GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

Habilitation Thesis

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GRIGORE T. POPA UNIVERSITY OF
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Researches regarding inflammation and cardiovascular diseases

- Habilitation Thesis -

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ABSTRACT

The habilitation thesis entitled "Researches regarding inflammation and cardiovascular diseases" presents my clinical research in the field of inflammation and cardiovascular diseases, and also some others subject related with these two main medical domains.

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

- Section I – Scientific achievements from the postdoctoral period;
- Section II – Future projects in the professional, academic and scientific field;
- Section III – References.

A short overview of my professional, academic and scientific activities has been introduced before section I, where I reviewed my studies and the main direction I have followed after my PhD thesis. I have also pointed the projects in which I have been involved and the results of my academic work, materialized in 22 bachelor degree thesis. The number of articles and abstracts and also my presence at conferences/congress have been mentioned.

The first section of the habilitation thesis entitled "Scientific achievements from the postdoctoral period" has four sub-sections, each of them having at least three chapters. In this section I have included the main ideas and results of 19 of the most important articles from my scientific activity. These articles are rated by Thomson ISI Web of Science Core Collection but also some of them are indexed by international databases. I have started with the researches related to inflammation and cardiovascular diseases in general, than I have continued with clinical and electrocardiographic features.

Sub-section *I.1. Researches regarding inflammation and cardiometabolic risk* starts with a introduction containing the main theoretical ideas about the link between inflammation and cardiovascular diseases. Two reviews *regarding the relationship between inflammation, cardiovascular diseases and chronic inflammatory rheumatic diseases* have been synthesized for a good understanding of the processes. *Researches regarding the inflammation, cardiovascular risk and metabolic syndrome* is the title of the next sub-chapter and contains literature data and the main ideas from two reviews and one research paper. The results of two others research works have been shown in *Researches regarding the inflammation, cardiovascular risk and periodontal disease* (indexed by international databases). This sub-section presents the results of 4 articles/reviews rated by Thomson ISI Web of Science Core Collection and 2 articles indexed by international databases.

Researches regarding inflammation and arrhythmogenic risk is the second sub-section of section I and includes information from four articles rated by Thomson ISI Web of Science Core Collection and one recent reasearch article indexed by international databases. Data about mortality risk imflamation and cardiovascular diseases, QT interval variations and associated consequences, early repolarization syndrome and clinical implications, the relationship between gastro-oesophageal reflux disease, atrial fibrillation and pathogenic mechanisms, volumetric assessment methods left atrium and anticoagulant therapy in patients with atrial fibrillation are

presented.

Researches regarding inflammation and the role of oxidative stress and interleukins in cardiovascular diseases is the third sub-section of my thesis and offers important informations about immune processes and inflammation. There are described metabolic consequences, chronic inflammatory response, development of vascular fragility, liver abnormalities, as well as the main mechanisms involved in degenerative pathology. This sub-section synthesizes meaningful data from 4 articles rated by Thomson ISI Web of Science Core Collection.

The last sub-section entitled *Researches regarding the rol of imaging in cardiovascular diseases* highlights important details about noninvasive imaging tool for assessing atheromatous plaque morphology and composition and the risk for major adverse cardiac events MACE (defined as all-cause mortality, cardiovascular death, myocardial infarction, repeated revascularization, repeated hospitalizations for cardiovascular related incidents, cerebrovascular events), data synthesized from an indexed item in the Thomson ISI Web of Core Collection.

Section II includes my future projects in the professional, academic and scientific field. Regarding the professional activity, my main purpose is improving the quality of care in hospital, I have identified several achievable goals in terms of professional activity which will be listed in this section.

Section III contains over 500 references used for this thesis and for the articles included. A considerable number of this references are new ones, from the last 5 years, meaning that the subject and the themes from my thesis are actual problems, that had not found solutions yet.

REZUMAT

Teza de abilitare „**Cercetări privind inflamația și bolile cardiovasculare**“ prezintă activitatea mea clinică și științifică în domeniul inflamației și ale bolilor cardiovasculare, precum și alte subiecte legate de aceste două domenii medicale principale.

Într-o primă etapă, pentru redactarea acestei teze, am studiat cu atenție recomandările Consiliului Național de Atestare a Titlurilor, Diplomelor și Certificatelor Universitare (CNATDCU). Am urmat instrucțiunile menționate mai sus și am structurat teza în trei secțiuni principale:

- Secțiunea I – Realizări științifice din perioada postdoctorală;
- Secțiunea II – Proiecte viitoare în activitatea profesională, academică și științifică;
- Sectiunea III – Referințe.

O scurtă trecere în revistă a activităților mele profesionale, academice și științifice a fost introdusă înaintea secțiunii I, unde am revizuit studiile și direcția principală pe care am urmat-o după susținerea tezei de doctorat. Am subliniat, de asemenea, proiectele în care am fost implicat și rezultatele activității mele academice, materializate în 22 teze de licență. Au fost menționate numărul articolelor, rezumatelor, precum și participările mele la conferințe / congrese.

Prima secțiune a tezei de doctorat intitulată „Realizări științifice din perioada postdoctorală“ are patru subcapitole, fiecare având cel puțin trei capitole. În această secțiune am inclus principalele idei și rezultate a 19 articole importante din activitatea mea științifică. Aceste articole sunt indexate de Thomson ISI Web of Science Core Collection, dar și unele sunt indexate în baze de date internaționale. Am început cu cercetările legate de inflamație și bolile cardiovasculare, în general, continuând cu particularitățile clinice și electrocardiografice.

Sub-secțiunea I.1. *Cercetări privind inflamația și riscul cardiometabolic* începe cu o scurtă prezentare care conține principalele idei teoretice despre inflamație și bolile cardiometabolice. Apoi, pentru o bună înțelegere a proceselor, am sintetizat un review despre inflamație, al cărui autor sunt. *Cercetări privind inflamația și sindromul metabolic* este titlul următorului subcapitol și conține date din literatură și principalele idei din două articole proprii. Trei lucrări publicate au fost introduse în subcapitolul *Cercetări privind inflamația și sindromul metabolic* și alte două în subcapitolul *Cercetări privind inflamația, riscul cardiovascular și boala periodontală* (indexate în baze de date internaționale). Această sub-secțiune prezintă rezultatele a 4 articole / reviewuri indexate în Thomson ISI Web of Science Core Collection și 2 articole indexate în baze de date internaționale.

I.2. Cercetările privind inflamația și riscul aritmogen se regăsesc în a doua parte a secțiunii I. Cuprinde un subcapitol teoretic și patru subcapitole cu informații din patru articole indexate ISI. Sunt prezentate informații despre riscul de mortalitate, variațiile intervalului QT și consecințele asociate, sindromul de repolarizare precoce și implicațiile clinice, legătura dintre boala de reflux gastro-esofagian, fibrilația atrială și mecanismele patogene, precum și metodele volumetrice de evaluare a atriului stâng.

I.3. Cercetările privind inflamația și studiul markerilor de stres oxidativ este a treia sub-secțiune a tezei și oferă informații importante despre procesele imune și inflamație. Sunt descrise consecințele metabolice, răspunsul inflamator cronic, dezvoltarea fragilității vasculare, anomaliile

hepatice, terapia periodontală şi markerii disfuncţiei renale, precum şi principalele mecanisme implicate în patologia degenerativă. Această sub-secțiune sintetizează date semnificative din 4 articole indexate ISI.

Ultima sub-secțiune *I.4. Cercetările privind rolul imagisticii în bolile cardiovasculare* evidențiază detalii importante despre modalităţile tehnice non-invazive de evaluare a morfologiei și compoziției plăcii ateromatoase şi riscul evenimentelor cardiace majore adverse MACE (definit ca mortalitate de toate cauzele, deces cardiovascular, infarct miocardic, revascularizare repetată, spitalizări repetate pentru incidente cardiovasculare, evenimente cerebrovasculare), date sintetizate dintr-un articol indexat în Thomson ISI Web of Core Collection.

Secțiunea II include proiecte mele viitoarele în domeniul profesional, academic și ştiinţific. În ceea ce privește activitatea profesională, scopul meu principal este îmbunătățirea calității îngrijirilor medicale oferite în spital. Am identificat mai multe obiective realizabile în ceea ce privește activitatea profesională care vor fi enumerate în această secțiune.

Secțiunea III include un număr de peste 500 de referințe bibliografice utilizate pentru această teză și pentru articolele incluse. Un număr considerabil de referințe sunt noi, din ultimii 5 ani, ceea ce înseamnă că subiectul și temele din teza mea sunt probleme reale, pentru care încă nu s-au găsit soluții.

OVERVIEW OF PERSONAL PROFESSIONAL, ACADEMIC AND SCIENTIFIC ACHIEVEMENTS

The habilitation thesis entitled “Researches regarding inflammation and cardiovascular diseases” presents my clinical research after the achievement of my PhD thesis entitled “Alcoholic dilated cardiomyopathy – clinical and experimental study”, under the coordination of Professor Adrian Cosovanu, which was confirmed by the Ministry of Education (Diploma No. 386/19.05.2004).

The approach in my doctoral thesis was the concept of "alcoholic dilated cardiomyopathy" in terms of inflammation, with the demonstration of the oxidative stress enzymatic profile, with a determining role in the induction of apoptosis and their correlation with the histopathological changes assimilated to a systemic disease. The approach and treatment in a doctoral thesis of alcohol dilated cardiomyopathy, both clinically and experimentally, is evidence of a major interest for this condition with wide multidisciplinary implications through the epidemiological dimension of alcoholism and by the inflammatory impact on myocardial function, work and vital prognosis. In addition to the multidisciplinary complex study of the 215 enrolled patients, the personal part is materialized by carrying out the first experimental biochemical, biophysical, anatomopathological (including electron-optic) and MRI study in Romania, a study that proves the value of the experimental model used and provides support pathogenic and anatomo-pathological experimental mechanisms of ethanol intoxication in the rat. An etiopathogenic model of the initial mechanisms of myocardial lesion development is therefore recommended, which is basically a diagnostic algorithm and the quintessence of this extensive clinical and experimental research. Practical usefulness of the work is required in the current work and allows development in complex research programs in the future.

Presently, I am associate professor at the Internal Medicine Discipline – Medical Department I, Faculty of Medicine, “Grigore T. Popa” University of Medicine and Pharmacy, Iasi. At the same time, I am working as senior physician in the field of cardiology and internal medicine at the largest hospital in the Moldavian region, Iasi Clinic Emergency County Hospital. Also I am the head of the 3rd Medical Clinic, at the hospital mentioned above. My professional activity, my academic activity and my scientific and research activity are in a closed correlation and I try to avoid neglecting any of them, by working all the time on subjects that covers my entire activities.

Professional activity

I have graduated in 1988 “Grigore T. Popa” University of Medicine and Pharmacy Iasi, Romania, Faculty of General Medicine (Diploma No. 1137/no.144/24.10.1988), after graduation starting clinical activity as trainee physician and later as a resident doctor in Internal Medicine.

I have finished this program and get my professional experience working in the field of Internal Medicine in 1994 and in the same year I have become specialist physician (Certificate No. 240/18.02.1994/ Adress No.4603/28.03.1994). In 1998, I have become senior physician in Internal Medicine (OMS 694/1998/Adress 2994/09.03.1999). In 1998, I have

finished my second speciality becoming specialist physician in Cardiology (OMS 1011/30.12.1998; Certificate Serial Number A No. 827/21.01.1999). Because my second speciality has a strong connection with my principal one, I have continued the activity in this field and in 2008 I became senior physician in Cardiology (OMS 1971/03.12.2008; Certificate No. 94/06.01.2009). In my daily practice I faced a complex pathology with multiple comorbidities which implies permanent professional development gaining competence in echocardiography in 1999 (Certificate C No. 001253/29.03.1999).

I think it is important to mention that since 2016 I have been the head of the 3rd Medical Clinic from Iasi Clinic Emergency County Hospital (Decision No. 2581/28.12.2015). A course on General Management at the "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania (Certificate E No. 0007595/1345/11.07.2006) was very useful for my leadership activity.

I have attended and finished postgraduate courses constantly in different cities of Romania (Iasi, Cluj-Napoca, Bucharest, Sinaia), but also in cities from other countries (Paris, Madrid, Viena, Roma, Barcelona, Munich, London, Amsterdam, Berlin). I am an active member in important Scientific Societies: Romanian Society of Internal Medicine, Romanian Society of Cardiology, Romanian Association of Algesiology, International Association for the Study of the Pain, Society of Physicians and Naturalists, American College of Cardiology, Member of EACVI, Romanina Society of Pathophysiology, Fellow of the European Society of Cardiology, Heart Failure Association of the ESC (HFA), European Society of Internal Medicine.

Academic activity

My teaching career has been started in 1991, as Junior Teaching Assistant, position held by competitive examination, at "Grigore T. Popa" University of Medicine and Pharmacy, Iași, Faculty of Medicine, Internal Medicine Department. In 1994 I have advanced to University Assistant. In 2002 I became Lecturer and in 2015 Associate Professor.

I am often a member of different committees: admission committees in bachelor programs and masters programs, specialist physician / primary school commissioner, member of committees for the submission of scientific reports within the doctoral thesis, scientific advisor in doctoral commissions, member of committees for teaching competitions for assistant professor, assistant, lecturer and so on. I have been regularly involved in coordinating the teaching and scientific activities of residents and students, and I have been coordinating and supervising some original papers presented at local student conferences or dedicated and even national ones, some of them receiving even awards.

During my academic activity I have coordinated 5 postgraduate courses and I have been lecturer at 7 postgraduate courses. Since 2002 I have coordinated an impressive number of 22 bachelor degree thesis, 4 of them in English. To help my students understand some aspects of the complex and enigmatic field of internal medicine and cardiology, I was a member of the college of authors of several scientific volumes. I was also the author of several chapters of books along with other colleagues.

Scientific activity

The results of my scientific and research activity have been published in articles

indexed by the Web of Science Core Collection and in other international databases. I have also disseminated the results local, national and international congresses, conferences, seminars and workshops. Also I have published books and book chapters, as principal author or as second author.

In the PhD thesis I studied alcoholic cardiomyopathy and markers of oxidative stress. I have continued my research with the study of inflammation in cardiovascular disease, as this field is not yet fully understood, is a subject of particular interest in the medical field, because cardiovascular disease represents a pandemic with the growth of the elderly.

As I mentioned in the section regarding my academic activity I have published 8 books, 3 as principal author and 5 as second author. Also, I have contributed to the publication of 18 book chapters. Until now, the results of my research activity have been highlighted in 19 articles rated by Thomson ISI Web of Science Core Collection, 53 articles indexed by international databases, 9 abstracts rated by Thomson ISI Web of Science Core Collection and 133 abstracts indexed by international databases. These articles have a total of 94 citations in Google Scholar.

I have participated at 33 International Conferences/Congress, 190 National Conferences/Congress and 41 Local Conferences/Congress.

During 2006-2008, I was the sub investigator of the project program with the title: *Quantifying the effect of simvastatin in systemic inflammation, of subclinical cardiac changes and early atherosclerosis in rheumatoid arthritis*- CESIA. The project was a complex study that had an experimental part on animal model and very important, a clinical part. The clinical part, included the observational clinical study of cross-sectional type that determined the component of subclinical cardiovascular dysfunction and the risk factors for the vascular disease at patients with rheumatoid arthritis and a randomized, double-blind, placebo-controlled study, conceived to evaluate the efficacy of simvastatin 10 and 40 mg/day at the patients with rheumatoid arthritis that had remissive treatment on a period of six months. Also, I was part at 5 clinical trials, some of them complex national trial.

Between 2010 and 2015, I was part of the team of 7 different projects, in two of them I was project manager:

- *Project ID POSDRU/174/1.3/S/149155 - Project Manager - President of the University Commission "Teaching Staff in the Pre-university and State University Education System - Promoter of Lifelong Learning", 2014-2015;*
- *Project ID POSDRU/160/2.1/S/139881 - Project Monitoring Officer - "Professional Counseling for Medical Students and Integrated General Practice and Dental Practice Program" Contract, 2014-2015;*
- *ISTEW 539194-LLP-1-2013-UK-ERASMUS-EQR - Improvement Science Training for European Healthcare Workers;*
- *Project ID POSDRU/86/1.2/S/63699 - Adaptation of the Superior Medical Dental Supply Offer to Labor Market and Society Based Knowledge*;
- *Project ID POSDRU/87/1.3/S/62208 - Center for training specialists and resources in oral rehabilitation, 2011-2013;*
- *Project ID POSDRU/81/1.2/S/62594 - MEDICALIS Educational Management and Quality Education in Information Society, 2010-2013.*

SECTION I
SCIENTIFIC ACHIEVEMENTS FROM THE POSTDOCTORAL PERIOD

I.1. RESEARCHES REGARDING INFLAMMATION AND CARDIOMETABOLIC RISK

I.1.1. Hallmarks

At the moment, cardiovascular diseases (CVD) are the leading cause of death worldwide. In the United States statistical data indicates that one of every three deaths is from CVD (1). Sadly, it kills more people than all forms of cancer and respiratory diseases combined and is the primary killer of women. According to World Health Organization – WHO, there are different types of cardiovascular disease, Table I.1.1 presenting those with the highest incidence and their specific feature (2).

Tabel I.1.1. Cardiovascular disease acording to WHO

Cardiovascular disease	**Particularity/ specific feature**
Coronary heart disease	Disease of the blood vessels supplying the heart muscle
Cerebrovascular disese	Disease of the blood vessels suppying the brain
Peripheral arterial disease	Disease of blood vessels supplying the arms and legs
Rheumatic heart disease	Damage to the heart muscle and heart valves from rheumatic fever, caused by streptococcal bacteria
Congenital heart disease	Malformations of heart structure existing at birth
Deep vein thrombosis and pulmonary embolism	Blood clots in the leg veins, which can dislodge and move to the heart and lungs.

Risk factors for CVD are numerous and are often generically classified as modifiable and nonmodifiable (3). Behavioral risk factors of heart disease and stroke have the greatest influence on these condition. Among them is important to mention: unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol. Their effects may show up in individuals as raised blood pressure, raised blood glucose, raised blood lipids, overweight and obesity. They are also named intermediate risks factors and can be measured in primary care facilities and indicate an increased chance of developing a heart attack, stroke, heart failure and other complications. Also, it is important to take into consideration a number of underlying determinants of CVDs which are a reflection of the major forces driving social, economic and cultural change – globalization, urbanization and population ageing. Poverty, stress and hereditary factors are other determinants of CVDs (2).

Atherosclerosis – ATH is defined as a process of continuous thickening and hardening of the walls of medium and large-sized arteries. This process is the result of fat deposits on the inner lining of arteries, ATH being considered a cholesterol storage disease. This pathological condition underlies several important adverse vascular events including coronary artery disease – CAD, stroke, and peripheral arterial disease, responsible for most of the

cardiovascular morbidity and mortality worldwide (4). In the past years, understanding the pathophysiology of CAD has experienced a remarkable evolution.

In the past, atherosclerosis has been seen as a degenerative disease caused by the continuous accumulation of cholesterol in the arterial intima and the idea that atherosclerosis is a principally lipid-driven disease has dominated the field of CVD many years. But, over the last few decades, the concept of atherogenesis has changed, because new evidences that atherosclerosis is linked to a chronic low-grade inflammation of the vessel wall have been strongly proved. The hypothesis that atherosclerosis presents features of an inflammatory disease has been first speculated since the 19th century, based on some theories made by Rudolf Virchow, Karl Rokitansky and others (5). The concept that inflammation may play an important role in atherosclerosis has been confirmed by valuable investigations that have concentrated their attention on inflammation, new understandings into mechanisms of disease being established (6-10).

The internal surface of blood vessels is lined by a layer called endothelium. This layer is forming an extensive cellular tissue which covers the entire vascular network, represented by major veins and arteries, minor vessels, arterioles and capillaries. Endothelium plays a main protective role in vascular homeostasis and has many endocrine, autocrine and paracrine functions, which is why it is responsible for synthesizing vasoconstrictor and vasodilator substances (11, 12). Shear stress carried by blood flow against endothelial cells has as a result the production of nitric oxide – NO. NO is related with two key aspects of O_2 supply and demand, first it regulates vascular tone and blood flow by activating soluble guanylate cyclase (sGC) in the vascular smooth muscle, and second, it controls mitochondrial O_2 consumption by inhibiting cytochrome C oxidase. In brief, NO main function is to maintain the blood vessel in a constant state of vasodilatation (13, 14).

An imbalance between vasodilatation and vasoconstriction will lead to an endothelial dysfunction, which is related to atherosclerosis and cardiovascular events. Moreover, this dysfunction represents an imbalance between mediators that regulate vascular tone and hemostasis (15).

The protective functions of endothelium are severely affected by dyslipidemia and arterial hypertension. These to main factors will cause first vascular damage, and in time will contribute to increase of oxidative stress and inflammation. Without doubt, inflammation is a key point in all of the stages of the atherosclerosis process, starting with the emergence of the lesion and finally leading to a coronary event (16).

There is a fundamental relationship between the chronic vascular inflammatory process and endothelial capacity to produce proinflammatory cytokines, factors and adhesion molecules (15).

The involvement of inflammation in atherosclerosis has lead to the discovery and research of inflammatory biomarkers for cardiovascular risk prediction. Nowadays, C-reactive protein – CRP is the main validated inflammatory biomarker, even if it has been used in clinical diagnosis for many decades. Soluble CD40 ligand, adiponectin, interleukin 18, and matrix metalloproteinase 9 are other important biomarkers monitorized, providing additional information for cardiovascular risk stratification and prediction (17).

Usually, ATH does not cause signs and symptoms until it severely tightens or totally blocks an artery. This represents a major problem, because many people don't know they have

the disease until they have a medical emergency, like a heart attack or stroke.

However, some people have some signs and symptoms of the disease, which depend on which arteries are affected. ATH affects coronary arteries, carotid arteries, peripheral arteries and renal arteries.

Coronary arteries supply oxygen-rich blood to your heart, and if plaque narrows or blocks these arteries will result a disease called ischemic heart disease. The most common symptom of ischemic heart disease is angina, other symptoms of ischemic heart disease being shortness of breath and arrhythmias. Also, plaque can be formed in the smallest arteries of the heart, in this case the disease is called coronary microvascular disease (MVD). The symptoms are similar with those of ischemic heart disease including angina, shortness of breath, sleep problems, fatigue and lack of energy.

The carotid arteries supply oxygen-rich blood to the brain, and if plaque narrows or blocks these arteries the symptoms are similar to those of a stroke: sudden weakness, paralysis, confusion, trouble speaking or understanding speech and so on.

Plaque also can form in the major arteries that supply oxygen-rich blood to the legs, arms, and pelvis, disease which is called peripheral artery disease, resulting insensibility, pain, and, sometimes, dangerous infections. The renal arteries supply oxygen-rich blood to the kidneys, causing chronic kidney disease. In time,this disease cand even lead to a slow loss of kidney function (18).

Inflammation is the primordial detection and alarm system of the body, aimed at the containment and elimination of foreign toxins and microbial pathogens. In recent years, chronic inflammation has begun to be considered a contributory factor in the development of numerous chronic diseases including CVD. The host inflammatory response and the mobilization of cells and soluble mediators is critical in commencement of immune responses and essential for host defense against infections. Moreover, the host inflammatory reponse is very important for minimizing or repairing tissue damage. When severe inflammation persists may has as a result the nitiation, progression, and degenerative features of chronic diseases like native atherosclerosis or the vasculopathy of autoimmune disorders like rheumatoid arthritis – RA. As a result of constant research, it has become increasingly apparent in recent years the fact that inflammation is a central mechanism involved in the entire life cycle of ATH (19).

The accumulation of lipids in the artery wall is not the only factor that characterize the atherosclerotic plaque, also the infiltration of numerous inflammatory cells, such as macrophages, T cells, mast cells, monocytes, and neutrophils play essential roles in mediating the inflammatory response in ATH. Another phenomenon is the formation by vascular smooth muscle cells of a fibrous cap composed mostly of collagen. Early lesions consist of subendothelial depositions of lipids, macrophage foam cells loaded with cholesterol and T cells. In time, a more complex lesion evolves, with apoptotic and necrotic cells, as well as cell debris, followed by cholesterol crystals forming a necrotic core in the lesion.

The study of inflammation biology in cardiovascular disease has focused a considerable attention on protein mediators, such as cytokines and chemokines, or on small molecules, such as the prostaglandins and reactive oxygen or nitrogen species (19). Together with growth factors, proinflammatory cytokines and chemokines serve to further elaborate this response in the vessel wall. Plaque growth can cause stenosis that can contribute to ischemia in the

surrounding tissue (20, 21).

The function of these proinflammatory cytokines, a group of cytokines produced particularly in response to inflammatory stimuli, is to communicate to surrounding tissue the presence of infection or injury. The most important proinflammatory cytokines are tumor necrosis factor (TNF)-α, the interleukins (ILs) IL-1, IL-6, IL-8, IL-12, and IL-18, and interferon-γ (IFN-γ). TNF and IL-1 are the principal mediators of the inflammatory response and are thought to have a critical role in orchestrating the local response through cell activation and triggering a cytokine cascade. Proinflammatory cytokines can enter the systemic circulation and produce immune cell activation and significant alterations in host physiology, such as fever and the acute-phase reaction (22-24).

I.1.2. Researches on the relationship between inflammation, cardiovascular diseases and chronic inflammatory rheumatic diseases

A. Background

Rheumatologists have long admitted that a variety of systemic inflammatory diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), ankylosing spondylitis, gout, psoriatic arthritis, and medium and large vessel vasculitides, are correlated with increased risk of cardiovascular diseases (CVD) (25).

It is well known that life expectancy of patients with RA is three to ten years less than that of the normal population. Even if it was already appointed the fact that cardiovascular mortality is higher in RA patients, the reasons for this remain ambiguous. Nowadays, ischemic heart disease (IHD) as a consequence of atherosclerosis is the most prevalent cause of death associated with CVD in patients with RA. Unfortunately CVD accounts an impressive percentage of all deaths in RA patients, more accurately, statistics show that the mortality rate is 30%, even up to 50% (26).

B. Published papers in this field

Together with physicians with a remarkable experience in rheumatology, I have studied the link between cardiovascular and rheumatic diseases. The interest in this field led to results published in two papers.

1. Rezuş E, Floria M, Grigoriu A, Tamba BI, **Rezuş C**. Cardiovascular risk factors in chronic inflammatory rheumatic diseases: modern assessment and diagnosis. *Current Vascular Pharmacology* 2015; 13 (6): 716-24.

Introduction

Chronic inflammatory rheumatic diseases (CIRD) are associated with an increased risk of atherosclerotic events and premature cardiovascular (CV) disease. Even if complex researches have been made, the precise nature of the relationship between local and systemic inflammation, their interactions with traditional CV risk factors, and their role in accelerating atherogenesis remains unresolved. Anti-inflammatory therapies may influence positively or

negatively the patient's general condition and understanding these influeneces is a real challenge (25).

An increase in the incidence of some traditional risk factors and also the presence of CV risk factors, which are the result of systemic chronic inflammation and/or various drug therapies, occur during ATS in CIRD. The occurrence of CV events 10 years earlier in CIRD diagnosis, especially in RA, suggests that joint inflammation, together with the immunologic and metabolic disorders specific to these conditions, are independent CV risk factors (27).

Traditional cardiovascular risk factors

There are a considerable number of risk factors like: obesity, dyslipidemia, type 2 diabetes mellitus (T2DM), metabolic syndrome (MetS), hypertension, physical inactivity, advanced age, family history of CVD, and tobacco have been associated with CVD in RA patients (26). Actually, patients with confirmed RA may act as an independent risk factor for CVD (28).

Hypertension (HTN) has a high prevalence in rheumatoid arthritis (RA) patients but unfortunately is often both underdiagnosed and undertreated in RA patients (28). Issues like systemic inflammation, sedentary lifestyle, obesity, and RA medication inhibit suitable blood pressure control in patients with CIRD (29). The mechanism by which systemic inflammation stimulates HTN is linked to high C reactive protein (CRP) levels. CRP regulates Ang-1 receptor expression and influences both the renin-angiotensin system and plasminogen activator inhibitor-1 induction, which increases fibrinolysis and atherothrombosis. Therefore, CRP plays a dual role in HTN. In HTN, the hemodynamic flow and elevated vascular parietal stress produce more adhesion molecules and inflammatory gene expression by endothelial cells, as well as a triggering of the inflammatory cascade in the arterial wall, with pro-inflammatory cytokine production and an acute phase response that includes higher CRP levels (28).

Dyslipidiemia: The HDL (decreased high-density lipoprotein) cholesterol is unable to protect the LDL (low-density lipoprotein) cholesterol against oxidation (pro-inflammatory HDL) (30, 31). The body fat mass index of patients with CIRD is also higher than expected for the same body mass index (BMI), age, sex and ethnic group (32). The pathophysiological mechanism for this seems to involve the pro-inflammatory cytokines (tumor necrosis factor – TNF and interleukin-6 – IL-6), which mediate rheumatoid cachexia development, involuntary muscle mass loss, and progressive body fat mass increase (32-34).

Special attention has been paid to anti-TNF therapy; however, its influence on the lipid profile is a controversial matter. While some studies have revealed an improvement in dyslipidaemia and a disproportionate increase in the total and HDL cholesterol levels, others have claimed that this therapy caused a “more atherogenic” lipid profile (35). Acute and chronic inflammation may lead to structural and functional changes of HDL, which render the particles proinflammatory. It seems that therapeutic agents that increase HDL levels may restrain the transformation of normal HDL into dysfunctional HDL (36).

Diabetes mellitus (DM) causes a lot of complications. Diabetic macroangiopathy, atherosclerosis secondary to DM may cause cerebral vascular disorder, ischemic heart disease, peripheral arterial disease, or other vascular diseases. These represent major causes of

death in patients with DM and gravely reduce their quality of life (37). An increased prevalence of metabolic syndrome (defined by central obesity, HTN, dyslipidaemia, and insulin resistance) has been reported in RA patients, and a direct correlation has been found with intima-media thickening in the carotid artery (38).

Sedentary lifestyle: Patients with CIRD are prone to a sedentary lifestyle because of their chronic musculoskeletal condition (pain, arterial stiffness, ankylosis, misalignment, tendon retraction etc). This lack of physical exercise leads to higher incidence rates for other CV risk factors: high BMI, central adiposity, HTN and dyslipidaemia (28, 35).

Nontraditional (inflammation-related) CV risk factors

The pro-atherogenic CIRD and vascular ATS share similar pathophysiological mechanisms that include pro-inflammatory cytokines, TNF α, and auto-reactive T cells. Systemic inflammation can induce vascular lesions and endothelial dysfunction through changes in NO production and secondary dyslipidaemia and can trigger the coagulation cascade. In addition to ATS plaque formation, the inflammatory process also causes complications from these phenomena (namely plaque rupture and thrombosis).

The pro-atherogenic effect of chronic systemic inflammation can be seen at two different levels (30):

- the endothelial dysfunction from the imbalance between the endothelial and inducible nitric oxide – NO synthases;
- the consequent excessive production of NO, imbalance in certain prostanoids, proatherogenic lipid profile support, and coagulation cascade activation (platelet activation and vascular inflammationmediated secretion of adhesion molecules, chemokines, and coagulation factors).

The predispo \sition for vascular dysfunction in CIRD is mediated by several paths: proinflammatory cytokines (TNFα, IL-1, and IL-6), acute phase reactants (erythrocyte sedimentation rate – ESR and CRP), chemokines (monocyte chemoattractant protein-1), thrombosis, adhesion molecules, cytotoxic response, insulin resistance, oxidized lipids and hyperhomocysteinaemia. All of these will cause vascular wall destruction, endothelial cell apoptosis, decreased NO production, increased platelet aggregation, smooth muscle cell proliferation, endothelial dysfunction and premature ATS (39).

Inflammation mediators: The local (synovial and vascular) and serum expression of inflammation mediators are high in both ATS and CIRD, the most remarkable of which are TNF-α, IL-1 and matrix metalloproteinases (40, 41). The increased inflammation levels found in the population without RA, which is reflected in the elevated CRP levels, increases individual myocardial infarction risk considerably. TNFα, a key factor in RA pathophysiology, increases the expression of adhesion molecules and IL-6 synthase and promotes endothelial dysfunction by reducing NO bioavailability (42). Indeed, its high level is a predicting factor for coronary event recurrence in AMI patients (43). TNFα overproduction in RA induces CD28 downregulation to LyTCD4+, which constitutes a pathogenic mechanism. Finally, TNFα is one of the factors that cause insulin resistance, increasing CV risk (44).

Prothrombotic markers: Prothrombotic markers (fibrinogen, von Willebrand factor, plasminogen activator inhibitor, and D-dimers) are independent CV mortality predictors and

are highly expressed in patients with CIRD (45). The mechanisms of prothrombotic propensity in chronic inflammatory diseases include an increase in platelet mass, low-level platelet activation, enforced by the interaction with leukocytes and the formation of proinflammatory cytokines, locally activated endothelium and an increased coagulant activity (46). CV and the factors associated with RA can alter the structure and function of platelets, starting from megakaryocytopoiesis. Hyperactive platelets target synovial membranes with subsequent local rheumatoid inflammation. Accumulating evidence suggests that DMARD decrease platelet activity (47). High mean platelet volume is associated with a variety of established risk factors, cardio and cerebrovascular disorders, and lowgrade inflammatory conditions prone to arterial and venous thrombosis. Active RA has low levels of mean platelet volume while lifestyle changes, antihypertensive or lipid lowering drugs and diet therapies may also affect mean platelet volume values (48).

Arterial stiffness: Arterial stiffness, which can be assessed with various techniques (e.g. pulse wave analysis or pulse pressure, the difference between systolic and diastolic arterial pressure), is currently considered an important CV risk factor (49). Increased arterial stiffness, which is correlated with the duration of the disease, quality of life, age and CRP values, was detected in RA patients (50, 51).

CIRD therapy and CV risk

The anti-inflammatory medications and DMARDs used to treat RA have been shown to increase CV disease prevalence in RA patients, although recent findings have suggested that some of these medications may be more cardioprotective than cardiotoxic. The effect of RA medications on atherogenesis promotion or suppression is complex and has only been partially clarified. There are several RA treatments that may theoretically promote ATS and/or atherothrombosis. Methotrexate and salazopyrin increase the serum level of homocysteine through folate depletion, and hyperhomocysteinaemia is associated with peripheral arterial and coronary ATS. However, this effect may be controlled through the concomitant administration of folic acid (33).

Glucocorticoids are associated with polymorphic CV risk because their effects on glucose, lipid and salt metabolism, water retention and immunologic function. CV risk is higher in patients with seropositive RA (68). Hafstrom et al. assessed the effect of small doses of glucocorticoids (prednisone $\leq$ 7.5 mg/day) on ATS, endothelial function and CV risk factors (HTN and dyslipidaemia) in patients with RA (69). They concluded that endothelial function was not affected by small prednisone doses. However, after administration for 4 years, the medication caused increases in systolic blood pressure and total cholesterol levels. Various studies have shown that suppression of inflammation by DMARDs, TNFα blockers, and corticosteroids may provide cardioprotection. Indeed, positive lipid profiles were found in patients that underwent traditional DMARD, TNFα blocker, and corticosteroid therapy (70).

CV risk management

Practical recommendations for CIRD therapy and CV risk management are presented in Table I.1.2. RA should be considered as a condition bearing a high CV risk. The same should apply to ankylosing spondylitis (AS) and psoriatic arthritis, although the evidence for these

conditions is poorer (52-56). The absolute CV death risk is considerable in the elderly and in men with RA, whereas the relative risk is higher in women with RA (53, 54). The chronic inflammation markers are independently associated with CV morbidity and mortality in RA (57, 58).

Tabel I.1.2. Practical recommendations for CIRD therapy and CV risk management

Therapeutic agent	Recommandations
Glucocorticoids	• Small doses • Minimal duration of treatment • Conduct cardiovascular risk factor screening and follow-up sessions (blood pressure, glycaemia, lipidaemia) at beginning of therapy and then periodically • Treat the cardiovascular risk factors (dyslipidaemia, glycaemic control, HTN treatment, smoking cessation, weight loss) • In patients with positive RF, stricter control of cardiovascular risk factors
NSAIDs	• Avoid administering specific COX2 blockers • Any nonselective NSAIDs should be individualized and with consideration of several factors (*e.g.* gastrointestinal • bleeding risk)
DMARDs	• Methotrexate and possibly sulfasalazine seem to be associated with a lower cardiovascular risk in RA patients • For specific DMARDs, observation of the disease activity control guides is necessary because there are no specific • cardiovascular risk management recommendations
Anti-TNFα therapy	• All research conducted so far has only involved subclinical forms of the vascular disease • There are no specific cardiovascular risk management recommendations

CIRD = chronic inflammatory rheumatic disease, COX2 = cyclooxygenase 2, DMARDs = disease-modified arthritis rheumatoid drugs, HTN = hypertension, RA = rheumatic arthritis, RF = rheumatoid factor, M = male, NSAIDs = non-steroid anti-inflammatory disease, TNFα = tumour necrosis factor alpha.

Adequate control of disease activity is vital for CV risk reduction; the best evidence is for anti-TNF and methotrexate therapy. Early TNFα blocker and methotrexate therapy have proven to be independently associated with a lower CV risk, to improve physical exercise, and to decrease HTN, obesity and DM risks (59-61).

Total and HDL cholesterol should be used in the SCORE model. Dyslipidaemia is associated with high CV risk in the general population (62), in whom the total/HDL cholesterol ratio (TC/HDL) is an important prognostic indicator (63). Patients with arthritis, especially those where an inflammatory disease is active, exhibit a high TC/HDL ratio and elevated triglyceride level (64). Statins can mediate some antiinflammatory effects with changes in vascular risk factors in the context of high-grade autoimmune inflammation (65). The atherogenic index has been suggested to be less susceptible to disease activity variation during long periods of time, making it more attractive to be used in CV risk prediction when compared to individual lipid concentrations (66). DMARDs, glucocorticoids, and TNF blockers decrease the TC/HDL ratio during the first months of therapy (67-69). The subsequent lipid profile improvement may also be a result of a decrease in disease activity,

improved diet and physical exercise.

CV risk assessments and interventions should observe national guidelines. While there are differences between countries (SCORE model, Framingham, etc.), there is no evidence that one model is better than the others. The therapy should be initiated when the systolic blood pressure > 140 mmHg and the LDL cholesterol > 2.5 mmol/L. The therapy should comprise antihypertensive drugs and statins; indeed, it is similar to the therapy regime administered to the general population.

The published paper offers an integrated view regarding cardiovascular risk factors in chronic inflammatory rheumatic diseases, based on the most recent concepts and hypothesis regarding the pathogenic mechanisms involved in accelerated ATS and the complexity of the resulting CIRD complications. Aggressive prevention and prompt treatment of all CV risk factors are mandatory to achieve efficient control over the inflammation and immunologic and metabolic disorders specific to CIRD. The current CV risk calculation, which consists of a sole assessment of traditional CV risk factors, underestimates the actual CV risk in people suffering from CIRD. The assessment and follow-up of both traditional and non-traditional ("disease-related") CV risk factors, as well as their classification in CV risk reduction categories are vital. Using imaging techniques, the early determination of the intima-media thickness in the carotid, flow-mediated vasodilation, and nitroglycerine-mediated vasodilation should be used for the diagnosis of endothelial dysfunction and ATS.

2. **Rezuș C**, Cardoneanu A, Dima N, Funingana Cumpata AJ, Rezus E. Myocardial ischemia in rheumatic inflammatory Diseases, *Romanian Journal of Cardiology* 2016; 26 (3): 263-268.

Nowadays, survival following a first myocardial infarct (MI) approaches around 90% and sadly, re-infarction occurs commonly and has a high mortality. Acute myocardial infarction is caused by rupture of an unstable coronary-artery plaque that appears as a single lesion on the complex medical imaging technique, called angiography. The plaque instability is sometimes caused by pathophysiologic processes, like inflammation, that exercise an adverse effects throughout the coronary vasculature resulting a multiple unstable lesions. After an ischemic event, atherosclerotic plaque grew faster and displayed higher protease activity (70, 71).

When we mention the term "inflammation" we need to refer to the recruitment of mononuclear cells found in blood, an increased expression of adhesion molecules, the production of matrix metalloproteinase and an increased release of proinflammatory cytokine (figure I.1.1).

Figure I.1.1. The main pathogenic mechanisms of atheroscleros

Due to the presence of inflammation, many inflammatory rheumatic disorders are associated with the inflammation of coronary arteries and with cardiovascular events such as: myocardial infarction, angina, coronary angioplasty and stoke. RA, SLE, giant cell arteritis and systemic vasculitis are often associated with coronary arteritis. Premature atherosclerosis occurs in RA, SLE, giant cell arteritis and also in infalmmatory myopathies (72-74).

Premature ATH followed by early deaths due to cardiovascular events or severe infections is found in SLE patients (75). Clinical trials using ultrasound at carotid artery level, sustained the early appearance of ATH plaques which were related to SLE activity which was quantified by the SLEDAI (SLE Disease Activity Index) (76). Another important fact related to cardiovascular risk is corticosteroid use which is considered to be dosedependent (77). Other factors involved in the occurrence of cardiac events are considered to be: high titer of anti DNA double-stranded antibodies, renal complications such as lupus nephritis, duration and disease activity (78).

Patients with RA have predisposition to premature ATH and also to a high prevalence of myocardial infarction (79-81). Clinical trials have highlighted a poor response to acetylcholine which leads to an impairment of endothelial vasodilatation and a limited number of circulating endothelial progenitors (82). Bergholm et al. found a defective relaxation of arterial smooth muscle due to an attenuated response to sodium nitroprusside (83).

Systemic sclerosis is another inflammatory rheumatic diseases, with a considerable risk of premature atherosclerosis. Figure I.1.2 presents the main mechanisms responsible for cardiovascular impairment are: endothelial cell injury induced by anti-endothelial antibodies, ischemia/reperfusion damage, immune-mediated cytotoxicity and an impaired vascular repair mechanism (84).

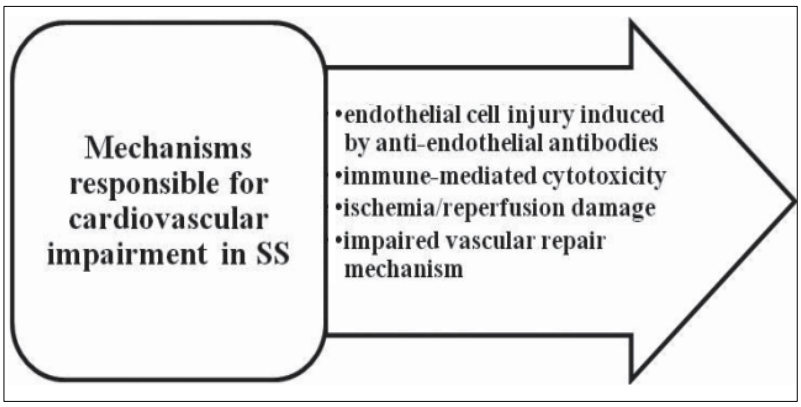

Figure I.1.2. Mechanisms responsible for cardiovascular impairment in SS

Systemic vasculitis of small, medium or large blood vessels is a chronic inflammatory disease in the rheumatic disorders subgroup and is characterized by accelerated atherosclerosis and an increased premature mortality rate due to cardiovascular events. Vascular injury has multiple mechanisms such as, activation of endothelial cells which causes increased expression of monocyte adhesion molecules and autoantigens, accumulation in an increased number of oxidized low density lipoprotein molecules and the formation of foam cells (85, 86).

In the case of some *Spondylarthropaties*, like *Ankylosing Spondylitis (SA)* and *Psoriatic Arthritis (PsA*), systemic inflammation represents the most important cardiovascular risk factor for developing a cardiac event. In AS patients, the genetic background represented by the HLA-B27 antigen increases the risk of a first-degree atrioventricular block (87). Regarding PsA, we can find accelerated atherosclerosis and arterial dysfunction due to prolonged inflammation leading to an increased secretion of Th1 cytokines, to the formation of foam cells or to endothelial dysfunction (88, 89). Furthermore, it has been revealed that persistently elevated erythrocyte sedimentation rate (ESR) is associated with the extension of atherosclerotic lesions (90).

In association with the treatment of rheumatic diseases, the use of synthetic modifying anti rheumatic drugs, mainly Methotrexate, can reduce mortality and cardiovascular risk (91). For instance, in RA, the treatment with Methotrexate, can reduce mortality with approximately 70% due to the decrease of inflammation and cardiovascular events (92, 93). Robertson et al. have proven that methotrexate increases the efflux of cholesterol from macrophages, decreasing the formation of foam cells and improving the dyslipidaemic profile (94).

The published paper suggests that patients diagnosed with inflammatory rheumatic diseases require special attention from both rheumatologists and cardiologists, due to the high cardiovascular risk associated with these diseases.

I.1.3. Researches regarding the inflammation, cardiovascular risk and metabolic syndrome

A. Background

The metabolic syndrome (MetS) represents a frequent disorder of the present age, with increased global prevalence, with complex etiopathogenesis and physiopathology, with great impact upon the patients, society and economy and which often is difficult to treat (95).

Literature data shows that the global prelevance of MetS of 5% in the normal-weight subjects, 22% in those with excess weight and 60% in the obese and it increases with age (10% in those aged between 20 and 29, 20% in those with ages comprised between 40-49 and 45% in those aged 60-69)(95-98). The prevalence varies between 8% and 43% in men and from 7% to 56% in women. In Romania, there is a national prevalence of excess weight of 33.1% and an obesity prevalence of 8.6%. The general prevalence in adults in the United States of America is of 34,7%, compared to the urban India of 33.5%, Malaysia with 27.5%, Brazil with 29.6% and 7.3% in China. From the point of view of the prevalence on gender, it varies, predominating in men during adolescence (10.9% vs. 6.29%), the ratio being inverted during the ages 20-39 (18% vs. 20%), reaching the adult age with a ratio predominant in women (42% compared to 51%) (95-98).

It is known the fact that the major cardiovascular events at the hypertensive patients are preceded by the development of the structural anomalies and the cardiovascular and asymptotic renal functions, of which the majority are recognized as significant independent predictors of the adverse cardiovascular results (99).

Cardiovascular disease is considered the principal cause of death and disease in persons

with diabetes mellitus (100). Patients with type 2 diabetes mellitus have a prevalence of ischemic heart disease which ranges from 10%-25% (100). Type 2 diabetes mellitus is a complex disease, characterized by disorders in the lipid profile, blood pressure and clotting factors. The traditional risk factors explain only 25% of the excess cardiovascular risk in patients with diabetes (100). This excess risk may by slightly explained also by the presence of insulin resistance.

MetS consists of a combination of impaired glucose metabolism, abdominal obesity, dyslipidemia, and hypertension. MetS contributes to the onset of cardiovascular disease. Unfortunately an insufficient number of studies have made a prospective analysis of the association between insulin resistance and cardiovascular death and disease in patients with type 2 diabetes mellitus (100).

B. Published papers in this field

Over time, I have published a number of three articles related to this theme, their main ideas being reproduced below.

1. Rezuș E, Leon MM, **Rezuș C.** Correlations between hyperuricemia and metabolic syndrome, *Rev. Chim. (Bucharest)* 2015; 66 (7): 1015-1018.
2. Gănceanu-Rusu AR, Mititelu-Tarțău L, Stătescu C, Boancă M, Poroch V, Lupușoru RV, Dima N, Bădescu C, Rezuș E, **Rezuș C**, Lupușoru CE, Study of dynamics of immunobiochemical parameters and pharmacological interferences in the metabolic syndrome, *Rev. Chim. (Bucharest)* 2018; 69 (6): 1493-1497.
3. Gănceanu-Rusu AR, Mititelu-Tarțău L, Stătescu C, Boancă M, Lupușoru RV, Dima N, Rezuș E, **Rezuș C**, Lupușoru CE, Evolution of biological parameters in the metabolic syndrome, *Rev. Med. Chir.* 2017; 121 (3): 638-644.

From the first two articles I have summarized theoretical data related to the components of metabolic syndrome, inflammatory markers and therapeutic impact.

Theoretical hypothesis

Hyperuricemia, defined as a serum urate (SU) concentration above the point of saturation of 6.8 milligrams per deciliter (mg/dL) or more is the most common biochemical abnormality associated with the development of gout, but is not a sufficient causative factor. Gout and hypeuricemia may be associated with increased cardiovascular risk, but analyses in different populations show conflicting results.

Triglycerides are lipid fractions, formed by combining glycerol with three fatty acid molecules. Alcohols have a hydroxyl (HO–) group. Organic acids have a carboxyl (–COOH) group. Alcohols and organic acids join to form esters. The glycerol molecule has three hydroxyl (–OH) groups. Each fatty acid has a carboxyl group (–COOH). In triglycerides, the hydroxyl groups of the glycerol join the carboxyl groups of the fatty acid to form ester bonds, as can be seen in figure I.1.3.

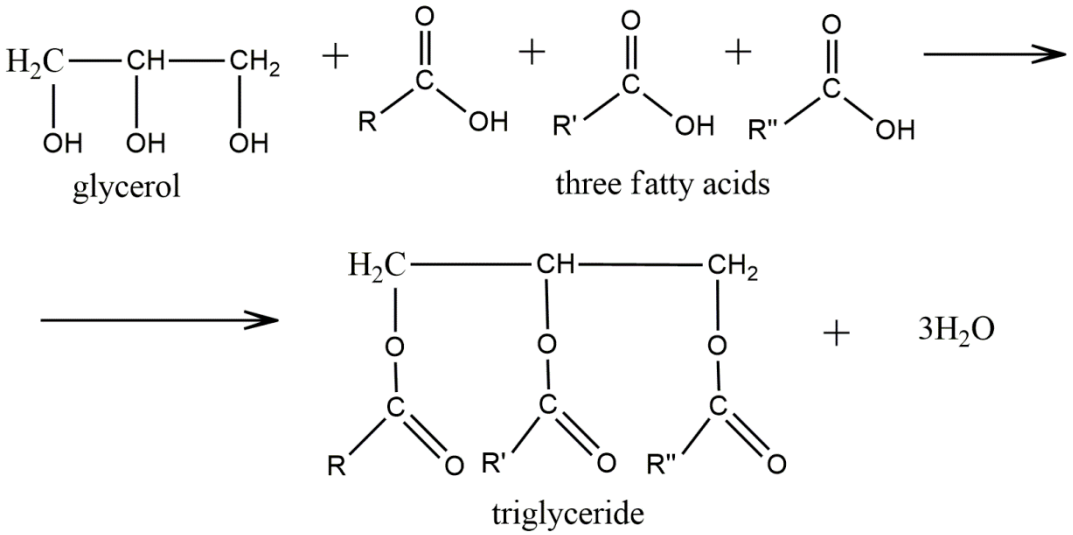

Figure I.1.3. Synthesis of triglyceride by esterification

The three fatty acids (R–COOH, R'–COOH, R''–COOH in the above equation) are usually different, but many kinds of triglycerides are known. The chain lengths of the fatty acids in naturally occurring triglycerides vary, but most contain 16, 18 or 20 carbon atoms. The level of triglycerides is very important because high levels stimulate the atherosclerosis process, and, by extension, the risk of cardiovascular diseases and stroke.

Another lipid fraction who plays an important role in the atherosclerotic process is HDL – cholesterol. HDL is one of the five major groups of lipoproteins, the smallest, which transport lipid around the body. Lipoproteins have central core of a hydrophobic lipid, encased in a hydrophilic coat of polar phospholipid, free cholesterol and apolipoprotein. HDL inhibits the atherosclerotic process.

Last clinical criteria used to establishe the diagnostic of metabolic syndrome is glycemia. Glucose is a monosaccharide with formula $C_6H_{12}O_6$ or H-(C=O)-$(CHOH)_5$-H, whose five hydroxyl (OH) groups are arranged in a specific way along its six-carbon back.

SOD, antioxidant metalloenzyme occurs in the conversion of superoxide radicals into water and hydrogen peroxide, subsequently decomposed to oxygen and water by the intervention of glutathione peroxidase and catalase (101). SOD determination was performed by spectrophotometric monitoring (at 505 nm) of superoxide anion generation by the participation of xanthine and xanthine oxidase.

After the interaction with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride, a Formazan- type substance was formed which showed a red reaction. It is believed that SOD activity is proportional to the degree of inhibition of the color reaction, in that a SOD unit represents that enzymatic activity for which the 50% inhibition of the color reaction is inhibited. Glutathione peroxidase (GPX) is an enzyme mediating the protection of proteins, lipids and nucleic acids from the action of oxidizing molecules, using electron donor glutathione, or in some cases, thioredoxin or glutaredoxin (102). Malondialdehyde (MDA) is a highly reactive enzyme mediating the biosynthesis of prostaglandins and thromboxane (103).

Glucocorticoids increase the activity of enzymes involved in the synthesis of fatty acids and contribute to lipoprotein secretion, inducing the hepatic gluconeogenic pathway (104).

The most prominent increase in serum cortisol values under experimentally induced forced effort conditions was found in the control group with cholesterol diet.

NSAIDs (nonsteroidal anti-inflammatory drugs) treatment reduced plasma cortisol levels but was statistically insignificant compared to the control group receiving cholesterol diet under the stress test.

Serum uric acid – UA is associated positively with C-reactive protein – CRP and the erythrocyte sedimentation rate – ESR, so it is a relationship between serum uric acid and markers of systemic inflammation. In clinical practice, hypeuricemia is an indication for investigating MetS criteria, and the presence of MetS is an indication for investigating the serum UA concentration (105-108).

Gout is an increased risk of cardiovascular disease beyond the potential contributions of hypeuricemia associated with a role due to the inflammation process shown in atherogenesis (109-111) and the development of thrombosis in a manner similar to other inflammatory rheumatic diseases associated with an increased risk of cardiovascular disease (rheumatoid arthritis or lupus) (112-114). So, the patient suffering an attack of gout is subject to mandatory assessment protocol to determine cardiovascular risk profile (115).

The serum UA concentration is positively correlated with the number of MetS criteria and the association between UA and MetS components, the relationship between serum uric acid and markers of systemic inflammation (ESR, CRP).

According to the appreciations of the European Cardiology Society, the European Atherosclerosis Society and the Hypertension European Society, the plasmatic cholesterol increase, especially of the low molecular density lipoproteins (LDLc) and of the low plasmatic level HDL cholesterol represents the risk factors for the atherosclerotic cardiovascular diseases. In case of HDLc's decrease it was shown a statistical differences according to sex, this decrease being lower in male patients. On the other hand, in case of hypertriglyceridemia, the triglycerides level was significantly higher in women (116).

It was proven that the hepatic transaminases, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and, to a lower degree, the gamma-glutamyl-transferase (GGT) represents markers of the hepatic disorder due to the infiltration of the fatty acids and of the inflammatory stimulations, these being associated with several components involved in the MetS physiopathology. The ALT's increased values are associated with the MetS's components (increase of the triglycerides, glycaemia, abdominal circumference, diastolic arterial pressure and low levels of the HDLc) (117). GGT is a sensible indicator, but with a low hepatic disorder specificity, suggesting the possibility of a link between it, the endothelial dysfunction and the cardiovascular risk (118).

The study carried out by Music et al (119) has shown that GGT was significantly higher in patients with type 2 DM and MetS than in type 2 DM without, while the ALT and AST did not present significant variations. Monitoring studies are required to determine if the hepatic enzymes can be used as hepatic component of the MetS.

The increased serum levels of UA were observed as being a prediction factor, independent from the apparition of the coronary arterial disease, thus indicating the involvement of its presence in the development of the vascular inflammation (120, 121).

The hyperuricemia is frequently associated with the arterial hypertension and it is present in 25 % of the patients with non-treated arterial hypertension, 50 % of the patients

under treatment with diuretics and more than 75% of the patients which suffer from malignant arterial hypertension (122).

To reach the therapeutic objectives, the European Society of Hypertension / European Society of Cardiology recommends as treatment: four classes of antihypertensive medication: inhibitors of the angiotensin-converting-enzyme inhibitors / angiotensin II receptor blockers, calcium channel blockers, beta blockers and diuretics. It is important the initiation of the MetS treatment for the reduction of the increased cardiovascular risk. The administered medication addresses the individual components of the syndrome. However, although the pharmacology therapy is often necessary, a very important element remains the lifestyle change, which represents the only full therapeutic approach which can reduce the resistance to insulin and the visceral obesity (123). The criteria defining the metabolic syndrome are shown in figure I.1.4.

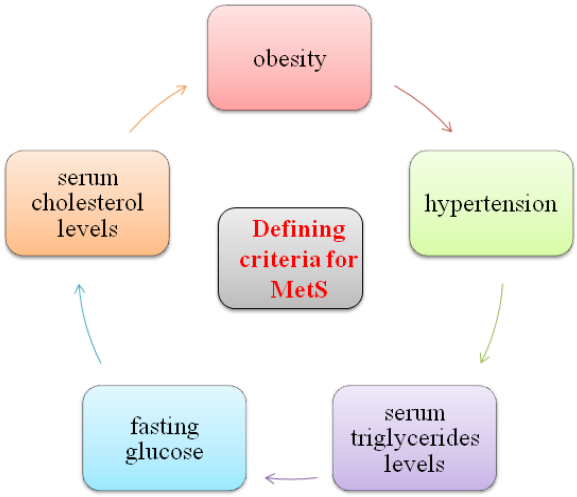

Figure I.1.4. Clinical Identification of the Metabolic Syndrome

Material and methods

In the first study, we investigated the medical records of 153 patients hospitalized in the Rheumatology Clinic within the Rehabilitation Hospital Iasi in the period 01 January 2014 – 31 July 2014. We selected only the patients who fulfilled at least three of the five criteria defining the metabolic syndrome (figure I.1.4). We used the criteria recommended by AHA/NHLBI (American Heart Association). Also, patients were diagnosed with gout or asymptomatic hyperuricemia. The patients were divided according to age groups, gender, gout and the presence of the metabolic syndrome as well as the essential hypertension grade and the number of clinical criteria used in establishing the MS diagnosis (3, 4 or 5 criteria). Triglycerides are lipid fractions, formed by combining glycerol with three fatty acid molecules. Another lipid fraction who plays an important role in the atherosclerotic process is HDL – cholesterol. Lipoproteins have central core of a hydrophobic lipid, encased in a hydrophilic coat of polar phospholipid, free cholesterol and apolipoprotein. There are five subfractions of HDL, types 2a, 2b, 3a, 3b, and 3c. The level of erythrocyte sedimentation rate (ESR) was also investigated.

The purpose of the second study was to investigate the pharmacodynamic effects of

associated ACE-NSAIDs administration on pressure values and markers of oxidative stress in rats with MetS. For experiments, Wistar white rats were used (weighing between 185-200g), with a uniform gender distribution. For induction of dyslipidemia, all animals have been subjected to cholesterol diet (0.2 g/kg body weight/day, 4 weeks). The animals were distributed in 9 groups (6 rats/ group) and received the following substances, single intraperitoneally injection, following protocol: Group M1 (control1): physiological saline - 0.5mL/100g body; Group M2 (control2): cholesterol diet; Group ENP: Enalapril -1 mg/ kg body weight/day; Group IND: Indomethacin - 1 mg/kg body weight/day; Group KET: Ketoprofen- 3 mg/kg body weight/day; Group NMS: Nimesulid - 1.5 mg/kg body weight/day; Group ENP+IND: Enalapril-1 mg/kg body weight/day + Indomethacin - 1 mg/kg body weight/day; Group ENP+KET: Enalapril - 1 mg/kg body weight/day + Ketoprofen - 3 mg/kg body weight/day; Group ENP+NMS: Enalapril - 1 mg/kg body weight/day + Nimesulid - 1.5 mg/kg body weight/day. Absolute blood pressure BP values were determined with the HAMEG sphygmomanometer. The physical exercise capacity analysis of rats after administration of the test substances was performed using forced treadmill exercise over a 10 min interval. This experimental model is used to evaluate the motor function and effort resistance of laboratory animals. To determine serum cortisol levels, blood was harvested in vacutainer without anticoagulant, with or without a separating gel. The serum level of the hormone was determined by the immunochemical method with electrochemiluminescence detection (ECLIA). Interleukin (IL)-1 acts on both T lymphocytes (with IL-2 production stimulation) as well as B lymphocytes (stimulating B lymphocyte proliferation and immunoglobulin production), IL-6 provides growth and differentiation of B cells, stimulates immunoglobulin production, promotes activation, growth and differentiation of T-cell; tumor necrosis factor (TNF-α), proinflammatory cytokine, is involved in cellular apoptosis (267, 268, 269). A venous blood sample (at least 0.5 mL of serum) was harvested in vacutainer without anticoagulant. IL levels were determined by an immunochemical method with chemiluminescence detection.

Because MetS is responsible for increasing the number of atherosclerotic cardiovascular diseases and for the increase of the mortality due to these disorders, compared to the general population, *the third article* presents a retrospective study, including 250 patients which fulfilled at least three of the five defining criteria of the metabolic syndrome. A retrospective study was carried out in the 3rd Medical Clinic of the "Sf.Spiridon" County Clinical Emergency Hospital of Iasi, and included 250 patients which fulfilled at least three of the five defining criteria (Figure I.1.4) of the metabolic syndrome. A percentage of 57% from the patients returned to our clinic for re-evaluation after 12 months of treatment. The inclusion and exclusion criteria are listed in tabel I.1.3.

Tabel I.1.3. Inclusion/exclusion criteria for patients

Inclusion criteria	***Exclusion criteria***
Age above 18 years	Pregnancy
Presence of the MetS	Patients with neoplasm
Absence of the chronic renal disease in terminal stage	Patients with major psychic/cognitive disorders
Absence of the severe hepatic insufficiency	Acute/chronic inflammatory disorders
	Hormonal treatment

Statistical data

The data from the three studies were centralized and statistically processed using the the same SPSS version 22.0 for Windows 10 and the ANOVA method. In the statistical analsis were used not only descriptive methods, but also the analytical ones at the 95% confidence interval (CI 95 %).

Results

The results of the first study indicated that the number of male patients (93, representing 60.8%) was significantly higher than the number of female patients (60, representing 39.2%) ($p < 0.01$). There were no significant differences based on age or sex. Individuals were predominantly late middle aged, 50-59 years old, average 55.89. Another criteria wa the value of uricaemia, 74 patients have gout (48.4%) and 79 have asymptomatic hyperuricemia (51.6%). Another studied parameter was HTN, which was present in 93 of the patients studied. Another parameter considered in the present study was dyslipidaemic syndrome. It was present in 77 patients. Moreover, hypercholesterolemia was present in 33 patients with gout and 44 patients with hyperuricemia. The statistical analysis have observed the cholesterol and HDL cholesterol levels. In the analysis of MS the value of triglycerides has been studied. The increase above normal triglyceride values was ascertained in 36 patients with gout and 41 patients with hyperuricemia. The difference between the two groups was not statistically significant. Obesity was also analyzed and the study batches average body mass index (BMI) was 29 kg/m2, with a variation between 18 and 49 kg/m2, without a significant difference ($p = 0.227$). It was present in only 27 of the patients (13 with gout and 14 asymptomatic hyperuricemia). Inflammatory syndrome has been seen in both the metabolic syndrome and rheumatic disease and has been also studied in our group. Thus, elevated ESR was found in 97 patients (47 with gout and hypeuricemia 50 with asymptomatic hypeuricemia), and the CRP increased in 40 patients (15 with gout and 25 asymptomatic hypeuricemia).

In the second study were evaluated serum titers of total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol of the analyzed rats. Indomethacin caused the highest cholesterol levels, which can also be observed with triglycerides. A significant difference of LDL-cholesterol was observed between the ENP + IND vs. IND, where it appears that angiotensin-converting-enzyme (ACE) administration had a role its decrease. Regarding the values obtained from the control group + cholesterol diet, they were significantly higher compared to the rest of the groups except for groups 4 and 7 (figure I.4.5). Following analysis of the distribution of lipid metabolism components, we can objectively claim that Enalapril has a beneficial effect on the decrease of total cholesterol, LDL cholesterol, triglycerides. Except for Indomethacin, the remaining NSAIDs used in the experiment did not significantly affect the values outlined in figure I.1.5.

The most prominent increase in serum cortisol values under experimentally induced forced effort conditions was found in the control group with cholesterol diet. NSAIDs treatment reduced plasma cortisol levels but was statistically insignificant compared to the control group receiving cholesterol diet under the stress test. The association of Enalapril with the studied NSAIDs decreased serum cortisol values, but was found statistically insignificant compared to both control groups, the cholesterol free group and the cholesterol diet control group, under stress conditions. The most pronounced effect was found for the combination of

Enalapril + Ketoprofen (figure I.1.6).

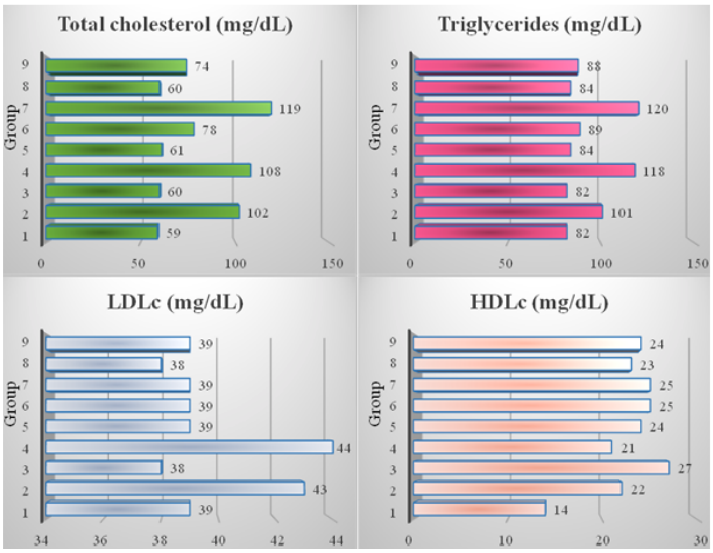

Figure I.1.5. Distribution of average values of lipid metabolism components in rats

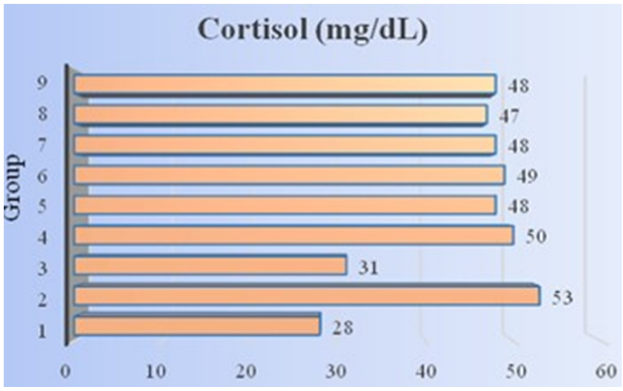

Figure I.1.6. Batch distribution based on serum cortisol value

The results of the third study have highlighted the clinical proof related to the inflammatory nature of the atherosclerotic process, represented by the increase of the circulatory levels of the inflammatory markers at the patients with cardiovascular diseases. Numerous epidemiologic studies have reported positive associations between the risk of the atherosclerotic and the fibrinogen's plasmatic levels, this parameter's high level being significantly associated with the cardiovascular risk, independent of the lipid profile. Thus, we can observe an improvement of the inflammatory process through the markers' decrease ($p = 0.001$), Tabel I.1.4.

Data from the tabel have proved a significant relationship between elevated C-reactive protein and fibrinogen levels and the presence of hypertension, isolated, or in combination with obesity, which confirmed that hypertensive patients presented a proinflammatory and procoagulant status, which implies an increased risk of cardiovascular disease.

After the 12 months of treatment and diet regime, it was obtained the improvement of the renal function. The patients with present inflammatory treated with Rosuvastatin had the

weakest response to the treatment, in the sense of this syndrome's improvement, as can be seen in figure I.1.7.

Tabel I.1.4. Evolution of the biological markers in patients with metabolic syndrome

Marker	Initial	Re-evaluation	p value for paired T test
Cholesterol	201.25 ± 53.06	186.35 ± 49.35	0.001
LDL	123.03 ± 44.12	112.21 ± 39.15	0.001
Serum urea	52.74 ± 17.2	51.78 ± 15.98	0.001
Creatinine	1.41 ± 0.54	1.35 ± 0.51	0.001
CRP	2.97 ± 3.59	2.61 ± 3.25	0.001
ESR	9.81 ± 7.07	8.54 ± 7.06	0.001
Uric acid	5.91 ± 1.51	5.7 ± 1.42	0.001
Fibrinogen	384.78 ± 114.1	358 ± 98.6	0.001
ALT	27.11 ± 22.61	21.57 ± 15.79	0.001
AST	22.92 ± 10.69	17.85 ± 8.07	0.001
GGT	42.45 ± 35.99	30.99 ± 23.48	0.001

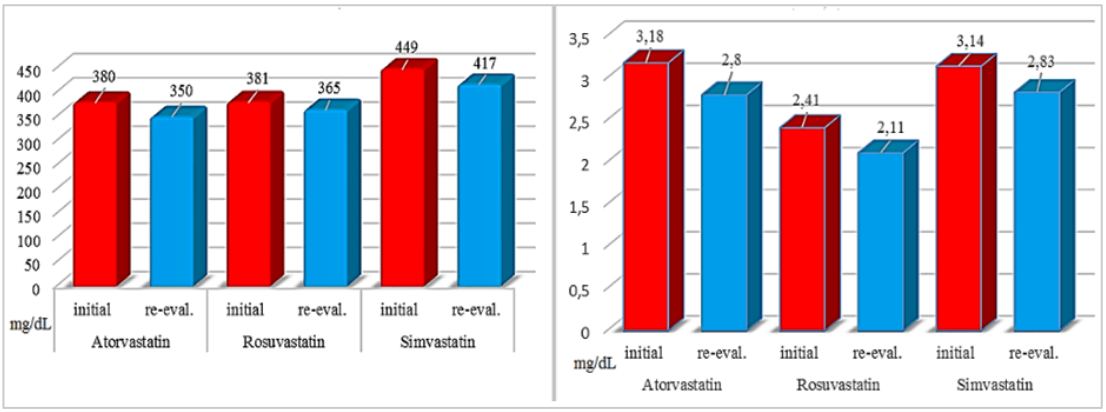

Figure I.1.7. Initial average distribution and the re-evaluation of the fibrinogen and C-reactive protein according to the type of administered statin

It has been observed a statistically significant relationship between elevated C-reactive protein and fibrinogen levels and the presence of hypertension, isolated, or in combination with obesity, which confirmed that hypertensive patients presented a proinflammatory and procoagulant status, which implies an increased risk of cardiovascular disease.

Considering the results of the various studies which have proved a direct relation between the increase of the uric acid's values and of the arterial hypertension at the level of the studied sample, it is observed the fact that not only in men, but also in women, the increased serum value of the uric acid was met significantly more frequently in the hypertensive subjects (figure I.1.8).

The patients treated with Candesartan had a decrease of the uric acid's values by 0,36 mg/dl in comparison with Perindopril, where the decrease was not statistically significant (figure I.1.9). A decrease of the CRP values compared to the initial moment can be observed in Figure I.1.10. The most important improvement can be seen in the case of patients which have received Perindopril.

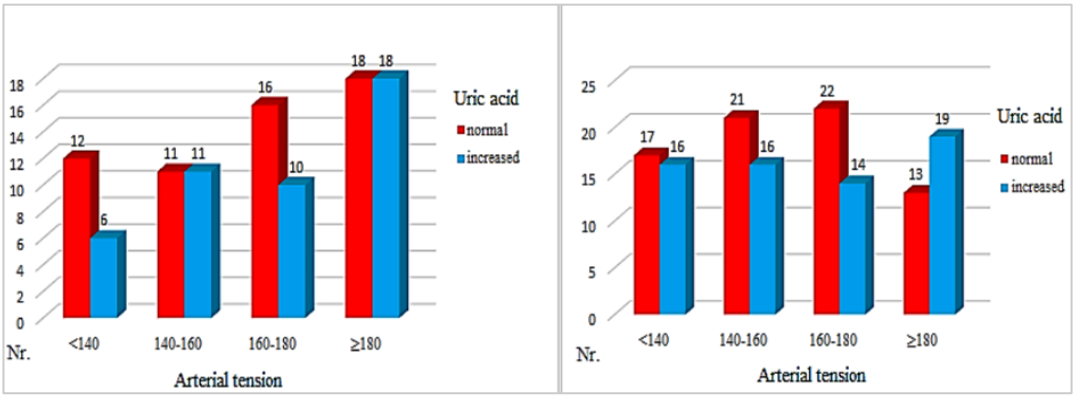

Figure I.1.8. Distribution of the initial values according to the AT and uric acid for men and women

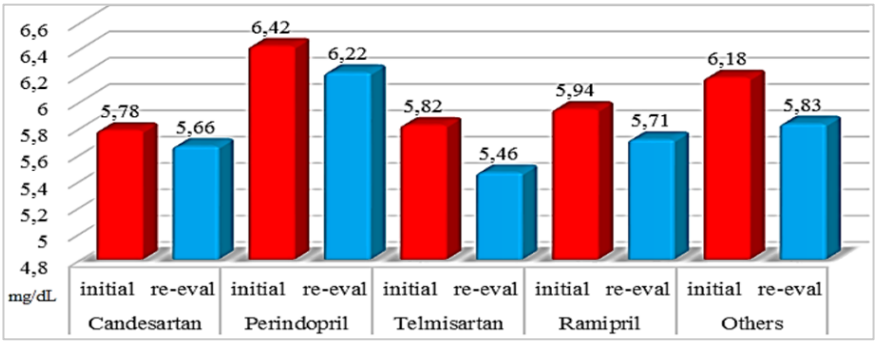

Figure I.1.9. The distribution of the initial and re-evaluation average values of the uric acid, according to the administered ACE/ARB type

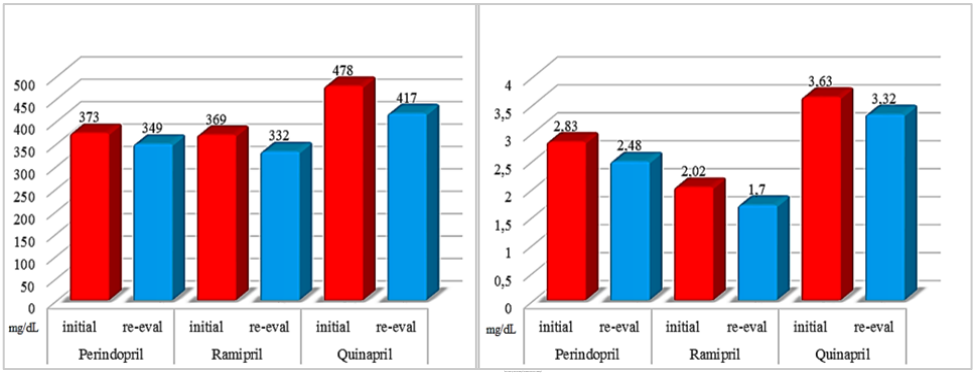

Figure I.1.10. Initial and re-evaluation average value distribution of the fibrinogen and C-reactive protein according to the administered ACE/ARB type

Discussions

The clinical proof related to the inflammatory nature of the atherosclerotic process is represented by the increase of the circulatory levels of the inflammatory markers at the patients with cardiovascular diseases. Numerous epidemiologic studies have reported positive associations between the risk of the atherosclerotic and the fibrinogen's plasmatic levels, this parameter's high level being significantly associated with the cardiovascular risk, independent of the lipid profile.

Local damage to tissue is followed by an inflammatory reaction, enhancing the acute phase response in which there is an increase in various plasma proteins, including fibrinogen, caeruloplasmin and CRP. Also, an increase in immunoglobulins will be observed in a later

stage. The most common methods used for detecting the acute phase respons are the measurement of ESR and CRP concentration. An increase in the CRP concentration occurs within 6 to 10 hours after tissue damage, reducing besides normal values as the inflammatory response decrease. Otherwise, an increase in the ESR reflects a rise in the plasma concentration of fibrinogen and possibly some immunoglobulins. Anaemia is another factor that can seriously influence ESR and may lead to confuse interpretation. CRP is a marker taken into account for early cardiovascular disease being correlated with ageing, even when there are no obvious clinical features. Also, ESR is influenced by incipient cardiovascular disease and, more precisely by changes in fibrinogen concentration (124).

Setting the values of the C-reactive protein is a practical modality to evaluate the presence of the inflammatory syndrome. The increase of the CRP values (> 3 mg/L) is a risk factor for the later apparition of the CVD. The medicine used for the treatment of the metabolic risk factors were efficient also regarding the reduction of the CRP serum levels (statins, fibrates, ACE, nicotinic acid).

Osei-Bimpong et al. have measured CRP and ESR from 295 blood samples from both male and female subjects. A normal consultation had not found clinically significant symptoms or abnormal physical sign and also, other pathology tests have given normal results. The results showed a mean ESR of 10 mm/1 h in both genders, below the age of 40 years. The values increased with age, to a mean of 18 mm by 60 years in both genders. Concerning CRP test, 95% of the samples in the >40 years group had CRP range of 0-18 mg/l compared with 0-10 mg/l in the younger subjects. The results of the study confirm that after the age of 40, there is a close association between age and the elevation of ESR, increasing constantly, especially after the age of 60. Even if CRP is less influenced by age, both ESR and CRP appear useful and reliable as a screening test (125).

Starting from the fact that inflammation has a key role in the pathogenesis of cardiovascular events; measurement of inflammation markers has been proposed as a method to improve the prediction of the risk of these events by Ridker et al (126). The autors conducted a prospective, nested case-control study among an impressive number of 28.263 apparently healthy postmenopausal women over a period of three years in order to evaluate the risk of cardiovascular events associated with base-line levels of markers of inflammation. The measured markers were: high-sensitivity C-reactive protein (hs-CRP), serum amyloid A, interleukin-6, soluble intercellular adhesion molecule type 1 (slCAM-1), homocysteine and several lipid and lipoprotein measurements. For the study, cardiovascular events were considered: death from coronary heart disease, nonfatal myocardial infarction or stroke, or the need for coronary-revascularization procedures. In the prospective assessment of 12 plasma variables, hs-CRP proved to be the strongest and most significant predictor of the risk of future cardiovascular events, and half of all cardiovascular events in the cohort occurred among women without overt hyperlipidemia. This is strong observation, which raises the possibility to add hs-CRP to standard lipid screening will generate an improved method for identifying persons at high risk for future cardiovascular events.

Cortisol secretion attests the activity of the hypothalamic-pituitary-adrenocortical axis, activity dependent on the diurn and metabolic rhythm, but also on the stress response. Cortisol is an important factor with role in atherosclerotic pathophysiological processes (metabolic imbalances and adiposopathy, insulin resistance, vascular and prothrombotic inflammatory

response). After the treadmill stress test of Wistar rats, significant increase in serum cortisol was observed in all groups receiving cholesterol, but a value close to that of the control group was obtained in rats given Enalapril. The complex interactions of ACE and NSAIDs, both from a pharmacodynamic point of view and in terms of producing changes in the body at different levels of apparatus and systems under stress, offer the possibility of complex experimental investigations with modern laboratory equipment, using standardized experimental models from the literature.

Comparing the results obtained in the second study with the literature data, a significant increase in IL-1β secretion in the control group with cholesterol was indeed noted compared to the control group with saline and an improvement in IL-1β secretion in animals test lots, which allows us to conclude that in the context of induced metabolic syndrome rats, ACE and/or NSAIDs administration significantly improves the process of chronic inflammation. At the same time, we have found a significant increase in serum cortisol levels along with elevated IL-1β levels, so we can anticipate new research directions on the cytokine study and correlations with the symptoms and values of classical markers. At the same time, we have found a significant increase in serum cortisol levels along with elevated IL-1β levels, so we can anticipate new research directions on the cytokine study and correlations with the symptoms and values of classical markers (127).

The objective of a retrospective study published by Mitu et al. (128) was to evaluate the levels of CRP and fibrinogen as markers of inflammation in patients with essential hypertension, with or without associated metabolic risk factors. Two hundred patients (110 women and 90 men) have been included and have been divided into five groups (control, hypertension, and respectively hypertension associated with obesity, or diabetes mellitus type II non-obese or obese type II diabetes). The monitored parameters were: anamnestic and anthropometric data, blood pressure and heart rate, blood glucose, lipid profile, fibrinogen, quantitative C-reactive protein and echocardiographic parameters have been reported and compared between groups. The highest values of CRP have been found in hypertensive and obese patients. Fibrinogen levels were significantly increased in all groups, proving the existence of an inflammatory syndrome, even in the absence of obesity or diabetes.

MetS is also found in patients with chronic renal disorders and probably has a role in the progression and development of the renal lesions, favoring the instalment of the renal disease in its final stage. Chronic renal disorders are a major global public health concern and their prevalence and incidence are steadily increasing mostly because of the growing burden of type 2 diabetes (T2D) and obesity worldwide. Only few studies examined the associations of MetS and the risk for albuminuria or proteinuria. The components of Mets may support the progression of renal damage mainly through the coexistence of several underlying pathological mechanisms such as increased oxidative stress, chronic inflammation, increased fibrogenic activity, and endothelial dysfunction (129*).*

In our study, we have demonstrated that inflammation within the metabolic syndrome decreased differently depending on the type of statin administered. Also, we demonstrated a direct relation-ship between the increased uric acid concentrations and arterial hypertension, its values being significantly higher in hypertensive individuals. Our study revealed that at patients which have received Perindopril was observed a significantly decrease of the CRP values.

I.1.4. Research regarding the inflammation, cardiovascular risk and periodontal disease

A. Background

Periodontal disease is one of the most common inflammatory diseases in adults, statistical data showing a prevalence of 3.9 billion people worldwide in 2010. The side effects of periodontal disease are disability, speech impairment, low self-esteem, all these leading to a reduced quality of life.The correlation between periodontal pathogens and inflammation has attracted the attention from researchers from various fields due to the potential influence of periodontitis on initiation and/or progression of several systemic diseases. The non-oral conditions often correlated with dental affections are: cancer, cardiovascular disease, type 2 diabetes, respiratory tract infection, adverse pregnancy outcomes, and neurodegenerative disease (130).

Bacterial species exist in complexes in the oral cavity, more precisely in the subgingival plaque, 5 major ones being usually observed using different analytical methods. *Bacteroides forsythus., Porphvromonas gingivalis* and *Treponema denticola*. are part of the first complex. The second complex includes members of the *Fusobacterium nucleaturm/periodonticum subspecies*, *Prevotella intermedia., Prevotella nigrescens* and *Peptostreptucoccus micros,* the species associated with this group are: *Eubacterium nodatum, Campylobacter rectus, Campylobacter showae, Streptococcus constellatus* and *Campylobacter gracilis. Streptococcus sanguis. S. oralis, S. mitis, S. gordonii* and *S. intermedius* are part of the third group. Three *Capnocytophaga species, Campylobacter concisus, Eikenella corrodens* and *Actinobacillus actinomycetemcomitans* serotype a. represent the fourth complex, while *Veillonella parvula*, *Actitwmyces odontotylicus. A. actinotnycetemcomitans* serotype b, *Selenomonas noxia* and *Actinomyces naeslundii* genospecies 2 (*A. viscosus*) the fifth complex (131).

B. Published papers in this field

I have published together with dentists and other collaborators, I have studied the similarities between cardiovascular risk and periodontal disease, and the results have been published in two papers.

1. Mârţu S, Nicolaiciuc O, Solomon S, Sufaru I Scutariu M, **Rezuş C**, Popescu E. The evaluation of the C reactive protein levels in the context of the periodontal pathogens presence in cardiovascular risk patients. *Rev. Chim. (Bucharest)* 2017, 68 (5): 1081-1084.

Introduction

The purpose of this study was to investigate the serum C-reactive protein (CRP) values in the presence of *A. actinomycetemcomitans, P. gingivalis*, *T. denticola* or *T. forsythia bacteria*, as an indicator of the cardiovascularn risk. It has been hypothesized that there is a complex relationship between periodontal disease and increased risk of acute myocardial

infection (132). Mechanisms are now under development in support of this hypothesis, linking transient bacteria, frequently in periodontal disease, with endothelial dysfunction, an event in the development of atherosclerosis. Periodontal infection induces local inflammation and several studies have demonstrated that patients with periodontal disease have high levels of systemic inflammatory mediators, such as CRP (133).

This inflammation often leads to gingival ulcerations and local vascular changes, which have the potential to increase the incidence and severity of transient bacteremia. Data from the Human Microbiome Project (134) suggests that there is significant diversity in the microfilm of both healthy and diseased periodontium. The research supports P. gingivalis as a key pathogen. The murine initial model showed that P. gingivalis could trigger changes in the amount and composition of oral microbiota, leading to periodontal bone inflammatory changes (135, 136).

The main identified pathogenic way was the subvertment of the complement, which led to the creation of a dysbiotic microbiota with the clinical signs associated with the disease. This has also been demonstrated in rabbits (137), where P. gingivalis caused the transition to an anaerobic microbiota and a global increase in bacterial load. Dysbiotic bacterial load may be of greater importance when analyzing a systemic inflammatory response than the presence of specific pathogens.

The incidence of atherosclerosis cannot be fully explained by classical risk factors (138). Consequently, the importance of infections as a potential cause of atherosclerosis has gained ground, supported by an abundance of epidemiological evidence supporting this notion (139). Infectious agents, including periodontal bacteria, have been implicated in the etiology of various vascular conditions through multiple mechanisms, including direct microbial invasion of endothelial cells.

In a clinical context, it is extremely difficult to determine the determinant factor of atherosclerosis for several reasons. First, the initiation factor is likely to be missed since the early phase of the endothelial lesion is usually asymptomatic (140). Second, atherosclerotic lesion is a common inflammatory response to several factors (141, 142), and some or all of these factors may be associated with the lesion. Thirdly, interventional studies evaluating the impact of periodontal treatment, with or without antimicrobial therapy, on systemic inflammation or endothelial dysfunction, showed mixed results, including lack of changes, transient worsening of signs immediately after treatment, or improvement in signs that did not persist in time (143, 144).

Despite these limitations, invasion of cardiovascular tissues by periodontal bacteria may have the potential to promote atherosclerosis.

Material and methods

The study consisted of 64 male and female subjects aged 55 to 75 years. Subjects were periodontally examined, with the determination of the probing depth, the periodontal attachment level, the number of present teeth (except the wisdom molars), with the diagnosis of periodontal disease.

Subgingival bacterial plaque sampling was performed from sites with the highest depth found in the patient. The sites of interest were isolated with cotton rolls and gently dried with the air spray. The bacterial plaque was harvested using a single Gracey curette (Hu-Friedy,

Chicago, IL, USA) from the base of the pocket to the coronary side. The samples were then placed in phosphate solution and immediately transferred for storage at -80°C until analysis. *P. gingivalis, A. actinomycetemcomitans, T. forsythia* and *T. denticola* were detected by real-time quantitative polymerase chain reaction (qPCR). Subjects were classified as having each pathogen present or not; this was an aggregate of all plaque samples, which means that if at least one sample for a subject was positive, the subject was reported to be positive for the pathogen.

For determination of C-reactive protein, venous blood samples were harvested and centrifuged. Small quantities were then frozen and stored at -80°C until analysis. CRP was measured using Quantex Biokit Reagents. Body Mass Index (BMI) was calculated as weight /height^2 (kg/m^2). Smokers have been classified as current smokers or not. Diabetes and hypertension were determined by specific measurements (blood glucose and blood pressure, correlated with patient history). A history of cardiovascular disease was recorded for subjects who had a previous myocardial infarction or an intervention such as angioplasty or bypass with stenting of the coronary arteries. Cerebral-vascular disease was recorded for subjects who had previously had a stroke. Material conditions were classified according to the type of home and lifestyle (rented or owned / mortgaged), the number of cars / vans / motorcycles in the household and the number of bathrooms and / or showers and toilets in the house.

Data for CRP values was not normally distributed; therefore the log converted values were used for analysis. Independent t tests were used to compare the transformed log means of CRP values based on the presence or absence of each separate pathogen. The association of each pathogen with both moderate and severe periodontal disease was analyzed by the chi-square test (Yates correction). Multiple regression analysis was performed in a sequenced design model in which categories of potentially complex variables were added to produce a fully-tailored final model for CRP-independent predictors. The significance level for all assays was set at $p < 0.05$. Statistical analyzes were performed with SPSS version 21 (IBM Corp, Armonk, NY, United States).

Results

Based on the statistical analysis, there were no significant differences in age, BMI, smoker status or CRP. The mean age of the 64 subjects was 72.5 years, with a range of 55-75 years. Mean BMI was 27.3 kg/m^2, with 21% of subjects classified as obese (BMI $\geq$ 30 kg/m^2). 21 subjects (30.40%) had mild periodontitis, 32 subjects (50.00%) had moderate periodontitis, and the remaining 11 subjects (19.60%) had severe periodontitis. These and other characteristics of the subjects studied are presented in Table I.1.5.

Tabel I.1.5. CRP values based on the presence or absence of the investigated pathogens

	Present pathogen		**Absent pathogen**		**p-Value**
	N	**CRP (mg/l) Mean**	**n**	**CRP (mg/l) Mean**	
P. gingivalis	30	2.03 (1.2-3.08)	34	1.53 (0.88-2.32)	0.003*
A. actinomycetemcomitans	12	1.87 (1.04-2.84)	52	1.70 (1.02-2.6)	0.82
T. forsythia	54	1.88 (1.15-2.64)	10	1.87 (0.86-3.6)	0.91
T. denticola	56	1.89 (114-2.76)	8	1.8 (1.05-2.61)	0.59

*Statistical significance ($p<0.05$)

The prevalence rates of the pathogens were: 45.0% *P. gingivalis*; 20.5% *A. actinomycetemcomitans*; 86.1% *T. forsythia*; 86.3% *T. denticola*. The median CRP was 1.5 (IQR 1.0-2.6) mg / L. We analyzed the risk levels according to CRP. Following this analysis, 11 subjects (19.6%) had a low risk (<1.0 mg / L); 15 (23.4%) have a medium risk (1.0-3.0 mg / L); and 38 subjects (57.0%) were in a high risk category (> 3.0 mg / L). There was a significant difference in CRP values between subjects who had P. gingivalis compared to those who did not (p = 0.003). There were no significant differences for any of the other pathogens (Table I.1.6). There was a significant association (p <0.001) for each of the four pathogens investigated with moderate periodontitis.

Tabel I.1.6. Prevalence of moderate / severe periodontitis depending on the detected pathogen

	Periodontis prevalence		**Odds ratio**	**(95% IC)**		**p-Value**
	Present pathogen	**Absent pathogen**				
Moderat periodontitis						
P. gingivalis	11	7	2.52	1.72	3.7	<0.001
A. actinomycetemcomitans	5	13	2.1	1.33	3.3	<0.001
T. forsythia	17	2	1.63	1.63	6.24	<0.001
T. denticola	18	1	2.73	2.73	14.03	<0.001
Severe periodontis						
P. gingivalis	5	4	1.75	1.117	2.82	0.01
A. actinomycetemcomitans	2	6	1.48	0.84	2.56	0.12
T. forsythia	8	1	4.17	1.37	11.65	<0.01
T. denticola	9	1	5.63	1.62	17.22	<0.01

The differences were also significant for severe periodontitis: P. gingivalis (p = 0.01), *T. forsythia* (p <0.01) and *T. denticola* (p <0.01), all associated with periodontitis, but not for A. actinomycetemcomitans (p = 0.12), as shown in Table I.1.7. Multiple regression analysis showed that the body mass index (p <0.001), current smoking (p <0.01), hypertension (p = 0.01) and the presence of *P. gingivalis* (p <0.01) are independent CRP predictors.

Tabel I.1.7. Group characteristics depending on the presence of p.gingivalis

	***P. gingivalis* – Present**	***P. gingivalis* – Absent**	**p-Value**
Age (years) – mean	73.8	73.2	0.02*
Present teeth – mean	18.1	19	0.08
BMI (kg/m^2) – mean	26.3	26.1	0.52
Cholesterol (mmol/L) – mean	5.7	5.7	0.98
Diabetes mellitus (n)	3	2	0.58
Arterial hypertension (n)	7	2	0.89
Smokers (n)	5	2	0.03*

*Statistical significance (p<0.05)

The presence of *P. gingivalis* was associated with a 1.20-fold increase in CRP (95%, confidence interval 1.04 – 1.37) in the fully-adjusted model. There were no significant associations between the presence of other periodontal pathogens investigated and CRP. The main finding of this study was that the presence of P. *gingivalis* in the subgingival plate was

significantly associated with the C-reactive protein level in a homogeneous group of 55-75 year-olds. This relationship remained significant after adjusting for various bias factors.

Discussions

Strong evidence has shown an association between periodontitis and cardiovascular diseases, such as atherosclerotic cardiovascular disease - CVDs, myocardial infarction, cerebrovascular disease and peripheral arterial disease. Nevertheless, it has been difficult to conclude a causal association between periodontitis and CVDs because both these conditions have common risk factors and display multifactorial etiologies. But, the consistency in the association between periodontitis and CVDs has been shown in some reviews. Also, multiple cross-sectional, cohort, and case-control studies have also proved a meaningful association between periodontitis and myocardial infraction. Unfortunately these results have not been totally proven (145).

Periodontal disease may, in fact, be just one of the few co-morbidities that develop on the basis of the interaction between microbial dysbiosis and other established risk factors, such as smoking. A good knowledge of the genetic basis of the interaction between the host and the microbe will be necessary to fully understand such mechanisms.

Nicolaiciuc et al. published an article which aimed to evaluate levels in serum and crevicular fluid (GCF) of TNF-α, IL-1β and IL-6, in order to explain the possible link between periodontitis and hyperlipidemia, and also the effects of conventional periodontal treatment by scaling and root planing on these pro-inflammatory molecules. The study involved a total of 40 patients divided into two main groups: the study group (n=26) and control group (n=14). The study group included patients with atherosclerosis and prescribed diet or antilipemic therapy with a drug from the statin class. Controls (C) were selected from systemically healthy subjects with chronic periodontitis. For both groups there were measured important serum decreases in TNF-α, IL-1β and IL-6 from baseline, and the decreases were more significant IL-1β for statin group. Significant decreases were also identified in the crevicular fluid for all cytokines, mostly for IL-6 in the statin group (133).

Katz et al. have also pointed toward a possible association between periodontal disease and increased risk of cardiovascular disease. The authors have considered the association of poor oral hygiene and atherosclerosis as an effect of chronic inflammatory disease on blood rheology. The study population included 151 men aged between 26-53 years (mean: 39 +/-5 years) classified as having coronary heart disease, like myocardial infarction and/or anginal syndrome with angiographic evidence of significant coronary disease, or suffer from atherosclerotic risk factors, like diabetes and HTN according to strict, well-established criteria. Blood levels of cholesterol and triglycerides were also determined. The severity of periodontal disease was assessed by the aid of CPITN score. Statistical analysis was performed with chi2 test and highlighted a significant association of CPITN score 4 with hypercholesterolemia and a possible association with coronary heart disease. In conclusion, the generation of higher cholesterol blood levels is related with a possible link between chronic periodontal inflammation and atherosclerosis (137).

Bernet et al. have studied the implication of periodontal inflammation in atherosclerosis and coronary heart disease – CHD. Because, an insignificant number of studies have correlated periodontal diseases – PD with angiographic measures of coronary atherosclerosis,

performed with Coronary angiography – CA, the aim of the study was to investigate this correlation. The authors made a prospective epidemiologic study, including 466 patients which have undergone coronary angiography and then were evaluated for PD. Other complex physical, laboratory, cardiac, and dental examination including dental x-rays have been performed for each patient. Periodontal disease and coronary angiograms were evaluated blindly by a dentist and by 2 cardiologists. A coronary stenosis greater than 50% was evaluated as CHD. A complex characterization and measuring of periodontal disease was performed with the Community Periodontal Index of Treatment Needs –CPITN. If more than to 2 sextants (segments dividing mandible and maxilla into 6) were recorded as having CPITN of at least 3 (signifying that sextant had periodontal pocket depth ≥ 3.5 mm), the patient was coded as having PD. A precent of 74.9%, representing 349 patients has been diagnosed with CHD after CA evaluation. The CHD patients had PD in 55.6% vs 41.9% in the non-CHD patients. As a final remark the obtained data indicate that PD represents a potentially risk factor that is both preventable and treatable with predictable treatment (146).

A study preformed by Kanaparthy et al. (147) evaluated the serum concentration of CRPs, used as a marker of periodontal disease as well as a risk indicator for CVD. The retrospective study included a total number of 45 subjects, with the mean age of 40 years. Based on the periodontal status, the subjects were divided into 3 equal groups which includes moderate and severe periodontitis: group I: control group, with attachment loss (AL) ≤ 2 mm and pocket depth (PD) < 3 mm; group II: Generalized aggressive periodontitis (AL ≤ 5 mm); group III: chronic periodontitis (AL ≥ 2 mm, PD ≥ 5 mm).

The measured clinical parameters were: plaque index, gingival index and bleeding index. Analysis of covariance was used for comparison of mean values between the groups (P value < 0.05). Broadly, the mean CRP levels were high in subjects with generalized aggressive and chronic periodontitis compared with controls. A statistically significant difference (P = 0.012) was found in the CRP level between groups I and II and between groups II and III, and between groups I and III. The results of the study indicated an increase in serum CRP levels in subjects with generalized aggressive periodontitis and chronic periodontitis in comparison with the controls. Of the four periodontal pathogens investigated, only the presence of *P. gingivalis* in subgingival bacterial plaque samples was significantly associated with a high level of CRP. Knowledge and understanding of the relationship between oral microbiota and both periodontal and systemic health will need to be further developed to fully elucidate the mechanisms of potential associations.

2. Sincar CD, Ioanid N, Rudnic I, Martu I, Solomon SM, Pavel LL, **Rezus C**, Martu S, Plesea Condratovici C. The biochemical effects of non-surgical periodontal therapy in patients with and without chronic renal disease, *Rev. Chim. (Bucharest)* 2017, 68 (3): 605-607.

Introduction

In the case of patients with chronic kidney disease – CKD an increased risk of cardiovascular morbidity and mortality has been observed in many years of research. In the

literature even exist a term for patients with both CKD and CVD – cardiorenal syndrome (CRS). Even if a large number of patients with chronic kidney disease do not reach the stage of renal failure, they have a significantly higher incidence of all cardiovascular co-morbidities. In patients with CKD the main biology substantiating CVD may be similar to that in patients without kidney disease, moreover, many more risk factors are involved as a consequence of renal dysfunction. Patients with kidney damage present a significant risk of cardiac arrhythmia and sudden death. The patients on dialysis are more predisposed to develop arrhythmias, especially atrial fibrillation and ventricular tachyarrhythmias. In time, the decline of kidney function will lead to impairment of electrolyte homeostasis, especially the levels of potassium and calcium from blood (148, 149).

Chronic renal disease is considered a global public health problem, mainly because of the high morbidity and mortality. A normal independent life is the main purpose of patients with chronic renale disease. Recovery integration means, specific of medicine, according to results of the studied analysis parameters, will ensure the success of multidisciplinary therapy approach to the management of these patients (150-152).

The aim of the study was to hypothesized that a part of chronic inflammatory response observed in patients with chronic renale disease, undergoing dialysis, is due to physiopathological reactions caused by the presence of chronic periodontitis, which, during the course of its evolution, induces an increase in the expression of inflammatory markers.

Material and methods

This study included patients over 18 years old who have not received in the last 6 months any periodontal, antimicrobial or anti-inflammatory treatment, and have not used steroids or immunosuppressive drugs. Patients were divided into two groups: the first group consisted of patients with chronic renale disease and periodontal disease who were undergoing periodontal treatment (test group) and the second group, control group, composed of patients without any systemic disease, but who experienced moderate to severe chronic periodontitis, also periodontal treated. Blood samples were taken for biochemical analysis (albumin, uric acid, creatinine and urea) at baseline and at 3 months after periodontal therapy.

For comparisons between the group with chronic renale disease and the control group was used Student T-test for independent samples or Mann-Whitney nonparametric test. For comparison before and after the periodontal therapy it has been used the t-test or Wilcoxon signed rank test. Analyses were performed using SPSS 13.0 computer program V.

Results

Chronic inflammatory response observed in patients with chronic renale disease is due to physiopathological reactions caused by the presence of chronic periodontitis, which, during the course of its evolution, induces an increase in the expression of inflammatory markers.

It is noted that the percentage of men is 63% and is significantly higher than women in the group with chronic renale disease. In the control group the percentage of men (55%) is lower than in the group without chronic renale disease but still predominant (Figure I.1.11 and Figure I.1.12).

For serum albumin, the average value before periodontal treatment is 4.90 g/dL for the group of patients without renale disease and 3.70 g/dL for the group of patients with renale

disease. The average values after periodontal treatment, are relatively enlarged and inverted for groups of patients studied, measuring 5.40 g/dL for the group of patients without renale disease and 4.15 g/dL for the group of patients with renale disease.

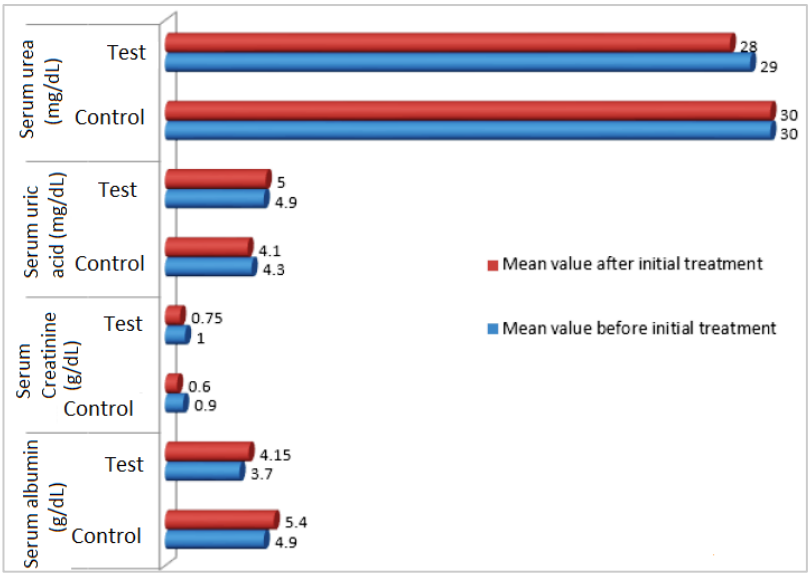

Figura I.1.11. Serum markers – before and after initial periodontal treatment

For the urinary urea, the average value before periodontal treatment is 25.9 g/24h for the group of patients without renale disease (control) and 24.5 g/24h for the group of patients with renale disease. The average values after periodontal treatment, are 23.5 g/24h for the group of patients without renale disease and 23.8 g/24h for the group of patients with renale disease. In the case of serum creatinine, the mean periodontal treatment before and after the show is respectively 0.60 g/dL for the group of patients without renale disease and 0.75 g/dL for the group of patients with renale disease. Mean before periodontal treatment, are relatively increased for groups of patients studied, measuring 0.90 g/dL for the group of patients without renale disease and 1.00 g/dL for the group of patients with renale disease.

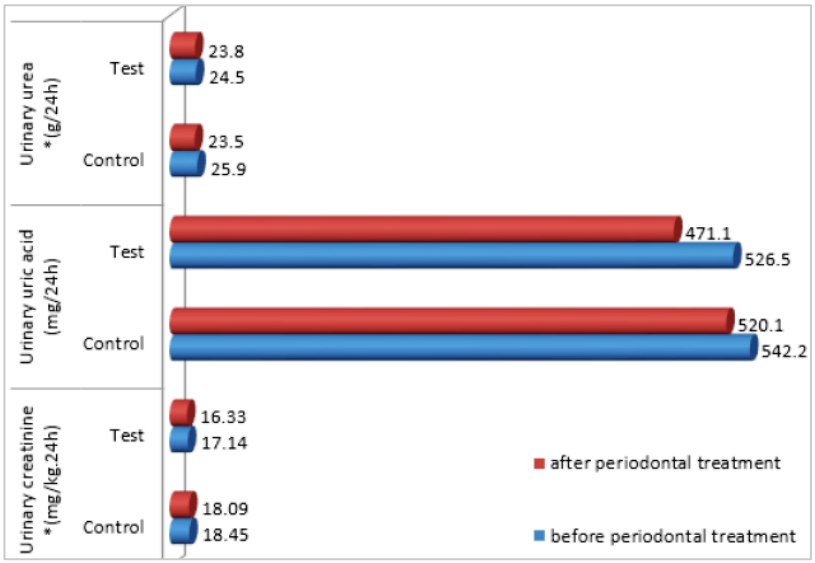

Figure I.1.12. Urinary markers – before and after initial periodontal treatment

For urinary uric acid, the mean values before periodontal treatment are respectively 542.2 mg/dL for the group of patients without renale disease and 526.5 mg/dL for the group of patients with renale disease.Values are relatively reduced after periodontal treatment for groups of patients studied, with values of 520.1 mg/dL for the group of patients without renal disease and 471.1 mg/dL for the group of patients with renal disease.

Discussion

CKD is defined as the presence of kidney damage or as a decreased kidney function – glomerular filtration rate. Noteworthy are the common risk factors between periodontitis and CKD. Among these factors the most significant are age, smoking, poorly controlled diabetes, or obesity. Also, both CKD and periodontis are major sources of morbidity and as independent risk factors for both cardiovascular diseases and all-cause-related mortality. Various data indicated a connection between both diseases based on biological hypotheses including continuous bacterial spreading from periodontal pockets through bloodstream to organs including kidney (153).

Older adults have an increased risk of chronic renal insufficiency. Chronic renal failure starts without symptoms, and as renal function decreases, blood pressure increases and urea accumulates, leading to uremia and fluid volume overload. Atherosclerotic cardiovascular disease, imbalances in bone metabolism, and chronic kidney disease have some common risk factors, such as the fact that renal insufficiency is known to increase osteoclast-related bone turnover and may influence bone metabolic parameters (151).

Our study evaluated the impact of periodontal therapy on biochemical markers and led for the first time a causal association between periodontal disease activity and their level. We included 56 patients with chronic periodontitis, 36 with chronic renale disease and 20 without systemic disease and with normal renal function (control group) (151, 154). Markers were evaluated before and 3 months after periodontal treatment. The effectiveness of periodontal treatment was confirmed by biochemical parameters improvement as correlated with other studies (155, 156).

An association between periodontal disease and renale disease is often found in studies using a population where the renale disease is already diagnosed. In these cases, duration of renal end stage and type of topical and systemic treatment administered to patients significantly affect the association. Therefore, we have shown that periodontitis may promote any detectable changes in renal function. Thus, by analogy, in our study the test groups and control groups were compared not only with each other but also comparative analyzes were performed based on the reference values of markers of renal dysfunction (157, 158). We think it could be plausible existence of a causal link between periodontal disease and chronic renal disease both by glomerular invasion by periodontal pathogens, directly and indirectly through systemic inflammatory effect caused by chronic periodontitis.

Important evidences support the contribution of the periodontitis to chronic systemic disease. For this reason, some results showed a considerable association between periodontitis and atherosclerosis, which can be explained by the circulation of the periodontal pathogens in the blood torrent, promoting the damage to the blood vessels endothelium and atherosclerosis. Actually, there is a bidirectional character of the relations between systemic inflammations and local, periodontal inflammations. Another important aspect correlated with this health

problem are reperesented by various acute and chronic infection that may lead to a renal inflammatory response (glomerulonephritis). Nowadays, the renal disease prevalence is increasing, most patients with renal diseases also present periodontal diseases (157).

Renal pathologies gradualy affect some peculiarities of the tissular reaction, manifested as belated curing and as a higher susceptibility to infections. Chronic renal insufficiency (IRC) a direct consequence of CKD, represents a progressive and irreversible loss of the function and number of nephrones, which leads to a decrease in the glomerular filtration ratio. Apart from the periodontal pathology, the drug systemic therapy specific to the periodontal disease strongly increases the risks for the patient suffering from a chronic renal pathology. The most used substances in the treatment of IRC are: acyclovire, acetaminophene, antibiotics from the group of aminoglycosides, tetracyclines and sulphonamides.

Hemodialysis and peritoneal dialysis are two techniques involved in the treatments of IRC patients. Both of them cause systemic modifications, oral complications and a modified saliva composition. Moreover, a decrease in the frequency of oral hygiene might negatively affect the oral health of the patients subjected to chronic dialysis, leading to numerous carious lesions, periodontontitis and other oral lesions. Also, the periodontal tissues may be affected inclusively by the manifestation of gingival hyperplasia in the patients immunitarily suppressed through renal transplant, which is also accompanied by an increased level of plaque, scale and gingival inflammation. The presence of non-diagnosed periodontites may have important effects upon the medical treatment of the patient with IRC (156).

Bayraktar et al. analyzed and compared periodontal parameters in chronic renal failure patients undergoing peritoneal dialysis therapy with a group of patients on hemodialysis treatment and healthy controls. The authors remarked that chronic renal failure patients on peritoneal dialysis therapy are more susceptible to periodontal diseases, in comparison with those on hemodialysis treatment. However, further studies on periodontal parameters of patients with peritoneal dialysis therapy are needed to get precise information on the oral health status of this patient group (152).

Considering that chronic inflammation is a risk factor for atherosclerotic diseases, cardiovascular patients with hypertension and diabetes, leading causes of chronic renale disease, it is plausible that immediate diagnosis of periodontal disease, followed by periodontal therapy should be an important preventive measure in chronic renale disease in daily clinical practice.

Our findings suggest that periodontal disease is more severe in patients with chronic renale disease and induce a systemic inflammatory response. Success of periodontal therapy reduce systemic inflammatory response and decreases levels of biochemicals markers indicating that this may be an important intervention therapy in patients with chronic renale disease. Further investigations are necessary in order to discover periodontal pathogens and/or level of biochemical markers before and after periodontal treatment in people with renal disease.

I.2. RESEARCHES REGARDING INFLAMMATION AND ARRHYTHMOGENIC RISK

I.2.1. Hallmarks

Commonly, an acute inflammation affects heart physiology causing rapid cardiac rate related with hyperthermia. In patients without heart problems, fever associated with acute common infections frequently provoke supraventricular ectopic beats, but incidence of sudden cardiac death and ventricular fibrillation or tachycardia is extremely rare in this population of patients. In case of those with risk of arrhythmic events, such as cardiomyopathies or channelopathies, hyperthermia as a consequence of acute common infections is associated with a higher rate of life-threatening ventricular arrhythmia occurrence. Also, common infections, principally viral or specific ones, will possibly involve the heart in the inflammation process resulting arrhythmias related directly to carditis (159).

A study performed by Ristic et al. (160) revealed histologic evidence of myocardial inflammation in 20% of patients with pericarditis, after endomyocardial biopsy. Ten patients had myocardium involvement, 4 suffering from ventricular fibrillation during follow-up and 2 patients died from arrhythmic events.

Cardiac arrhythmias is an abnormality or disturbance in the normal activation sequence of the myocardium and may be indicate a structural heart disease and the cause of significant cardiovascular complications and sudden cardiac death. This disfunctions are characterized by irregular rhythm of heartbeat which could be either too slow (<60 beats/min) or too fast (>100 beats/min) and can happen at any age. They start when the electrical signals to the heart that coordinate heartbeats are not working properly. Actually, some people experience irregular heartbeats, which may feel like a racing heart or fluttering. Many of them are inoffensive, but if they are particularly abnormal, or result from a weak or damaged heart, arrhythmias will lead to dangerous and even potentially fatal symptoms (161-163).

Atrial fibrillation – AF is the most frequent arrhythmia in clinical practice, affecting millions of patients worldwide. Statistics showed that about 7 million of patients in Europe and USA endure from AF. Sadly, the number of patients suffering from this dysfunction will multiply up to 2.5 times until 2050. A real challenge will be represented by the increasing of optimal stroke prevention and rhythm management in these patients (164).

This disease increases with aging and with the presence and severity of underlying heart disease, especially congestive heart failure and valve disease. The correlation with aging is attributed to the increasing life expectancy worldwide. More precisely, it occurs in less than 1% of persons aged 60 to 65 years but in 8% to 10% of those older than 80 years, with prevalence higher in women than in men (164-166). It is often associated with other conditions including sleep-disordered breathing and hypertension (164). Atrial fibrillation is due to structural and/or electrical atrial remodeling, thereby promoting abnormal mechanisms of atrial depolarization. These changes may induce diverse pathophysiologic mechanisms and AF may represent a phenotype for multiple disease pathways. However, the potential mechanisms leading to AF are still not entirely understood (163). Diabetes mellitus, arterial hypertension, obesity, metabolic syndrome, coronary heart disease and heart failure are the most common risk factors and diseases that predispose for the development of AF (164).

I.2.2. Research regarding the inflammation and mortality risk

A. Background

When assessing an electrocardiogram, increased attention should be paid to the QT interval, because the risk of malignant arrhythmias and sudden death are often associated with an aberrant QT interval. This interval is measured from the beginning of the QRS complex to the end of the T-wave; it should be revised for heart rate to enable comparison with reference values. Physicians often encounter problems in determining the correct range determination of the QT interval, and its value. Even if in clinical practice, computerized analysis and interpretation of the QT interval are widely available, these might well overestimate or underestimate the QT interval, an unnecessary treatment may be given to patients. Difficult situations occur also in case of manual assessment of the QT interval, even of rather straightforward ECGs (167).

The long QT syndrome – LQTS is a multi-factorial condition of myocardial repolarization predisposing to life-threatening ventricular arrhythmias and it can be classified as congenital or acquired. Recently, inflammation and immunity have been more and more recognized as new factors significantly involved in modulating ventricular repolarization. The presumed underlying mechanisms of inflammation are complex but essentially cytokine-mediated. They include both direct actions on cardiomyocyte ion channels expression and function, as well as indirect effects resulting from an increased central nervous system sympathetic drive on the heart. In terms of autoimmunity increasing evidence demonstrates that autoantibodies may affect myocardial electric properties by directly cross-reacting with the cardiomyocyte and interfering with specific ion currents as a result of molecular mimicry mechanisms. Moreover, new data highlighted the involving of inflammation and immunity in modulating the clinical expression of congenital forms of LQTS, possibly triggering or enhancing electrical instability in patients who already are genetically predisposed to arrhythmias (168).

B. Published article in this field

Review articles summarize, analyze and discuss the current state of primary research on a particular topic in a scientific discipline and are very useful before starting any experimental research or clinical trial.

Rezuş C, Moga VD, Ouatu A, Floria M. QT interval variations and mortality risk: Is there any relationship? *Anatol J Cardiol*. 2015; 15 (3): 255-8.

Introduction

Prolongation of the QT interval is associated with early after depolarizations. Early after depolarizations of sufficient amplitude can generate premature action potentials that lead to cardiac arrhythmias, which can progress to ventricular fibrillation and sudden cardiac death. By contrast, shortening of the QT interval is associated with exaggerated heterogeneity of repolarization in time and space. This exaggerated heterogeneity of the action potential duration creates a substrate for functional reentry similar to that of the long QT syndrome but

with has tened recovery and reduced refractoriness in the ventricle. Arrhythmias are more likely to be malignant in short, compared with long, QT syndromes (169, 170).

Frequently, reference ranges for the QT-interval in the general population are expressed in terms of QTc, a corrected form of the QT interval (171-174). Bazett's formula is the most commonly used method to calculate QTc and adjusts for the heart rate, although it tends to underestimate the duration of repolarization when the heart rate is particularly slow (or overestimate when the heart rate is fast). The electrocardiographic QTc is approximately normally distributed in the general population. Normal values for the QTc range from 350 to 450 ms for adult men and from 360 to 460 ms for adult women; however, 10%-20% of otherwise healthy persons may have QTc values outside this range. Marked prolongations in the QT interval may be caused by genetic disorders (e.g., long QT syndrome), pharmacological agents (e.g., antiarrhythmics, antipsychotics, and antibiotics), electrolyte abnormalities (e.g., hypokalemia and hypomagnesemia), and their interactions. Other factors associated with QT interval length variability include age, sex, hypertension, body mass index, medication usage, low-calorie diets, serum potassium levels, and common genetic variants (175). Finally, withinperson variability and measurement error are additional sources of variability in the QT interval length.

Heart rate correction of the QT interval is fraught with problems. Manual measurements of the QTc interval are better than digital (with the 12SL algorithm) because the latter may lack a prolonged QTc interval diagnostic statement (176). Therefore, automated measurements should be manually confirmed (Figure I.2.1), both in daily clinical practice and for scientific purposes.

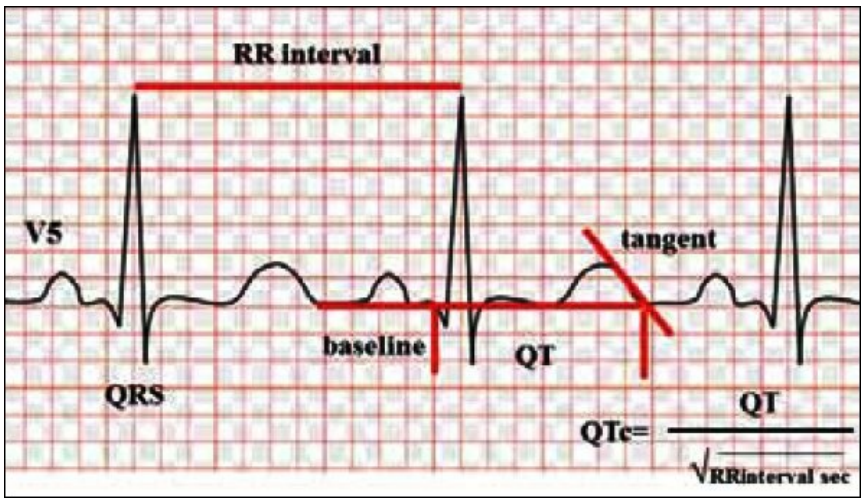

Figure I.2.1. The QT interval starts at the onset of the Q wave and ends where the tangent line for the steepest part of the T wave intersects with the baseline of the electrocardiogram (in V5 or DII lead)

Mechanism of ventricular arrhythmias in QT interval variations

Prolongation of the QT interval is associated with early afterdepolarizations, in which an abnormal depolarization occurs during phase 2 or 3 of the action potential before repolarization has been completed. Early afterdepolarizations of sufficient amplitude can generate premature action potentials that lead to cardiac arrhythmias. This can progress to ventricular fibrillation and sudden cardiac death. Longer QT intervals reflect longer

ventricular action potentials and a reduction in the repolarizing reserve that is associated with exaggerated spatial and temporal heterogeneity of electrical recovery of the ventricle (177). This allows for the development of functional reentry, in which still-activated regions of the ventricular myocardium re-enter and reactivate regions with shorter action potentials, producing polymorphic ventricular tachycardias (such as torsade de pointes) (175).

Similar to QT prolongation, shortening of the QT interval is not uniform in time and space in the ventricle (175), producing an exaggerated heterogeneity of repolarization. Arrhythmias are even more likely to be malignant in short, compared with long, QT syndrome (178). However, less is known regarding shortened QT intervals, especially at the population level.

QT interval variations and mortality rate

In literature, there are many papers describing QT interval variation and mortality in patients with cardiac or non-cardiac pathologies. Increased levels of B-type natriuretic peptide are associated with prolongation of the action potential in the ventricular myocardium. It seems that a B-type natriuretic peptide augmentation in patients with heart failure is associated with an increased risk of sudden cardiac death only in patients with QTc interval prolongation (179). The paced QTc interval appears to be a more useful marker for predicting increased total mortality and cardiac mortality than the intrinsic QTc interval in patients with indications for a permanent pacemaker (180). A 50-ms increase in the QTc interval is associated with doubling in the probability for all-cause mortality in patients with rheumatoid arthritis. The association of QTc with C-reactive protein levels could indicate a potentially hazardous interplay between inflammation and arrhythmogenesis (181). A high prevalence of prolonged QT interval duration has been observed in hemodialysis patients. In a case series of these patients, QTc seemed to be associated with total mortality and sudden cardiac death (182). QTc prolongation is also associated with increased mortality in patients with sickle cell diseases (183). Intubation and respiratory arrest are independently associated with the QTc interval in acute methadone-intoxicated patients presenting to the emergency department; indeed, QTc could be a potential predictor for adverse outcomes related to acute methadone intoxication (184).

Short QT syndrome is an uncommon inherited autosomal dominant cardiac channelopathy. It is correlated with malignant ventricular and atrial arrhythmias, atrial fibrillation being one of the most frequent complications. The first criteria for short QT interval involve a corrected QT interval of <330 msec. Another important one is family history of sudden death, vindicating a heightened index of suspicion for ventricular tachycardia in otherwise healthy patients who have syncopal episodes in the context of previous documentation of atrial fibrillation. Also, a short or even absent ST segment, and narrow T waves may indicate the syndrome (185, 186).

Therefore, there are consistent associations between QT interval variation and an increased risk of mortality. At the population level, these associations are substantial and comparable in magnitude to the effect of other traditional cardiovascular risk factors (187). QT interval prolongation may be associated with conditions affecting autonomic tone or left ventricular structure, including left ventricular hypertrophy or myocardial infarction (188). By contrast, the QT interval may simply be a marker for the severity of an underlying clinical or

subclinical cardiac disease (189). However, most studies adjusted for blood pressure levels or the presence of hypertension and either excluded or adjusted for the presence of a pre-existing coronary heart disease (187, 188). Furthermore, a direct link has been established between genetic variations in the QT interval length and sudden cardiac death, indicating that QT prolongation could be a direct causal contributor to mortality risk (190). However, one meta-analysis study provided evidence of substantial heterogeneity in the methodology used to study QT and mortality relationships across studies. When combined with the genetic findings related to genetic variability in the QT-interval length and mortality, the QT-interval length is still thought to be a determinant of mortality in the general population (175).

Although the risk of mortality increases with longer and shorter QT interval durations compared with the population average, until now there is still no clear threshold for this risk. Some studies have shown progressive associations between the QT-interval duration and mortality rate (191-193), whereas others have shown either U-shaped or non-significant associations (194-196). More than 20 studies have demonstrated an association between the QTc interval and all-cause and cardiovascular mortality in large samples from the general population with dose- effect responses even within the normal QTc interval range (197). In a large study (72 subgroups from 173,529 Danish primary care patients aged 50–90 years) analyzing the 5-year risk of all-cause, cardiovascular, and non-cardiovascular mortalities based on the QTc interval, the authors found that either the prolongation or shortening of the QTc interval (defined as ≤379 ms), especially in women, is associated with a worse prognosis (198).

Another important issue related to QT interval and mortality rate in clinical practice is the proarrhythmia risk of drugs.

Drug-induced ventricular tachyarrhythmias may have different causes, not only cardiovascular drugs, but also non-cardiovascular drugs, and even non-prescription agents. The side effects of these drugs are arrhythmic emergencies and even sudden cardiac death. Proarrhythmia is defined as the aggravation, or the occurrence of a new arrhythmia during therapy with a drug at a concentration usually considered non-toxic. The occurrence of polymorphic ventricular tachycardia can be elevated by the effects of various cardiovascular and non-cardiovascular drugs. The most important groups are antiarrhythmic drugs, antimicrobial agents, and antipsychotic and antidepressant drugs (199).

The pharmaceutical industry has focused on developing new approaches to assess the proarrhythmia risk during the discovery phase of potential new drugs (200, 201). Recently, Fossa et al. (202) developed a methodology that could validate the sensitivity of the QT interval using drugs in early development that enhanced diagnosis of long QT syndromes by reducing the variability and allowing adequate definition of normal limits. The QT beat-to-beat methodology and electrocardiogram (ECG) restitution could be used for future analyses to potentially quantify the arrhythmogenic vulnerability from temporal irregularities (203).

From a clinical perspective, the most important aspect is how the QTc interval-based predicted risk of mortality affects patient management. The RR–QT interval trend co-variability could be a novel index for the sudden cardiac death risk stratification (204). In addition to the QTc interval itself, other resting conduction and repolarization ECG indices such as heart rate (205), P-wave duration (206), and frontal T-wave angle (207) are increasingly associated with all-cause and cardiovascular mortality and morbidity in the

general population. However, these ECG indices may not be causally related to mortality; all these parameters could reflect an arrhythmogenic substrate. We should also be aware of the risk potential of cardiovascular and non-cardiovascular drugs that may further lengthen the QTc interval. Currently, recognition of acquired QTc prolongation is poor according to a study that assessed whether prescribers checked for arrhythmic risk with QT-prolonging medications (208). Finally, normal values for ECG indices may differ between individuals from different ethnic backgrounds (209); geographic factors should be taken into consideration when determining risk assessment models.

Shortened and prolonged QT interval durations, even when found within normal reference ranges and calculated using the most recent normative standards, are associated with an increased risk of cardiovascular disease mortality. However, there is a need for more standardized methods for measuring and reporting QT-interval measurements, population characteristics, and sudden cardiac death to more precisely estimate the magnitude of the increased risk associated with the QT interval variation.

I.2.3. Research regarding the inflammation and electrocardiography changes

A. Background

Early repolarization is a medical term that has been used for more than 50 years. A percentage of 1% to 13% of the overall population presents an early repolarization pattern. Until 2008, this electrocardiographic pattern was considered benign. Various recent studies proved an association between early repolarization and sudden cardiac death arrest due to idiopathic ventricular fibrillation, called early repolarization syndrome – ERS in cardiology pratice. In ERS patients, current imbalances between epicardial and endocardial layers result in dispersion of depolarization and repolarization. An early repolarization pattern is frequently found on ECGs surface in many patients, the majority of individuals with ERS will remain asymptomatic and the isolated presence of an early repolarization pattern does not require further intervention. The contradiction between frequently found early repolarization patterns in general population, low incidences of sudden cardiac deaths related to ERS, but fatal, grave consequences in affected patients are huge clinical challenges. There is a need for more precise tools for identification of this minority of patients (210, 211).

B. Published papers in this field

Early repolarization syndrome was the theme of another review, important data related with mechanisms of early repolarization, electrocardiographic diagnosis of ERS risk stratification of VF in patients with ERS have been summarized.

Rezus C, Floria M, Moga VD, Sirbu O, Dima N, Ionescu SD, Ambarus V. Early repolarization syndrome: electrocardiographic signs and clinical implications. *Ann Noninvasive Electrocardiol.* 2014; 19 (1): 15-22.

Introduction

The aim of this review was to summarize the current electrocardiographic data concerning ERS with clinical implications.

Primary electrophysiological disorders with known or unknown ionchannel abnormalities are responsible for 10% of sudden cardiac deaths – SCDs. Long and short QT syndrome, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia are known ionchannel abdnormalities, while early repolarization syndrome – ERS and idiopathic ventricular fibrillation – VF are unknown (212–215).

In most of the cases, the ECG pattern of early repolarization is a benign phenomenon, which is observed predominantly in teenagers, young adults, male athletes, and the black race. However, in recent years, it has emerged as a marker of risk for idiopathic VF and SCD (215). The universally accepted criterion for the diagnosis of early repolarization involves the presence of an elevated junction between the end of the QRS complex and the beginning of the ST segment (like the J point). Specifically, this condition is characterized by an elevation from baseline in the ST segment of ≥ 1 mm or 0.1 mV in at least two adjoining leads on standard 12-lead ECG. In particular, early repolarization in the inferior ECG leads has been associated with idiopathic VF and has been termed as ERS. Several clinical entities can cause ST-segment elevation, including asthenic habitus, acute pericarditis, ST- segment elevation myocardial infarction, Brugada syndrome, congenital short QT syndrome, and idiopathic VF. Therefore, ECG and clinical data are essential for differential diagnoses. Although there are similarities between ERS and Brugada syndrome, there is insufficient evidence to support their association.

The potential arrhythmogenicity of early repolarization has also been demonstrated in experimental studies (216-218). In fact, a high prevalence of early repolarization was reported in patients with idiopathic VF in 2007 and 2008 (219). Although these findings have supported the need for careful evaluation of individuals displaying ERPs, especially those with syncope or ventricular arrhythmias and/or family history of sudden death, it might not be reasonable to consider early repolarization as a general risk marker for SCDs in the general population. Even in athletes, early repolarization was found to only minimally increase their arrhythmic risk.

Prevalence of ERS

The prevalence of ERS in the general population varies from <1% to 13%, depending on age (predominant in young adults), race (highest among black populations), sex (predominant in males), and the criterion used to measure J-point elevation (0.05 vs. 0.1 mV) (215, 220, 221). In patients with documented idiopathic VF and a structurally normal heart, the overall prevalence of ERS is approximately 31%. However, the prevalence of the ERS pattern with J- wave elevation >= 0.2 mV in patients with idiopathic VF was found to be 16% (220). The prevalence of early repolarization is significantly higher than previous estimates among asymptomatic young adults, and the majority of early repolarization regressed by middle age. It seems that black race, lower body mass index, lowerserum triglyceride levels, and longer QRS duration are independently associated with maintenance of early repolarization over time (222).

Mechanisms of early repolarization

Although the J wave has been described as synonymous with early repolarization abnormalities, the mechanistic understanding of the J-wave signature on surface ECG remains incomplete. In fact, it is unclear whether J waves are more strongly associated with depolarization or repolarization abnormalities.

Despite of some similarities observed between ERS and Brugada syndrome (like gender, arrhythmia triggers, and response of early repolarization to sodium-channel blockers) there is a differential mechanism between early repolarization in the inferolateral leads and ST elevation in the right precordial leads (223). Ajmaline significantly decreases the J-wave amplitude in early repolarization and prolongs the QRS width significantly less than in patients with Brugada syndrome (224); In addition, there is a heterogeneous response to isoproterenol (225).

Although ERS, idiopathic VF, and Brugada syndrome share common ECG features, they display remarkably different clinical consequences. Early repolarization is a benign ECG finding characterized by a distinct J wave and ST segment in left precordial leads (V4–V6). In contrast, idiopathic VF and Brugada syndrome are the leading causes of SCD in young South–East Asian males and are characterized by J wave and ST-segment elevations in the inferior and right precordial leads, respectively.

An arrhythmogenic platform could be created by disproportionate amplification of the repolarizing current in the epicardial myocardium due to either a decrease in the inward Na^{+} or Ca^{2+} channel currents or an increase in the outward potassium currents mediated by ion channels (such as Ito, IK-ATP, and/or IK-Ach). The trigger and substrates driving phase 2 reentry and ventricular tachycardia/VF eventually produce transmural dispersion in the duration of cardiac action potentials (220).

It is likely that the J-wave signature is coincident with phase 1 of the cardiac action potential in the epicardial region of the ventricular myocardium and precedes phase 1 in endo- and midmyocardial cells, generating an early gradient in repolarization currents within the ventricles (226). In fact, the J wave should be considered as a repolarization phenomenon rather than a late depolarization event because of its slower inscription and its spontaneous/rate-dependent fluctuation in a morphologic pattern or amplitude within stable QRS complexes (i.e., increased pattern at slow heart rate, decreased pattern at faster heart rate). In addition, its amplitude varies concurrently with the ST segment (220).

The occurrence of J wave related arrhythmias is mediated by phase 2 reentry. The stability of the action potential dome in the ventricular epicardium is dependent on the prominence of the action potential phase 1 notch (227). Also, it has been suggested that differences in Ito density and Ito-mediated epicardial “spike and dome” are the underlying mechanisms that lead to the distinct clinical consequences of these syndromes. Indeed, when Ito is prominent, a complete loss of the dome can result from either decreased inward currents or increased outward currents, which can consequently lead to a phase 2 reentry that is capable of initiating VF in either idiopathic VF or Brugada syndrome. When Ito density is reduced, as in ERS, partial depression of the dome occurs without the development of phase 2 reentry. Thus, a strong genetic component could underlie the ERP. In this regard, it has been suggested that ERS is polygenic and influenced by environmental factors (220). Moreover, ERS could be inherited through autosomal dominant trans- mission and might be considered a real inherited arrhythmia syndrome. Familial investigation can be

facilitated by using the Valsalva maneuver to reveal the electrocardiographic pattern in family members (228).

There were identified reductions in heart rate and cardiac conduction in patients with idiopathic VF associated with early repolarization related to SCN5A mutations; it seems that these mutant channels did not generate any currents. SCN5A is a disease gene for idiopathic VF associated with early repolarization (229).

An ERP can develop during the radiofrequency ablation of the left accessory pathway; the mechanisms might be increased vagal tone due to chest pain or direct vagal stimulation (230).

However, the exact mechanisms that drive early repolarization remain unknown. For this reason, future research should be aimed at identifying the various underlying mechanisms involved in early repolarization as well as characterizing depolarization abnormalities as potential risk stratifiers.

Electrocardiographic diagnosis of ERS

ERS is defined as an elevation of the J point (the junction between the end of the QRS complex and the beginning of the ST segment) and/or ST segment by at least 0.1 mV from baseline. J-point elevation can manifest as either QRS slurring (at the transition from the QRS segment to the ST segment) or notching (a positive deflection inscribed on the terminal S wave), resulting in ST-segment elevation with upper concavity and prominent T waves in at least two contiguous leads (Figure I.2.2) (220). In benign ERS, reciprocal ST-segment changes are only possible in aVR and the ST segments when T-wave patterns display a relative temporal stability.

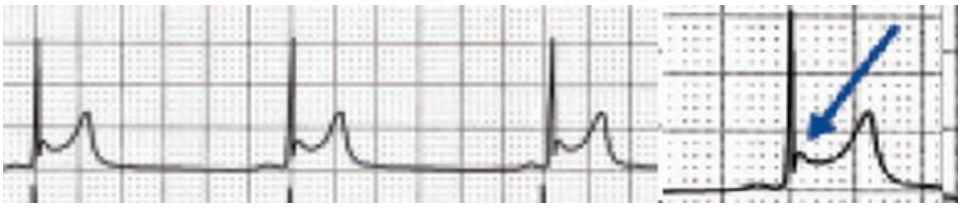

Figure I.2.2. Electrocardiographic pattern of J-point elevation in early repolarization

Accurate measurement of early repolarization is dependent on a sharp transition from the terminal QRS complex to the ST segment. This is usually straightforward in notched early repolarization, but frequently unclear in cases of slurred early repolarization. Therefore, when there is a gradual transition at the end of the QRS complex, definition of the J point is more subjective. In this situation, the discrepancy between repeated QRS interval measurements has been shown to approach as much as 40 ms. In addition, the QRS complex may begin and end at different times in different ECG leads. In this regard, leads that display the earliest QRS wave are often distinct from those showing QRS ending last, with up to a 20-ms difference (231). Thus, what may appear as notching of the terminal QRS and early repolarization in one ECG territory may look like QRS fragmentation and conduction delay in another.

Patients with idiopathic VF do not display structural heart disease based on echocardiographic biventricular dimensions and function. In addition, they do not have detectable coronary artery disease or known repolarization abnormalities.

Idiopathic VF is a low prevalence condition, which is possibly familiar and is

characterized by the occurrence of VF events in young individuals, particularly males. It occurs in the absence of structural heart disease in subjects with otherwise normal ECG readings, even when high right accessory leads and/or ajmaline injection are used.

VF can be dramatic, predominantly occurring at night during vagotonic predominance when J waves are >2 mm in amplitude. These ST/T abnormalities are dynamic, inconstant, and reversed with isoproterenol. These changes are characterized by the presence of convex upward J waves, which display either horizontal/descending ST segments or a "lambda-wave" ST shape (232) and occur in the absence of hypothermia, ischemia, or electrolytic disorders (figure I.2.3). Convex up ward J waves, with horizontal/descending ST segments or "lambda-wave" ST shape are suggestive of idiopathic VF with early repolarization abnormalities (233).

Premature ventricular contractions with very short coupling and "R on T" phenomenon are characteristics with two patterns: when originate from right ventricular outflow tract, left bundle branch block morphology; and from peripheral Purkinje network, left bundle branch block pattern. The combination of J waves with horizontal/descending ST segment it seems to improve the ability to distinguish patients with idiopathic VF from controls matched by gender and age (234).

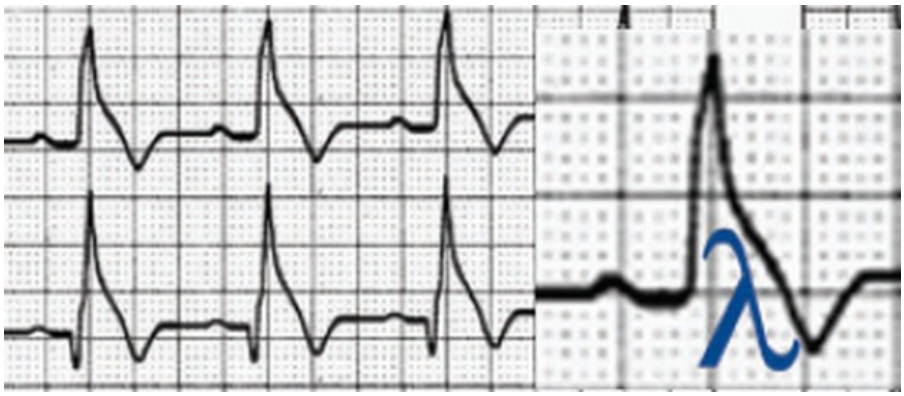

Figure I.2.3. Downsloping ST-segment elevation is present in inferior leads and labeled as "lambda-wave."

Cases of primary electrical disorders should be excluded if the QT interval corrected for heart rate (QTc) is <340 ms (short QT interval) or >440 ms (long QT interval) at baseline prior to arrhythmia (212). Notably, ERS and Brugada syndrome seem to share some similar electrocardiographic characteristics, clinical outcomes, and risk factors. In addition, they display common arrhythmic platforms related to amplification of Ito-mediated J waves. Although Brugada syndrome and ERS differ with respect to magnitude and lead location for abnormal J-wave manifestation, they both represent conditions within the phenotypic spectrum of J-wave syndromes.

Brugada syndrome is an inherited cardiac disease first described in 1992. Patients with the condition exhibit a characteristic electrocardiographic pattern consisting of a J wave that mimics a right bundle branch block with typical ST-segment elevation (≥ 0.2 mV) in at least two precordial leads (V1–V3), which occurs in the absence of intervention or following infusion of a sodium-channel blocker (214). Although this ECG signature was believed to be a normal repolarization variant for more than three decades, the syndrome is now known to be

associated with a high incidence of life-threatening ventricular tachyarrhythmias and is responsible for numerous sudden deaths in young adults worldwide. Moreover, the ERP can be detected with inferolateral leads in Brugada syndrome patients; however, this is a rare finding (235). Therefore, Brugada syndrome should also be excluded during diagnosis of ERS.

In addition, patients with catecholaminergic arrhythmias, which are defined as arrhythmias that occurring during catecholamine infusion or exercise testing, should be included as differential diagnoses. Notably, J-point elevation and ST elevation in V2–V4 are commonly observed in highly trained athletes, and confusion should be avoided when examining such individuals. Anterior ST-segment elevation during myocardial infarction can be difficult to differentiate from ERS on the ECG. However, R-wave amplitude is lower, ST-segment elevation is greater, and QTc is longer for subtle anterior ST-segment elevation myocardial infarction versus early repolarization (236). Also, the ERS pattern may coexist with a number of cardiac or extracardiac conditions, such as hypothermia.

ERS can be divided into three subtypes (237):

- Type 1, which is predominantly characterized by an ERP that is detected with lateral precordial leads, is prevalent among healthy male athletes and rarely seen in VF survivors;
- Type 2, which is predominantly detected through the inferior or inferolateral leads, is associated with a higher level of arrhythmia risk than type 1 ERS;
- Type 3, which involves ERPs that are observed globally through the inferior, lateral, and right precordial leads, is associated with the highest level of risk for malignant arrhythmias and often associated with VF storms.

ERS patients had an abnormal repolarization dynamics with a continuously depressed diurnal and nocturnal adaptation of the QT interval to the heart rate (238); this might provide a substrate for reentry and be an important element for developing VF. It seems that there is a possible relationship between hypokalemia and VF in ERS (239).

Risk stratification of VF in pateints with ERS

Although ERS is a common entity, unexplained SCD in young adults is very rare. For this reason, the incidental discovery of a J wave on routine screening should not be interpreted as a marker of “high risk” for sudden death because the odds for this fatal disease would be approximately 1:10.000 (240). However, close follow-up should be offered to patients that display ERS along with a personal history of unexplained syncope and/or a family history of unexplained SCD.

The magnitude of the J-point elevation might constitute a discriminator of risk. In this regard, a J-point elevation >0.2 mV seems to be rare in the normal population (241). Also, it must be noted that the magnitude of J-wave elevation can fluctuate, even without drug provocation or exercise, which means that a low-magnitude J wave should not be considered as a static entity (242).

In normal subjects, early repolarization is mostly confined to the inferior leads, lateral leads, or left precordial leads. Almost half of all patients with ERS and VF (so-called malignant early repolarization) displayed an ERP in both inferior and lateral leads (i.e. a much

more diffuse repolarization abnormality) (215). Moreover, in a small study, it was recently observed that left precordial terminal QRS notching was more prevalent in malignant variants of ERS than in benign cases (243). Nevertheless, more work will be needed to determine whether this information could be used as a tool for risk stratification.

The accentuation of repolarization before the onset of arrhythmia and the origin of triggering beats from the region of early repolarization underlie the link between ECG pattern and the site of malignant arrhythmia. The early repolarization abnormality can either be limited to a single region in the ventricles or extend beyond, involving more than one region simultaneously. In patients diagnosed with idiopathic ventricular arrhythmias and early repolarization, an alarm should be raised if the origin of the arrhythmia, as identified by the morphology of VF—initiating ventricular foci, is concordant with the location of early repolarization (215).

It appears that patients with idiopathic VF and J waves have a high incidence of late potentials, which show a circadian variation with night ascendancy (242). Therefore, detection of these late potentials using a signal-averaged system and 24 hour Holter electrogram could represent a useful technique for identifying those at high risk for arrhythmia.

As transmural dispersion of repolarization markers, Tpeak-Tend interval and Tp-Te/QT ratio are significantly increased in patients with J-wave syndromes compared to age and sex-matched uneventful early repolarization (244, 245).

Patients with early repolarization do not seem to display significantly higher inducibility in comparison to those without early repolarization. Moreover, electrophysiologic study is less sensitive for risk stratification of symptomatic patients (215).

L-type calcium channel mutations are detected in a high percentage of probands with J-wave syndromes associated with inherited cardiac arrhythmias, suggesting that genetic screening of Cav genes may be a valuable diagnostic tool in identifying individuals at risk (246).

The presence of early repolarization seems to increase the vulnerability to fatal arrhythmia during acute myocardial ischemia (247-249); this could provide a plausible mechanistic link between this ECG pattern and higher arrhythmic mortality of middle-aged/elderly patients (250). In addition, early repolarization could be an independent predictor of occurrences of VF in the very early phase of acute myocardial infarction (249).

Many questions about the pathogenesis of J-wave patterns, and the associated magnitudes of risk, remain unanswered, especially in regard to the risk implications in certain high-prevalence subpopulations such as athletes, children, and adolescents. To estimate the magnitude of mortality and SCD risk associated with J-point elevations and J waves, in what has become known as ERPs, is still a problem. The patterns of J-point elevations and J waves or ERPs appear to reflect a continuum of risk for arrhythmias. The estimated cardiac mortality risk associated with the corresponding electrocardiography pattern (the highest risk in patients with extensive repolarization abnormalities, followed by the horizontal/descending ST segment >0.2 mV in inferior leads, horizontal/descending ST segment 0.1–0.2 mV in inferior leads, horizontal/descending ST segment of 0.1 mV in lateral leads, and the lowest for rapidly ascending ST segment with tall R waves in inferolateral leads) seems to be inversely with the estimated prevalence of the ERP in general population (251).

Early repolarization: benign or malignant?

In the general population, the benign form of ERS is associated with younger age, increased ECG evidence of voltage criteria for left ventricular hypertrophy, and lower heart rate/blood pressure, which is indicative of a younger, healthier, and physically active phenotype. In contrast, the malignant form of ERS, involving horizontal/descending ST-segment variation, is associated with older age and increased ECG signs of coronary artery disease (245). In addition, benign early repolarization is associated with a significantly shorter QTc interval, whereas malignant early repolarization is associated with a significantly longer QRS duration. Thus, these early repolarization types likely represent two different processes. Classical benign early repolarization appears to reflect earlier onset of repolarization, whereas malignant early repolarization might reflect abnormal depolarization, possibly due to a subtle underlying structural disease (252).

It appears that we can continue to consider classical Wasserberger ER with a rapidly ascending ST segment as a benign finding (253). J-point elevation with a horizontal ST segment was suggested as a malignant feature of the ERP; the prevalence of ERP with a horizontal ST segment is higher in patients with aborted sudden cardiac arrest (254). It is still unable to determine who is at significant risk when presenting with slurred or notched ER morphology unless they have already suffered a cardiac arrest (255).

ERS has emerged as a marker of risk for idiopathic VF and sudden death. However, the incidental discovery of a J wave on routine screening should not be interpreted as a marker of "high risk" for sudden death. Nevertheless, close follow-up should be offered to patients that display ERS along a personal history of unexplained syncope or a family history of unexplained sudden death. There are still major unanswered questions relating to our limited ability to determine which individuals with common electrocardiographic variant are at risk for sudden death.

I.2.4. Research regarding the anticoagulant therapy in patients with atrial fibrillation

A. Background

Taking into consideration the recent growth in the elderly population, the number of patients with AF and the number of patients indicated for anticoagulant therapy have been increasing.

Warfarin was the only oral anticoagulant used in clinical practice for more than fifty years. Recently, new oral anticoagulants were introduced and used in increasing quantities (256). Oral anticoagulation is essential in the management of thromboembolic risk in patients with non-valvular atrial fibrillation – NVAF.

Non-vitamin K antagonist oral anticoagulants – NOACs treatment has proven to be as effective as vitamin K antagonist – VKAs treatment, in NVAF patients (257).

B. Published paper in this field

The aim of one of my recent studies was to determine the level of information of patients with atrial fibrillation (AF) on oral anticoagulant treatment.

Rezus C, Baicus C, Badescu C, Floria M, Oatu A, Ganceanu-Rusu R, Tanase DM, Dima N. Medical and social apprehension of oral anticoagulation in patients with atrial fibrillation. *Romanian Journal of Cardiology* 2019; 21 (26): 3829-3834.

Introduction

Anticoagulant therapy stands for one medical field that undergoes rapid development and evolution. Quite frequently, the fear of not having a hemorrhagic episode triggers the failure to recommend the anticoa-gulant treatment or the ungrounded ceasing of such anticoagulant treatment in the proximity of some invasive maneuvers, with hemorrhagic potential.

AF, duly associated with the increased incidence of morbidity and mortality, has been correlated with a lower quality of life and an enhanced risk of developing a cardiac insufficiency and cerebral – vascular strokes. While several studies have assessed the passive influence of anxiety and of other psychological disorders on the cardiovascular system, the interrelation of AF and anxiety remains evasive. This condition evolution impairs personal satisfaction and the quality of life. The explanation and directionality of one such correlation brings a justification as for a further evaluation (258, 259).

The CHA_2DS_2-VASc scoring has been developed so as to stratify the risk of a stroke. According to the guidelines of the European Society of Cardiology (ESC), participants with CHA_2DS_2-VASc scoring of >2 require oral anticoagulant treatment, those having CHA_2DS_2-VASc scoring of 1 require either oral anti-coagulants or platelet anti-aggregating, and those with CHA_2DS_2-VASc scoring of zero shall fail to require any antithrombotic medication (260). By help of HAS-BLED scoring, one may calculate the likely risk of bleeding. Patients showing a HAS-BLED scoring of ≥3 are deemed to show an increased risk of bleeding and one recommends prudence and a periodical evaluation after the due initiation of the anti-thrombotic therapy (261).

Material and methods

One hundred and fifty-five patients with a diagnosis of AF, have replied to a questionnaire on the social – demographic conditions and the education level, so as to for one to be able to establish the latter's influence of the incidence of failure to comply with the indications and the complications occurred during treatment.

The statistical analysis has been carried out by using SPSS 22.0 software (Statistic pack for Social Sciences, Chicago, Illinois). Data have been expressed as an average ± standard-deviation (SD) or the number of cases with a percentage, for the constant and listed variables.

Results

The average age of patients has been 70.75 ± 10.57 years old, the minimum age being 31 and the maximum age being 90. Of the aforementioned, within the age group <50, the

percentage has been 3.23% (n=5); within the age group: 51-60 the percentage has been 9% (n=14); within the age group 61-70 the percentage has been 38.6% (n=59); within the age group 71-80 the percentage has been of 32.9% (n=51) and within the age group >80, the ratio has been 16.77% (n=26).

Within the present study, 71.6% (n=111) of the patients enrolled in the study were married and a significant percentage 22.6% (n=35) have stated that they were either a widow or a widower. Starting from the idea that education helps us become aware of the risk we expose ourselves to when we deny getting a treatment, 26.5% (n=41) of the patients had graduated gymnasium education, 24.5% (n=38) had graduated the elementary school, 40% (n=62) had graduated high school and 9% (n=14) had graduated a faculty. Of the aforementioned, the largest number of patients who were under anticoagulant treatment, were high school graduates, with a share of 30.32% (n=47), as compared to the gymnasium education graduates group, who stood for the majority of the group of those patients undergoing an anticoagulant treatment, with a share of 14.84% (n=23) respectively (Figure 2).

Most of the patients diagnosed at the beginning of the study were high school graduates, married (84.21%). In terms of subjects of a known diagnosis, the predominance has also been held by married high school graduates, while at the opposite end, holding the lowest percentage there were the gymnasium education graduates, with a share of 7.69%. Subject to the type of anticoagulant treatment being accepted, we may observe that Acenocoumarol is preferred by patients from the rural environment, most probably given the latter's lower cost, while people from the urban environment enrolled in our study prefer the apixaban.

Acenocoumarol is mainly administered in our lot of subjects, with a share of 9,03% (n=14) in gymnasium education graduates, 10.97% (n=17) in general educa-tion graduates, namely 16.13% (n=25) in high school graduates, while faculty graduates prefer the Apixaban in a majority share of 9.68% (n=15) (Figure I.2.4).

Another relevant issue, as well as possibly a risk factor for the occurrence of complications during the administration of the anticoagulant treatment might be represented by each single subject's physical and social activity. We've divided our group subject to the type of activity, namely active (aged <65) and passive (aged >66). Acenocoumarol is once again preferred by the elderly group, although monitoring the therapeutic efficiency is cost – consuming, not to mention the additional visits paid to the doctor's office (figure I.2.4).

The stroke risk stratification has been accomplished by the CHA2DS2-VASc scoring: 41.3% (n=64) have had the CHA2DS2-VASc scoring of ≥5, of the afo-rementioned, 40.6% (n=26) were undergoing thera-py by Acenocoumarol, and the rest of 59.4% (n=38) were undergoing therapy by NOAC. The assessment based on HAS-BLED scoring has shown that 74.83% (n=116) of the people under questioning showed an HAS-BLED scoring of ≥3 points, three quarters being aged over 65.

Within the present study, the complications that have been most frequently met in patients suffering from atrial fibrillation and who underwent anti-thrombotic treatment, namely the stroke and major bleeding, have held the highest percentage among high school graduates in the urban environment, in terms of strokes the percentage being 30% and 44% was for major bleeding. The lowest incidence in terms of the-se complications has been noticed in subjects who had graduated the gymnasium and who lived in the urban environment, namely 1.6%, respectively 1.53% (figure I.2.5).

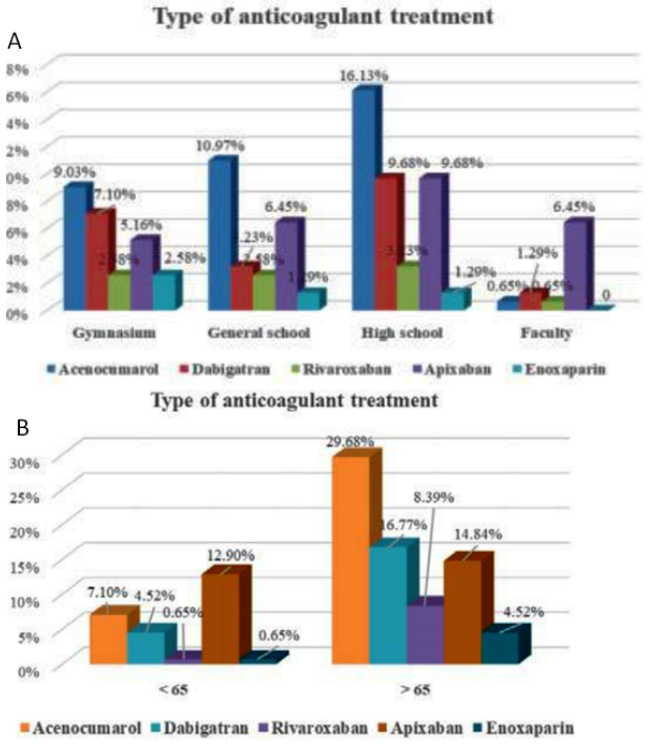

Figure I.2.4. The structure of the lot, subject to the type of oral anticoagulant treatment and the educational level (A); Patients' distribution subject to age group and the type of oral anticoagulant treatment (B)

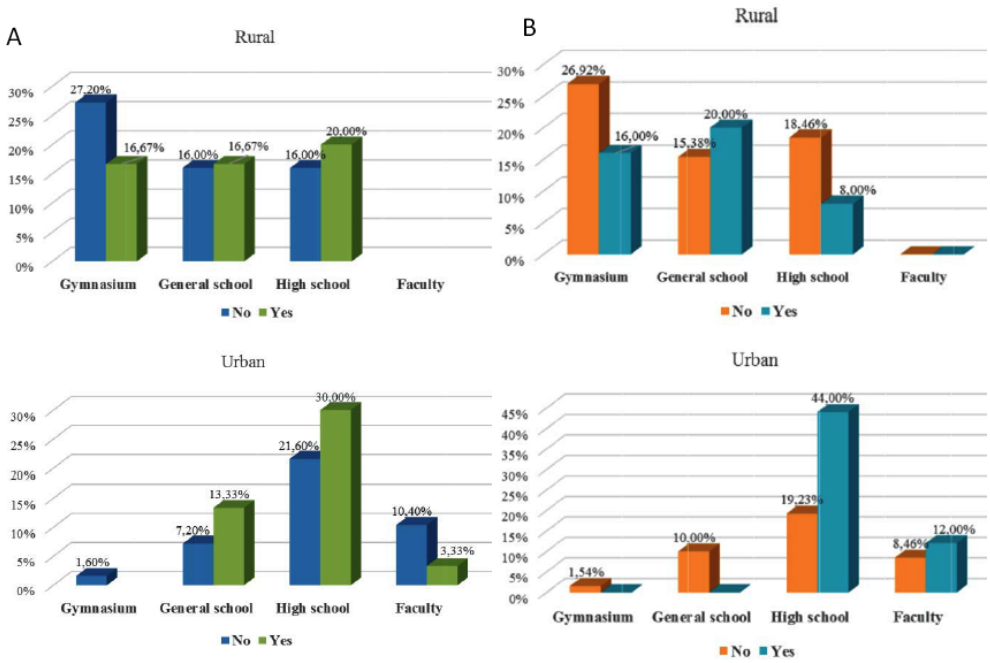

Figure I.2.5. A. Patients with stroke (in the past); B. Patients with major bleeding (in the past).

Only 12.9% of the patients suffering from AF have stated that one can detect AF by regularly taking one's pulse. It should be noted that almost one in three patients (38.1%) had no idea that AF can cause pulmonary embolism and strokes.

Less than half of these patients (38.1%) knew that risk factors such as overweight can facilitate AF. Moreover, only 34.8% of the patients having received anti-thrombotic drugs

were aware of the eventual hemorrhagic complications that are associated with the therapy.

Unfortunately, the information on analgesics that can be safely used in combination with the anticoagulant therapy is not sufficiently clear and precise, since only 27.7% know they can use those of the type of Acetaminophen. In the event of any surgery, most likely 62.6% of the patients would not check with their physician in terms of the eventuality of any adjustment of the anticoagulant therapy.

Relative adherence to treatment in rural patients resulted in under-treatment of oral anticoagulant therapy (52.23%), and therapeutic INR in the same patient population was found in 2.98% of cases. In urban patents, there is a better treatment compliance, as evidenced by the higher proportion of patients with therapeutic INR (7.46%) (figure I.2.6).

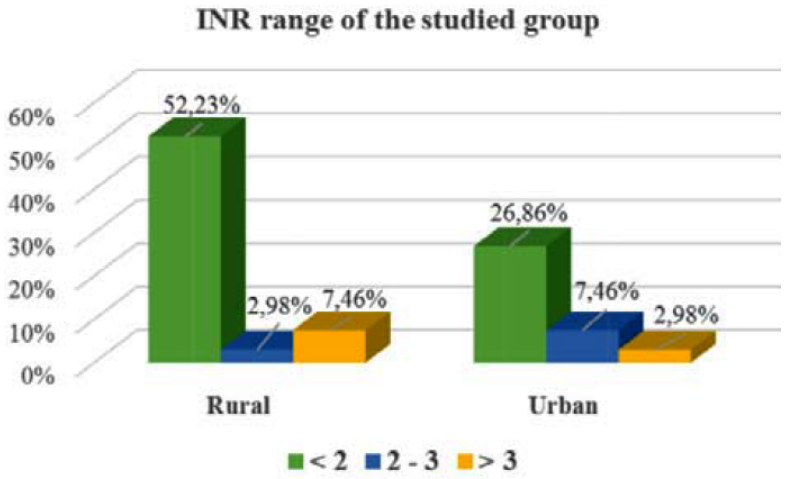

Figure I.2.6. Ischemic and hemorrhagic events in relation to provenance environment

Discussions

In all the chronic diseases, accurate assessment of medication and strategies to stop medication discontinuation are essential for an efficient treatment.

Oral anticoagulant (OAC) therapy is used to prevent thromboembolic events in AF patients. Medication adherence and persistence, in order to ensure efficacy and safety, represent major challenges for stroke prevention with OAC (VKAs and NOACs). Adherence refers to patient choice to properly comply with the doctor's prescription for medication. Persistence with medication is defined as the time from initiation to discontinuation and should be followed in order to increase the success of any prescription (257).

The literature informations have proven that in patients suffering from atrial fibrillation and who undergone treatment with warfarin or NOAC, the major bleeding risk has been significantly lower than in patients treated by NOAC (262).

One recently cross-sectional conducted study on patients' satisfaction with the warfarin – based treatment included 100 persons who attended a pharmacist-run urban anticoagulation clinic at Howard University. The primary outcome evaluated was willingness to switch to a new oral anticoagulant. Other studied variables were social demographics, clinic factors, patient knowledge and satisfaction. In order to estimate patient knowledge, satisfaction and willingness to switch, different questionnaires have been used: the modified anti-clot treatment survey (ACTS), the oral anticoagulant knowledge survey (OAKS), and a validated

willingness to switch survey.In correlation with our study, it is important to mention that the majority of participants were retired/disabled (59%). Patients from the anticoagulation clinic had low knowledge of their warfarin therapy, were overall satisfied with warfarin treatment, but they have been willing to consider using a new oral anticoagulant which was more convenient. The major barrier for taking this turn has been represented by the cost and the necessity to administer some of the most re-cent agents twice a day, unlike once every day as it was the case of warfarin. The major barrier for taking this turn has been represented by the cost and the necessity to administer some of the most recent agents twice a day, unlike once every day as it was the case of warfarin (263).

The present questionnaire approaches one of the most significant issues regarding the AF management and the anti-thrombotic therapy, including not only theoretical questions, but also such questions as related to the management of various possible cases in anticoagulant patients. Whereas the main focus of AF refers to preventing the thromboembolic stroke, we focused on the possible side effects, the drug interac-tions and the relevant of one good adherence to the anticoagulant treatment.

The anti-thrombotic treatment should be applied in due compliance with the current guidelines, and the risk-benefit ratio shall be assessed for each and every single patient, as concerned. The hemorrhagic risk shall dominate the clinician's logics and reasoning in terms of the anticoagulant therapeutic approach to the detriment of any potential benefit whatsoever (264).

The result of the numerous clinical trials confirm the fact that patients have significant benefits as a result of one judicious anticoagulant treatment (265, 266). Knowing how the anticoagulant medication works, as well as knowing the protocol and the necessary medication required in hemorrhagic emergency cases, it's what gives the physician the surety he needs upon making the calls. All these facts bring a series of benefits to the patient in terms of vascular aging, as well as the length and quality of the latter's life (267, 268).

Lane et al. have published a cross-sectional, prospective survey of 936 adult patients (mean age 54.3 years; 37.2% female, from different countries: USA, Canada, Germany, France and Japan) with AF on OACs treatment. Three groups were involved in the study: AF with recent stroke (≤ 6 mounths); new onset AF (≤ 6) without recent stroke; established AF (7-24 mounths) without recent stroke. As in our study, the patients were grouped according to educational level: no school-leaving certificate, high school diploma, community college and university/technical college and some open-ended questions determined their stroke knowledge, assessed according to a predefined scoring system. As a general remark, the authors observed that stroke knowledge was similar in all patients regardless of educational level. In particular, a greater proportion of patients with college education were 'always' or 'often concerned' about stroke, whereas a greater proportion of those with low levels of education were 'never concerned' about stroke. Also, the study revealed that educational level had no effect on self-reported OAC adherence or patient preference to be involved in OAC treatment decisions (265).

The main causes of NOAC's under-utilization in the present study were: modest economic status, high cost of NOAC, limited access to medical information of rural population and reduced educational level.

Our study underlined the need to take action and conduct an intervention at the education level among the patients suffering from AF, particularly those showing a high risk of stroke. The results shall be used for the purpose of drawing up a series of information and prevention programs which have proven to be an absolute requisite, so as to enhance the quality of life and increase life hope among the relevant population, as concerned.

I.2.5. Research regarding the inflammation, arrhythmias and gastro-oesophageal reflux disease

A. Background

AF, with a prevalence of 1–2% of the general population and a number of cases expected to double or even triple within the next 2-3 decades related to the ageing of the population, an inappropriate control of cardiovascular risk factors like hypertension and potentially better treatment options of other conditions like coronary heart disease or heart failure. The relationship between gastrointestinal symptoms and arrhythmias, first described under the name of 'Roemheld gastrocardiac syndrome', is based on an oesophago-gastric stimulus able to induce arrhythmia-related symptoms. An association between AF and disorders of the gastrointestinal tract has been reported in many studies; this association is due to the close vicinity of the oesophagus and the left atrium, gastroesophageal reflux disease – GORD and oesophagitis being the most commun gastrointestinal diseases found in AF patients. It is estimated that the presence of GORD might increase the risk of AF by 39%. GORD patients may have episodes of AF triggered by defaecation, abdominal bloating, alcohol, cold water, and fatty food consumption (269, 270).

B. Published paper in this field

Considering the fact that GORD and oesophagitis are among the most common gastrointestinal diseases found in AF patients, I have published a review related with this subject together with experienced gastroenterologists.

Floria M, Barboi O, **Rezuș C**, Ambarus V, Cijevschi-Prelipcean C, Balan G, Drug VL. Atrial fibrillation and gastro-oesophageal reflux disease – controversies and challenges. *Curr Pharm Des*. 2015; 21 (26): 3829-3834.

Introduction

GORD, one of the most frequent benign disorders of the upper gastrointestinal tract has an increasing prevalence (varying between 0.8-40%). It is defined as symptoms or complications related to the reflux of gastric content (271-277).

The pathophysiology of GORD is multifactorial. An abnormal oesophagogastric junction (EJ) function (pathologic transient low oesophagus sphincter (LOS) relaxation,

hypotensive LOS or impaired clearance) and/or anatomy (hiatus herniation) are the main pathophysiological factors. Abnormal gastric secretion and impaired gastric emptying may also contribute to GORD. Age related changes and abdominal obesity appear to be important components that contribute to alterations of the EJ (278).

According to the Montreal classification, GORD is classically grouped in oesophageal syndrome and extraoesophageal syndrome (figure I.2.7) (271). Chest pain syndromes together with typical reflux syndromes are considered part of the oesophageal syndrome. According to this classification, the established extraoesophageal manifestations are chronic cough or laryngitis, bronchial asthma and dental erosions, while the proposed manifestations are sinusitis, pulmonary fibrosis, pharyngitis and recurrent otitis (278).

The relationship between GORD and AF was observed for the first time in 1952 (279). At that time the induction of chest pain and/or cardiac dysrhythmias by an irritant oesophago-gastric syndrome was called Roemheld gastrocardiac syndrome. Due to the fact that the esophagus and atria are adjacent and have identical nerve innervations, it has been emphasized that AF appearance could be related to the development of GORD (280-286).

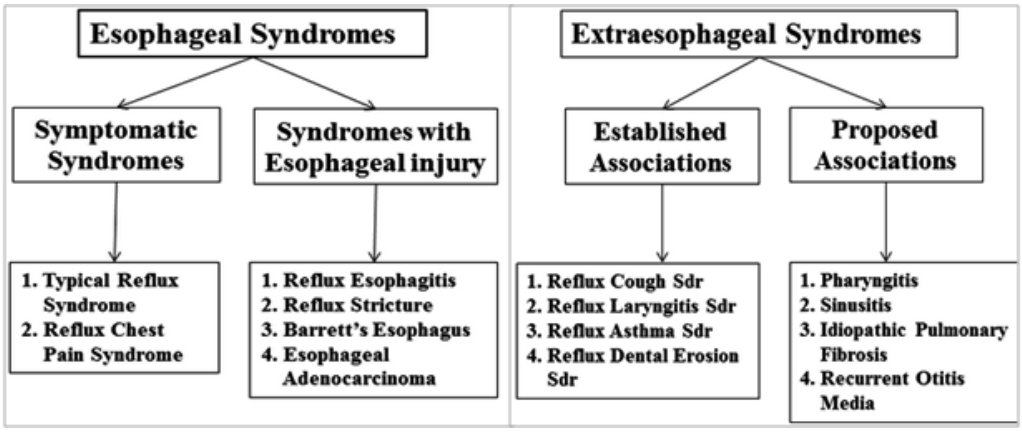

Figure I.2.7. The Montreal classification of gastro-oesophageal reflux disease

However, GORD also seems to be independently correlated with AF initiation. We used the keywords as AF and GORD to find and analyze the evidences of this potential association, published after 1952. Despite the experimental and clinical data supporting this association, as detailed in this review, AF is not included as a possible extraoesophageal manifestation of GERD according to the Montreal classification (271).

Possible mechanisms of atrial fibrillation associated with gastro-oesophageal reflux disease

Sympatho-vagal imbalance is one of the principal mechanisms suspected to be involved in the association of AF with GORD. Both components of autonomic nervous system play a role in AF pathophysiology. However, the cholinergic component seems to be critical for AF appearance (287). Electrical activation of the ganglionic plexi situated on left atrial posterior wall (near the oesophagus) induces AF initiation (287, 288). The majority of AF patients with GORD have triggered AF, and these patients have a positive vagal response during radiofrequency ablation (289). Gastro-oesophageal reflux disease could induce vagal nerve

stimulation (290-292). It seems that AF initiation is related to vagal nerve overstimulation and vagal nerve-mediated parasympathetic stimulation (293-295). In effect, the vagal nerve overstimulation involved in GORD development is also responsible for AF promoting in patients with GORD.

The second mechanism that seems to be implicated in the association of GORD with AF is hiatal hernia. Mechanical function alteration of the EJ may result in symptomatic or asymptomatic reflux disease. Obesity, neuromuscular dysfunction and oesophageal fibrosis may exacerbate and perpetuate progression of the disease through an overt hiatal hernia. The latter has an important role in gastro-oesophageal reflux, as it has an impact on most of the underlying pathways involved. By modifying the LOS pressure or relaxation, oesophageal clearance and acid pocket position, hiatal hernia is associated with a more severe GORD (287). Nearly all patients with severe reflux disease have hiatal hernia (296).

Obesity, particularly central obesity, is an important factor in the etiology of reflux, as well as in AF. Obesity could increase the abdomino- thoracic pressure gradient inducing hiatal hernia thereby increasing the rate of flow of reflux when sphincter opens. Dysrhythmias are common in upper gastrointestinal endoscopy due to the manipula- tion of the upper gastrointestinal tract. The possible mechanisms that could create this association between hiatal hernia, acid reflux and atrial arrhythmias are mechanical effects on the left atrial posterior wall that are either related to the mechanical effect of the food passage or left atrium compression by the hernia (295). However, these purely mechanical effects are hypothetical and have not yet been confirmed by scientific data.

Another possible mechanism that should be considered a participating factor in arrhythmias is inflammation (297, 298). Inflammation via cytokines is known to provoke calcium channel dysfunction, which is a cornerstone of AF and is known to be highly sensitive to reactive oxygen species (298). Mediators of inflammation have been implicated in the alteration of connexin integrity that plays a crucial role in gap junctions, which are inherent to electrical conduction. Finally, inflammatory pathways regulate extracellular homogeneity and are strongly linked to fibrosis, through the production of reactive oxygen species, cytokines, matrix metalloproteinases and growth factors (229). All of these modifications could contribute to left atrial electrical and structural remodeling witch mean initiation and persistence of AF.The inflammation could be a cause or a consequence of AF (229-304). The patients with AF had increased inflammatory cell infiltration in the atrial myocardium (300, 301). Proliferation and activation of epicardial fibroblasts could induce the loss of cardiomyocytes and alter gap junctions that favor the occurrence of AF through a non-uniform decrease in conduction velocities (305).

Local inflammation seems to be involved in the pathophysiology of both AF (305) and GORD (306). The high sensitivity C-reactive protein level is correlated with the incidence, defibrillation efficacy (307), recurrence (308) and prognosis of AF (309). Recurrent acid reflux also induces persistent low-grade inflammation and the secretion of inflammatory cytokines (310-312). It is speculated that local inflammation of the oesophagus may increase the risk of triggered atrial activity due to the close position of the oesophagus with the left atrium posterior wall (where are localized pulmonary veins) (313). Because inflammation seems to play a role in both AF and GORD, this pathophysiological mechanism may be a common link between these diseases.

In patients with ischemic heart disease the stimulation of lower oesophagus by gastric acid reflux may determine a significant reduction in coronary blood flow. This condition is called cardiooesophageal reflex (314). Another proposed mechanism is chronic atrial ischemia (315). It is well known that GORD is common in patients with coronary artery disease (316). It was observed that short-term treatment with proton pump inhibitor may reduce myocardial ischemia. A phenomenon known as "linked angina" could be due to the coronary perfusion decreasing by acid-derived cardio-oesophageal reflex (314). However, 51% of patients with noncardiac chest pain have GORD. Notably, chest pain identical with ischemic cardiac pain can be induced by GORD. As such, GORD can determine chest pain that resembles ischemic cardiac pain, without accompanying heartburn or regurgitation (271).

It seems that AF severity may decrease after GORD treatment (281-283). The successful treatment of hiatal hernia with a Nissen fundoplication may convert paroxysmal AF to a normal sinus rhythm (283). Epigastric pain, inflammation and the AF attacks either decreased in frequency or was completely eliminated after treatment with proton pump inhibitors. Treatment with proton pump inhibitors is successful in decreasing AF symptoms in 78% of cases with AF and GORD. In addition anti-arrhythmic treatment was discontinued in 28% of the patients. The therapy with proton pump inhibitors may decrease arrhythmia symptoms (confirmed by Holter monitoring) due to the gastric acid suppression (282). Patients with symptoms of GORD requiring proton pump inhibitors seem to have a higher arrhythmogenic risk of AF (281).

Studies regarding association between atrial fibrillation and gastro-oesophageal reflux disease

In the oldest study on 14 healthy volunteers was shown that oesophageal stimulation could modulate autonomous nervous system by amplifying vagal activity and decreasing sympathetic activity (317). In some patients with idiopathic supraventricular arrhythmias and GORD, neutralization of the gastric acid seems to improve reflux disease and related symptoms (318). In addition patients with lone AF, proton pump inhibitors seem to reduce not only GORD-related but also paroxysmal AF-related symptoms (319).

In a large retrospective study of more than 160,000 patients the relative risk of a diagnosis of AF was increased with 39% by the presence of GORD. This relationship remained even after correction of risk factors for AF (286). The authors of this study have used a database containing all health care encounters for patients who received ambulatory care. In a multicenter survey with 188 consecutive subjects designed for GORD screening using a scale for GORD symptoms, AF alone was showed significant correlation with GORD (320).

GORD was independently correlated with a high risk of AF in the largest prospective epidemiological study of one million patients (276). The diagnosis of GORD was made using the ICD-9 codes from a database. Therefore GORD or AF prevalence could be underestimated (due to asymptomatic patients).

Only one study, which was based on a self-report questionnaire and included more than 5,000 patients, concluded that GORD did not involve higher risk for AF after exclusion of other risk factors. However, this study did find that patients with more frequent GORD had a slightly higher AF risk (285). In this study, oesophagitis increased the risk of AF, but this

relation was not maintained when controlling for other risk factors. The authors concluded that no association was found between GORD and AF and also that this association requires further studies. Unfortunately, this study did not use an objective method like endoscopy to diagnose GORD. It is well known that GORD could be asymptomatic.

Atrial fibrillation and gastro-oesophageal reflux disease: the cardiologist perspective

Atrial fibrillation is associated with an increasing morbidity and mortality that continue to remain unacceptably high despite all efforts aimed at improving its management. Therefore the etiology of AF was placed in the foreground for the first time by the Third Consensus Conference of the Atrial Fibrillation Competence Network/European Heart Rhythm Association (321).

In a large majority of patients with AF we can find pathologies like hypertension, obesity or diabetes mellitus as substrate for left atrial remodeling. Most common substrates for both AF and GERD are obesity and aging. In addition, both AF and GERD are associated with other pathologies like sleep apnea or diabetes mellitus. It seems that among traditional cardiovascular risk factors, GORD could be an independent risk factor for AF. Also, we think that AF should be considered as possible extraoesophageal syndrome in the GORD classification (271).

Sympatho-vagal imbalance is one of the principal mechanisms of AF associated with GORD (289). Although both autonomic nervous system components play a role in AF, the cholinergic component seems to be more important for spontaneous initiation of AF. Majority of AF patients with GORD have triggered AF. During radiofrequency ablation, these patients may have positive vagal response (305). Gastro-oesophageal reflux could be only a trigger for AF in paroxysmal AF. Probably in these patients the therapy with proton pump inhibitors decreases arrhythmia symptoms (proved by Holter monitoring). Less known, not only GORD may trigger AF, but also AF may determine the occurrence of GORD.

For cardiologists and especially electrophysiologists the relationship between esophagus and left atrium have a different significance because it seems that GORD is more frequent after AF ablation. This complication of radiofrequency ablation was first described in 2001 (322). After that more and more oesophageal injury has been reported with delivery of radio-frequency lesions at the left atrium posterior wall in catheter ablation procedures for AF (323-327).

Erythema of the oesophagus seems to be a common finding in patients undergoing pulmonary vein antrum isolation procedures, with important clinical relevance. In addition it seems to be a correlation between reflux-like symptoms and oesophageal lesions (328). Using endosonography peri-oesophageal injury was detected in 27% of patients undergoing pulmonary vein isolation (329). Oesophageal thermal lession during catheter ablation for AF could be minimized by oesophageal temperature monitoring; multiple factors such as patient characteristics and specific strategies for radiofrequency energy delivery also merit consideration (328). Initiating proton pomp inhibitors in these patients might facilitate recovery of oesophageal wall injuries produced during radiofrequency catheter ablation (330). However, this complication of AF ablation has another type of pathogenic mechanism. Most important in this relationship is the proximity of oesophagus with posterior wall of the left atrium.

The published review highlighted the fact that cardiovascular involvement in GORD is less assessed. For this reason is mandatory to extend the research in this field for better understanding the relationship of AF with GORD. Gastro-oesophageal reflux disease seems to be independently associated with increased risk of developing atrial fibrillation. Even this relationship is still controversial the clinicians should be aware of this possible association. Identification and appropriate treatment of gastro-oesophageal reflux in patients, particularly in those with lone AF, may decrease the use of anti-arrhythmic agents, which often have complex side effects and the potential for producing pro- arrhythmic effects.

I.2.6. Research regarding the inflammation, arrhythmias and volumetric assessment methods

A. Background

The reservoir function of the left atrium – LA is defined as the LA filling during systole of the left ventricle and is regulated by atrial compliance. In patients with AF, mechanical remodeling of the LA consists in atrial contractility decreasing and atrial compliance increasing, which leads to LA enlargement. Radiofrequency ablation is an important interventional procedure for the restoration of normal sinus rhythms in AF patients (331).

Morphologic changes in AF, a frequently encountered arrhythmia that carries the risk of cerebral infarction and increases cardiovascular mortality, have been associated with LA size. It is not clear yet if AF results in atrial dilation or if LA enlargement causes AF (332). Measured by M-mode echocardiography, LA enlargement is associated with cardiovascular disease and is a risk factor not only for AF, but also for stroke, and even death (333). A widerange of LA sizes has been also observed in patients with dilated cardiomyopathy (334).

In the past decades, echocardiographic methods and techniques have improved and increased significantly. Due to the introduction of higher frequency transducers, harmonic imaging, fully digital machines, left-sided contrast agents, and other technological advancements, the progress in image quality has been considerable. Standardization of measurements in echocardiography has been inconsistent and less successful, compared to other imaging techniques and consequently, echocardiographic measurements are sometimes perceived as less reliable (335).

B. Published paper in this field

Assessment of volume in relation with LA shape alteration before and after pulmonary veins PV isolation, was the purpose of a study published in *Journal of Interventional Cardiac Electrophysiology*, an international journal regarding studies related with cardiac arrhythmias and rhythm management.

Floria M, Blommaert D, Lacrosse M, Ambarus V, Dormal F, Dabiri Abkenari L, Jamart J, **Rezuş C**, Cozma D, De Roy L. Assessment of left atrial shape and volume in structural remodeling secondary to atrial fibrillation. *J Interv Card Electrophysiol*. 2009; 25 (3): 167-170.

Introduction

In persistent and paroxysmal AF-LA geometry and shape could change and a single linear dimension such as antero-posterior diameter may not be representative of LA size. (332–334). Thus the measured LA size may be misleading and LA volume determination should be used in both clinical practice and research (335).

Routine echocardiography can not adequately assess the largest LA dimensions (336) and therefore the ellipsoid formula is known to underestimate LA volume (337). Magnetic resonance imaging and CT could be more adequate methods for atrial size evaluation.

Progressive LA dilation is associated with asymmetrical structural remodeling and shape changes. LA dilatation may thus modify the ellipsoidal shape into a more trapezoidal one because of atrialization of pulmonary veins in AF patients (338). Therefore, the assessment of LA volume taking into account LA shape in structurally remodeled atria in AF patients could avoid underestimation with the ellipsoid formula by echocardiography or overestimation by standard CT measurements. A comparison of LA volume obtained by echocardiography and CT, in relation with shape alteration in structural remodeling has not yet been performed.

Material and methods

Forty patients with paroxysmal AF underwent radiofrequency ablation for isolation of pulmonary veins. The ablation procedure consisted of endocardial ostial isolation of each pulmonary vein antrum by radiofrequency energy using a Lasso™ catheter (Biosense Webster, CA, USA) and an irrigated tip Celsius™ Thermo Cool (Biosense Webster, CA, USA).

We analyzed the influence of LA shape in volume assessment by means of two different imaging methods, echocardiography and CT scan. We evaluated the evolution of LA volume using the ellipsoid formula applied both to conventional echocardiography (E_{EL}) and computed tomography (CT_{EL}). We also performed measurements on CT using the truncated cone formula (CT_{TR}) and either ellipsoid or the truncated cone formula according to LA shape ($CT_{TR/EL}$). LA shape was defined as trapezoidal if basal dimension, at the level of the atrium-pulmonary vein junction, was greater than the mitral annular dimension. In the opposite situation it was considered as a classical ellipsoidal shape. LA volume was calculated in the 40 enrolled patients with AF, before and 3 months after ostial endocardial pulmonary vein isolation. We thus performed a total of 80 measurements with each method: E_{EL}, CT_{EL}, CT_{TR} and $CT_{TR/EL}$.

Echocardiographic evaluation of left atrial volume

LA volume measurements were done using two-dimensional (2D) echocardiography with an IE33 machine (IE33 Echocardiography System, Philips Healthcare, The Netherlands).

Considering the LA as a prolate ellipse we applied the biplane dimension-length formula:

$$\text{LA volume} = 4\pi/3 \cdot (d/2) \cdot (l/2) \cdot (t/2) \quad (1)$$

The two orthogonal diameters (t=medial-lateral and l=superior-inferior) were measured in the apical four chamber view and the antero-posterior diameter (d) in the parasternal long axis. All echocardiographic measurements were performed at end-ventricular systole, at maximal LA size. We optimized each view in order to avoid: an underestimation of LA volume by foreshortening of the major length of the LA, inaccurate assumption of the mitral annulus boundary, loss of lateral resolution of the LA wall in the apical view or dropout of the interatrial septum or anterior wall. All measurements by echocardiography (E_{EL}) were performed by the same operator, before and at 3 months after ostial endocardial AF ablation.

Computer-tomographic evaluation of left atrial volume

All patients underwent cardiac contrast enhanced CT (Siemens Somatom Senzation 64-Slice Configuration, Siemens AG, Germany) for evaluation of the morphology of the left atrium, less than 24 h prior to ablation and at ± 3 months after this procedure. We applied the ellipsoid formula to CT and echocardiography regardless of left atrial shape. When taking into account LA geometrical changes we used either the ellipsoid or the truncated cone formula ($CT_{TR/EL}$).

For the ellipsoidal shape, the two orthogonal diameters were measured on the greatest LA section, after we obtained an optimal contour in transverse axis by tracing the endocardial border cavity (figure I.2.8). The third dimension was calculated by the number of LA slices. Each of these CT slices was performed at 1 mm. The truncated cone formula was as follows:

$$\text{LA volume} = \pi.\text{Lal} \cdot (\text{LAa}^2+\text{LAb}^2+\text{LAa.LAb})/12 \quad (2)$$

with the LAb dimension measured at the base of the atrium and the LAa at the mitral annular level (figure I.2.9), on the greatest LA area (at end-ventricular systole), in transverse section views. Likewise, LAl was measured as the longitudinal dimension.

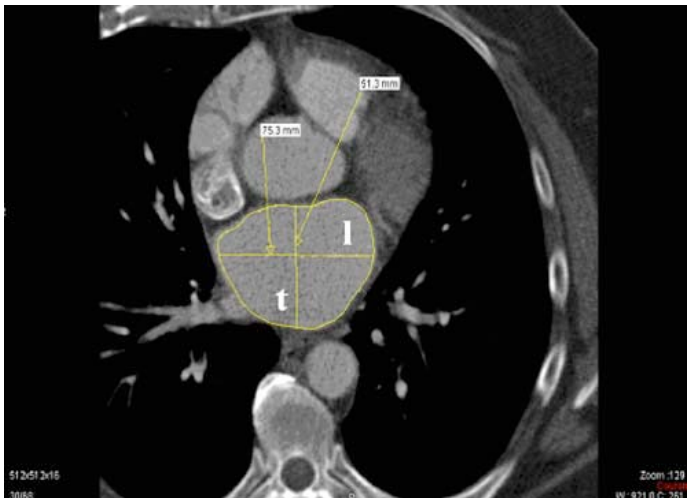

Figure I.2.8. An ellipsoidal shape on computer-tomography (transverse axis); the two orthogonal diameters are marked (t=transverse, l=longitudinal)

We performed the measurements avoiding an overestimation of LA volume on CT, by the following: the inclusion of part of LA appendage volume, the incorporation of the pulmonary veins or overlap and the duplicate measurements of the multiple sequence scans. All measurements on computer-tomography (CTEL, CTTR and CTTR/EL) were performed by the same operator, before and at 3 months after antral endocardial AF ablation.

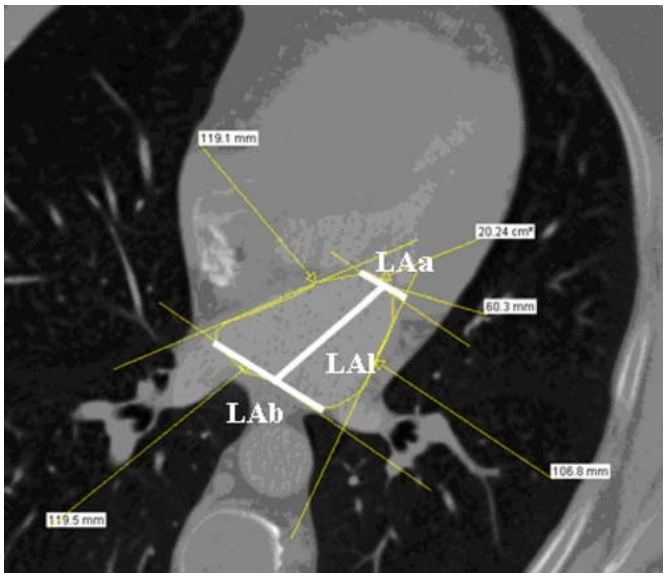

Figure I.2.9. Computer-tomography image in transverse axis displays the trapezoidal shape of the left atrium (LAa=annular dimension, LAb=basal dimension, LAl=longitudinal dimension)

Statistical analysis

Data are presented as frequency distributions and simple percentages. Continuous variables are expressed as mean ± standard deviation. Statistical analysis was performed using SPSS 10 for Windows (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered significant. We calculated the Pearson's correlation coefficient between the echocardiographic method and the computer-tomography methods and afterwards we compared the correlation coefficients.

Results

Forty patients were consecutively included in this study. Mean age was 56±11 years and 77.5% were men. Mean LA volume of the measurements for E_{EL}, CT_{EL} and CT_{TR} was 53 ml, 71 ml, and 65 ml, respectively. In order to assess reverse LA remodeling we compared mean LA volume by the ellipsoid method (E_{EL} and CT_{EL}), before and 3 months after ablation: 57.9±24.7 ml vs. 50.8±20.8 ml and 67.33±29.37 vs. 60.01±21.42 ml, respectively ($p<0.001$). Seventy seven and a half percent of patients were in stable sinus rhythm. There was no significant reverse remodeling in patients (n=9) who continued to have AF at 3 months after the procedure, 60.7±21.3 ml vs 58.2±19.7 ml, $p>0.05$ and 73.2±20.2 ml vs. 71.5±17.7 ml, $p>0.05$ respectively for E_{EL} and CT_{EL}.

All mean LA volumes, irrespective of imaging method or formula were >50 ml, probably due to AF structural remodeling. The actual shape of the LA was evaluated on CT scan and showed a trapezoidal shape in 61 out of 80 measurements (76%). A significant

reversal of trapezoidal shape, found in 37 out of 40 pts (92.5%) before ablation compared to 24 out of 40 (60%) after ablation was observed at 3 months after the procedure.

Irrespective of the LA formula, each measure of LA volume by computer-tomography (CT_{EL}, CT_{TR} and $CT_{TR/EL}$) was significantly correlated with EEL ($p<0.001$), tabel I.2.1. However the correlation coefficient between EEL-CTTR/EL, before ablation, was stronger than the correlation coefficient between EEL-CTTR ($p<0.001$). At 3 months after ablation and reverse remodeling, with 77.5% of patients in stable sinus rhythm, we found nearly the same correlation coefficients ($p<0.001$).

Tabel I.2.1. The correlation coefficients between E_{EL} and CT methods

	CT methods	E_{EL}
Before ablation	**CT_{EL}**	**0.779***
	CT_{TR}	**0.614***
	$CT_{TR/EL}$	**0.837***
After ablation	**CT_{EL}**	**0.753***
	CT_{TR}	**0.603***
	$CT_{TR/EL}$	**0.608***

Discussions

Left atrial enlargement is an important predictor of cardiovascular events like atrial fibrillation, stroke, heart failure and mortality. In clinical practice are used a significant number of methods of left atrial size assessment by echocardiography, from the simple antero-posterior diameter in the parasternal long axis view to the more complex ellipsoid, area-length and Simpson's method of estimating left atrial volume. Real-time three-dimensional (3D) echocardiography is also used in clinical practice to assess LA volume. The 2D and 3D measurement of LA volume are sometimes compared with reference standards such as magnetic resonance imaging (MRI) and CT (339).

Mathematically, LA volume can be calculated from the antero-posterior, supero-inferior, and latero-lateral LA diameters. After a relevant monitoring over 20 months, Casio et al. observed that in patients without AF recurrence, appendage size decreased and the opening of the appendage to the LA became more eccentric. Also, they estimated a decreased LA volume, that did not return to normal. On the other hand, in patients with AF recurrence, LA size and appendage size increased and unfortunately, the opening of the appendage became less eccentric (336).

The aim of a similar study, published by Rodevan et al. was to establish the accuracy and reproducibility of left atrial volume measurements by three-dimensional (3D) echocardiography compared to 2D biplane and monoplane measurements. Left atrial volumes of 18 unselected cardiac patients were achieved using magnetic resonance imaging (MRI). The measured volumes were compared with those obtained with different echocardiographic methods, such as a multiplane 3D method based on 90 images acquired by apical probe rotation, a simplified 3D method using only the three standard apical views, and 2D biplane and monoplane methods. The main results have proved that the echocardiographic methods significantly underestimated maximum left atrial volumes as obtained by MRI by 14-37%. The authors also assessed the accuracy, defined as 1 SD of individual estimates around this

systematic underestimation, was 25 to 27% for all methods, except for the 2D 2-chamber monoplane method (37%). As a final remark, all echocardiographic methods significantly underestimated left atrial volumes as obtained by MRI.

It has been shown that the largest LA surface in AF patients is frequently situated just under the upper PVs (340). In AF patients, LA dilation is a marker of LA structural remodeling, and is associated with dilation of the pulmonary vein antrum. This results in a larger LA surface that changes LA shape into a more trapezoidal one. Because LA volume calculated by the CT truncated cone formula correlates significantly with each of the other ellipsoidal methods, it seems appropriate for assessing remodeled LA volume. The better correlation of EEL with CTTR/EL than with CTEL, before ablation, could be explained by the geometrical changes which occur in structurally remodeled atria. EEL was not best correlated with CTTR probably because not all LA follow the same degree of dilatation and shape change. In our study, in patients without AF recurrences, there was a significant reverse LA remodeling at a mean of 3 months after ostial endocardial pulmonary vein isolation. Perhaps the LA returns to a more ellipsoidal shape after AF ablation, in stable sinus rhythm and after reverse remodeling. This could be an explanation for the better correlation between EEL and CTEL than with CTTR or CTTR/EL at 3 months after AF ablation. There was no significant reverse remodeling in patients (n= 9) who continued to have AF at 3 months after the procedure. LA shape thus seems important in LA volume assessment for precise evaluation of LA volume in the context of AF ablation.

In practice, despite a frequent trapezoidal shape modification, echocardiography with the ellipsoid biplane dimensionlength formula remains an adequate method for assessment of LA volume, although implying a certain degree of volume underestimation. This method can still be used because it is less costly, avoids irradiation exposure and is easier to use in daily practice.

A study published by Kishima et al (341) analyzed the hypothesis that AF leads to a morphologic change in the left atrial appendage – LAA and investigated the characteristics of LAA morphology in patients with and without AF. The left atrial appendage – LAA is derived from the left wall of the primary atrium, which is formed during the embryonic development. Compared with LA proper, it has different development, ultrastructure and physiological characteristics. The LAA is located within the confines of the pericardium in close relation to the free wall of the left ventricle. Due to that localization, its emptying and filling may be significantly affected by left ventricular function. Patients with atrial fibrillation and mitral valve disease have a predilection to form throumbus in the LAA (342).

The study involved 225 patients (persistent AF [PeAF], n = 76; paroxysmal AF [PAF], n = 70; control, n = 79]) who underwent echocardiography and CT. The patients were categorized in two groups (chicken wing [CW] or non-chicken wing [non-CW]) using CT. The first results of the study showed that the prevalence of non-CW-type LAA was 39.5%, 15.7%, and 8.9% in the PeAF, PAF, and control groups, respectively. Also, PeAF patients have a higher prevalence of non-CW-type LAA than those in the PAF group and control group (P = .0014 and P <.0001). Moreover, all patients were divided into 5 groups based on the type of cardiac rhythm (PeAF, PAF, or sinus rhythm) and left atrial volume index (cutoff value; 34 mL/m(2)): group A (Control), group B (PAF/Small-LA), group C (PAF/Large-LA), group D (PeAF/Small-LA), group E (PeAF/Large-LA). The prevalence of non-CW-type LAA

was 9%, 14%, 17%, 29%, and 41% in groups A, B, C, D, and E, respectively. Presence of persistent AF was associated with a higher prevalence of non-chicken wing-type LAA. The conclusion of the study was that remodeling in patients with persistent AF can lead to a change in LAA morphology (341).

The well-known overestimation by computer-tomography and underestimation by echocardiography using the ellipsoid formula for the assessment of left atrial volume could be, at least partly, explained by changes in the geometry of remodeled atria. In atrial fibrillation, left atrial structural remodeling and left atrial shape are related. Therefore, for precise assessment of left atrial dilatation in atrial fibrillation patients, a trapezoidal left atrial shape would probably be more appropriate. However due to an acceptable correlation between methods, echocardiographic ellipsoid formula could still be used in clinical practice because it still remains the easiest available tool. Changes in LA shape and morphology after ablation may predict how reverse remodeling might correlate with a better outcome after AF ablation.

I.3. RESEARCHES REGARDING INFLAMMATION AND THE ROLE OF OXIDATIVE STRESS AND INTERLEUKINS IN CARDIOVASCULAR DISEASES

I.3.1. Hallmarks

Nitric oxide (NO) is a signal molecule that plays a key role in the pathogenesis of inflammation, exhibiting an antiinflammatory effect under normal physiological conditions (343). It is also considered to be a pro-inflammatory mediator, which induces inflammation due to its over-production under pathological conditions and is a strong neurotransmitter in neuronal synapses and contributes to the regulation of apoptosis (344). Moreover, NO is involved in the pathogenesis of inflammatory disorders of the joint, intestine and lungs (345-347). Therefore, the use of NO inhibitors represents an important therapeutic progress in the management of inflammatory diseases.

Aging is an inevitable process in the human body that is associated with a multitude of systemic and localized changes. All these conditions have a common pathogenic mechanism characterized by the presence of a low-grade proinflammatory status. Inflammaging refers to all the processes that contribute to the occurrence of various diseases associated with aging such as frailty, atherosclerosis, Alzheimer's disease, sarcopenia, type 2 diabetes, or osteoarthritis. Inflammaging is a systemic, chronic, and asymptomatic process.

I.3.2. Researches regarding inflammation and nitric oxide effects

A. Background

NO is part of the radical entities known as reactive oxygen species (ROS). It contains one nitrogen atom which is covalently bonded to an oxygen atom with one unpaired electron. Concerning the fact that NO reacts with oxygen and heme-iron containing groups which reduce NO to more stable nitrate compounds, the bioavailability of this ROS in certain tissues

is very low and the biological actions are limited temporally and spatially close to its site of synthesis. Furthermore, NO is lipid soluble, meaning that it is highly membrane permeant (348).

NO is a versatile molecule that has key roles in a variety of biological processes including immune defenses, inflammation and neurotransmission (348). More precisely, even if NO plays a positive role in many cellular process, excessive NO can damage cells and organs and also it can interact with the intermediate components of ROS, which further elevate oxidative stresses and provoke inflammation (345).

B. Published paper in this field

Starting from the fact that the use of NO inhibitors represents an important therapeutic progress in the management of inflammatory diseases I have published an *in vivo* research study related with this area of interest.

Bucă BR, Mititelu-Tarțău L, **Rezuș C**, Filip C, Pinzariu AC, Rezuș E, Popa GE, Panainte C, Lupușoru CE, Bogdan M, Pavel L, Lupușoru RV. The effects of two nitric oxide donors in acute inflammation in rats. *Rev. Chem. (Buchares)* 2018, 69 (10): 2899-2903.

Introduction

NO is synthesized by many cell types that participate in the immune processes and inflammation (348). The main enzyme involved is the inductive isoform nitric oxide synthase type 2 (NOS-2), which produces a sustained NO synthesis (349, 350). The expression NOS-1 and NOS-3 is constitutive, calcium/calmodulin-dependent and generates NO in the picomolar-nanomolar range. NOS-2 in macrophages is induced by the stimulatory action of tumor necrosis factor alpha (TNFα), interleukin-1 (IL-1), interferon gamma (IFNα), endotoxin or lipopolysaccharide (LPS), and generates large amounts of NO in the micromolar range for a prolonged period. The level of NOS2 may reflect the state of inflammation (345). However, the role of NO in non-specific and specific immunity in vivo, in immunologically mediated diseases and in inflammations is poorly understood. NO does not act via a receptor, the specificity of the target cell depends on its concentration, chemical reactivity, proximity to target cells and the way target cells are programmed to respond (348).

The aim of this study was to evaluate the effects of two nitric oxide donors in experimental-induced acute paw inflammation in rats.

Material and methods

Adult male Wistar rats (weighting 200-250 g), from our University bio-base, were used in the research. The following drugs were used: nebivolol, S-nitroso-glutathione (Sigma Chemical Aldrich Co.), which were dissolved in saline, and the solutions were extemporaneously prepared. The animals were randomly distributed in 4 groups of 5 rats each, treated intraperitoneally as follows:

- Group 1 (coded SS): saline solution 0.1mL/100 g body weight control;
- Group 2 (coded IND): 150 mg/kg body weight indomethacin;

- Group 3 (coded NEB): 1 mg/kg body weight nebivolol;
- Group 4 (coded GSNO): 1 mg/kg body weight S-nitroso-glutathione.

The effects of nitric oxide donors were investigated using the experimental model of acute hind paw inflammation, induced after intraplantar injection of carrageenan in rats. The subcutaneous administration of 0.2 mL 1% carrageenan (CA) was accompanied by swelling, with a maximum intensity after 3-5 h and maintained for about 24 hours after the irritant agent administration. Indomethacin was used as a positive control drug in the experiment, having known anti-inflammatory effects in various acute and subacute inflammatory model in rodents (351, 352). The degree of local inflammatory edema and its duration was assessed by using a plethysmograph (PanLab Apparatus). We measured the posterior paw volume according to the following scheme: before the induction of edema (initial volume at moment zero), at 24 h and 3 days after the inflammation was developed (353, 354).

The results were expressed as percentage of reduction in inflammation, compared to initial volume in control animals. The level of edema evolution was calculated by determining the percentage of rat paw volume increase (%PVI) using the following formula:

%PVI = (determined paw volume - initial volume) x 100 / initial volume

The anti-inflammatory activity was evaluated by calculating the percent inhibition of paw edema (%PIE) according to the equation:

%PIE = (%PVI control -%CVL treated) x 100 / %CVL control

The influence of nitric oxide donors on blood parameters, specific inflammatory and immune markers was evaluated prior to the induction of inflammation, at 24 hours and 72 h in the experiment. To assess the blood count, 2 mL of venous blood were taken from the retroorbital plexus of the animals, under general anesthesia with enflurane. The HEMAVET 950, an automatic analyzer, operating on the principle of fluorescence flow cytometry, was used for hematological investigations. The evaluation of the complement fractions C3 and C4 activity was based by Hartmann-Brecy technique (consisting of hemolysis with serum complement of sensitized erythrocytes). The blood levels of interleukin IL-6 and tumor necrosis factor alpha (TNFα) were also assessed.

Results

We assessed the effects of two nitric oxide donors, NEB and GSNO on the acute inflammatory process in rats with experimental-induced paw edema after local administration of carrageenan. Subcutaneous injection of 1% carrageenan solution on the plantar surface of the hind paw induced an acute inflammatory reaction associated with visible changes such as enlargement, redness and local pain, suggested by animals licking and biting the paw. The inflammatory process was developed and progressed, reaching the highest intensity after 6 hours, thereafter gradually slowed over time, maintaining clear manifestations for about 72 hours after chemical irritation of the paw.

During the experiment the gradual increase of the polymorphonuclear neutrophils (PMN) percent and the progressive reduction of the percentage of Ly was revealed in control group. The treatment with IND, NEB and GSNO induced a continuous increase in the percentage of PMN and a decrease in the percentage of lymphocytes (Ly) in carrageenan-induced paw

inflammation test in rats (table I.3.1).

Tabel I.3.1. The effects of NEB and GSNO on the leukocyte formula elements in the carrageenan induced paw inflammation in rats

		PMN	LY	E	M	B
SS	M0	17.62 ± 1.70	72.15 ± 1.47	2.40 ± 0.27	4.84 ± 1.02	0.10 ± 0.07
	24 h	32.64 ± 2.36	63.76 ± 3.40	1.32 ± 0.27	2.42 ± 0.41	0.00 ± 0.00
	72 h	33.70 ± 1.85	61.92 ± 1.87	1.20 ± 0.16	3.20 ± 0.54	0.00 ± 0.00
IND	M0	13.40 ± 1.30*	78.20 ± 0.20*	1.80 ± 0.80	6.55 ± 0.65	0.05 ± 0.05
	24 h	28.40 ± 3.10*	61.20 ± 4.50*	4.70 ± 0.60*	5.30 ± 0.80*	0.00 ± 0.00
	72 h	33.55 ± 6.25	61.05 ± 5.15*	2.20 ± 0.50	3.10 ± 1.60	0.10 ± 0.00
NEB	M0	17.20 ± 2.50	75.30 ± 2.10	1.45 ± 0.65	6.05 ± 1.05	0.00 ± 0.00
	24 h	28.60 ± 5.20	61.70 ± 3.00*	5.00 ± 2.30*	4.60 ± 0.10*	0.10 ± 0.00
	72 h	28.70 ± 0.10	64.25 ± 0.05*	2.05 ± 0.55	4.95 ± 0.75*	0.05 ± 0.05
GSNO	M0	17.10 ± 1.90	75.60 ± 3.00	1.00 ± 0.40*	6.25 ± 0.65*	0.05 ± 0.05
	24 h	23.40 ± 0.90	68.05 ± 0.25*	1.75 ± 0.05	6.75 ± 1.15*	0.05 ± 0.05
	72 h	29.70 ± 1.60*	63.90 ± 1.40*	1.75 ± 0.45	4.55 ± 0.65*	0.10 ± 0.00

The treatment with IND and with NEB resulted in an increase of PMN, eosinophils (E) and monocytes (M) percent, and a decrease in the percentage of Ly, statistically significant (*p<0.05) compared to control group at 24 h. The percentage of PMN decreased and the percentage of Ly increased, statistically significant compared to SS group after 72 h. Intraperitoneal injection of GSNO was associated by a decrease in PMN percent (*p<0.05) and an increase in Ly and M percent (*p<0.05), statistically significant compared to control at 24 and 72 h in the experiment (table I.3.1). No significant variation of the reactive C protein values between IND, NEB, GSNO and control groups at 24 hours and 72 h was noted.

Discussions

Carrageenan (CA) - induced edema is considered a highly reproducible model of acute inflammation. Annamalai et al. investigated the local and systemic expression profiles of various inflammatory cytokines following the subplantar injection of CA in male Wistar rats, the same type and the same gender being in our study. Using an antibody array, serum and paw tissue were examined for the level of 19 specific inflammatory cytokines. Moreover, enzyme-linked immunosorbent assay (ELISA) was use in order to quatify the CA-elicited level of key inflammatory cytokines, cytokine-induced neutrophil chemoattractant (CINC)-2, CINC-3, interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α. Edema was peaked 3 h postinjection of CA in hind paw. Among the specific cytokines studied, CA significantly ($p < 0.05$) elicited in paw tissue, the levels of CINC-2, CINC-3, IL-1β, IL-6, βNGF, TNF-α, and VEGF and in serum that of CINC-2 and CINC-3. Another important observation was related to the levels of CINC-2, CINC-3, IL-1β, IL-6, and TNF-α in tissue and CINC-2 and CINC-3 in serum, upregulated in CA treated rats when compared to control, quantified by ELISA. The data from this study corroborates the distinct pattern of inflammatory cytokines involved during CA-induced acute inflammation and also provide new evidence on elevated expression of rat CXC chemokines (355).

A rat model of carrageenan-induced paw edema has been also used by Abd-Allah et al. in a study concerning the mast cells and pro-inflammatory cytokines roles in assessment of

grape seeds extract anti-inflammatory activity. The researchers used grape seeds extract alone or in combination with IND, the drug used also in our study, orally administrated for 10 days prior or as a single dose after edema induction in rat's left hind paw by sub-plantar single injection of carrageenan to assess the prophylactic and therapeutic anti-inflammatory activities of both through the estimation of selective inflammatory mediators and oxidative damage-related biomarkers as well as tissue mast cell scoring. The results indicated a marked reduction on the inflammatory mediators, edema volume and oxidative by products in edema bearing rats' prophylactic and treated with grape seeds extract and IND. It was remarked that IND may induce some toxicological impacts which minimized when administered together with GSE, meaning that GSE is a safe antioxidant agent with anti-inflammatory property (356).

Due to its particular properties, which allow it to be very soluble and diffuse easily through biological membranes, nitric oxide exerts important actions influencing multiple intracellular processes (357). Being an intercellular signaling molecule, it plays a determining role in the immune system, also contributing to the generation of the peroxinitrites, free oxygen radicals, which have cytotoxic effects, causing tissue damage and apoptosis (358, 359). NO exerts a functional role in different pathological processes in the body, such as: leukocyte adhesion, transmigration, proliferation, expression of cytokines. The generation of NO in inflammatory states and during the infective processes is due to inducible NOS intervention (348).

The effects of nitric oxide donors NEB and GSNO on experimentally induced paw inflammation were objected by the significant decrease in local edema, as well as by the influence exerted on blood elements, and on the specific serum inflammatory and immune markers. The use of NEB and GSNO induced substantial anti-inflammatory effects especially at 24 h in this model of experimental-induced hind paw inflammation. Nebivolol exhibited more pronounced anti-inflammatory effects than GSNO, but less intense than indomethacin at certain times during the experiment.

Our research study highlighted the fact that treatment with nebivolol and S-nitroso-glutathione produced anti-inflammatory effects on local acute inflammation in the carrageenan-induced paw edema experimental model in rats.

I.3.3. Researches regarding inflammation and enzymatic systems in hepatic dysfunction

A. Background

The liver is the center of some complex biochemical processes, the hepatic functional tests showing the particularity of the non-invasive evaluation of patients (in the daily practice there are numerous cases in which the patients refuse invasive explorations, sometimes in situations with vital risk) (360-363).

The prognosis in patients with cirrhosis of the liver is hard to anticipate but it usually depends on the frequency and significance of the complications. Chronic inflammation and activation of the hepatic stellate cells (HSC), programmed to induce fibrogenesis and

angiogenesis, represent the first step in the development of cirrhosis. Alcohol, virus and even smoking, recently hypothesized to be a cofactor, can cause the liver injury that lead to necroinflammation. All these factors may lead to vascular changes within the liver lobule with endothelial dysfunction and deposition of extracellular matrix (ECM), the results being the disappearance of the fenestrations of the hepatic sinusoids. The progressive fibrosis will lead to qualitative and quantitative changes of the ECM distribution in liver. Also, an excessive accumulation of fibrotic tissue and an overall change in liver structure will result. The endothelial dysfunction is most likely related to insufficient release of vasodilatators, such as NO (364).

B. Published paper in this field

Based on the fact that there exist a direct interaction between heart and liver function correlated with a wide range of acute and chronic diseases affecting both the heart and the liver, I have published a study focused on the analysis of enzymatic systems in the severe hepatic dysfunctions.

Dima N, Rezus E, Singeap AM, Trifan A, **Rezuș C**. Analysis of enzymatic systems in the severe hepatic dysfunction. *Rev. Chim. (Bucharest)* 2016; 67 (5): 948-952.

Introduction

The hepatic enzymatic screening was performed by determining the values of the hepatic transaminases, ALAT (alaninaminotransferasis) and ASAT (asparatamino-transferasis) – enzymes which catalyze the reversible reaction of transfer of the amino group of aminoacids to α- cetoacids, determination of the alkaline phosphatases, lactate dehydrogenase, and dosing the alcohol consume markers (gamma-glutamyl transpeptidase, erythrocyte medium volume and high-density lipoprotein cholesterol – HDLc (365). Standard testes in compliance with the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) have been used (366).

Knowing the localization of the enzymes at the level of different cellular organelles is very important, both in understanding the metabolic processes, as well as for interpreting the laboratory results. The enzymatic equipment is different from one organ to another (367).

Enzymatic systems involved in the severe hepatic dysfunction

Alaninaminotransferasis (ALAT), an enzyme with predominantly hepatic distribution, is localized only on cytoplasmic level (unilocular) catalyzing the transamination from alanine to α-cetoglutaric acid, with the generation of the pyruvic acid and the glutamic acid (glutamic - pyruvic transamination) (365, 367).

Asparataminotransferasis (ASAT), localized on a cytoplasmic and mitochondrial level (bilocular), catalyzes the transamination reaction from the aspartic acid to the α-cetoglutaric acid by forming oxaloacetic acid and glutamic acid (365, 367), as glutamic-oxaloacetic transamination ecuations, represented in figure I.3.1.

The serum concentration of these lesional enzymes varies depending on several factors:

the number of hepatic cells affected the severity of damage on each hepatocyte, damage speed, speed of elimination from the serum (t 1/2) of the respective enzymes. Associated to enzymatic individual growths variations of the ratio between them interfere. Thusly, the Ritis rapport (ASAT/ALAT) is modified depending on the etiology of the cirrhosis of the liver. Gamma-glutamyl transpeptidase (GGT), ubiquitous enzyme, insures the trans-membrane transport of amine acids and peptides. At a cellular level, it is predominantly situated in the membrane, its distribution being specific at the level of the same locus where alkaline phosphatase is also present. The serum activity increase of GGT is parallel to the quantity and duration of the alcohol abuse. This enzymatic behaviour allows the differentiation between heavy drinkers and occasional drinkers. The high serum values of the enzyme plead for the association with chronic hepatic suffering. With a broadly appreciated specificity, 50-100%, and a sensitivity between 25-65%, GGT can be considered as an enzyme which susceptibly and specifically reacts both during the ingestion of small quantities of alcohol and in chronic alcoholism, having a marker role (368).

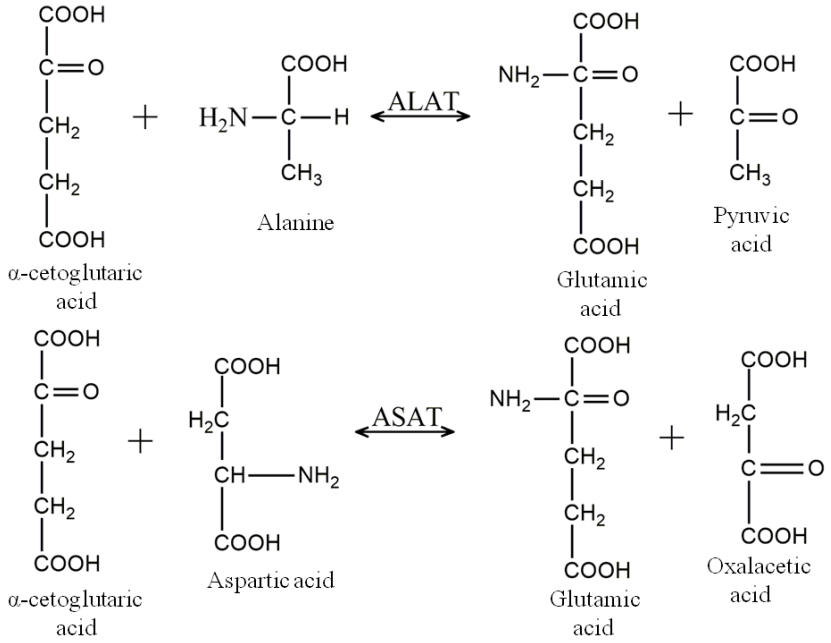

Figure I.3.1. Glutamic-oxaloacetic transamination ecuations

The determination of GGT activity is performed using L-α-glutamyl-3-carboxy-4-nitroaniline (367), as underlayer enzymatic mechanism of gamma-glutamyl trans-peptidase (figure I.3.2). Alkaline phosphatase, enzyme included in the intermediary metabolism enzyme family, is an esterase which acts at alkaline *p*H (*p*H = 8.6), performing the hydrolysis of phosphate esters (365, 367), as enzyme mechanism of alkaline phosphatase (figure I.3.3). High density lipoprotein cholesterol (HDLc) transports excess cholesterol from different tissues in the liver. Under the influence of even small doses of alcohol, through an enzymatic induction process, this lipoprotein increases in the bloodstream, being generated at a hepatic level. Cirrhosis of the liver leads to a decrease in HDLc levels (369).

The metabolic consequences of alcohol consumption have cascading effects, reflecting the nature of the molecule and its degradation. It is small and easily miscible in lipids and

water, thusly being rapidly distributed in the tissues. Its metabolization is compulsory, as another excretion method does not exist (with the exception of small quantities eliminated through respiration and urine). Also, it is not subjected to feedback control. The degradation happens especially at a hepatocyte level. In the same manner as fuel, alcohol supplies 29.5 kJ/6 to 7kcal/g, but the process is dependent on oxygen, resulting in the undesirable production of hydrogen ions. During the metabolic process from alcohol to acetaldehyde and further from acetaldehyde to acetate, the nicotinamide adenine dinucleotide (NAD) acts as a hydrogen acceptor and the resulted NADH+ alters the redox state of the cell, with multiple metabolic effects (370, 371). Some of the effects of alcohol on the hepatocyte depend on dose and type of ingestion, acute or chronic. Alcohol degradation is mainly catalyzed by alcohol dehydrogenase, a non-microsomal enzyme, and only a small part is controlled by the microsomal enzyme oxidizing system (372).

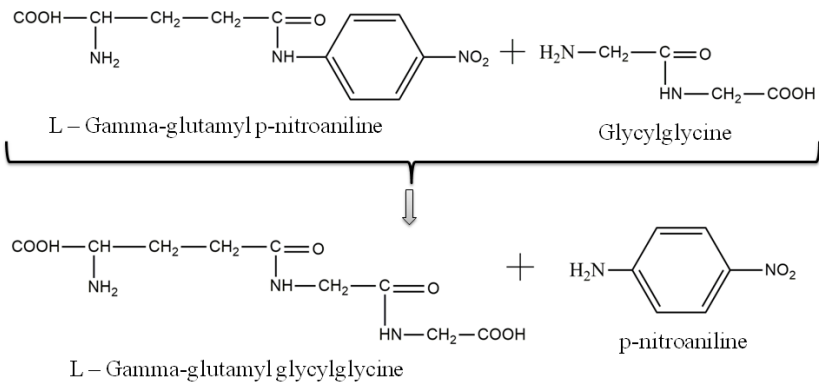

Figure I.3.2. Enzymatic mechanism of gamma-glutamyl trans-peptidase

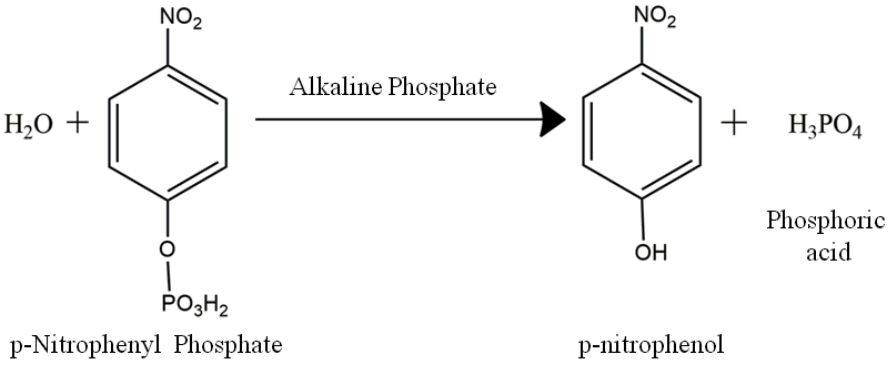

Figure I.3.3. Enzyme mechanism of alkaline phosphatase

Three enzymatic systems are involved in alcohol oxidation:

- alcohol dehydrogenase (ADH) located in the cytoplasm (the soluble fraction of the cell);
- the microsomal ethanol oxidizing system (MEOS), located in the endoplasmic reticulum;
- catalase, located in peroxiredoxins.

There are numerous pieces of evidence that the major biochemical lesion produced by alcohol is acetaldehyde accumulation. This molecule produces a vicious circle, disturbing exchanges at a mitochondrial level, essential for the elimination (disposal) of reduction equivalents. The peroxidation of membrane lipids increases and the glutathione decreases, these being decisive factors in the production of cellular deterioration (373).

I.3.4. Researches regarding the link between inflammaging, degenerative diseases and cardiovascular risk

A. Background

Aging represent the factor with the greatest impact on chronic diseases. This observation has been completly proved in large population studies. Some evidence indicates that the accumulation of multiple chronic disease in some older individuals results from the distribution expected based on the compound probability of having each single disease. The consequences of aging on the development of the aging phenotypes can be approximately synthesized into four principal domains: changes in body composition; imbalance between energy availability and demand; dysregulated signaling networks that maintain homeostasis; and neurodegeneration with impaired neuroplasticity (374).

Inflammaging is a condition described by increased levels of blood inflammatory markers. This condition presents high susceptibility to chronic morbidity, disability, frailty, and premature death.Genetic susceptibility, central obesity, increased gut permeability, changes to microbiota composition, cellular senescence, NLRP3 inflammasome activation, oxidative stress caused by dysfunctional mitochondria, immune cell dysregulation, and chronic infections are all part of its complex mechanism. Clinical trials suggest that inflammaging is a risk factor for chronic kidney disease, diabetes mellitus, cancer, depression, dementia, sarcopenia and others (375).

Without doubt, inflammatory processes are crucial factors to the development and complications of cardiovascular diseases. Complex studies has highlighted that the specific targeting of these processes in experimental models attenuates myocardial and arterial injury, reduce disease progression, and even promote healing. Unfourtunately, the translation of these remarks and the demonstration of specific efficacy in clinical practice have shown disappointing results. One of the major limitation could be the fact that the instruments presently used to quantify inflammation are not precise enough and do not provide specific information like disease site, activity, or discriminate between functionally important activation pathways (376).

Comorbidities such as CVD or osteoporosis – OP occur more frequently in aging population, idea supported by evidence proven that patients with CVD have an increased risk of bone loss (377, 378, 379). These two conditions develop even more frequently in patients with inflammatory rheumatic diseases such as RA and SLE. Even if rheumatologists are successful in treating their patients according to the treat-to-target design, mainly in RA, it is evident that this single treatment plan may not cover all comorbidities. A study published by Mhuircheartaigh et al. (380) highlighted that the risk of a CV event is 80% higher in RA patients after a fracture but is likely to be even higher in RA patients with high disease

activity and lower in patients in clinical remission (381).

B. Published papers in this field

Considering the fact that inflammaging is a process intensely studied nowadays, and is linked with degenerative joint and cardiovascular diseases, I have published a review related to this topic.

1. Rezuş E, Cardoneanu A, Burlui A, Luca A, Codreanu C, Tamba BI, Stanciu GD, Dima N, Bădescu C, **Rezuş C**. The link between inflammaging and degenerative joint diseases. *Int J Mol Sci*. 2019; 20 (3): 614.

Introduction

Inflammation is characterized by the presence of systemic low-level inflammation due to the excess secretion of cytokines with a pro-inflammatory role. Along with these, the aging of the body also presents an imbalance of the immune system that leads to up-regulation of immune responses. Older age also shows a decrease in apoptotic processes. All of these mechanisms seem to be incriminated in the pathology of age-related disorders (Figure I.3.4).

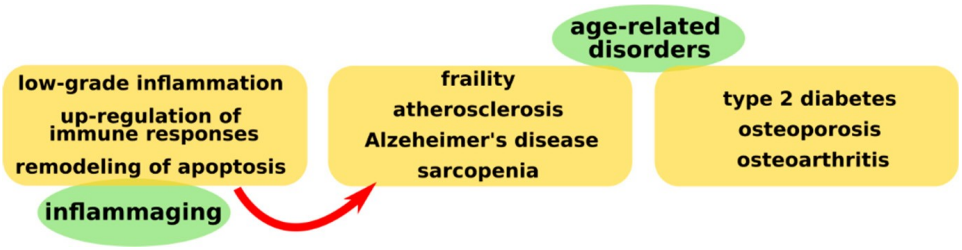

Figure I.3.4. This figure depicts the main mechanisms implicated in inflammaging, as well as the main associated diseases with this process.

The term *inflammaging* was first used in 2000 by Franceschi and refers to all the processes that contribute to the occurrence of various diseases associated with aging. Inflammaging represents a low-grade inflammatory status and together with the up-regulation of the immune response, as well as with the remodeling of apoptosis, contributes to these age-related disorders (382). Inflammaging is systemic, chronic, and asymptomatic.

Osteoarthritis – OA and many age-related degenerative joint diseases are correlated with aging mechanisms such as the presence of an inflammatory microenvironment and the impaired link between inflammasomes and autophagy (383).

The link between aging and articular cartilage

Aging is responsible for the senescence of chondrocytes and for the specific modifications that appear in the structure of the cartilage (384).

The anabolic processes are slowed down, and the catabolic ones accelerated. Significant changes in cell phenotype have been observed. Cells modification of the normal shape with a flattened one, altered secretory capacity and synthesis of collagen type X has been also noted. A decrease in specific secretion products, such as glycoproteins, proteoglycans or type II

collagen, was also highlighted.

The aging of articular cartilage is characterized by a decrease in cellularity, dehydration, decreased elasticity and solubility, and decreased proteoglycan molecule sizes. On the other hand, an increase in chondrocyte size, cartilage stiffness, protein content and glycosylation products were observed.

Moreover, the cartilage suffers from changes in blood flow and, secondarily to this, from the modifications in chondrocyte activity, overall leading to joint cartilage destruction (figure I.3.5). It has been shown that decreased blood flow results in poor nutrition, as well as the disruption of chondrocyte function and fluctuating oxygen levels promoting a pathological augmentation in metabolic activity (385, 386).

In addition, in cases of prolonged hypoxia, chondrocytes release high amounts of proinflammatory cytokines and reactive oxygen species (ROS), which contribute to the development of a proinflammatory microenvironment (387).

Chondrocyte telomere instability as well as apoptosis may also be bolstered by the presence of ROS (388). Moreover, oxidative stress induces a reduction of extracellular matrix (ECM) components by chondrocytes, leading to an alteration of cartilage structure and the subsequent decline of the tissue's mechanical properties, with the appearance of fissures and fragmentation (389).

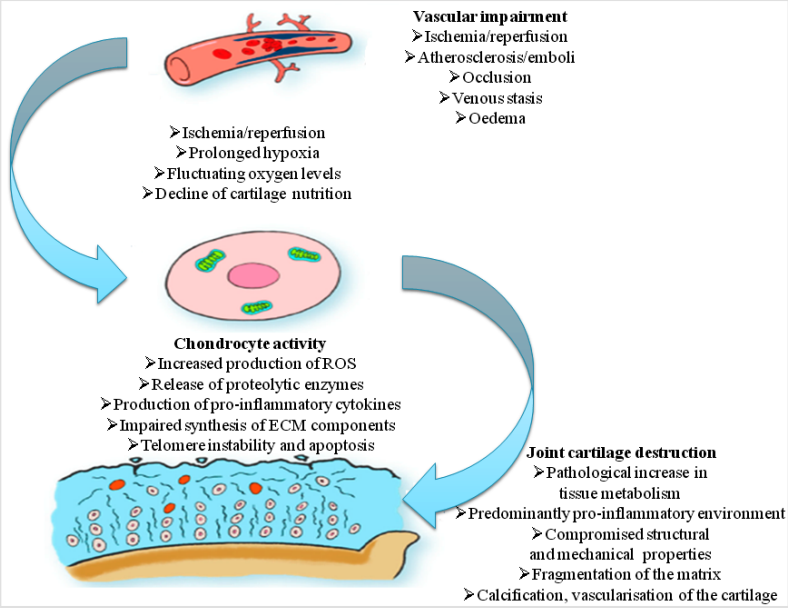

Figure I.3.5. Vascular impairment leading to a disruption in chondrocyte activity and the subsequent destruction of joint cartilage

Mechanism of inflammaging and implications in OA

Current data supports the multifactorial etiologyof inflammaging, including increased number of pro-inflammatory cytokines, oxidative stress, immunosenescence, autophagy, or cellular DNA damage (figure I.3.6).

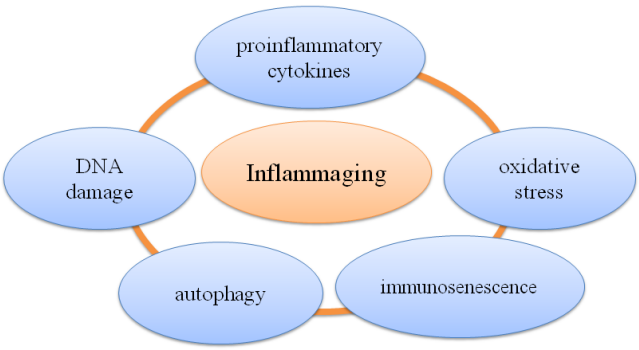

Figure I.3.6. The main multifactorial mechanism related to inflammaging

Systemic low-grade inflammation is the main pathogenic factor for chronic disorders related to aging, including OA (390, 391). Data from the literature highlighted increased levels of IL-6 and CRP) in patients with knee OA and correlated them with the progression of the disease (392, 393). Other studies demonstrated a relationship between the levels of pro-inflammatory cytokines and physical symptoms such as articular functionality or the level of the pain (394–397). In older cases with knee OA, physical mobility decreased directly proportional to the increase of soluble receptors for TNFα (398). Furthermore, elevated levels of CRP and TNFα have been associated with higher pain levels in patients with knee arthritis (399). Low-grade inflammatory status refers to an imbalance between pro- and anti-inflammatory cytokines. The most important proinflammatory cytokines involved in the process of inflammaging are tumor necrosis factor α – TNFα, interferon γ – IFNγ, and interleukins (IL)—IL-1, IL-6, IL-15, IL-18, respectively (400). These molecules can have pleiotropic effects, stimulating immune reactions. Data from the literature sustain the role of genetical changes in this susceptibility to inflammaging.

The polymorphisms in the promoter C/G 174 on the IL-6 gene is related to immune-inflammatory responses and affect serum IL-6 concentrations (401). Furthermore, the polymorphism of toll-like receptor 4 and of IL-10 can influence the inflammatory mechanisms (402). The association of low levels of IL-10 with increased levels of IL-6 can improve the ability to fight pathogens (403).

Figure I.3.7. shows the link between the three processes involved in the pathogenesis of the aging process characterized by a low-grade systemic inflammation.

The etiology of immunosenescence includes genetic, environmental, and immune factors. The damage of innate immunity refers to monocytes, neutrophils, natural killer, and dendritic cells and is characterized by the reduction of phagocytosis and superoxide production. The damage of acquired immunity includes B and T lymphocytes and determines thymus atrophy, increased proinflammatory cytokines, and autoreactivity. High levels of proinflammatory cytokines including IL-6, IL-1, and TNFα have a crucial role in the aging process by creating an inflammatory environment in most of the organs and body tissues. Systemic low-grade inflammation can determine stem cell aging through the activation of the signaling pathways (NF-kB, TOR, JAK/STAT). An important role in the process of replicative senescence and age-related diseases belongs to DNA damage response, which is directly related to telomere shortening. DNA damage response favors proinflammatory status through its action on stem cells, fibroblasts, or macrophages, thus exacerbating the phenomenon of inflammaging.

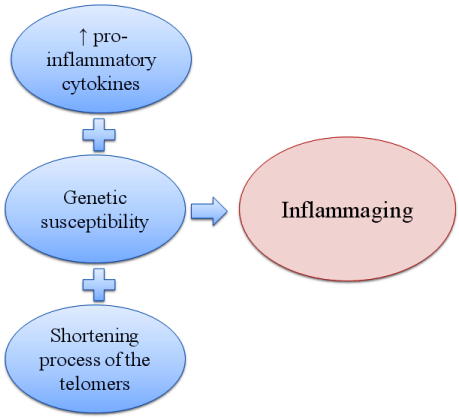

Figure I.3.7. The link between pro-inflammatory cytokines, genetic susceptibility, and DNA changes that occurs with inflammaging

Oxidative damage through the accumulation of reactive oxygen species (ROS) leads to what is believed to be a remodeling of the immune system to which the body tries to adapt, but in failing to do so, as is the case of elderly patients, predisposition to chronic inflammatory conditions appears (382).

Data sustain the link between oxidative stress, inflammaging, and immunosenescence (404, 405). Mitochondria are considered to be a source of oxygen metabolites during oxidative phosphorylation. The accumulation of the metabolites of oxygen can determine the damage of nucleic acids, proteins, or lipid membranes, inducing apoptotic mechanisms and deoxyribonucleic acid (DNA) damage, especially increasing the risk of cancer (406). There is a relation between the function of immune cells and the redox state. High levels of antioxidants can decrease the oxidative stress and slow down the aging process (407, 408). Directly related to this, the literature speaks about the oxidation-inflammatory theory of aging (409).

The accumulation of oxygen metabolites could accelerate the process of cellular aging and increase apoptosis by decreasing the adenosine triphosphate (ATP) levels and increasing the porosity of the cellular membranes (410).

Aging of the body is associated with an increase in the oxidative phosphorylation process, which results in the accumulation of oxygen metabolites. Reactive oxygen species include peroxides, superoxide, hydroxyl radical, singlet oxygen, and alpha-oxygen. All of these have important roles in cell signaling and homeostasis processes. The most important harmful effects of reactive oxygen species on the cell are damage of DNA or RNA, oxidations of polyunsaturated fatty acids in lipids (lipid peroxidation), oxidations of amino acids in proteins, and oxidative deactivation of specific enzymes by oxidation of co-factors.

Oxidative stress is also responsible for accelerated apoptosis and cellular damage, which in turn leads to the emergence of various pathologies associated with aging (Figure I.3.8). Mitochondrial dysfunction plays an essential role in the appearance of low-level systemic inflammation that characterizes aging processes.

Aging of the body is associated with an increase in the oxidative phosphorylation process, which results in the accumulation of oxygen metabolites. Reactive oxygen species include peroxides, superoxide, hydroxyl radical, singlet oxygen, and alpha-oxygen. All of these have important roles in cell signaling and homeostasis processes.

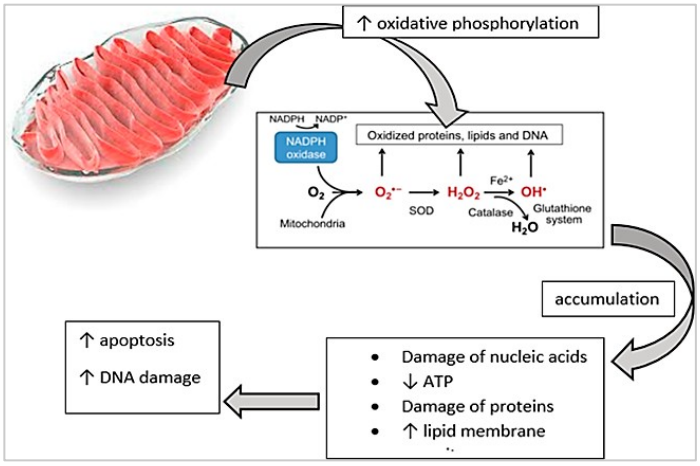

Figure I.3.8. Representation of mitochondrial changes due to increased oxidative phosphorylation with respect to DNA modification and apoptosis linked to the theory of aging

The most important harmful effects of reactive oxygen species on the cell are damage of DNA or RNA, oxidations of polyunsaturated fatty acids in lipids (lipid peroxidation), oxidations of amino acids in proteins, and oxidative deactivation of specific enzymes by oxidation of co-factors. All these structural and genomic changes cause an increase in apoptotic processes and affect genetic transcription, leading to shortening of telomeres.

The definition of cellular senescence refers to the mechanism that leads to an irreversible loss of the proliferation of somatic cells (411). Furthermore, experimental studies demonstrated that senescent cells, though a precise pathway which involves the release of certain mediators and the stop of proliferative activity, can determine their clearing and tissue regeneration (412, 413). The etiology of immunosenescence includes genetic, environmental and immune factors. The damage of innate immunity refers to monocytes, neutrophils, and natural killer and dendritic cells and is characterized by the reduction of phagocytosis and superoxide production. The damage of acquired immunity includes B and T lymphocytes and determines thymus atrophy, increased proinflammatory cytokines, and autoreactivity (414).

On the other hand, systemic low-grade inflammation can determine stem cell aging through the activation of the signaling pathways (NF-κB, TOR, JAK/STAT) (415). Senescent cells can have a negative effect on NF-κB activity only in cells actively involved in inflammation (416). This is possible due to the ability of senescent cells to express two microRNAs (mir-146a, mir-146b) (417). Figure I.3.9. schematically illustrates all the mechanisms that determine the phenomenon of immunosenescence leading to various systemic disorders strictly related to aging process of the organism.

Autophagy and cellular apoptosis

Autophagy is a cellular mechanism which maintains normal function and homeostasis of the cells through removal of abnormal substances via lysomal degradation. It is an important cellular homeostatic mechanism implied in the removal of altered or dysfunctional organelles and macromolecules, being increased by different types of stresses (418, 419). In chondrocytes and cartilage affected by OA, autophagy processes are at high levels in order to regulate changes in OA-like gene expression by modulation of apoptosis and reactive oxygen species, especially during the initial degenerative phase (420).

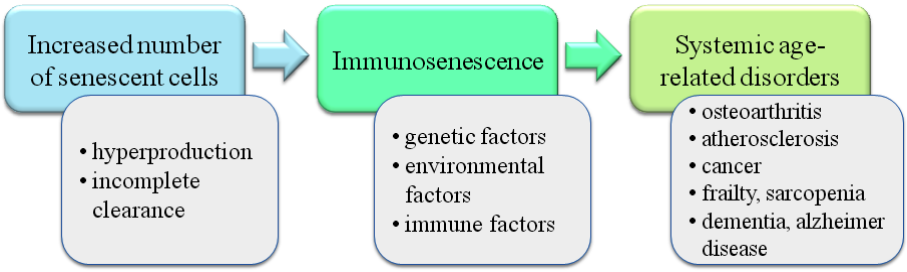

Figure I.3.9. The mechanism of age-related disorders

Cellular apoptosis is a very well controlled process in the body, having an important role throughout life. Initiation of apoptosis can be made using two pathways. The intrinsic pathway is based on cellular stress and intracellular signals that cause initiation of programmed cell death, while the extrinsic pathway refers to signals received from other cells (421, 422). Both mechanisms eventually trigger caspase activation.

The published review underlines the fact that aging is an inevitable process in the human body which is associated with a multitude of systemic and localized changes. All these conditions have a common pathogenic mechanism characterized by the presence of a low-grade proinflammatory status.

2. Tanase DM, Gosav EM, Radu S, Ouatu A, **Rezus C**, Ciocoiu M, Costea CF, Floria M. Arterial hypertension and interleukins: potential therapeutic target or future diagnostic marker? *International Journal of Hypertension* 2019; 3159283, 17 pages.

Introduction

Arterial hypertension – HTN is a major CVDs risk factor and multifactorial disease, affecting 30-40% of the population and causing 7.5 million deaths worldwide (423). Despite numerous non-pharmacological measures to prevent it or to slow it down, HTN prompts 62% of strokes and 38% of heart diseases in developing countries (424). Increasing evidence reveals HTN as a chronic inflammatory state (425, 426).

Metabolic/chemical, mechanical (wall stretch), or infectious endothelial aggressions trigger complex immune reactions, leading to a proinflammatory state (427,428). Inflammation, in turn, promotes endothelial dysfunction and atherosclerosis through reactive oxygen species (ROS), a downstream product of cellular and soluble immune factors (429-431). Consequently, ROS stimulates proinflammatory cytokine secretion, increasing IL-6 expression and decreasing NO availability (432). Studies have shown that inhibition of these ROS led to blood pressure reduction through endothelial function improvement via increased nitric oxide (NO) production (431, 432).

RAS and Proinflammatory Cytokines

The implication of renin-angiotensin-angiotensinogen system – RAS in the pathogenesis of HTN has been long known. Interestingly, several immune cells (T lymphocytes, dendritic cells, and macrophages) express angiotensin 1 receptors (AT1R). By binding to AT1R, angiotensin II determines immune cells differentiation and subsequent

proinflammatory cytokine production, such as IL-6, IFN-γ, and TNFα (426). In addition, by acting on P-selectins and adhesion molecules, it increases leukocytes adhesion and migration. Moreover, angiotensin II impacts the immune system even in the absence of vasoconstrictor effects. In fact, it seems that angiotensin II contributes not only to HTN development, but also to HTN-mediated organ damage. In turn, proinflammatory cytokines, such as TNFα, determine increased angiotensin converting enzyme (ACE) production, which contributes to inflammatory-mediated HTN (430, 431).

ROS Regulation via Inflammation

It is known from preclinical models that the imbalance between reactive oxygen species – ROS production and degradation is involved in the HTN mechanisms. Because excessive ROS enhances cellular processes like differentiation and apoptosis and controls vascular tone and endothelial function, it contributes to endothelial dysfunction (433). Increased ROS generation and reduced antioxidants levels (nitric oxide) lead to oxidative stress. ROS production involves both cellular and mitochondrial levels, with the latter being the main endogenous source. ROS molecules including xanthine oxidoreductase, uncoupled NO synthase (NOS), nicotinamide adenine dinucleotide phosphate (NADPH) nitric xanthine oxidase (NOX), and mitochondrial respiratory enzymes play a role in the HTN development (433, 434). The production of mitochondrial ROS depends on the activation of the mitochondria adenosine triphosphate (ATP) – sensitive potassium channels (mKATP), opening of mitochondrial permeability transition pore (mPTP), and the pH gradient in the inner membrane.

Both end-organ dysfunction and HTN could be a result of the mitochondrial oxidative stress, which is the outcome of overproduction of mitochondrial superoxide and reduced SOD2 function. Interchanges between mitochondrial oxidases and NOXs are highly implicated in cellular ROS production (435).

The proinflammatory state of hypertensive patients is supported by many studies that revealed increased serum levels of C-reactive protein (431). Moreover, these patients had histological arterial wall inflammation attributed to proinflammatory cytokines secretion (430, 431).

Endothelial Dysfunction in Hypertensive Patients

The innermost layer of the blood vessels, the endothelium, was first considered a passive barrier between blood and vascular wall. The arterial wall can synthetize both endothelium-derived relaxing factors (EDRF) like NO, prostacyclin (PGI_2), and hydrogen sulfide (H2S) and constrictor factors including angiotensin II (Ang II) and endothelin-1 (ET-1) (436).

Cardiovascular risk factors such as dyslipidaemia, obesity, and diabetes, through their inflammatory state, promote endothelial dysfunction. Mast cells, T lymphocytes, dendritic cells, activated neutrophils, and platelets interact to produce an inflammatory response, with increased production of proinflammatory cytokines, ROS, and adhesion molecules (429).

In some autoimmune diseases such as psoriasis or rheumatoid arthritis (437. 438) anti-endothelial antibodies cause endothelial cells to release adhesion molecule and chemokines. Elevated serum levels of IL-6 lead to increased hepatic inflammatory markers production

such as CRP (439) and increase vascular permeability, cell apoptosis, and thrombosis (440) (figure I.3.10). Moreover, the link between endothelial dysfunction and inflammatory cytokine production has been emphasized by studies that attribute increased IL-6 production to mechanical endothelial stretch in hypertensive individuals, with a subsequent decrease in NO production (432).

Low level chronic inflammation increases the concentrations of markers and of inflammatory cells, leading to increased production of C-reactive protein (CRP) by the liver, in response to interleukin-6 (IL-6), which provokes a reduction in vasodilation and an increase in vascular damage.

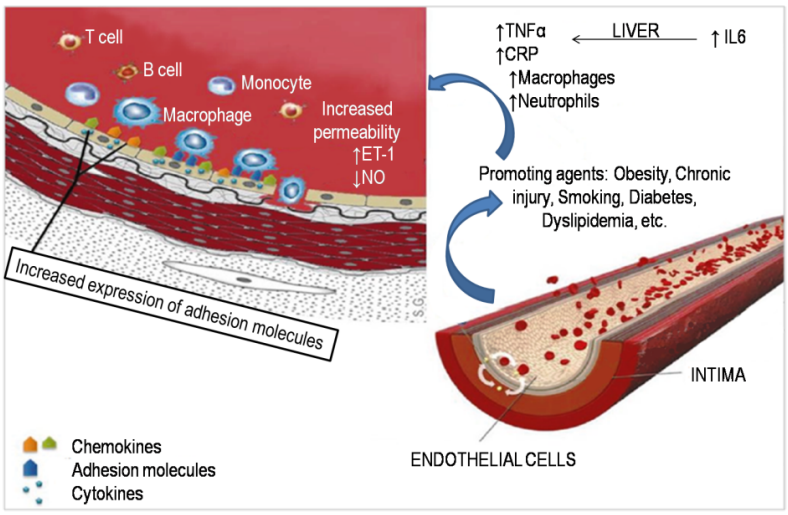

Figure I.3.10. Etiology of the inflammatory process

Role of Interleukins in Inflammation and HTN Development

Cytokines are involved in several cellular processes, ranging from inflammation to both tissue damage and regeneration. They induce immune cells recruitment and activation and play an important role in arthrosclerosis development (441, 442). Proinflammatory stimuli determine mast cells cytokine production, triggering endothelial expression of adhesion molecule such as vascular cell adhesion protein 1 (VCAM-1), P- selectin, and plated activating factor (PAF). Leukocyte recruitment and adhesion promote vascular and extracellular matrix remodeling through increased fibrosis and hypertrophy with a subsequent reduction in vascular lumen diameter (443, 444).

Apart from their effect on blood pressure, interleukins, as key mediators of inflammation, seem to play a role in end-organ damage in hypertensive individuals (442, 443). Both immune and endothelial cells contribute through their interaction to the inflammatory state in hypertensive patients. The balance between proinflammatory (IL-1β, IL-6 IL-12, IL-18, IL-17, or IL-23) and anti-inflammatory cytokines (IL-4, IL-10) is tightly regulated and directly involved in CVDs, not only in HTN development per se, but also in mediating hypertensive end-organ damage such as ventricular remodeling and renal and cerebral involvement (445, 446). In this inflammatory-mediated HTN process, adaptive or innate immune cells produce proinflammatory cytokines through different signaling pathways (Figure I.3.11).

Cytokines play an essential role in the inflammatory processes. As their role in the development or progression of cardiovascular diseases such as HTN or atherosclerosis (Table I.3.2.) continues to be researched, new possible therapeutic paths concerning these interleukins may be discovered.

Tabel I.3.2. Cytokines, cytokine receptors, and their vascular impact

Interleukine	***Receptor***	***Cell source***	***Cell Target***
IL-1α,β	Type I IL-1r, Type II IL-1R	Monocytes/macrophagefibroblast, endothelial cells, B cells, epithelial cells including thymic epithelium	All cells
IL-4	IL-4α, common γ	Mast cells, T cells, basophils	Endothelial cells, T cells, B cells fibroblast, NK-cells, monocytes, macrophages
IL-6	IL-6r, gp130	Fibroblast, endothelial, Monocytes/macrophages, most epithelial cells including thymic epithelium	Hepatocytes, macrophages monocytes, T cells, B cells, epithelial cells
IL-10	IL-10r	T cells, B cells, monocytes macrophages, keratinocytes, mast cells	T cells, B cells, NK cells, mast cells, monocytes macrophages
IL-17	IL-17r	CD4+ T cells	Endotheliumepithelium, fibroblast, macrophages
IL-23	IL-12 Rb1/IL23R	Macrophages, other cell types	T cells

HTA-arterial hypertension, ATS-atherosclerosis, ST-stroke, IM-myocardium infarction, CHD-coronary heart disease, AF-atrial fibrillation, CH-cardiac hypertrophy, LVD-left ventricule dilatation, HTP-pulmonary hypertension, UA-unstable angina, CHF-chronic heart failure.

Proinflammatory interleukins

IL-1 is considered to be an "early-response" cytokine, involved in energy homeostasis and inflammation, connected to metabolism mechanisms (447). Recent observations linked elevated levels of CRP as an indirect marker of IL-1 activity in the context of low-grade inflammation to HTN development (448).

IL-6 is a pleiotropic cytokine, with both proinflammatory and anti-inflammatory effects (449) and multiple physiological roles. 30% of circulating IL-6 originates in adipose tissue. IL-6 promotes B cells differentiation, T cells expansion and activation, and acute-phase response regulation. Given its effects, it is now considered an important cardiovascular risk biomarker (450, 451).

IL-6 may be involved in the pathogenesis of HTN through its effects on vascular inflammation and stiffness and endothelial dysfunction. Moreover, it stimulates arterial wall collagen synthesis, inhibits its degradation, and stimulates fibrinogen production. Interestingly, increased IL-6 levels have been found in atherosclerotic plaques. IL-6 may even show promise as a biomarker; several studies have proposed increased IL-6 and TNF-α serum levels as independent risk factors for the development of high blood pressure in apparently healthy patients. A correlation between plasma levels of IL-6 and TNF-α with coronary endothelial dysfunction was found in hypertensive patients (452-455). IL-6 also plays an important role in pulmonary hypertension and fibrosis (456-458).

IL-23 is stimulated by antigen-stimulated macrophages and dendritic cells. It enhances T helper cells development Tn17/ThIL-17) which secrete IL-17. IL-23 acts also in an

autocrine/paracrine manner by promoting the release of other proinflammatory cytokines like IL-1, IL-6, and TNF-α.

The IL-17 family is formed by 6 members ranging from IL-17A to IL-17F. Despite several monoclonal antibodies targeting IL-17A, IL-17F, or the IL-17RA being used as autoimmune disease treatment (i.e., psoriasis), their effect on blood pressure has not been established. There are 3 types of Th cells: Th1 cells which produce interferon-g (IFN-g), Th2 cells which produce IL-4 and IL-5; and IL-13, a third type of Th cells that secrete IL-17A, IL-17F, and IL-22 (459-461).

The latter have a controversial role in inflammation with both atherogenic and protective roles being observed (462). IL-17- and IL-23-producing cells have been shown to be involved in the pathogenesis of atherosclerosis (427, 462). IL-17, by stimulating proinflammatory cytokine production, fibroblast proliferation, and profibrotic gene expression, plays a role in remodeling after post-myocardial infarction (463).

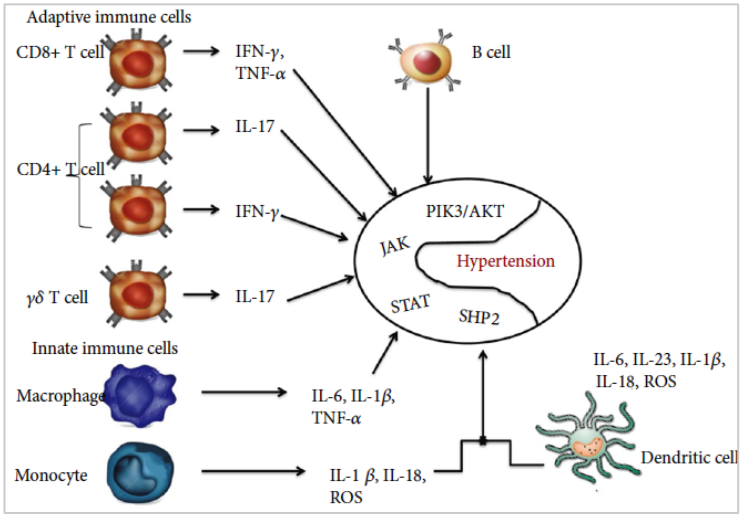

Figure I.3.11. The inflammatory-mediated HTN process, immune cells via different signaling pathways

There are more cytokines involved in the inflammatory mechanisms of HTN, like those described by Buemia et al.; they showed that in patients with essential hypertension, IL-8 and ICAM-1 significantly increased the Ca^{2+} dependent K^{+} outflow in red blood cells (464). IL-12, mainly secreted by macrophages, is an inducer of Th-1 type cellular immune response (465). Moreover, IL12B*A/*A genotype was less frequent in patients with a history of stroke and IL12B genotype had increased risk of stroke. Authors thought IL-5, with its anti-inflammatory and prothrombotic cytokine, mediated atherogenesi (466). Kaibe et al. showed that Il-15 serum concentration were higher in hypertensive patients with severe organ damage (467). HTN, through its effects on the vascular wall, leads to increased macrophages and mature IL-18 cells production. The subsequent natural killer and T cell maturation together with IL-2 secretion determines IL-4, IL-10, IL-13, and IFN-γ release (468)

Anti-inflammatory cytokines (IL-4, IL10) and HTN

IL-10 is known to be the main anti-inflammatory cytokine, produced by Th1, Th2, Th17, and epithelial cells and keratinocytes. It binds to its receptor, activating the IL-

10/JAK1/STAT3 cascade. Hence, STAT3 phosphorylation is crucial for IL-10 pathway (469). Its anti-inflammatory effects consist of suppressing IL-1, IL-6, IL-12 and TNF, HLA class II, and adhesion molecules. It up-regulates a tissue inhibitor of MMP-1 expression, leading to atherosclerotic plaque stabilization (470). Another pleiotropic cytokine secreted by T and B cells is IL-4. It can also be detected at the feto-maternal interface. It inhibits inflammatory cytokine production, increases MHC class II and CD23, and promotes immunoglobulin E and G1 production (471). Piyali et al. showed that IL-4/IL-10 cotreatment during gestation in mice with preeclampsia normalized blood pressure and endothelial function and decreased the IL-6, IFNγ, TNFα, and TGF levels (472).

Inflammation as a potential therapeutic target in arterial hypertension

There has been consistent evidence over the past years that inflammation is a key element in the pathophysiology of hypertension, leads to, progression, and development of end-organ damage. While hypertensive patients have been shown to present with increased levels of proinflammatory cytokines such as IL-1β, IL-6, IL-8, IL-17, and TNFα, antihypertensive treatment seems to determine a reduction in their concentration.

Several drugs already used in cardiovascular pathologies have been shown to lower serum inflammatory cytokines level (473). Table I.3.3. summarizes the anti-inflammatory effects of cardiovascular drugs and their potential hypotensive mechanisms.

Tabel I.3.3. Anti-inflammatory effects of cardiovascular drugs

Drugs	Effects on inflammatory cytokines	Antihypertensive mechanism	Proposed references
Statins	↓ IL-1β ↓ IL-6 ↓ MCP-1 ↓ ICAM-1 ↓ MMP-2 ↓MMP-9 ↓ hs-CRP ↓ PAI-1 ↑ NO	NF-κB inhibition AT1R downregulation HMC CoA inhibition (G protein coupled signalling inhibition) PPAR-γ inhibition Upregulates NO synthase	(473, 475, 477)
ARBs/ACEIs	↓ IL-1β ↓ IL-6 ↓ TGF-β (losartan) ↑ NO (AT2R)	NF-κB inhibition AT1R downregulation Decreased ACE synthesis	(432, 475)
Calcium channel blockers	↓ MMP-2 ↓ MMP-9 ↓ IL-1β ↓ IL-18 ↓ CRP ↓ MCP-1 ↓ ICAM-1	Protein kinase pathway (MMP-2)	(477, 478)

Statins are the most frequently used lipid-lowering agents, both in primary and secondary prevention of cardiovascular events (473–476). Apart from their effect on serum cholesterol and nonsteroidal isoprenoids synthesis through the inhibition of 3-hydroxyl-3-methyl-glutaryl coenzyme A reductase (HMGCR), they present a range of pleiotropic effects such as plaque stabilization, antithrombotic and anti-inflammatory effects, and endothelial

function enhancement (476). There has been a debate on whether statins have blood pressure lowering effects and if these effects can be attributed to their ability to reduce serum inflammatory cytokines levels and to oppose endothelial dysfunction (473).

Apart from statins, *first-line antihypertensives affecting the renin-angiotensin-aldosterone system* (RAAS), angiotensin converting enzyme inhibitors (ACEI) and aldosterone receptor blockers (ARBs), respectively, have shown anti-inflammatory effects in addition to their blood pressure lowering effects. Patients having received valsartan showed reduced LPS stimulated IL-1β levels. Several studies agree that hypertensive patients showed increased IL-1β secretion under stimulation with lipopolysaccharide – LPS (475, 477, 478). IL-6, IL-8, TGF-β, and TNF levels were determined in 286 hypertensive patients and CAD patients treated with captopril, atorvastatin, losartan, aspirin, clopidogrel, metoprolol, or nitrocontin in varying doses and combinations (478). The results showed decreased IL-6 levels and increased TGF-β in patients treated with higher doses of the aforementioned drugs except for metoprolol. There are several proposed mechanisms for ARBs and ACEIs anti-inflammatory effects.

Amlodipine, a calcium channel blocker, seems to possess the same anti-inflammatory effects as ARBs and ACEIs, decreasing serum levels of proinflammatory cytokines in hypertensive patients. Matrix metalloproteinases (MMPs) are endopeptidases secreted by myocardial fibroblasts and inflammatory cells that promote pressure-overload induced myocardial remodeling in hypertensive patients (479, 480).

Immunosuppressants effects on blood pressure levels

Given that conventional antihypertensive therapies have shown anti-inflammatory effects, it is questionable whether the ability to inhibit proinflammatory cytokines such as Il-6, IL-1, TNF, and IL-17 could contribute to their blood pressure lowering effects. If so, the question arises as to whether anti-inflammatory drugs could lower blood pressure in hypertensive patients, thus offering a new therapeutic option in arterial hypertension.

The published results have shown that treating hypertension is not limited to blood pressure control. One of the most important goals is preventing/counteracting end-organ damage. Taking into consideration that inflammation plays an important role in developing/maintaining both hypertension and its subsequent end-organ damage, hypertensive patients may benefit from immunosuppressive therapies as a new therapeutic option.

I.4. RESEARCHES REGARDING THE ROLE OF IMAGING IN CARDIOVASCULAR DISEASES

I.4.1. Hallmarks

All imaging technologies have experienced significant improvements in the last decades. The use of imaging to study biology and uncover biomarkers of human disease supports *in vivo* the phenotyping of diseases and offers an opportunity for early diagnosis of disease. The deep details of a disease mechanisms and the shrewdness of the responses to treatment are essential for its understanding and treating.

Nowadays, imaging is a fundamental tool not only for showing in detail the pathogenic mechanisms, but also for developing therapeutic strategies. Furthermore, transformations in technical capabilities, spatial and temporal resolution, and processing speed have led to many advanced imaging tools, already integrated in the continuum of patient care, offering a real opportunity for clinical translation (481, 482).

Echocardiography, nuclear imaging, computerized tomography – CT, and cardiac magnetic resonance – CMR imaging are the most used techniques in clinical practice. In nuclear imaging, the recent cameras with cadmium-zinc-telluride – CZT detectors have enhanced sensitivity and performance.

Positron emission tomography – PET agents provide better measurements of blood flow. CT imaging has improved the number of slices obtained at once to cover a larger area of the heart and also temporal resolution that is crucial in cardiac imaging, allowing imaging with much lower radiation and better accuracy.

In CMR, can be determined the quantitation of collagen, scar burden, and its distribution, which can be merged with perfusion imaging and anatomy. Echocardiography has evolved to transesophageal studies, 3-dimensional (3D) real-time acquisition, and tissue Doppler and speckle tracking technologies (482).

Coronary computed tomography angiography – CCTA and invasive coronary angiography – ICA are other two imaging techniques used often in clinical practice. Coronary angiography, is traditionally performed in patients with chest pain and significantly elevated troponin, symptoms that usually indicate a coronary artery disease – CAD.

CCTA is often preferd because has plenty of advantages compared to ICA; CCTA is a non-invasive procedure that can be performed quickly with relatively few risks. On the other hand, ICA is expensive, requires admission, and carries risks of several serious complications, such as MI, stroke, and even death. For exemple, in patients with chest pain and positive troponin, with signs or symptoms not usual for ischemia or a deficiency of cardiovascular risk factors, CCTA is sufficient to exclude CAD (483).

CCTA has the ability to provide a three-dimensional visualization of the epicardial vessels. This is a very important characteristic because in that way, a useful method to study the coronary artery anatomic variation is provided.

The physicians can clearly see the characteristics of coronary artery distribution in the peri-crux area and evaluate its dominance (484).

I.4.2. Researches regarding inflammation and noninvasive techniques for assessing atheromatous plaque morphology and composition

A. Background

Atherosclerosis of the carotid arteries is one of the risk factor for the development of ischemic stroke. Presence of intraplaque hemorrhage and a lipid-rich necrotic core are important characteristics of the vulnerable plaque, predisposed to rupture and causing an ischemic event (485).

An efficient quantification of carotid plaque morphology, in terms of geometry and tissue composition, may be useful for the prevention of future stroke and assess plaque progression or regression in response to medical risk factor modification (486).

B. Published paper in this field

Together with physicians with a valuable experience in cadiology, I have published an article concerning the impact of coronary plaque geometry on plaque vulnerability and its association with the risk of future cardiovascular events in patients with chest pain.

Ratiu M, Chitu M, Benedek I, Benedek T, Kovacs I, Rat N, **Rezuș C.** Impact of coronary plaque geometry on plaque vulnerability and its association with the risk of future cardiovascular events in patients with chest pain undergoing coronary computed tomographic angiography—the GEOMETRY study Protocol for a prospective clinical trial. *Medicine (Baltimore)*. 2018; 97 (49): e13498.

Introduction

CCTA has emerged as a valuable noninvasive imaging tool for assessing atheromatous plaque morphology and composition, and several CCTA features have been validated as reliable indicators of the plaque-associated risk. However, the role of lesion geometry as a CCTA feature of plaque vulnerability has not been investigated so far.

Atherosclerosis represents the underlying condition leading to myocardial ischemia, ultimately resulting in acute coronary syndromes – ACS and having devastating consequences on public health. The vast majority of ACSs are the consequence of a sudden modification in coronary plaque morphology leading to plaque rupture or erosion, the 2 principal mechanisms triggering an acute coronary event (487).

CCTA-based imaging-derived markers associated with vulnerability have been validated as reliable indicators of the plaque-associated risk. Such key CCTA features of plaque vulnerability identified by previous studies include positive remodeling (PR), low-attenuation plaque (LAP), napkin- ring sign (NRS), and spotty calcifications (SC) (488, 489). Interestingly, intravascular ultrasound studies demonstrated that ruptured coronary plaques are usually eccentric (490). A combined intravascular ultrasound (IVUS) and optical coherence tomography (OCT)-based study reported that lesions demonstrating plaque erosion had a greater plaque eccentricity index than those with plaque rupture or calcified nodules ($P < .001$ and $P < .001$) (491).

Whitin this context, the primary objective of the study is to evaluate the association between different patterns of plaque geometry and the risk for major adverse cardiac events MACE (defined as all-cause mortality, cardiovascular death, myocardial infarction, repeated revascularization, repeated hospitalizations for cardiovascular related incidents, cerebrovascular events) during a 2-year follow-up.

The second objective of the study is to evaluate the association of plaque eccentricity with plaque vulnerability and plaque progression after 2 years of follow-up.

Material and methods

Study design

GEOMETRY is a prospective, non-randomized, cohort, single center study to investigate the relationship between plaque eccentricity, plaque vulnerability, and the risk for MACE in order to validate plaque eccentricity as a new CCTA marker of coronary plaque vulnerability.

Population and time

GEOMETRY will include patients with chest pain and pre-test probability of coronary artery disease between 15% and 85%, referred for CCTA (according to the recommendation of the guidelines of the European Society of Cardiology) (492). Also, the patients must be able to provide informed consent to be over 18 years of age.

As exclusion criteria will be considered: patients with pre-test probability of CAD >85% or <15%; electrocardiographic evidence of ST-segment elevation acute myocardial infarction; presence of pre-existing CAD including prior myocardial infarction; history of coronary artery revascularization; atrial fibrillation or other irregular rhythm; unwillingness or incapacity to provide informed consent; allergy to iodine contrast media; inability to tolerate beta-blocker medication; renal insufficiency (serum creatinine values higher than 1.5 mg/dL) or renal failure requiring dialysis; pregnant women or lactation; active malignancy or malignancy within the last 5 years prior to enrolment; conditions associated with an estimated life expectancy of under 2 years; coronary calcium score >1000.

One thousand patients who meet the selection criteria will be included in the trial, conducted from November 2018 to August 2019, followed by a 24-months follow-up, and will be divided into 2 groups, namely patients in whom screening CCTA analysis identifies only non-eccentric coronary plaque (group 1) and patients in whom CCTA analysis reveals the presence of at least 1 eccentric significant coronary plaque producing a significant luminal narrowing (group 2). Figure 1.4.1. illustrates the GEOMETRY diagram with the flowchart that will be used in the study.

Procedures and outcome assessment

In all patients clinical data including sex, age, comorbidities, history of coronary artery disease, stroke, peripheral arterial disease, diabetes, smoking status, as well as clinical status and laboratory tests (creatinine, total cholesterol, low-density lipoprotein [LDL]-cholesterol, triglycerides) will be recorded. All patients will undergo CCTA scanning of the coronary arteries at screening and will be enrolled in the study if CCTA reveals the presence of at least 1 obstructive coronary plaque in any coronary artery.

CCTA scanning protocol

CCTA will be performed with a 128-slices single source CT scanner with retrospective electrocardiographic gating, at a tube voltage of 100 kV, a gantry rotation time of 330 ms and a collimation of 128 × 0.6. Oral beta-blockers will be administered to all patients with a heart rate >65 beats/min in order to achieve the desired heart rate, and 0.4 mg nitroglycerin will be administered sublingually 2 minutes before scanning in order to obtain a coronary vasodilatation and thus a superior image quality. Contrast agent will be injected with a flow rate of 5 mL/s in the antecubital vein, adapted to patient body weight. Contrast administration will be followed by a flush of 50 mL saline solution with the same flow rate.

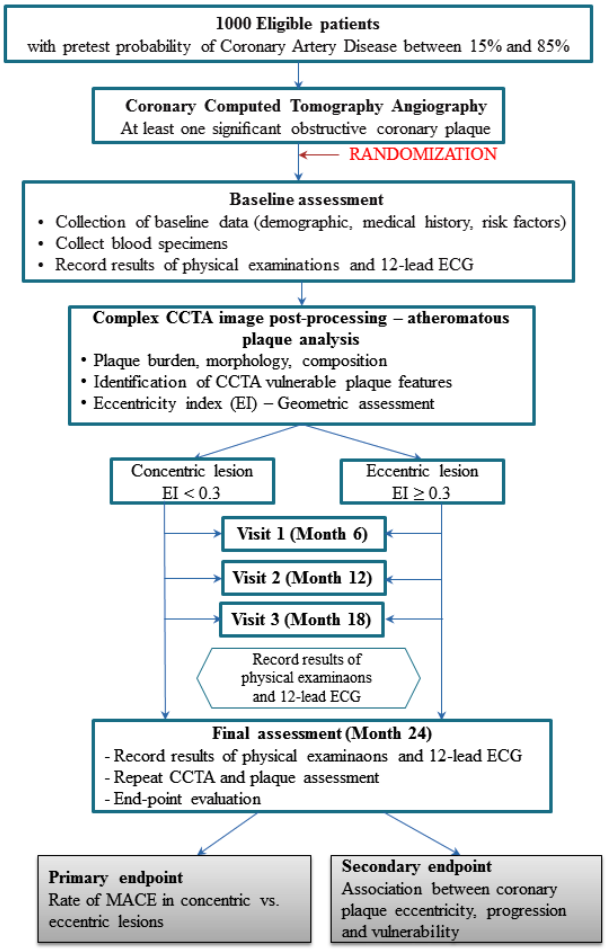

Figure I.4.1. Flowchart diagram of GEOMETRY study

Plaque reconstruction and analysis

All reconstructed CCTA images will be further evaluated semi automatically using a three-dimensional (3D) contour detection algorithm (Syngo.via Multimodality Workplace, Siemens, Frontier–Coronary Plaque Analysis platform, Siemens, Erlangen, Germany). All coronary vessels with a diameter of at least 2 mm will be assessed using the 19 coronary segments model.

Quantitative evaluation of the atheromatous lesions will include plaque length, vessel volume, lumen volume, plaque volume, and the severity of luminal narrowing. The volumetric measurements will be performed using as reference the proximal and the distal extremities of the plaques, and assessment of plaque composition will include determination

of dense calcium and non-calcified plaque components, such as lipid rich and fibrotic tissue.

Qualitative assessment of plaque characteristics will investigate the presence of vulnerability features inside the coronary plaque: PR, LAP, SC, and NRS.

Study definitions

In this study, a significant coronary plaque is defined as a plaque producing at least 50% luminal narrowing. The remodeling index (RI) represents the ratio between the cross-sectional area at the site of maximum stenosis divided by the average of the proximal and distal reference cross-sectional areas, and PR is defined as a remodeling index of 1.1 or greater (493). Plaques having at least 10% of their content non-calcified, with a CT density of <30 HU will be classified as LAP (494).

Cross-sectional plaque eccentricity will be assessed according to the location of the circulant lumen in a transverse section at the level of maximum stenosis. The cross-sectional eccentricity (CE) index will be calculated as follows: (maximum wall thickness – minimum wall thickness)/maximum wall thickness. Eccentric lesions will be defined as having a CE index of 0.3 or greater, whereas concentric lesions will be defined as having a CE index of <0.3. Longitudinal plaque eccentricity, defined as stenosis with an abrupt narrowing of the proximal or distal edge, will be assessed according to the location of maximum stenosis in a longitudinal section. The longitudinal eccentricity (LE) index will be calculated as the ratio of distance between the proximal end of the lesion and the place of maximum stenosis to the lesion length (495).

Outcomes

The primary outcome of the study is represented by the rate of MACE during follow-up. Secondary outcome refers to coronary plaque vulnerability associated to plaque eccentricity, based on the number of vulnerability markers in eccentric versus concentric plaques.

As results, we establish the protocol for a prospective cohort, single center study which aims to study the correlation between coronary plaque eccentricity, lesion vulnerability, and 24-months MACE rates in patients with suspected coronary artery disease referred for CCTA for coronary luminal evaluation. The main contribution of this study is to incorporate the evaluation of coronary plaque eccentricity in routine analysis during coronary computed tomography angiography in order to identify lesions with a more vulnerable phenotype using a noninvasive method.

Discussions

I have always considered that prevention should be prioritized when discussing cardiovascular disease, particularly coronary ischemic disease, responsible for a significant morbid-mortality among patients with atherosclerotic vascular damage.

Atheroma plaque rupture with subsequent thrombosis is a major event that turns coronary atherosclerosis into a condition that puts the patient's life at risk. The multiple evidence which accumulated over time in studies across large batches of patients, concludes that important predictors of atheroma plaque stability are its composition and configuration. The risk of rupture of the atheroma plaques depends more on the composition of the plaque than on its volume. Most complicated plaques are soft, lipid-rich, with a thin fibrous hide and

an important inflammatory component. Small cracks remain clinically silent, but extensive ones have clinically and biologically significant resonance, with unstable angina, myocardial infarction, or sudden cardiac death. Also, the need for detection and classification of coronary lesions is outlined in order to stratify the risk of patients with ischemic coronary artery disease (known or presumptive) to develop fatal and non-fatal coronary events.

To assess the morphology of an atheroma plaque and to obtain information on the degree of vulnerability of coronary atherosclerotic plaque, a multimodal imaging approach may be adopted, consisting of modern techniques such as intravascular ultrasound, optical coherence tomography, coronary angioscopy, infrared spectroscopy,computerized multidetector tomography and magnetic resonance imaging. Early access to non-invasive (non-invasive) methods that highlight unstable atherosclerotic lesions would have a major impact on the prevention of acute coronary events, often accompanied by adverse long-term vital and prognostic risk.

Accurate quantification of atherosclerotic lesions is essential both for optimal therapeutic timing and choice of appropriate therapeutic strategy (interventional vs. surgical). When the therapeutic option is the minimally invasive technique, precise measurement of atherosclerotic lesions becomes even more necessary because in this case an inadequate assessment of the atherosclerotic substrate can be associated with serious complications such as a distal coronary dissection to the stent implant site or periprocedural myocardial infarction. Myocardial necrosis after implantation of the stent is possible, through multiple mechanisms such as occlusion of a collateral, incomplete coverage of the lesion of the stent, distal embolization of atherothrombotic material, all of which can lead to an unfavorable prognosis of the patient. In this context, the use of high sensitivity and specificity imaging techniques makes it possible to precisely quantify coronary degenerative changes, allow for the establishment of an appropriate post-procedural technique and monitoring.

In this study we hypothesize that lesion geometry has an impact on plaque vulnerability and could be associated with several well- established vulnerability features such as SC, LAP, presence of non-calcified plaques, and lipid-rich atheroma.

Previous postmortem studies demonstrated that plaque rupture and erosion occur more frequently in eccentric atheroma and launched the hypothesis of plaque eccentricity as a feature significantly associated with plaque vulnerability (496).

Costopoulos et al. (497) recently demonstrated that plaque rupture occurs in the regions exposed to increased plaque structural stress, which is determined by plaque composition, architecture, and geometry. In their study, the authors reported that plaque structural stress increases with lumen eccentricity ($r = 0.32$, $P = 0.001$). It has been suggested that hemodynamic forces acting on vascular endothelium can initiate the atherosclerotic process and a proatherogenic shear stress profile can be associated with distinct transformation of plaque phenotype toward increased vulnerability (498-500).

At the same time, endothelial shear stress is directly associated with plaque geometry, a baseline low endothelial shear stress being an independent predictor of substantially increasing plaque eccentricity in the study of Papafaklis et al (501) (odds ratio [OR]= 2.33, P =.003) and in the study of Puri et al. (502). This shows a complex inter-relation between plaque geometry, hemodynamic profile, and plaque vulnerability.

Andreini et al. (503) published a study which highlighted the long-term prognostic role

of multidetector CCTA in patients with suspected CAD. Even if there is a significant amount of data supporting the prognostic role of CCTA for crucial adverse cardiac events in the intermediate term, its long-term prognostic role in patients with suspected CAD was not completly studied. A considerable number of 1304 patients were analyzed with CCTA for detecting the presence and assessing extent of CAD, monitoring the disease extension and coronary plaque scores. Patients were divided in 2 study groups according to the presence of normal coronaries and nonobstructive (<50%) and obstructive (>50%) coronary lesions. The endpoints of the study were the composite rates of hard cardiac events and all cardiac events, including late revascularization. From the study were excluded 70 patients, their CTA data being uninterpretable. For the remaining patients, clinical follow-up (mean 52 ± 22 months) was obtained for 97%. In patients with normal coronary arteries, cumulative event-free survival was 100% for hard events and all kind of events. In patients with nonobstructive CAD, percentages were 88% for hard events, respectively 72% for all events. In patients with obstructive CAD, the results showed 54% for hard events and 31% for all events. Multivessel CAD was associated with a higher rate of hard cardiac events.

However, to the best of our knowledge, GEOMETRY will be the first CT-based study investigating a potential direct association between plaque geometry and vulnerability features. Our hypothesis is that plaque eccentricity, exposing the atheroma to an increased circumferential stress, may be responsible for plaque rupture. Coronary angiography by computerized tomography using state-of-the-art devices allows the assessment of not only the vascular lumen (occlusion or accurate quantification of stenosis) but also a non-invasive morphological evaluation of the presence and severity of coronary atherosclerosis. The possibility of identifying and evaluating the positive remodeling phenomenon is an indisputable advantage of this non-invasive imaging method. Positive remodeling atherosclerotic plates generally have a large lipid core, are rich in macrophages, both of which are attributable to vulnerable plaques. Also, the identification of positive remodeling can be used as a marker of vulnerable plaque.

Various recent studies reported the role of CT-derived features for characterization of the functional significance of a coronary plaque (504, 505). In a recent study, Kang et al (495) demonstrated that coronary lesion geometry has a direct impact on the functional significance of a coronary stenosis, showing that complex coronary lesions are independently associated with reduced fractional flow reserve (FFR) values and that lesion eccentricity is the most significant independent predictor for low FFR.

In the GEOMETRY study presented here, we extended this hypothesis to investigate for the first time the impact of lesion geometry on plaque vulnerability and the association between geometric distribution of atheromatous plaques and the related risk of future cardiovascular events.

GEOMETRY will be the first CCTA-based study that will investigate the impact of geometric distribution of coronary atheromatous plaque on the future risk of cardiovascular events and on the rate of plaque progression, introducing and validating a new potential feature of plaque vulnerability represented by plaque geometry.

SECTION II
FUTURE PROJECTS IN THE PROFESSIONAL, ACADEMIC AND SCIENTIFIC FIELD

II.1. Perspectives in the academic activity

Until now, I have held internal medicine and cardiology lectures for medical students, as well as lectures on cardiovascular rehabilitation for students of the faculty of Medical Bioengineering. My interest in providing quality information for medical students and internal medicine trainees is supported by a strong belief that remarkable teaching can lead to a more profound understanding of cardiovascular conditions and their management. I have ensured that both students and trainees have access to relevant material regarding the pathomechanisms, assessment, and treatment of cardiovascular diseases. In 2012, the release of the book „Arithmology in daily practice“ marked the beginning of my endeavor to supply the local student bodies with updated textbooks. There followed other books, either as author of chapters or as a member of the team of authors of many prestigious treaties, of which I mention: Medical Therapeutics (released in 2014), Compendium of Internal Medicine (released in 2014), Pain therapy – current issues (released in 2014), New concepts in gastroenterology and hepatology (released in 2016), Interdisciplinary approach to HIV infection (released in 2018), Certainties and controversies in infectious pathology (released in 2018), Thrombosis and antithrombotic therapy (released in 2018).

Improving my teaching system as well as the medical students' and trainee doctors' learning strategy represents a priority. The main perspectives in my academic work involve the following:

- Creating an online platform with shared information (images, case reports, articles, and textbooks) available for medical students and the trainees in our center, with the possibility of introducing a new evaluation method (interactive case reports with multiple choice questions);
- Updating the content of lectures in order to provide the newest relevant and reliable information on cardiovascular disease management (evaluation, diagnosis, treatment, protocols);
- Releasing new and updated editions of the internal medicine textbooks;
- Acquiring anatomical teaching models (flexible heart models, ultrasound training models, cardiopulmonary resuscitation) and anatomical software;
- The introduction of internal medicine and cardiology flash cards (questions and answers) and infographics on specific topics (clinical evaluation, overview of certain cardiovascular conditions, therapeutic difficulties, rehabilitation);
- The implementation of the "Mind Mapping" learning approach (a new concept which involves the visual organization of up-to-date information in the form of an interactive tree-like structure);
- Further strengthening the relationship between the academics in my center by involving them in different projects and by encouraging them to form a team in order to obtain an effective teaching strategy, unique to our center;

- Setting clear and reachable goals for students, trainee doctors, and academics, as well as encouraging teamwork;
- Organizing workshops, debates on specific topics (ex. „Involvement of the heart in autoimmune pathology“) and clinical case report presentations with students and trainee doctors;
- Encouraging students and trainee doctors to present their work in congresses;
- Assisting 4th year medical students in writing their final thesis;
- Inspiring and supporting internal medicine trainees to participate in the annual sessions of the Internal Medicine Summer School.

II.2. Perspectives in the scientific activity

So far, my scientific work focused both on cardiovascular conditions, as well as associated comorbidities. I have created a small but effective research team in my center, while also cultivating relationships and participating in projects with other medical specialities (gastroenterology, diabetes and nutrition, rheumatology, immunology, neurology, anaesthesia and intensive care medicine). Our center was also involved in several clinical projects, in an effort to relieve patients suffering and contribute to the growth of medical knowledge.

Moreover, I am interested in supplying our center with the newest available devices required for the diagnosis and monitoring of cardiovascular conditions (electrocardiography, echocardiography, speckle contrast imaging system, medical thermograph, bioelectrical impedance device and others). I expect to do so by forming reliable partnerships with the administrative departments of the „Grigore T. Popa“ University of Medicine and Pharmacy and that of the „Sf. Spiridon“ Emercency Clinical Hospital, as well as by accessing further funding (grants, projects).

In general, the future perspectives of my scientific work include the following objectives and activities:

- Encouraging an interdisciplinary approach to cardiovascular pathology;
- Conducting further research in the field of autoimmunity (clinical and immunological aspects in ischemic heart disease, heart failure, cerebrovascular disease);
- Conducting studies on the clinical correlates of inflammaging and immunosenescence;
- Carrying out research on pain perception and optimal management, pain medication consumption among cardiovascular pathology;
- Collection and analysis of scientific data related to the health-related quality of life of heart failure patients;
- Additional research on the impact of biological therapy in internal medicine and cardiology;
- Testing the biopsychosocial approach to the assessment of patients with cardiovascular conditions;
- Accessing international data bases to optimize the management of patients with cardiovascular disease;
- Encouraging the participation in congresses and the publishing activity of my

research team, in order to disseminate relevant data and contribute to the growth of scientific knowledge;

- Cultivating the relationship between my center and the Department of Biostatistics of the „Grigore T. Popa“ University of Medicine and Pharmacy, in an effort to obtain reliable results and a correct reporting of the team's findings;
- Further creating interdisciplinary scientific teams in order to give response to multifaceted medical issues;
- Attracting clinical studies in our center;
- Emboldening my team to engage in projects or apply for temporary positions (internships) in other centers in order to strengthen their knowledge and acquire new skills;
- Further providing PhD students with the resources and support they require, and encouraging them to participate in congresses, presenting their work;
- Accessing additional funding for my team's research activities (analysis kits, devices, access to information, participation in congresses);
- Engaging students and trainee doctors in scientific activities in order to familiarize them with research-related aspects such as data collection and analysis, article writing, as well as thorough literature review;
- Establishing a common strategy with the research team and assigning projects according to the members' main strengths;
- Working on a schedule (time management - setting clear objectives and deadlines);
- Supervising and ensuring the compliance with the ethical and deontological aspects of clinical research conducted in my center;
- Preserving a transparent approch toour research in order to secure scientific integrity.

In particular, in the light of my previous achievements and based on the significant expertise that I encountered in the field of inflammation-oriented research, my plan for further scientific development includes the following 4 main directions of research:

The link between inflammation and acute coronary syndromes

The link between inflammation and cardiovascular diseases is well known, being proved that an increased inflammation could be responsible for progression of the atherosclerosis process. However, the role of inflammation in triggering an acute coronary event has not been elucidated so far. It is not clearly demonstrated in present whether inflammation is a trigger for atheromatous plaque vulnerabilization, plaque rupture and acute myocardial infarction, or it is rather the consequence of the myocardial infarction, being well known that in the following days after the infarction there is an increase of inflammation, triggered by the acute event. At the same time, an exacerbated inflammatory response in the next phase is responsible for enhancing myocardial repair after ischemic injury.

One of my main scientific goals is to unravel the mechanisms via which inflammation could contribute to an increased risk of acute myocardial infarction in patients with atheromatous coronary plaques. To achieve this goal, I plan to develop a research project in cooperation with several cardiology centers from the entire country.

Within this project, we will study the inflammatory biomarkers from the systemic circulation (hsCRP, interleukins, tumour necrosis factor) in parallel with adhesion molecules

(V-CAM, I-CAM, etc) in patients with acute coronary syndromes (unstable angina or recent myocardial infarction).

My aim is to develop a panel of specific biomarkers that could characterize inflammatory responses directly associated with an increased risk for atheromatous plaque rupture, in order to provide the clinician a useful prediction tool that would help to identify patients at risk for acute myocardial infarction. This would provide a novel diagnostic system for identifying vulnerable patients.

The role of systemic inflammatory diseases in cardiovascular diseases

An exacerbated systemic inflammation could represent a significant risk factor for atherosclerosis progression. Many diseases that associate increased inflammation have been associated with an increased frequency of acute coronary syndromes. However, the exact mechanisms involved in the complex pathway from inflammation to atheromatous plaque formation or even rupture are still under investigation. The goal of this research direction that I plan to develop in a close future is to study the correlations between several diseases characterized by increased inflammation, cardiovascular risk and severity of cardiovascular diseases.

Among the diseases that I plan to study I mention:

- Parodontal diseases, in which bacteria located in the parodontal pockets release in systemic circulation several inflammatory mediators leading to enhanced systemic inflammation.
- Systemic sclerosis, a diseases characterized by increased systemic inflammation.
- Alteration of gut microbiota, in which inflammatory mediators originating from the perturbation of gut microbiota are released in the systemic circulation determining a complex systemic inflammatory response.

In order to elucidate the link between these diseases and cardiovascular diseases, I plan to enroll a large number of patients in 3 different studies (one study for patients with parodontal disease, one study for patients with systemic sclerosis and one study for patients with alteration of gut microbiota, each of them with the corresponding control lot), and to study the incidence of traditional or surrogate markers of atherosclerosis or cardiovascular risk in the group of patients versus controls. Of major interest will be also the determination of inflammatory biomarkers (hs-CRP, interleukins, tumor necrosis factor, periostin) in patients with above mentioned conditions who have already developed atherosclerosis, as demonstrated by presence of atheromatous plaques at coronary angiography, cardiac computed tomography or carotid ultrasonography.

Epicardial adipose tissue and inflammation - a major player in cardiovascular diseases

In the recent years, many publications have described the role of epicardial adipose tissue as a depot of inflammatory mediators. It seems that epicardial adipose tissue represents an active organ, releasing inflammatory factors in the systemic circulation. The hypothesis of my third research direction is that various cardiovascular diseases are associated with an increased amount of epicardial adipose tissue, that could play a significant role in their progression. Such diseases include coronary arterial disease, hypertension, heart failure or various types of cardiac arrhythmia.

In order to elucidate this hypothesis, I plan to develop a research project in common with several centers highly specialized in cardiac imaging, and to study the link between epicardial fat volume (determined by routine echocardiography or by more advanced imaging techniques, such as cardio CT or cardiac MRI), and severity of the cardiovascular disease in patients with the above-mentioned diseases. At the same time, in patients with documented atheromatous plaques in the coronary arteries, I plan to study the reliability of different representations of epicardial fat accumulation: global epicardial fat, pericoronary epicardial fat and periplaque epicardial fat (the one located in the immediate vicinity of a coronary plaque), for predicting the vulnerability degree of the coronary atheroma and the consequent risk for cardiovascular events.

The link between epicardial fat and atherosclerosis will be further studied taking into account the possible inflammatory biomarkers via which the epicardial fat could influence the systemic vulnerability, such as hsCRP, interleukins, matrix metalloproteases and also adhesion molecules.

Perivascular adipose tissue in atherosclerosis

Another research direction will be represented by the study of perivascular adipose tissue, represented by the fat accumulated around the major arteries, and its role in development of atherosclerotic process at this level. This intriguing hypothesis is very new in this field and the potential role of perivascular fat in the progression of systemic atherosclerosis has not been elucidated so far. In order to test this challenging hypothesis, I will develop a complex algorythm for investigating the cardiovascular patient, including ultrasound at the major vessels (carotid and iliofemoral arteries), in order to correlate the amount of perivascular fat with the traditional markers of atherosclerotis at this level (intima-media thickness, plaque volume, remodeling index) and with the systemic inflammatory biomarkers (hsCRP, etc) and with their progression in time.

SECTION III. REFERENCES

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