rasagiline) increase the amount of dopamine in the basal ganglia by inhibiting the activity of monoamine oxidase B (MAO-B), a dopamine-degrading enzyme. As dopamine agonists, their MAO-B inhibitors produce more effective PDOD symptoms. There are few studies on their efficacy at advanced stage, suggesting that they are useful in reducing fluctuations between on-line and off-line periods. An initial study indicated that selegiline in combination with levodopa increased the risk of death, but this was later disproportionate.

Tremor is the key motor symptom in Parkinson's disease, present in 95% of patients. The tremor is accentuated or initiates rest and emotional situation, intense stress, concentration or fatigue. This is the most noted symptom at the neurology consultation.

Muscle rigidity is evident in the majority of cases, with a frequency of 91%. This muscular tension disorder determines the attitude characterized parkinsonian patients (fuga attitude) with a slight flexion in all joints. Daily activities (performing body hygiene, lifting a

chair) are performed with difficulty, demanding according to gravity, personal care. When rigidity is accentuated, lumbar, posterior cervical pain may appear.

Muscular hypertonia is objectified through: cogwheel sign, Noica sign, exaggerated posture reflex, palmo-mental reflex, sharp oral reflex, exaggerated nasopalpebral reflex, plantar skin reflex, decreased abdominal skin reflex or abolit.

Bradykinesia is present in the majority of cases, a proportion of 90%, it is shown as slow, in different grade, daily activities. The difficulty of walking appears in the initiation of walking or absence of automatic movements, by small hesitant steps, difficulty or blockage of return. Bradykinesia is visible by micrograph (small writing, illegible), hypomimic (decreased movements imitate), rarely blinked eyes and hypophony (voice decreased).

Postural instability is less common, but it appeared in an important stage in the evolution of the disease, because postural instability is difficult to treat and a source of disability in an advanced stage of the disease.

The most common non-motor manifestation encountered are digestive disorders with a percentage of 24%, followed by cardiac manifestations, represented by orthostatic hypotension 25%.

The digestive manifestations are present by:

- *Deglutition disorders - dysphagia for solids and liquids, occurring as a result of inability to push the pharyngeal food balls and that the inability to contract the upper esophagus, appears late in the course of the disease, with intermittent, more rarely permanent appearance.*
- *Sialorrhea, appears due to the inability to swallow saliva, which accumulates in the oral cavity, it is correlated directly proportional to the severity of the dysphagia.*
- *Slowing intestinal transit (constipation).*
- *inability to push the pharyngeal food balls and that the inability to contract the upper esophagus, appears late in the course of the disease, with intermittent appearance more rarely permanent.*

The most common non-motor manifestations are followed by a percentage of 24%, followed by cardiac manifestations, represented by orthostatic hypotension 25%.

Urinary disorders are of 1% frequency, and are present by:

- *Frequency of urination*
- *Urinary retention disorders;*
- *Difficulty of complete and incomplete urination;*

Disturbances of thermoregulation are present by hyperhidrosis;

Sleep disorders are manifested by the difficulty of sleeping and the inability to mention sleep, with frequent nocturnal awakenings. Most often occurs reversal of the sleep-wake rhythm (nocturnal insomnia and exaggerated daytime sleepiness).

Cognitive conditions have a high share of 48%. Cognitive conditions can also occur in patients recently diagnosed with Parkinson's disease. In MP are frequent affections of executive functions, such as planning and decision-making ability, working memory, language, Visio-spatial ability (especially the perception and interpretation of visual information), reaction time, and 'Warning. Since Parkinson's disease is a progressive disease, cognitive conditions worsen in a short time (Sharma et al., 2019).

Depression is the most common form of psychic manifestation in Parkinson's disease, with a frequency of 56% in the group studied, characterized by a state of sadness, loss of hope for a long period of time, losing the interest for activities made for pleasure.

The dementia is present by the disorders of recovery, confusion incas of recognition and computation, disorientation in an advanced stage with a frequency of 13%. Throughout the study, psychotic symptomatology is present by a frequency of 16%.

Novel compounds such as L 4 (IC50 = 0.11 .mu.M), L8 (IC50 = 0.18 .mu.M), L16 (IC50 = 0.27 um) and L17 (IC50 = 0.48 .mu.m) had selectivity and MAO inhibitory activity B similar to Selegilina. These or others could improve the treatment of Parkinson's disease.

Parkinson's disease still affects cerebral circulation and especially the ventricular system. A new model called Modified Gray Wolf Optimization (MGWO) was proposed based on the traditional Wolf Wolf Optimizer (GWO), which acts as a search strategy for selecting features. It uses different types of data sets related to voice, handwriting (spiral and meander) and speech. The presentation algorithm contributes to prediction of Parkinson's disease with an estimated accuracy of 94.83%, a detection rate of 98.28% (Li et al., 2019).

Data from our study shows that MRI equipment used with even less than 3T power may indicate some brain damage with a direct or indirect diagnosis of Parkinson's disease. Once those aspects have been detected and recorded, remote tracking and assessment of a patient's prognosis is much easier and more accurate.

The current optimal treatment consists in the continuous administration of L-Dopa derivatives via the portable Duodopa (PDP) pump without feedback. Closed loop control for PDP thus provides a fully automated drug infusion without breaking down, by infusion proportional to the reduction in plasma dopamine levels. This results in the alleviation of side effects caused by incorrect doses in drug therapy.

3.3.5. Final remarks

The results of our study show at least a temporary improvement in the quality of life of the patients in the study group following the initiation of progressive release levodopa treatment correlated with the administration of dopamine inhibitors. MRI technology is indispensable in achieving a differential diagnosis but, monitoring the response of dopaminergic centers to medicative treatment. We believe that the use of advanced radiological techniques such as 3T IRM or PET-SCAN can significantly enhance the results of Parkinson's disease.

3.4. Polineuropathy

3.4.1. Introduction

Unbalanced diabetes mellitus produces, through different mechanisms, altered nerve conduction in the central and peripheral nervous system. Pathological changes of the peripheral nervous system, commonly referred to as "diabetic neuropathy", are classified by Dyck et al., in: distal symmetric polyneuropathy with sensory, vegetative and motor

involvement; proximal symmetric neuropathy; asymmetric focal neuropathy (multiple neuropathies); asymmetric neuropathy combined with distal symmetric polyneuropathy (Argoff et al., 2006).

Chronic sensitive-motor symmetric diabetic polyneuropathy (mixed polyneuropathy) starts insidiously at the distal level (fingers) and evolves slowly proximally. It initially affects somatic and autonomous slim fibrous fibres and then the myelinated thick fibres, and in advanced phases motor fibres as well. The affection of the thin sensory fibres causes alteration of thermal and pain perception, and the affection of thick fibres leads to proprioceptive and vibratory sensitivity and osteo-tendon reflexes disorders. Acute sensory polyneuropathy (acute painful polyneuropathy) has a much lower frequency and is characterized by pain of varying but usually intense nature with nocturnal exacerbation. It can be produced or exacerbated by banal stimuli (allodynia). This form of polyneuropathy occurs after long periods of glycaemic imbalance, with significant decreases in weight, or along with the initiation of insulin therapy and has a naturally regressive evolution. The objective neurological changes are minor compared to the intensity of the symptomatology.

The simplest and most used classification divides diabetic neuropathies into symmetrical and asymmetric. Symmetric variants include autonomic polyneuropathies and neuropathies, asymmetric variants group mononeuropathies, multiplex mononeuropathies, plexopathies, “entrapment” syndromes, radiculopathies and cranial neuropathies (Bandeira et al., 2014; Bandeira et al., 2012).

The pathology of diabetic neuropathy and its interpretation has been a continuing source of controversy. It cannot yet be asserted that nerve damage is primary or secondary to neural degeneration where demyelination or axonal loss is primary or the main lesion. The pathology of diabetic neuropathy has been described as having a metabolic or ischemic mechanism. What is certain is that diabetes mellitus has the potential to induce pathological changes in cellular and non-cellular components of peripheral nerves) (Boyko et al., 2006; Boulton 2006; Boulton et al., 2008).

Electronic microscopy studies have shown significant loss of non- myelinated fibres. The characteristics of the degenerative changes of these fibres include axon atrophy, accumulation of vesicular elements, and damage to the tubular and filamentary elements of the cytoskeleton. Complete degeneration results in the vaping or denervation of Schwann cell subunits surrounded by basal lamina. Although the low density of the non-myelinated fibres represents the quantitative reflexion of the modified denervation, Schwann cell subunits are considered to be a better indicator in this context (Boulton et al., 2008).

The arterial vascularisation of the peripheral nerve trunks (*vasa nervorum*) is made up of the endoneurial intrinsic vessels and extrinsic vessels at the epinerve and perinerve level. In diabetes, histological changes were described at the level of all vascular components. At the endonerve level, thick-walled vessels and with narrow-lumen calibre were studied closely. Qualitative and quantitative studies have demonstrated endothelial cell hypertrophy and hyperplasia with diminished vascular lumen. Fenestrated endothelial cells, a feature normally present only in the epineural vessels, were seen in the endoneural vessels as a result of the loss of endothelial cell junction. The occlusion of microvascular lumen resulting from endothelial hyperplasia or from fibrin plugs was investigated without confirmation of the studies performed (Brownlee 2005; Caralis and Bakris 2005; Brodsky 206).

Epineural capillary abnormalities include hyperplasia and basal lamellar thickening. The intima of epineural arterioles is thickened in diabetic neuropathy. However, despite these changes, endoneural micro-vessels are much more affected than the epineural ones with basal lamina thickening, endothelial cell hypertrophy and lumen narrowing (Bower 2008).

In the average of the denerved arterioles, structural changes have been reported, such as glycogen growth, smooth muscle cell edema, cell detritus accumulation, collagen deposits.

As for the etiopathogenesis of diabetic neuropathy in the research literature, several theories are issued: vascular, metabolic, which may be interfere with other pathogenic mechanisms (Chan et al., 2009; Dieter 2009).

These mechanisms explain the development of microvascular complications related to plasma glucose levels and the possibility of preventing them by maintaining a near-euglycaemic level in the long term. Metabolic lesions affect small vessels, the so-called *vasa nervorum*, the vessels that nourish the peripheral nerves. The longer these nerves (namely those from the lower limbs), the greater the risk of being affected, and therefore, typical diabetic neuropathy occurs especially in the lower limbs (Du and Li 2005; De Meira et al., 2014).

On the one hand, this lesion is angiopathic, and on the other hand there are metabolic deviations. Because of the metabolic imbalance, an increased intracellular glucose concentration occurs in a non-insulin dependent pathway; the higher the concentration of glucose in the extracellular medium, the more intracellularly it penetrates (Borba 2015; Fisher 2007).

Excess glucose is metabolized in sorbitol and then fructose. In the diabetic, excess sorbitol accumulates in the basal membrane of the capillaries by altering the vasa nervorum resulting in a microangiopathy with consecutive hypoxia.

Excessive activation of aldol reductase induces a deficiency of myoinositol in Schwann cells by altering nerve conduction.

Oxidative stress and abnormal glycosylation of structural proteins are also known pathogenic mechanisms.

Current studies address other therapeutic targets: neurotrophic factors, insulin receptors for advanced glycation compounds (AGE), phenotypic sodium channel switching, Poly (ADP-ribose) polymerase (PARP).

Subjective sensitivity disorders represent distal paraesthesia, initially in the lower limbs, then including the upper limbs; sometimes pain, more or less violent, with a feeling of constriction or burning occurs (Heidrich 2005; Jude 2006; Jeong 2012).

Motor disorders usually occur later in the course of the disease, it also starts initially at the lower limbs, symmetrically, with the paresis of the antero-external area of the calves and stepped walking; more rarely the deficit is primarily interested in the posterior area of the calves or thighs (Mohler 2008; Park 2004).

Trophic disturbances are frequent and early, manifested by dry skin, squamous, changes with hair loss, ribbed nails, breakable nails.

Vegetative disorders consist of: vasomotor disorders of intestinal motility, hyper or hypo-transpiration, hypersalivation, orthostatic hypotension, Argyl-Robertson sign, etc.

Cranial nerve lesions are rare in diabetic neuropathy, occurring only in cases with an important metabolic imbalance and affecting ocular motricity and the optic nerve with predilection (Peltz et al., 2012; Sahli et al., 2005; Sivanandam et al., 2012).

Polyneuropathy is characterized by distal, symmetrical and bilateral distribution of manifestations of interest initially for the lower and then upper limbs and early loss of superficial or profound sensitivity. With the progression of the disease, there is a particular muscular impairment in the small muscles of hands and feet.

The first symptoms that appear are paraesthesia then associated with pain, which has different characters: deaf, startling, tenacious, nocturnal, sometimes becoming a real cause of disability. Usually occurring as bilateral manifestations, symptoms include numbness, paraesthesia, burning sensation, feeling of vibration in the legs, tingling sensation, severe hyperesthesia and pain.

In diabetic patients, due to the collagen glycation process, there is a progressive decrease in the elasticity of collagen-containing tissues; this change is manifested by limitation in joint mobility due to thickening of skin and periarticular structures; there is a flexion deformation of the hand called cheiroarthropathy, which is recognized by the so-called "sign of prayer".

A particular condition of lower limb neuropathy is that of the "painful - painless" foot in which, in addition to pain and paraesthesia mentioned by the patient, there is a decrease in the sensitivity to pain and of the proprioception one at the objective examination (Spacek 2010).

A feature of the peripheral arterial involvement in diabetic patients is the frequent association of medium and large artery calcification (mediosclerosis or Mönckeberg disease), which is attributed to the affection of the sympathetic nervous system, similar changes being observed in sympatheticomized patients.

Foot ulceration caused by diabetic neuropathy, trauma, and peripheral vascular disease may result in a life-threatening infection or endanger the integrity of the affected limb; foot infections are common in patients with diabetes and are associated with high morbidity and increased risk of lower limb amputation.

Knowing the causes and mechanisms that increase the risk of foot injuries and then their production in patients with diabetes is indispensable for the effective application of prevention and treatment measures. The diabetic foot injury occurs as a consequence of the interaction of several factors (Spittel 2004; Suzuki et al., 2013).

Charcot arthropathy normally causes obvious foot deformations, thus severely affecting its functionality, causing unusually large pressure loads during walking. The presence of calluses is a predictive element of the occurrence of ulceration.

Mobility of the joint is defined as the movement of that joint, and is related to age, gender, and ethnic origin; limitation of joint mobility in the foot and ankle in diabetic patients leads to an increase in planting pressure, being closely related to the occurrence of ulceration with the same localization.

In the pathophysiology of diabetic foot ulceration, there are multiple mechanisms, including neuropathy, peripheral vascular disease, foot deformities, the presence of abnormal pressures and long duration of diabetes evolution (Suganthi et al., 2014; Tesfaye 2006; Tesfaye et al., 2005).

"Diabetic foot" is a concept, a working tool designed to draw attention to the need for an integrated approach to the causes and management of one of the most common, expensive and invalid complications of diabetes, lower limb amputations.

Lower limb obliteration arteriopathy is the most common form of peripheral vascular affection in patients with diabetes mellitus. This consists of the progressive reduction of blood flow in the lower limb arteries due to the progressive narrowing of their lumen produced by atherosclerotic plaques. In pathogenic arteriopathy four pathogenic processes occur: atherogenesis, arterial thrombosis, arterial embolism and arterial spasm.

The main lesion in obliterative arteriopathy is atheroma or atheromatous plaque which consists mainly of cellular diffusion with various lipids and especially cholesterol deposited in the intimal layer of the artery (Tesfaye et al., 2010; Treece et al., 2004; Zimny et al., 2004).

The most characteristic symptom of chronic and progressive arterial obstruction of the lower limbs is intermittent claudication, that is, a pain at the level of the buttock, thigh, calve or leg that occurs while walking and disappears within a maximum of 10 minutes of stopping the effort.

Polyneuropathies are a group of diseases that occur by including in a pathological process the nerve endings that are furthest from the spinal cord, practically the nerve endings distributed to the muscles of the fingers of the anatomical hands and feet are affected.

The clinical examination includes: palpation of the arterial trunk allows at the same time to highlight different parietal indurations, different sinuosities and even arterial aneurysms; the calculation of the systolic index has value in the diagnosis of some arteritis; the Rotschow posture test is appreciated by many authors because it is easy to execute and provides data on large arterioles (inaccessible to palpation), the vascular end and collateral circulation; measuring the skin perfusion pressure is a method by which the blood flow is estimated in the ischemic limbs; the Tarfis-Samuel test consists of placing the patient on the back and raising the calves at 65 degrees, then the pedalling movement is performed for 10 minutes.

During diabetic neuropathy, the electrophysiological exam shows the presence of the eventual signs of denervation in the muscle areas that may be affected by polyneuropathy. These consist of spontaneous denervation activity (fibrillation and slow denervation potentials) and debilitation of the voluntary route (neurogenic deficit index).

Medical thermography is unique in its ability to visualize some of the physiological and metabolic processes that take place in the human body; it is the method of determining skin temperature that consists in capturing skin images with an infrared-sensitive film and is based on the fact that warm objects emit infrared radiation. The warmer a body is, the more it emits a greater amount of infrared radiation.

Through the thermographic method, the temperature is monitored and recorded allowing thus the thermal flow visualization. There are three types of thermography: Liquid Crystal Thermography (LCT); Infrared Thermography (IRT); Microwave Thermography MWT).

Thermography has been successfully used in detecting and characterizing the following neurological disorders: irritation and compression of nerve roots, peripheral nerve lesions, and occlusive diseases of cerebral vessels, migraine, spinal cord injuries and pain syndrome differentiation.

The advantageous features of thermography (painless, non-invasive, without adverse biological effects, is objective, produces a dynamic recording) make it suitable for characterizing neurological dysfunctions.

Diabetic neuropathy affects lower limbs much more frequently than other parts of the body. The thermography applied in diabetic foot monitoring provides useful information about skin temperature and peripheral vascularisation. The thermographic method as a method of paraclinical diagnosis in diabetic polyneuropathy in current practice.

3.4.2. Material and method

The study batch consisted of 40 diabetic patients admitted to the Neurology Clinic of the Clinical Recovery Hospital and Diabetes Clinic between January 2014 and December 2018 that were investigated by means of the cutaneous thermography that completed the usual algorithm of the investigations.

Thermography provides a picture of temperature variations on the surface of the skin by capturing the infrared radiation emitted by it. The diabetic patient with multiple comorbidities, with subclinical or clinical disorders of the peripheral vascularisation, plantar temperature may show changes in the direction of decrease.

Thermographs have been performed on patients with diseases that have successfully allowed to perform thermographic scans on the areas of interest (lower limbs, trunk, upper limbs, cephalic extremity).

Being particularly sensitive to the temperature differences of various parts of the body, the thermograph may show areas of slightly modified temperature, the temperature that from a medical point of view may be the result of an inflammatory or other process.

Thermographic scanning involves capturing images remotely without the subject being touched with any object while the images are captured. First, a general scan will be done, tracking all possible affections. From certain areas highlighted by the thermograph, images are taken and analysed. Afterwards, all the thermograms obtained from scanning are interpreted and a conclusion is reached, establishing the final diagnosis.

Thermography is a method of non-invasive imaging investigation, with no side effects and no contraindications for the patient. Unlike other imaging investigation methods, medical thermography is completely risk-free for the patient and for the thermographer.

It is a diagnostic method by which the temperature differences from the surface of the body are visualized, corresponding to physiological or pathological changes both from the surface ad the depth of the skin. By analysing these temperature differences, a diagnosis can be made; is a contactless, non-invasive, non-irradiating method that can be repeated as many times as needed, being basically a special photo of the body's thermal energy. Temperature gives us information about the physiological processes that are taking place, and these processes are disturbed long before the structural changes of an organ appear. The thermograph captures the infrared light radiation. Infrared radiation is emitted by the warmth of the human body, and the thermograph translates it into an image made of a series of colours from the visible spectrum.

Infrared spectrum radiation is emitted by the heat of the human body. For certain diseases, there are well-defined areas on the surface of the body that change their

temperature. There is a difference in temperature as compared to neighbouring areas or a certain temperature difference as compared to the normal value. This temperature difference is called the "temperature gradient" and may indicate a possible affection of sub-tegumentary structures in that area.

Selected patients submitted at the entrance to the study investigations of nerve conduction velocity performed in outpatient facilities (electriophysiology practices) or during admission to the Clinic of Neurology of the Clinical Recovery Hospital.

Patient selection criteria were: identification of patients suffering from diabetic polyneuropathy, establishment of epidemiological data on gender, average age of symptomatology, living conditions and social context of patients with diabetic polyneuropathy, determination and analysis of variability of clinical manifestations of this pathology; evaluation of the impact of diabetic polyneuropathy on the patient's quality of life, identification of the main pathologies associated with diabetic polyneuropathies and the search for possible correlations in terms of both their etiology and the way of life of the patients included in the study, the analysis of diagnostic possibilities in case of diabetic polyneuropathy; role of thermography in diabetic polyneuropathy.

The study group consisted of 20 diabetic patients admitted to the Neurology Clinic of the Clinical Recovery Hospital and the Diabetes Clinic. The casuistry was divided into two study batches (Batch I - 20 patients with type 2 diabetes and Batch II - 20 patients with type 1 diabetes), depending on the type of diabetes.The age of the patients ranged from 20 to 81 years, the average of the group being 58.90 ± 12.37 years, being homogeneous between the batches analysed.Compared to study batches, the mean age in men was higher than that observed for women in type 1 diabetics, which is not maintained in the batch of type 2 diabetic patients, but the differences were not statistically significant.

3.4.3. Results

Distribution by place of origin revealed a slightly higher frequency of patients with type 2 diabetes mellitus.

The distribution according to living with someone factor outlined the homogeneity of the study groups, 36.7% of the patients in the first batch and 40% of the second batch lived alone.

Of the comorbidities, we have diabetic retinopathy established in the history of 57.8% of patients with type 2 diabetes and of 59% of type 1 diabetics, statistically insignificant distributions from a statistical point of view. Chronic kidney disease was significantly more common in patients with type 2 diabetes. HTA was present in the personal history of 83.6% of patients with type 2 diabetes and of 80% of type 1 diabetes. Dyslipidaemia was significantly more common in patients with type 2 diabetes.

In the personal pathological history, limb ulcerations were noted in only 15.3% of patients with type 2 diabetes with an estimated risk of 1.46 times higher. The presence of signs and symptoms was noted more frequently in the lower limbs in both type 2 diabetes (49.3%), but especially in those with type 1 diabetes (79%), statistically significant differences.

The case study showed a burning sensation or numbness in the lower limbs / feet, both in patients with type 2 diabetes (66.2%) and in those with type 1 diabetes (78%). We also noted paraesthesia, lower limb weakness, lower limb cramps, lower limb pain.

From the pathology associated with diabetic neuropathy, type 1 diabetics more frequently noted ulcers present in 19% of subjects on the left foot and in 21% on both feet compared to the current incidence of ulcerations in patients with type 2 diabetes of only 15.3%, ulcerations that cause relative risk approximately 2 times higher.

Hyperkeratosis, as a neuropathy associated disorder, did not show significant differences on types of diabetes. The "hammer fingers" were significantly more common in patients with type 2 diabetes. Amyotrophy did not show significant percentage differences depending on the type of diabetes (24.3% vs. 39%).

In the studied case, 80% of the type 2 diabetic patients and 60% of the type 1 patients have peripheral pulse in the left limb, which is also noticeable in the right foot with 78.6% and 59% respectively, insignificant percentage differences from a statistical point of view.

Achilles reflex in patients with type 2 diabetes remained absent after retest in 37.3% of patients, both on the left and right feet. In patients with type 1 diabetes, the absence of the Achilles reflex was noted in 21% of patients.

In the case study, it is noted that approximately ½ of the patients (53%) recorded nerve conduction velocity below 43 m /s on the sural nerve and below 39 m /s on the peroneal / tibial nerves.

Thermography in patients with conditions present at the time of study - by drawing the ROC curve, on the case study studied, it is noted that SPOT, regardless of the tested foot, is a good predictor of neuropathy in patients who also associated ulcerations at the time of study - left foot (AUC = 0.670; IC95%: 0.576-0.783) and right foot (AUC = 0.756; IC95%: 0.661-0.852). SPOT thermography is a good predictor of neuropathy in patients who have associated osteolysis at the time of study; SPOT, regardless of the foot tested, it is a good predictor of neuropathy in patients who have associated hyperkeratosis at the time of study; SPOT thermography is not a good predictor of neuropathy in patients who have associated amyotrophy at the time of study.

SPOT reassessment – in patients in group I, patients with type 2 diabetes, mean SPOT values decreased by 6.7% in the left foot and increased by 0.3% in the right foot.

In patients in batch II, patients with type 1 diabetes, mean SPOT values decreased by 37.6% in the left foot and increased by 1.9% in the right foot.

A characteristic of diabetic neuropathy is symmetry of symptoms and signs (in both feet), and our results indicate that the etiology in diabetic patients may be mixed, which should be investigated further in clinical practice.

3.4.4. Discussions

Medical thermography can be considered a step forward in preventive medicine. The term medical thermography refers to a non-invasive investment for the patient.

Thermography has been successfully used in the detection and characterization of the following neurological disorders: irritation and compression of nerve roots, peripheral nerve

lesions, occlusive diseases of cerebral vessels, migraine, spinal cord injuries and pain syndrome differentiation.

The advantageous features of thermography (painless, non-invasive, without adverse biological effects, it is objective, produces a dynamic recording and is no more expensive than other diagnostic procedures) makes it suitable for diagnosis of neurological disorders.

In 2002, Saldo - Butkovic et al. carried out a characterization of the thermal models in the case of neurologically normal patients and those who had peripheral neural structures in the lumbosacral region with particular reference to the anterior femoral area. The authors conclude that it was an isothermal imbalance that altered the inclination angle of the isothermal lines relative to the horizontal line and in the presence of isotherm. This may be a clear sign of injury to the peripheral nervous system. They proposed introducing this diagnostic criterion into clinical practice.

Thermography can be used to identify a local affection and determine its extent. It is a useful and sensitive technique for detecting local radio-induced conditions, especially in early and latent phases when there are no relevant symptoms.

Infrared thermography is superior in terms of diagnostic possibilities in partial body exposure, especially in the extremities.

In 2003, Stulin et al., published a paper debating the problem of use of thermography in the field of neurology, but they have not come to any conclusion. They mentioned, however, the use of thermal imaging in the recognition of pre-cancerous and cancerous conditions in mammary, articular diseases and in the diagnosis of arterial occlusion. In the same year, Carbone et al., published another paper on new possibilities for investigating and detecting arteriosclerotic lesions in carotid arteries, and cited among them intravascular thermography as well.

3.4.5. Final remarks

One of the types of images that have developed over the last decades is represented by thermal imaging. They are captured by infrared cameras and their development over the past few years has been remarkable; they can indicate small changes in body temperature.

There are studies on the use of thermography in: diagnosis of cancer, diagnosis of muscle damage, diagnosis of ischemia in patients with diabetes and evaluation of muscle recovery.

The study has shown that there is a diabetic foot injury both from the angle of polyneuropathy and the presence of peripheral arterial disease at different stages of evolution, even if clinically the patient had a pulse in the peripheral arteries.

SECTION II - PATTERNS AND FORESIGHTS OF FUTURE RESEARCH

Patterns

2.1. Directions and principles for further research stroke

Stroke is the leading cause worldwide of death and disease burden. **Post-stroke studies** have been conducted and they excluded survivors and caregivers from underserved communities. It can be argued that the impact of stroke on survivors and caregivers from underserved communities may be greater.

Using qualitative exploratory research design, we intend to perform a database for the needs of post-stroke recovery and readjustment among stroke survivors and stroke caregivers from an underserved community. Then, using this database we have to evaluate and conduct regional and national protocols in the field of post-stroke management of the patients.

A substantial proportion of patients with an ischemic or hemorrhagic stroke have long-term disabilities, such as weakness, speech difficulty, or gait disorders. In addition, cognitive dysfunction and depression are present in at least one third of stroke survivors. To identify unmet needs, including those directly related to stroke care, investigators surveyed patients or their proxies at least 2 years after the initial stroke event.

We have to questions on the following five categories: body functions, activities and participation, environmental factors, secondary prevention, and post-acute care.

There is important to underline that personal, economic, and socio-cultural nuances play a role in how post-stroke recovery is lived and experienced. Study findings highlight the importance of policies to support family-centered and system-level advocacy in post-stroke care.

Persons with stroke and their caregivers identified social support, resources, and knowledge as the most salient factors associated with stroke recovery. Perceived barriers to recovery included: physical and cognitive deficits, mood; medication issues; lack of support and resources; stigma, culture, and faith. Health care providers identified knowledge/information, care coordination, and resources in the community as key to facilitating stroke recovery outcomes.

These findings shall serve as an useful reminder of what our patients need from us and from the overall healthcare system. Patients especially sought information on secondary prevention, which includes items such as education on stroke, information on diet, education on medication use, and self-management tools. Optimizing factors such as diet and physical activity and taking medications properly are crucial for best practices in secondary prevention and to prevent readmissions.

Along with the rising global burden of disability attributed to stroke, costs of stroke care are rising, providing the impetus to direct our research focus towards effective measures of **stroke prevention**. We intend to discuss and establish strategies for reducing the risk of

the emergence of disease (primordial prevention), preventing the onset of disease (primary prevention), and preventing the recurrence of disease (secondary prevention).

Our focus includes national strategies, campaigns, and measurements of the effectiveness of preventive interventions, with an emphasis on low-income and middle-income regions of our country. Our findings will reveal that effective tobacco control, adequate nutrition, and development of healthy cities are important strategies for primordial prevention, whereas *polypill* strategies, use of mobile technology (*mHealth*), along with salt reduction and other dietary interventions, are effective in the *primary prevention of stroke*.

An effective collaboration between various health-care sectors, government policies, and campaigns can successfully implement secondary prevention strategies, through surveillance and registries, such as the WHO's non-communicable diseases programmes, across high-income and low-income countries.

Until now, little is known about the prevalence and nature of *mobile application adoption in patient* centered clinical practice, let alone about its effectiveness in changing healthcare outcomes.While there are many mobile applications in fields such as diabetes management, there are still usability and integration issues among almost all of them.

There have been relatively few randomized controlled trials involving mobile applications, although some have shown promise in managing cardiovascular disease, lung disease, ordiabetes mellitus. Additionally, there hasbeen at least one quality improvement project studying mobile technology assistance in blood pressure control for stroke survivors.

Because there havebeen no large-scale randomized control trials specifically involving stroke prevention we can say that mobile applications have the potential to change the healthcarelandscape, leading to improved outcomes while reducing cost. We intend to introduce this on our patients and mobile technology is going to be implemented in a meaningful way. In this context it is imperative that this technique to be grounded in clinical regional trials.

2.2. Directions and principles for further research in epilepsy

Epilepsy is a chronic neurological illness and about 3% of the United States population will be diagnosed with epilepsy at some. Multiple studies across cultures have found that, despite its prevalence, epilepsy is poorly understood by people with this disorder as well as health professionals. Negative portrayals in the media contribute to this situation.

Due to these, significant barriers to successful treatment persist, including stigmatization, limited patient knowledge of the disease, and poor communication with the health care provider, as well as inaccurate patient perception of adherence to treatment.

The persistence of such barriers to care, diagnosed epilepsy or even repetitive seizures can greatly influence both health and quality of life. Researchers have found that psychosocial factors can contribute enormously to patient and patient families' views about a person with epilepsy's level of health or ability to adhere to treatment.

One such factor is a patient's relationship with the care provider, who understands a patient's epilepsy experience and has the opportunity to build confidence and motivation and thereby ensure successful treatment.

Continuing misperceptions about prognosis and treatment indicate that there is a disjunction between patient and provider beliefs about knowledge needs relating to epilepsy treatment.

In order to close this gap and help providers better understand how to address barriers to epilepsy treatment, their opinions on epilepsy must be assessed. To date, however, most studies of care providers have been conducted in the United Kingdom or Australia.

Based on thse facts, our first goal in epilepsy is to lead serial trials on care providers of these patients.

This clinical condition is commonly considered an archetypical network disease, with seizures and interictal activity generated and spreading in networks involving one or both hemispheres. There are imaging evidences suggesting that epilepsy affects both structural and functional brain network properties. Thi suggests that even in idiopathic/genetic generalized epilepsy, there is a certain level of focality both in resting-state imaging as well as for generators of epileptiform activity and seizures. These structural and functional network properties are investigated using brain connectivity analysis.

Brain connectivity can be categorized into structural, functional and effective connectivity. Structural connectivity refers to the white matter connections in the brain and can be examined in vivo with MRI measuring the motion of water along the axons. Functional and effective connectivity entangle the neuronal communication between brain regions. These types of connectivity can be calculated when signals are sampled over multiple time points, such as brain activity recorded via EEG, MEG, but also fMRI, or PET.

The electrical activity of active neurons can be recorded at the scalp surface as voltage differences across EEG electrodes, while the neuronal currents in the brain generate magnetic fields that can be measured outside the scalp surface by the *MEG sensors*.

Compared to other neuroimaging clasical techniques MEG have a superior temporal resolution but an inferior spatial resolution. Despite this inferior spatial resolution, the temporal resolution and the fact that EEG and MEG directly measures neuronal activity makes them highly valuable techniques to study functional and especially effective connectivity.

We intend to combine neuroimaging techniques with high spatial resolution with a technique with high temporal resolution, such as EEG-fMRI, as a valid approach to examine slow changes in blood supply based on spiking activity or at rest and, therefore, provides an excellent validation for localization accuracy of source connectivity.

The benefits of interictal, ictal, or resting state connectivity as a predictor of disease evolution have not been formally studied and the same needs for validation and comparison of methods are needed apply here.

Regarding vagal nerve stimulation, there was studied the source activity and connectivity of the P300 response with the vagal nerve stimulation system on/off and found that good response to therapy was correlated with specific patterns of source activity and connectivity, mostly involving the limbic system, insula, and the orbitofrontal region. However, the study only investigated EEG after implantation of the vagal nerve stimulator.

M/EEG source space connectivity techniques allow studying temporal patterns and it could be used to investigate network aspects of specific phenomena such as focal slowing and link specific patterns to specific forms of epilepsy. The source space connectivity

patterns could shed more light on how these M/EEG patterns are generated in the brain, and distinguish which patterns of focal slowing are potential surface correlates of epileptogenic activity from deeper regions.

2.3. Directions and principles for further research in Parkinson's disease

Parkinson's disease is known to be caused by complex interactions between environmental factors and a genetic predisposition. Environmental factors include exposure to pesticides and toxins, heavy metals and accumulation of iron and/or manganese in the brain.

The gut-brain health and function are impaired in Parkinson's disease, often a decade before motor symptoms are diagnosed. This theory sumarise the peripheral and central nervous system pathology, gastrointestinal symptoms experienced by many Parkinson's patients, the route by which gut-brain dysfunction may occur and changes in gut microbiota that are associated with disease expression.

We consider future gut-based treatments to prevent or slow down the progression of Parkinson's disease and explore whether this knowledge may highlight biomarkers to be included in complex algorithms in the future to assess a person's risk of developing Parkinson's disease.

Patients are receptive to outpatient team-based palliative care services to address psychosocial issues, adjustment to illness (particularly at diagnosis and with progression), nonmotor symptom control, and advance care planning as an adjunct to usual care. Future research is needed to develop and test the effectiveness of palliative approaches to improve the care of patients with Parkinson's disease.

Therapeutic Perspectives in Neurology highlights the cutting-edge translational and clinical research into the treatment of neurological disorders. Potential and highly promissing topics include: advances in neurostimulation, state-of-the-art interventional neuroradiology, breakthroughs in revascularisation, progress in gene therapy, prospects of regenerative medicine, new therapeutic targets and discoveries in targeted treatment and novel pharmacological and cellular treatments.

Our perspective are focused on approaching immune reconstitution therapies as concepts for durable remission in multiple sclerosis, the role of inflammatory syndrome in migraine, the turnover of proteins linked to neurodegenerative diseases, the use of roboyic devices in the dementia services and support, and on chronic traumatic encephalopathy.

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