GRIGORE T. POPA UNIVERSITY OF
MEDICINE AND PHARMACY IASI

Habilitation Thesis

Associate Professor
ELENA REZUȘ, MD, PhD

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Researches regarding inflammation and rheumatological diseases

- Habilitation Thesis -

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Contents

ABSTRACT

The habilitation thesis entitled "Researches regarding inflammation and rheumatological diseases" presents my clinical research in the field of inflammation and rheumatology, 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 135 bachelor degree thesis and 5 master 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 five sub-sections, each of them having at least three chapters. In this section I have included the main ideas and results of 27 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 rheumatic diseases in general, than I have continued with the most frequent inflammatory rheumatic diseases.

Sub-section *I.1. Researches regarding inflammation and inflammatory rheumatic diseases* starts with a short introduction containing the main theoretical ideas about inflammation and rheumatic diseases. Then, a review about inflammation have been synthesized for a good understanding of the processes. *Implications of peroxide radicals in the inflammatory rhematic disease* is the title of the next sub-chapter and contains literature data and the main ideas from two reviews. Two published papers have been introduced in the sub-chapter *Cardiovascular risk in chronic inflammatory rheumatic diseases* and another one in *Tuberculosis Infection in Rheumatic Diseases* (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.

I.2. Researches regarding rheumatoids arthritis is the second sub-section of section I and has one theorethical sub-chapter and five sub-chapters with information from seven articles, 5 articles rated by Thomson ISI Web of Science Core Collection and 2 articles indexed by international databases. Informations about the factors that may influence this disease, the treatment received from the patients affected by rheumatoid arthritis, the impact of

proinflammatory cytokines of rheumatoid polyarthristis, the cervical spine lesion of the patients and the link between rheumatoid arthritis and periodontal diseases.

I.3. Researches regarding ankylosing spondylitis is the third sub-section of my thesis and offers important informations about some molecular biology techniques involved in ankylosing spondylitis diagnosis, the connection between inflammatory bowel diseases and ankylosing spondylitis and neurophysiological abnormalities reported in patients with ankylosing spondylitis: evoked potentials visual evoked potentials (VEPs) and brainstem auditory evoked potentials (BSAEPs). This sub-section synthesizes meaningful data from 2 articles rated by Thomson ISI Web of Science Core Collection, 2 articles ISI proceedings and 2 articles indexed by international databases.

I.4. Researches regarding other rheumatic disease: systemic scleroderma, systemic lupus erythematosus and gout highlights important details about the three mentioned diseases in the title of this sub-section, bringing together the results of 6 articles, 4 rated by Thomson ISI Web of Science Core Collection and 2 articles indexed by international databases. Gout has been treated together with another interesting theme for 21st century in the field of medicine: the metabolic syndrome.

The last sub-section *I.5. Researches regarding the relationship between plantar pressures of the elderly* includes, after a short presentation of literature data about this theme, data from two ISI proceedings articles with interesting results. A classification of the ederly foot type based on plantar footprints has been first made and then a comparative anthropometric study regarding the foot of the ederly female population has been achieved.

Section II include 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 include a number of 250 bibliographic 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 hadn’t found solutions yet.

REZUMAT

Teza de abilitate "Cercetări privind inflamația și bolile reumatologice" prezintă activitatea mea clinică și științifică în domeniul inflamației și reumatologiei, precum și alte subiecte legate de aceste două domenii medicale principale.

Într-o primă etapă, pentru redactarea acestei teze a fost studiat 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;
- Section 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 implicată și rezultatele activității mele academice, materializate în 135 teze de licență și 5 teze de masterat. 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 cinci subcapitole, fiecare având cel puțin trei capitole. În această secțiune am inclus principalele idei și rezultate a 27 de 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 reumatismale, în general, continuând cu cele mai frecvente boli reumatice inflamatorii.

Sub-secțiunea I.1. *Cercetări privind inflamația și bolile reumatismale inflamatorii* începe cu o scurtă prezentare care conține principalele idei teoretice despre inflamație și bolile reumatismale. Apoi, pentru o bună înțelegere a proceselor, am sintetizat un review despre inflamație, al cărui autor sunt. *Implicațiile radicalilor peroxizi în boala reumatismală inflamatorie* este titlul următorului subcapitol și conține date din literatură și principalele idei din două articole proprii. Două lucrări publicate au fost introduse în subcapitolul *Risc cardiovascular în bolile reumatismale cronice inflamatorii* și un altul în subcapitolul *Tuberculoza în bolile reumatismale* (indexat 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 artrita reumatoidă este al doilea subcapitol al secțiunii I. Are un subcapitol teoretic și cinci subcapitole cu informații din șapte articole, 5 articole indexate ISI și două articole indexate în baze de date internaționale. Informații despre factorii care pot influența această boală, tratamentul primit de la pacienții afectați de artrita reumatoidă, impactul citokinelor proinflamatorii în poliartritei reumatoidă, leziunea coloanei cerviale la pacienți având această afecțiune și legătura dintre artrita reumatoidă și bolile parodontale.

I.3. Cercetările privind spondilita anchilozantă este a treia sub-secțiune a tezei și oferă informații importante despre unele tehnici de biologie moleculară implicate în diagnosticarea spondilitei anchilozante, legătura dintre bolile inflamatorii intestinale și spondilita anchilozantă și anomaliile neurofiziologice raportate la pacienții cu spondilită anchilozantă: potențiale evocate (VEP) și potențialul evocat auditiv (BSAEP). Această sub-secțiune sintetizează date semnificative din 2 articole indexate ISI, 2 articole ISI proceedings și 2 articole indexate în baze de date internaționale.

I.4. Cercetările privind alte boli reumatismale: sclerodermia sistemică, lupusul eritematos sistemic și guta evidențiază detalii importante despre cele trei afecțiuni menționate în titlul acestei sub-secțiuni, reunind rezultatele a 6 articole, 4 indexate în Thomson ISI Web of Core Collection și 2 articole indexate prin baze de date internaționale. Guta a fost tratată împreună cu o altă temă interesantă pentru secolul 21 în domeniul medicinei: sindromul metabolic.

Ultima sub-secțiune *I.5. Cercetările privind relația dintre presiunile plantare ale vârstnicilor* include, după o prezentare succintă a datelor din literatură despre această temă, date din două articole ISI proceedings cu rezultate interesante. Inițial a fost făcută o clasificare a tipului de picior al vârstnicilor pe baza amprentelor plantare și apoi a fost realizat un studiu antropometric comparativ cu privire la piciorul populației de gen feminin.

Secțiunea II include proiecte mele viitoarele în domeniul profesional, academic și științific. În ceea ce privește activitatea profesională, scopul meu principal este îmbunătățirea calității îngrijiriilor 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 250 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, care încă nu au găsit soluții.

OVERVIEW OF PERSONAL PROFESSIONAL, ACADEMIC AND SCIENTIFIC ACHIEVEMENTS

The habilitation thesis entitled “Researches regarding inflammation and rheumatological diseases” presents my clinical research after the achievement of my PhD thesis entitled “Reactive arthritis-Aspects of the inflammatory process”, under the coordination of Professor Stefan Dumitriu, which was confirmed by the Ministry of Education (Diploma No. 3951/15.04/2001).

The approach in my doctoral thesis was the concept of "reactive arthritis" in terms of inflammation - the most common pathogenic process in rheumatic diseases and also in terms of nosological entity in a variety of clinical forms. In briefly, the general part provides important data on the etiology of reactive arthritis presented in relation with the entrance gate, and the pathogenesis explaining the role of genetic field, the gateway, immunity and infection outbreak. Also, there are presented diagnostic methods of reactive arthritis, especially highlighting the clinical and laboratory features depending on the microbial agent trigger, and the principles of treatment: symptomatic, curative, background therapy, anti-infective, perspective and recovery ones. The personal part, in addition to complex multidisciplinary study of the 150 patients enrolled, is materialized by conducting the first study of oxidative metabolism in reactive arthritis, using 4 antioxidant systems: erythrocyte superoxide dismutase, reduced glutathione, glutathione peroxidase and lipid peroxides. The survey reveals that changes in oxidative metabolism in arthropathy are convergent with clinical manifestations and offers new exploring possibilities of inflammatory syndrome in reactive arthritis. It confers a deeper understanding of the concept of reactive arthritis, recommending a diagnostic algorithm for both rheumatologists as well as for other specialties facing osteoarticular pathology in localized or systemic infection.

Presently, I am associate professor at the Rheumatology. Rehabilitation, Physical Medicine and Balneology Discipline, 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 rheumatology and in the field of recovery, physical medicine and balneology at one of the largest hospitals in the Moldavian region, Iasi Rehabilitation Hospital. Also I am the head of the 1st Rheumatology 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 1992 “Grigore. T. Popa” University of Medicine and Pharmacy Iasi, Romania, Faculty of General Medicine (Diploma No. 1500/no.25/23.10.1992), and next year I have started the training program required to qualify as a rheumatologist.

I have finished this program and get my professional experience working in the field of Rheumatology in 1996 and in the same year I have become specialist physician (Certificate No. 2707/18.12.1996/ Adress No.83/7.01.1997). In 2001, I have become senior physician in Rheumatology (OMS 538/07.08.2001/Adress 8504/21.08.2001).

In 2002, I have finished my second speciality becoming specialist physician in Recovery, Physical Medicine and Balneology (OMS 1024/2002; Certificate Serial Number A No. 042564/2002/, 14923/12 2002). Because my second speciality has a strong connection with my principal one, I have continued the activity in this field and in 2017 I became senior physician in Recovery, Physical Medicine and Balneology (OMS 988/30.08.2017; Certificate No. 14018/30.08.2017). I have often faced diseases of endocrine-metabolic bone pathology, so I have completed my professional activity with a complementary specialization in this field (Certificate C No. 013740/2, 2004/15470) and I have been also certified as Clinical Densitometrist (CCD), by The International Society for Clinical Densitometry – ISCD (September 30, 2006 – September 30, 2011).

I think it is important to mention that since 2010 I have been the head of the 1st Rheumatology Department from Iasi Rehabilitation Hospital (Decision No. 368/01.11.2010). A course on General Management at the "Grigore. T. Popa" University of Medicine and Pharmacy Iasi, Romania (Certificate E No.0007596/1347/11.07.2006) 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 (Prague, Czech Republic, Split Croatia, Genoa Italy).

I am an active member in important Scientific Societies: Romanian Society of Rheumatology, Romanian Society of Internal Medicine, Romanian Association of Algesiology, International Association for the Study of the Pain, Romanian Society of Rehabilitation and Physical Medicine, Society of Physicians and Naturalists. Very important to mention is the fact that I am coordinator of EUSTAR center no. 202 (The European Scleroderma Trials and Research group).

Academic activity

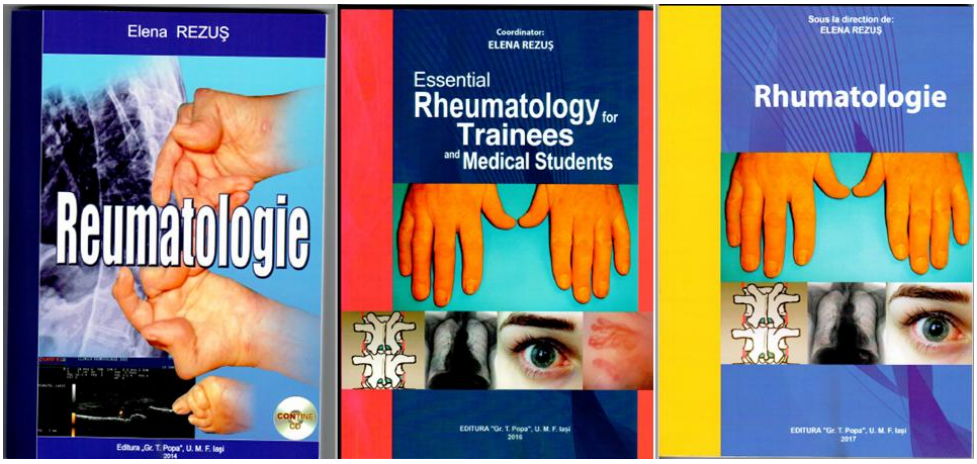
My teaching career has been started in 1992, as Junior Teaching Assistant, position held by competitive examination, at "Grigore. T. Popa" University of Medicine and Pharmacy, Iași, Faculty of Medicine, Physiotherapy – Rheumatology Department.

In 1995 I have advanced to University Assistant. I have continued my activity in the department were I started my academic activity but I also started to have didactic activities at the Faculty of Balneo-Physio-Kinesiotherapy and Medical Recovery, form the same University. In 2003 I became Lecturer and in 2015 Associate Professor.

I am often member in different commissions: admission commission of bachelor programs and master programs, commission for the specialist/ senior physician, member in commissions for the presentation of scientific reports within doctoral thesis, member in commissions for didactic contests for graduate assistant, 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 21 postgraduate courses.

Since 2002 I have coordinated an impressive number of 135 bachelor degree thesis, 19 of them in English and 5 master degree thesis. To help my students understanding some aspects concerning the complex and enigmatic field of rheumatology I have publish a book about this branch of medicine in three different editions, as principal author, each of them in a different language: Romanian, English and the last one in French. Also, I have been author at 5 more books together with other colleagues, and also some book chapters.



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 my PhD thesis I have studied reactive arthritis and aspects of the inflammatory process. I have continued my research with the study of inflammation and arthritis, because this field is not yet fully understood, is a topic of huge interest in the medical field, since arthritis greatly affects the elderly and in the last decade world's population is ageing.

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 23 articles rated by Thomson ISI Web of Science Core Collection, 81 articles indexed by international databases, 81 abstracts rated by Thomson ISI Web of Science Core Collection and 55 abstracts indexed by international databases. These articles have a total of 190 citations in Google Scholar.

I have participated at 40 International Conferences/Congress, 124 National Conferences/Congress and 52 Local Conferences/Congress.

During 2006-2008, I was the coordinator 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.

Between 2010 and 2016, I was part of the team of 3 different projects:

- *Project PN-II-PT-PCCA-2013-4 - Partener 1 - Complex formulations based on liposomes and cyclodextrin for transdermal pain therapy (NANODERMA), 2014-2016* – the project was part of the efforts of the scientific community to improve percutaneous pain therapy by developing new gel formulations with topical application for transdermal controlled release of drugs induced by improved bioavailability with beneficial effect on pain control. The main purpose was to improve the functional model, the methods of preparation and the effectiveness of the use of analgesic gel formulations with topical application.
- *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.*

Important to mention is the fact that I was part at 18 clinical trials, some of them complex international trials, involving patients form the whole world. For example, the results of one of this international trial have been highlighted in an article with 43 citations in Google Scholar. The main ideas of this article will be presented in section I, chapter I.4.

SECTION I
SCIENTIFIC ACHIEVEMENTS FROM THE POSTDOCTORAL PERIOD

I.1. Researches regarding inflammation and inflammatory rheumatic diseases

I.1.1. Introduction

Rheumatic diseases are defined as chronic inflammations of joints, muscles, tendons, ligaments and bones, with clinical correspondence of swollen, redness, stiffness, warmth and pain (1), about 5% of the population worldwide suffers from a chronic inflammatory rheumatic disease . Over 100 entities rheumatic diseases are identified, however their etiopathology is complex and still incompletely clarified (2). In rheumatic diseases, a wide range of disorders is involved. In the literature there are many classifications of these diseases, one of the simplest methods of classification being based on their nature. Hence rheumatic diseases can be divided into autoimmune, autoinflammatory, and degenerative/metabolic disorders (3).

Autoimmune rheumatic diseases constitute a mixed group of conditions characterized by immune tolerance breakdown and production of auto-antibodies and of a number of substances responsible for lesions in several body structures. In the case of autoimmune rheumatic diseases joints and muscles are mainly affected, the most common diseases are: systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), inflammatory myopathies, systemic sclerosis (SSc), systemic vasculitis, Sjögren's syndrome (SS) and so on (4,5).

During the last decade, knowledge about the pathophysiological background of rheumatic diseases has significantly increased, especially of the immune-mediated inflammatory diseases (6). As mentioned above, this classification is based on their nature, many other classifications being made, because in fact inflammation contributes to all nontraumatic musculoskeletal diseases, both orthopaedic and rheumatic diseases, leading to pain and disability in millions of people worldwide. Inflammatory processes play a very important role in tissue integrity and homeostasis, the processes of healing and recovery after traumatic injuries, surgical procedures, infections and other adverse stimuli being driven by inflammatory pathways and mediators (7).

In pathology, the concept of rheumatic inflammatory diseases was introduced in the 5th decade in order to define a few diseases with vascular system and connective tissue injuries and damage to the internal organs (kidneys, heart, lungs and spleen). This group of diseases includes Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Systemic Scleroderma (SS), Dermatomyositis, Polyarteritis, Systemic Vasculitis and mixed connective tissue disease. The causes of rheumatic inflammatory diseases are not yet fully understood. In the evolution of the lesions we can distinguish the following physiological phenomena: aberrant immune response – hyper-γ-globulinemia, high titers of circulating autoantibodies, autoimmune vasculitis and fibrinoid degeneration of connective tissue. Clinically, most diseases evolve with multiple manifestations at both a mucocutaneous and a musculoskeletal level as well as at the level of other internal organs such as the cardiovascular, pulmonary, renal, nervous, ocular systems, etc (8).

I.1.2. Researches regarding the role of chemical and molecular biology in inflammation

Published paper in this field

Macovei L. A. , Birsan M., Teodor V. I., Cristofor A. C., Ioanid N., **Rezus E.**, *On the Role of Chemical and Molecular Biology in Inflammation Research,* Rev. Chim. (Bucharest), 68 (4), 2017, 786 – 788.

As specified above, most rheumatic diseases have an inflammatory cause, and for a good understanding of inflammatory processes at the chemical level and at the level of molecular biology can be a real help in the stages of diagnosis and treatment of the mentioned diseases, important information about this subject being summarized in this review. The stages of inflammation and the development of inflammation research are the main subjects of the review.

Stages of inflammation

Inflammation is one of the most widespread pathological processes underlying many diseases that differ in their clinical presentation. The inflammatory response was first described by Paracelsus and it included the following stages: swelling (tumor), fever (calor), change in color (rubor), pain (dolor) and functional impairment of the tissue (functio laesa).

In normal conditions, the inflammatory response is tightly controlled by the body, but the excessive inflammatory response by itself can lead to diseases (e.g. atherosclerosis and rheumatoid arthritis). The causes of inflammation are extremely varied. The inflammation occurs mostly as a result of the action of various exogenous factors, such as infectious agents (bacteria and toxins), mechanical forces (impact or injury), thermal damage (burns) or chemical agents (the effects of strong acids or bases). Endogenous causes of inflammation are represented by tissue necrosis, thrombosis, infarction, extended hemorrhage, salt deposits and certain disturbances in the trophic function of the nervous system (symmetrical centrogenous inflammations) (9, 10).

Inflammation has three main stages, which are closely related to each other and evolve simultaneously:

- tissue dystrophy (alteration);
- impairment of blood circulation (transudation of fluid and migration of leukocytes);
- multiplication of cellular elements (proliferation).

Dystrophy is a metabolism and nutrition disorder of tissue function and structure, which is more evident in the area where the tissue was exposed to the harmful agent. The alteration of the physicochemical properties of the inflamed tissue leads to changes of tissue colloids and especially to changes in proteins. Their degree of dispersion and their ability to attract and retain water lead to an increase in coloidosmotic pressure or in the oncotic pressure of tissue colloids. To outskirts outbreak inflammatory oncotic pressure gradually decreases towards the periphery of

the inflammatory site. Phenomena related to increased metabolism and their subsequent physicochemical changes are found in the inflammatory area, such as accumulation of ions (increased concentration of hydrogen ions – hyper-ionic); increased osmotic pressure (hyper-tonic); increased oncotic pressure (hyper-oncotic). All these changes are the result of the trophic disorders of the tissues and they influence the magnitude of changes in the cells of the inflamed tissue.

Disorders of blood circulation are seen right from the beginning, as a reflex response, due to the effect of inflammatory agents on the receptors from the damaged area. A brief spasm occurs. The vascular spasm and subsequently the pallor of the damaged tissue area are the result of the excitation of vasoconstrictor nerves. This stage is followed by vessel dilation (arterioles and capillaries) and increased capillary bed volume, leading to an increased inflow of blood into the inflamed tissue area. Vessel dilation, which is influenced by various factors, is initially caused by a reflex following the action of a harmful agent. The increase in the concentration of hydrogen ions is also very important. Products of metabolism and products of tissue injury exert the most intense vasodilation. Changes in blood vessels of the inflamed tissue area occur immediately after the blood flow slows and stasis develops.

Proliferative or productive processes are slightly manifested phenomena of multiplication of cell elements. They occur almost simultaneously with the alteration processes found in the periphery of the inflammation area. These phenomena are highly active in the later stages of the inflammation. The inflammatory agent and the products generated by disintegration and disturbed metabolism, which accumulate in tissues, play an important role in developing proliferation (11).

The development of inflammation research

In the early period of scientific study of pathology, Rudolf Virchow and Julius Cohnheim had elaborated two theories related to the process of inflammation.

According to *Virchow (Cellular pathology, 1858),* inflammation was in essence an increase in the biological activity of cell parts that begin to show an intense nutrition and multiplication based on the liquid component of blood (the so called nutritional irritation), in response to excitation of tissue. Virchow explained the emergence of an increased number of cells in the inflammation area as an increase in local tissue elements (12).

Suppurative exudate consisted of elements removed from the tissue. Other phenomena observed during inflammation, such as vascular or exudative processes, played a secondary and subordinate role. All the inflammatory processes fell into two categories: interstitial inflammation, with predominant manifestations of the connective stroma and parenchymal inflammation, with manifestations of the specific elements of the organ. The concomitant existence of altering, exudative and proliferative processes in the affected tissue area was characteristic to the inflammatory reaction.

The vascular theory of Cohnheim (1885) places special emphasis on the vascular reaction that occurs during inflammation. The increase in vessel permeability, slowing of blood flow and local increase in blood pressure, determine exudation and migration, which, according to this

theory, occur passively. On the other hand, changes found during inflammation in the tissue itself are of minor importance. The reflex influences were not considered directly related to the occurrence mechanism of the inflammatory process. According to this theory, even the inflammatory process of the avascular tissues is determined by vessels, albeit from neighboring tissues, from which the solid components of blood migrate towards the site of action of harmful agents (13).

After the infectious agents of inflammation were discovered**,** *the biological theory on the process of inflammation occurred (1892)*. It revolutionized the concepts related to the mechanisms of the inflammatory reaction. The main role in inflammation was assigned to white blood cells, phagocytes that are involved in all inflammatory processes. This is when the method of compared pathology was first used. A reasonable interpretation of the origin of inflammation and the research of this process by using phylogeny proved to be extremely valuable (10).

The process of nutrition, i.e. embedding and digesting harmful agents, underlies the inflammatory response. In organisms with complex structure, cells of mesenchymal origin such as microphages and macrophages play this phagocytic function. Granulocytes migrated from the blood vessels into the inflammation area fall into the category of macrophages. They are able to incorporate and digest mostly microbes. Macrophages are large amoeboid cells, which are formed from the tissue cells, the so-called leukocytoid cells, during the inflammation process. They are able to incorporate and digest particles resulting from cell disintegration and even erythrocytes. Phagocytes help the body to dispose of harmful substances that permeate it. This is the defense mechanism of the inflammatory reaction, which developed as part of evolution and natural selection. All other phenomena observed during inflammation (e.g. vascular reaction) are only of secondary importance, as they help phagocytes to enter the inflammation area.

The *evolutionary theory on inflammation based on Darwin's theory* lasted for many decades and determined the development of the theory on inflammatory and the immune status. The method of comparative pathology helped to explain the role of inflammation as an adaptation and defense mechanism and also to associate the inflammatory reaction with the immune response. Although inflammation is not fully explained by this theory, the evolutionary concept is still very important, as it explains the role of the body's adaptation mechanism in the formation of an inflammatory response. Furthermore, the biological theory is the starting point of the modern theory on the role of mesenchymal tissues in inflammation.

The *phagocytosis theory* explained for the first time the interdependence between the inflammation area and the body seen as a whole. Inflammation was considered not only a local response, but also a general reaction of the body against the action of a harmful agent. It was the phagocytic theory that moved the focus of research towards changes in hematopoiesis and blood composition during inflammation and towards the influence exerted by the immunological properties of the organism on the development of the inflammatory process.

The emergence of physical chemistry and biochemistry and the use of their achievements in the field of pathology have enabled a deeper understanding of the inflammatory process. A physical chemical orientation was used in the research of inflammation, leading to concepts based on a *physical chemical approach of inflammation (Schade).*

The physical chemical orientation in the study of inflammation has explained several aspects playing a role in this process. This theory even proposed a link between different phenomena observed during the process of inflammation. But the question remains, if the physicochemical changes represent the primary contributing factor to inflammation. All the physicochemical changes characteristic to the inflammation process can be described only if the inflammatory process has already occurred. Therefore, these observations are secondary. Physicochemical theory has another major limitation in explaining inflammation in that it tries to subject the inflammatory process to the laws of physical chemistry and colloidal chemistry. This mechanistic approach ignores the biological aspect of the inflammatory response.

According to the so-called *vasomotor theory*, the rigorous limit between inflammation and noninflammatory vascular disorders disappears. By emphasizing the crucial role of vasomotor nerves in the inflammatory process, this theory could not provide a qualitative description of the inflammatory reaction as a whole. Some research highlighted the role played in inflammation by disturbances of the trophic function of the nervous system, in addition to changes in the modulation of the vascular lumen. When acting on the tissue, the harmful agent induces a reflex response in the form of disorders of tissue metabolism, such as dystrophy and acidosis, with all their consequences. The importance of trophic reflexes in the development of the inflammatory process is also proved from the fact that their abolition prevents or mitigates inflammation. The argument that the inflammation may occur even in enervated tissues was brought against the neurogenic theory.

I.1.3. Researches regarding the link between inflammaging and degenerative joint diseases

A. Background

Over time, it has been demonstrated that aging is an important contributing factor to the development of osteoarthritis (OA). The mechanisms responsible involves a multitude of factors and may include inflammaging. Age-related inflammation can be both systemic and local (14).

Articular cartilage, a highly specialized connective tissue of diarthrodial joints, provides a smooth, lubricated surface for articulation and facilitates the transmission of loads with a low frictional coefficient. Unlike most tissues, blood vessels, lymphatics, and nerves are absent from the structure of cartilage. Its structure is represented by a dense extracellular matrix – ECM (composed of water, collagen, and proteoglycans, with other noncollagenous proteins and glycoproteins) with a sparse distribution of highly specialized cells called *chondrocytes*. This tissue is subject to a harsh biomechanical environment. Because articular cartilage has a limited capacity for intrinsic healing and repair, its preservation and health are paramount to joint health. Cartilage components help to retain water within the ECM, which is critical to maintain its unique mechanical properties (15).

B. Published paper in this field

Rezuș E., Cardoneanu A., Burlui A., et al. The Link Between Inflammaging and Degenerative Joint Diseases. Int J Mol Sci. 20 (3), 2019, 614.

Aging process is associated with the appearance of various pathologies such as frailty, atherosclerosis, Alzheimer's disease, sarcopenia, type 2 diabetes, or osteoporosis, all these conditions having a common pathogenic mechanism characterized by the presence of a low-grade proinflammatory status (16, 17). The term *inflammaging* was first used in 2000 by Franceschi and can be defined as all the processes that contribute to the occurrence of various diseases associated with aging, representing 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 (18).

I.1.3.1. 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, with the main changes being represented in figure I.1.1. (19).

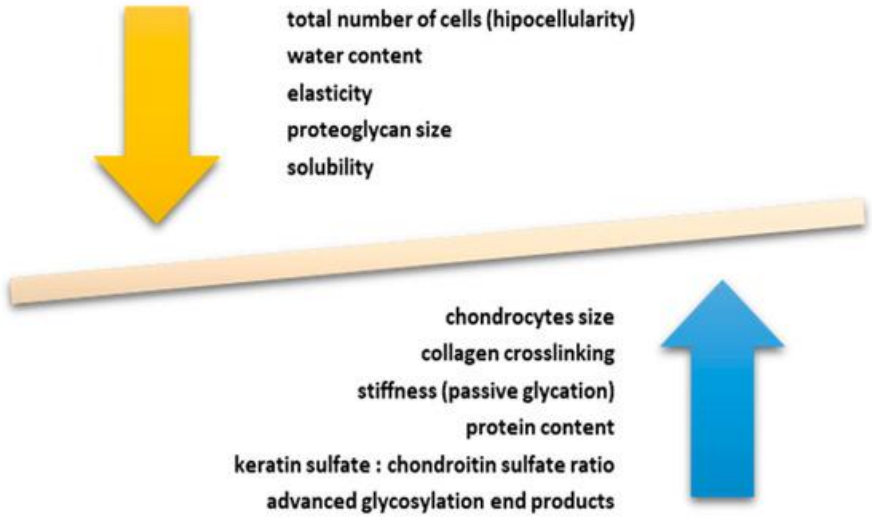


Figure I.1.1. Main changes in cell phenotype have been observed

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

Studies have 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 (20, 21). 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 (22). Chondrocyte telomere instability as well as apoptosis may also be bolstered by the presence of ROS (23). 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 (24).

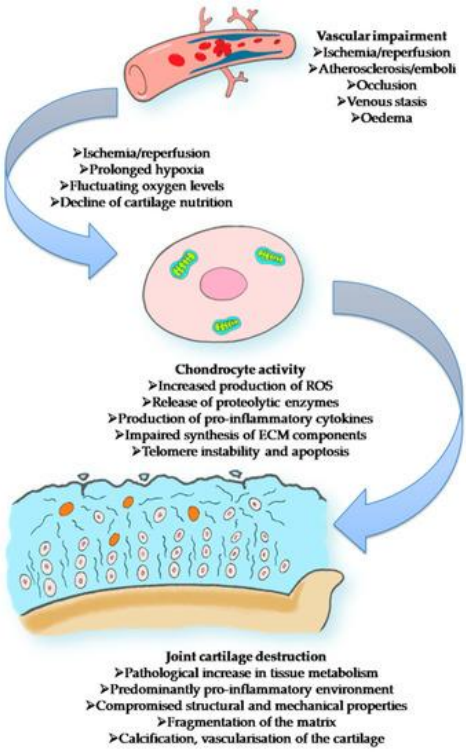


Figure I.1.2. Vascular impairment leading to a disruption in chondrocyte activity and the subsequent destruction of joint cartilage.

I.1.3.2. Mechanism of Inflammaging and Implications in OA

The mechanisms of inflammaging are not fully understood. However, current data supports its multifactorial etiology, including increased number of proinflammatory cytokines, oxidative stress, immunosenescence, autophagy, or cellular DNA damage, further detailed in figure I.1.3.A. (14, 16, 17). The aging process of the body is complex, influencing numerous cellular, immunological, and even genetic mechanisms. Thus, a proinflammatory status characterized by an excess secretion of proinflammatory cytokines such as interleukins (-1, -4, -6, -15), alpha

tumor necrosis factor, or gamma interferon was revealed. Along with this inflammatory phenotype, an increase in the oxidative stress has been highlighted, which results in the accumulation of oxygen metabolites. Furthermore, in the aging process, there is a decrease in the autophagy capacity, which determines pronounced proinflammatory responses and mitochondrial damage. DNA damage response is directly related to telomere shortening and favors proinflammatory status through its action on stem cells, fibroblasts, or macrophages, thus exacerbating the phenomenon of inflammaging.

Intrinsic factors in the aging process in association with extrinsic factors such as mechanical overload or different chemical stimuli act on articular cartilage. As a consequence, an inflammatory environment characterized by increased proinflammatory cytokines, chemokine, and activated proteinase occurs locally. All these lead to the aging process of chondrocytes (chondrosenescence), which favors the appearance of degenerative joint modifications (figure I.1.3.B).

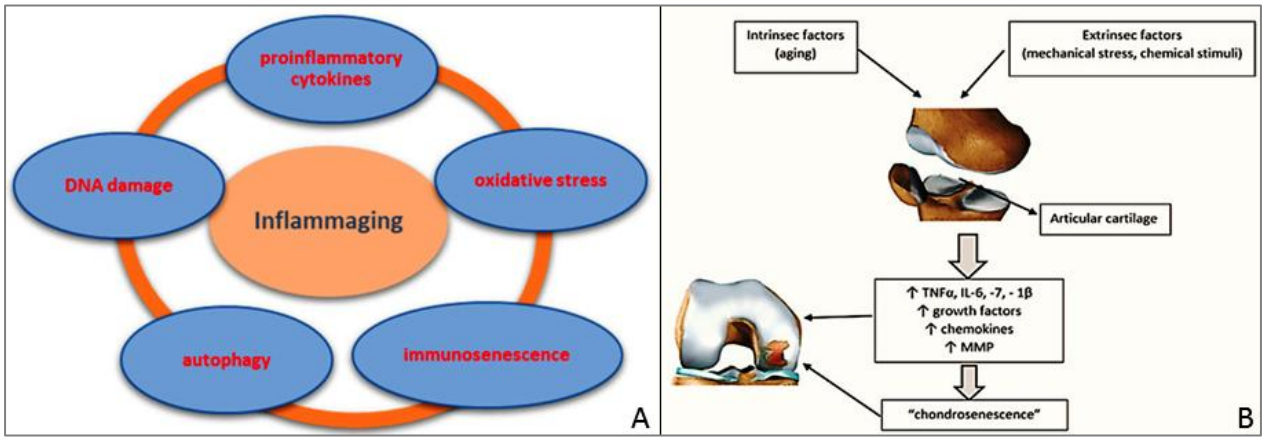


Figure I.1.3. A. The main multifactorial mechanisms related to inflammaging; B. Mechanisms of osteoarthritis in aging.

I.1.3.2.1. ProInflammatory Cytokines

Low-grade inflammatory status refers to an imbalance between pro- and anti-inflammatory cytokines (figure I.1.4.A). 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 (25). These molecules can have pleiotropic effects, stimulating immune reactions.

The most important cytokine in age-related disorders seems to be IL-6, being associated with chronic morbidity, mortality, and disability (26, 27). Furthermore, studies proved that IL-6 can be considered a predictive marker of inflammaging (27), being called the cytokine of geriatricians (28). High levels of proinflammatory cytokines including IL-6, IL-1, and TNF have a crucial role in the aging process by creating an inflammatory environment in most of the organs and body tissues (29). A study that included old horses highlighted increased levels of TNF, IL-15, IL-18, and IL-1 (30). The fragility, due to the overproduction of proinflammatory

cytokines, associated with physical inactivity, hormonal changes, and diet deficiencies, causes a favorable environment for the appearance of osteopenia and sarcopenia (31).

I.1.3.2.2. Oxidative stress

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 (16). Data sustain the link between oxidative stress, inflammaging, and immunosenescence (32, 33). 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 (28, 34), as shown in figure I.1.4.B.

Complex data about the link between oxidative stress and inflammatory process will be detailed in the next section.

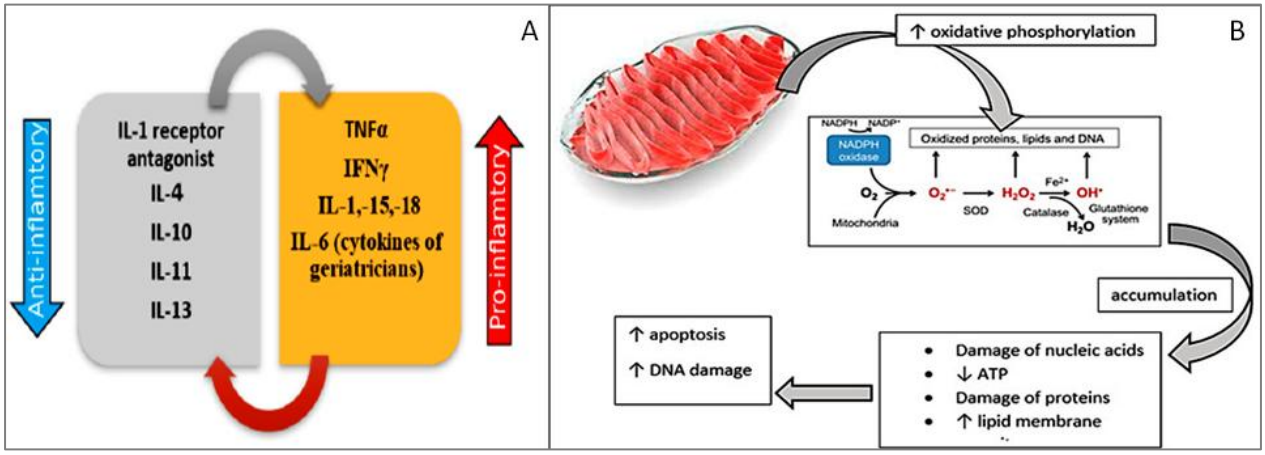


Figure I.1.4.A. Modifications related to anti-inflammatory and proinflammatory cytokines in the process of inflammaging; B. Representation of mitochondrial changes due to increased oxidative phosphorylation with respect to DNA modification and apoptosis linked to the theory of aging.

I.1.3.2.3. Immunosenescence and DNA Damage

Cellular senescence is defined as the mechanism that leads to an irreversible loss of the proliferation of somatic cells (35). Senescent cells can determine their clearing and tissue regeneration though a precise pathway. This pathway involves the release of certain mediators and the stop of proliferative activity (36, 37), process which is affected in old tissues, leading to the accumulation of these senescent cells (38). These cells have an important role in aging through the secretion of matrix-degrading proteins and proinflammatory cytokines, which is called “senescence-associated secretory phenotype” (39). 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 (40).

I.1.3.2.4. Autophagy

Autophagy is a cellular mechanism which maintains normal function and homeostasis of the cells through removal of abnormal substances via lysomal degradation (41). This process stops inflammasome accumulation, thereby reducing systemic inflammation and increasing longevity. In the aging process there is a decrease in the autophagy capacity which determines pronounced proinflammatory responses and mitochondrial damage (42).

In the elderly, the defects produced by this cellular mechanism are associated with accumulation of adipose tissue around and within the organs. Adipokines play a proinflammatory role, and more particular, leptin, has endocrine and paracrine roles (43). This highly studied adipokine stimulates the production of proinflammatory cytokines, activates natural killer lymphocytes and monocytes and transforming them into macrophages (44). The decrease in autophagy contributes to the inflammation, especially through direct participation in the formation of the proinflammatory state. First, it stimulates the oxidative stress by mitochondrial damage and second, it favors the formation of adipokines with an important role in inflammation.

I.1.3.2.5. Cellular apoptosis

Apoptosis is a programmed cell death (45), having an important role in many chronic disorders including OA, many studies shown an increase in apoptosis, particularly in RA parients (46, 47, 48). 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 (49, 50). Both mechanisms eventually trigger caspase activation. When apoptosis is affected it leads to the accumulation of dysfunctional cells, reducing the immunological space and thus promoting cancers or infections, which, in other ways, could be reduced through the correct modulation of the immune system (51).

In conclusion, this study summarized the main mechanisms implicated in inflammaging and the connection it has with degenerative joint diseases.

I.1.4. Implications of peroxide radicals in the inflammatory rheumatic disease

A. Background

According to Chiurchiu et al. (52), inflammation represents one of the best known pathophysiological processes and represents a well-conserved mechanism evolved by vertebrates as an adaptive and defensive response to tissue injury and invasion of microorganisms that might attempt to colonize the host. Even if its definition seems simple, inflammation is instead an

elaborate network of cellular and molecular events, at the core of which, a plethora of pre-formed or newly synthesized mediators is carefully arranged in order to obtain specific temporal and spatial responses. Certainly, the most important mediators are endogenous lipids, not only to be implicated in all phases of inflammation, but also to be involved in the regulation and fine-tuning of its course. Lipids are not only the most important constituents of cell membranes and a very efficient sources of energy, they are also the key pathophysiological mediators of several intercellular and intracellular processes. Due to their pivotal role in immune regulation, inflammation, and maintenance of tissue homeostasis, they have been termed "bioactive lipids", or biologically active lipids.

Oxidative stress occurs in many autoimmune diseases, along with the excess production of free radicals: reactive oxygen species (ROS) and reactive nitrogen species (RNS) (53). Free radicals are molecules or any chemical species with an unpaired electron in the outer orbit and are capable of independent existence, which often produce a highly reactive free radical. In biological systems, the most common source of free radicals is oxygen which in term reacts with nitrogen and produces nitrogen, ROS and RNS. These two free radicals are produced in human cells under physiologic and pathologic conditions and both include radical and non-radical species (54).

B. Published papers in this field

1. Macovei L.A., Matei M. N., Nechita A., Leata R., Chiscop I., Ilie M., Arbune M., **Rezus E.**, *Peroxide Radicals Implications In The Inflammatory Rheumatic Disease*, Rev. Chim. (Bucharest) 66 (9), 2015, 1516-1520.

2. Macovei L.A., Debita M., Ilie M., Moisei M., Chiscop I., Cardoneanu A., **Rezus E.**, *The Role Of Antioxidative Enzyme Mechanisms And Of Oxygen Free Radicals In Reumatoid Inflammatory Processes*, Rev. Chim. (Bucharest), 66 (10), 2015, 1645-1650.

The following two articles synthesize important data related with the relationship between the peroxide radicals and the inflammatory rheumatic disease and also the roles of antioxidative enzyme mechanisms in rheumatoid inflammatory processes.

Theoretical hypothesis, theoretical results and applications

The biologically active lipids in the acute or chronic inflammation. The Arachidonic Acid and the Inflammation

The inflammation is a type of response developed by the body to various injuries. A large number of research works are focused on inflammation field, however recent reports have provided important data for understanding the pathogenesis of this process (55, 56, 57).

We consider that the active lipid system is the key stone in the pathogenesis of acute inflammation, as in the chronicity, the rehabilitation or reorganization of the tissues (57). The active lipid system defines the eicosanoids (C20 fatty acids and their metabolites) as biologically

mediators, embracing prostaglandins, thromboxanes and leukotrienes. High synthesis of eicosanoids corresponds to one of the biological cascade of inflammation. Their biosynthesis is enzymatic reactions regulated by ciclooxygenase, lipoxygenase and cytochrome P450 mono-oxygenase that incorporate one molecule of oxygen into the polyenoic acids (58). Also called essential fatty acids, the polyenoic acids are polyunsaturated monocarboxylic acid-long chain, such as the arachidonic, linoleic and linolenic. Since there are no endogenous precursors or cellular reservoirs, essential fatty acids are discharge from the phospholipid of the membrane, in a turnover process with various triggers. Phospholipid precursors are released by the enzymatic action of the phospholipase A2, either the damage of the cell membrane or of the internal organelles membranes induces, enzymatic activation (59, 60).

Prostaglandins are derivatives of arachidonic acid and has been the most studied. We regard as the prostaglandins play the role at membrane level of continuous adaptation of the cellular response to exogenous factors (59). Prostaglandins E (PGE) are the main factors involved in the pathway of the acute inflammatory process, inducing heavy vasodilatation, permeabilization of the microvessel walls and increasing the phagocytosis process (57, 61, 62). Moreover, prostaglandins are able to act like local hormones with brief duration, named autacoids.

Deeping understanding of the inflammation mechanism evidences the involvement of lipid autacoids. Certainly, we notice that inhibitors of each metabolic pathway of the linoleic, linolenic and arachidonic fatty acids achieve the anti-inflammatory effect (62, 63).

Activated inflammatory cells from the inflammation site generate chemically reactive oxygen species, that can initiate lipid peroxidation, figure I.1.5. The phospholipids in the cellular membranes are primary targets of chemical and enzymatic oxidation, inducing serious damages of the cellular membranes and necrosis. Oxidized lipids as oxygenated arachidonic acid products, the prostaglandins, leukotrienes, and thromboxane A2, have potent effects throughout the inflammatory and reparative responses (64).

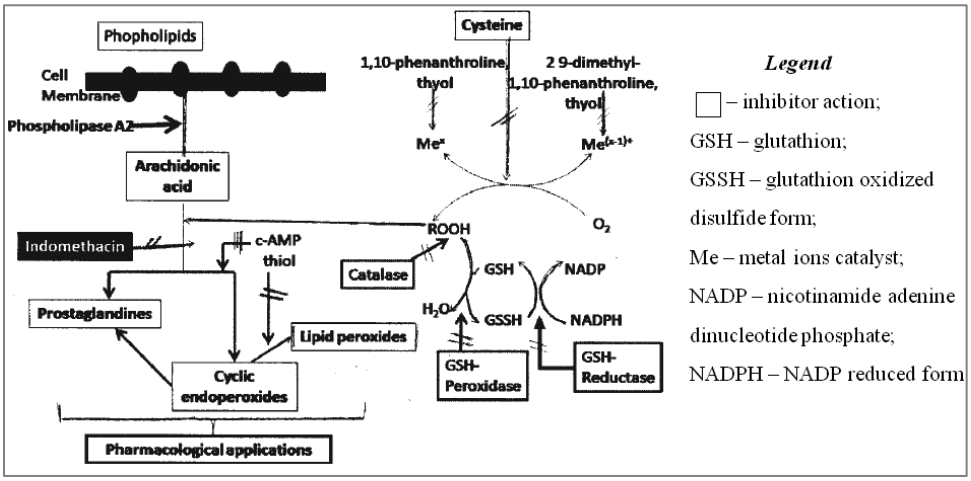


Figure I.1.5. The chart of the presumptive interactions between the arachidonic acid and the natural mediators or drugs, considering the role of the arachidonic acid in the inflammations and the interaction with certain anti-inflammatory agents

Administering the arachidonic acid parenterally intensifies the biosynthesis and has a vasodilator effect through the release of a contracting factor. If we accept that AGPN peroxidation in the membranes is a reaction associated with the changes of cell membranes in inflammation, then administering the arachidonic acid amplifies the inflammatory process, and the nonsteroidal anti-inflammatory drugs inhibit the action of this acid.

Unfortunately, because of the inflammatory process, the effect of administering the arachidonic acid is not clear, first of all by the contradictory action of the prostaglandins, endoperoxides and prostacyclins of favoring or inhibiting the reactions in inflammations.

Competing with prostaglandins production, the enzymes glutathione peroxidase and catalase decompose the excess of peroxides. Catalase is not directly involved in the decomposition reaction of the lipid peroxides. Actually, this enzyme antagonizes the effects of the arachidonic acid, such the aggregation of the thrombocytes and the contraction of the small vessels. The applications of these results are focused to detect and to quantify the free radical species, mainly the phagocytic products of macrophages (65). Referring to the advanced rheumatic diseases, there are observed increase in the lipid peroxidation and low plasmatic level of sulfhydryl (-SH) groups (66).

Free radicals

Free Radicals (FR) are molecules that derive from incompletely oxidized compounds that have undergone partial burning. Their oxygen groups are able to act in the surface of the cellular membranes or organelles, to initiate aggressive oxidation reactions and to damage the cells (67). The most active free radicals are ions: superoxide (O_2^{2-}), peroxide (O^{2-}), hydroxide (OH^-), nitric oxide (NO^-). The sources of aggressive oxidants as peroxides and superoxides are the hydrogen peroxide (H_2O_2) and the metabolites of fatty food, as lipid peroxides.

The first stage is initiation, consisting in production of free radicals derived from the R substance, which is subjected to peroxidation.

$$R\cdot + O_2 \rightarrow ROO\cdot$$
$$ROO\cdot + RH \rightarrow ROOH + R\cdot$$

The emergence of free radicals in an organic substance is due to one or more external (high temperature, humidity, light, ionizing radiations, UV) or internal factors (slightly oxidative impurities, for instance aromatic compounds, such as diphenols). Disassembly of some free radicals forms the most effective initiator:

$$2ROOH \xrightarrow{O_2} R\cdot + ROO\cdot + H_2O$$
$$ROOH \xrightarrow{h\upsilon} R\cdot + HO_2^-$$

Propagation is the second stage of peroxidation, characterized by continuation of free radical formation. The most important reactions are:

$$R\cdot + O_2 \rightarrow ROO\cdot$$
$$ROO\cdot + RH \rightarrow ROOH + R\cdot$$

Termination is the third stage, and represents the consequence of reactions between various free radicals that produce inactive compounds:

$$R\cdot + R\cdot \rightarrow R \text{ --- } R$$
$$R\cdot + ROO\cdot \rightarrow \text{inactive products}$$

In normal circumstances, there is a balance between ROS production and their clearance. In various pathologic states, the balance is destroyed, either by ROS supra production, by diminishment of their annihilation capacity, or both. ROS alterations of cellular compounds are followed by annihilation of activity of nucleotide coenzymes, change of redox function, disturbance of the activity of thiol depending enzymes, covalent attachment of proteins and lipids, change of lipid metabolism, protein alterations leading to the increase of protein turnover, lipid peroxidation (which alters the structure and function of cell membrane), and transportation disturbances. ROS exerts tissular toxic actions by direct and indirect mechanisms.

Direct mechanisms – addressed to nucleic acids, producing DNA denaturation, involving breaks in chromosomes (clastogenic effect), with major consequences on multiplication, transmission and replication or genetic message. Also directly, the radicals act on collagen, which is degraded by depolymerisation of mucopolysaccharides, hyaluronic acid and procollagen microfibers.

Indirect mechanisms – target the lipid peroxidation. Polyunsaturated fatty acids contained by membrane phospholipids are extremely vulnerable to the action of ROS, due to the presence of double bindings. An autocatalytic reaction takes place, which in the end leads to the alteration of membrane integrity, until their complete lysis.

Protection systems against the action of the free radicals

The protection of antioxidant system involves the enzymatic and non-enzymatic pathways. Because the antagonist protective system is promptly initiated, the lipid peroxidation is difficult to be proved in vivo, either on the individual cells, or on the global organism. However, the presence of antioxidant system himself confirms the theory of lipid peroxidation (68).

Superoxide – dismutase (SOD) SOD is the first enzyme in the enzymatic chain that protects against O_2 toxicity; it catalyzes a dismutation reaction, through which a tetravalent reduction of O_2 in H_2O_2 occurs:

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

SOD are metal-containing enzymes depending on metal as manganese, copper or zinc, that are produced in the eukaryotes aerobe cells, but also in the facultative aerobe bacteria. Recognized as the main enzymatic factor that is able to acquire the tolerance to oxidative stress, SOD has been described since 1969. Nowadays, there are described three types of SOD, based on the type of metal from the catalytic center: the manganese-containing enzyme is most abundant in mitochondria, while the zinc or copper forms are predominant in cytoplasm. The role of SOD is to catalyze the conversion of two superoxides into oxygen and hydrogen peroxide, that is substantially less toxic that superoxide. Actually, the catalytic action of SOD is to accelerate over 1000 folds the dismutation of the superoxide in the hydrogen peroxide (55, 69, 70).

Catalase is an enzyme widely disseminated in the tissues, localized in the peroxisomes of mammalian cells (62). *Catalase* acts in the reactions of hydrogen peroxide conversion and uses hydrogen peroxide to oxidize toxins, including phenols, formic acid, formaldehyde and alcohols. It major antioxidant role is the catalysis the chemical decomposition of hydrogen peroxide into two molecules of water and one molecule of oxygen:

$$H_2O_2 + H_2O \rightarrow O_2 + 2\ H_2O$$

Considering that H_2O_2 toxicity is 1000 times higher in the presence of transition metals, it is understandable why the aerobic organisms, implicitly the human body, need two major defence classes with complementing role: catalase and enzymes associated with glutathione. Catalase has as prosthetic group 4 hem nuclei that coordinate one atom of Fe^{3+} at a 248.000 molecular mass. Elucidation of the action mechanism of catalase provoked numerous controversies, left without an answer. The catalase and peroxidase activity starts with the formation of complex I between the first molecule of H_2O_2 and heminic Fe (figure I.1.6) (71, 72).

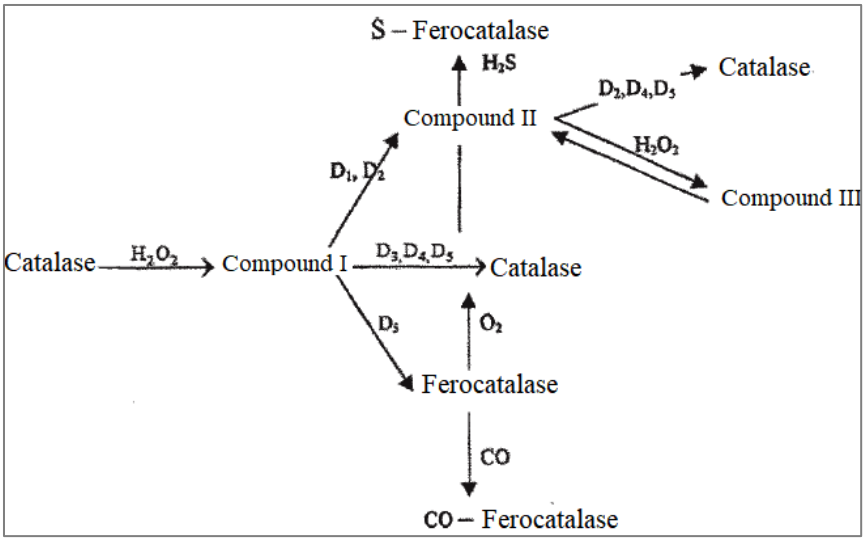


Figure I.1.6. Catalase activity and peroxidase activity of catalase

Glutathione peroxidase (GSH-Px) is one of the catalyzer enzymes involved in the degradation of organic hydroxiperoxide provided during the normal metabolic course. Also called the "life protein" glutathione is a bioactive polypeptide found in the most cells of the human body, acting as a coenzyme. Glutathione synthesis occurs in the liver, based on cysteine, glutamine and glycine amino acids (69, 70). The activity of GSH-Px surrounded by the physiological conditions is difficult to be quantified, since for the same substrate there are multiple competitions with other enzymes. Owing to the high specificity of it substrate, the antioxidant potential of GSH-Px is superior than SOD and catalase. The importance of GSL-Px in detoxification process is based on the ability to decomposes the peroxide radicals, the hydroperoxides (mainly-the lipid peroxides) and even H2O2. The role of *GSH-Px* is to protect the proteins, lipids and nucleic acids, regarding the free radicals (24, 29, 30). This reaction consists of an electron donation, that is dislocated from the molecules of glutathione, thioredoxin or glutaredoxin (73).

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

or

$$R\text{-}OOH + 2\ GSH \rightarrow R\text{-}OH + GSSG + H_2O$$

Hydroperoxides (ROOH) are sources of peroxides, as H_2O_2 or any peroxide derived from the nucleic acids, polyunsaturated or steroid fatty acids. Glutathione (GSH) is the reduced monomeric compound, and GS–SG represents glutathione disulfide (74).

Non-enzymatic systems comprise the liposoluble vitamins E and A. Vitamin E is the generic name of four compounds of tocopherol, characterized by the common tocol structure and different position of methyl groups: alfa, beta, tau, and delta. Alfa tocopherol contains a chroman ring with 2-methyl-6-hydroxy substitution and a saturated radical containing 16 atoms of carbon. The antioxidant effect of vitamin E is more powerful *in vivo* than *in vitro*. Strategically place of alfa-tocopherol in the membranes presents the ability to block the lipid peroxidation (75).

Phagocytosis is a complex process used to remove pathogens, cell debris and other foreign particles, figure I.1.7.A. The polymorphonuclears have multi-lobed nucleus and a very short life, while the mononuclear phagocytes have a large nucleus and a long life. Appointed by the chemotactic factors, the phagocytes are activated and recruited, moving towards the inflammation area, but first arrive the polymorphonuclears and next the macrophages (62). Phagocytes adhere to the bacteria and ingest them inside the phagosome, using oxygen-dependent bactericidal mechanisms that activate the NADPH – oxidase and produce peroxide derivates (70). The release of these reactive species requires to couple the phagocytosis with the glycolysis, as energy resource, figure I.1.7.B. Intracellular respiration increase, because augmented oxygen consumption is required for its activation and producing the free radicals (57, 65).

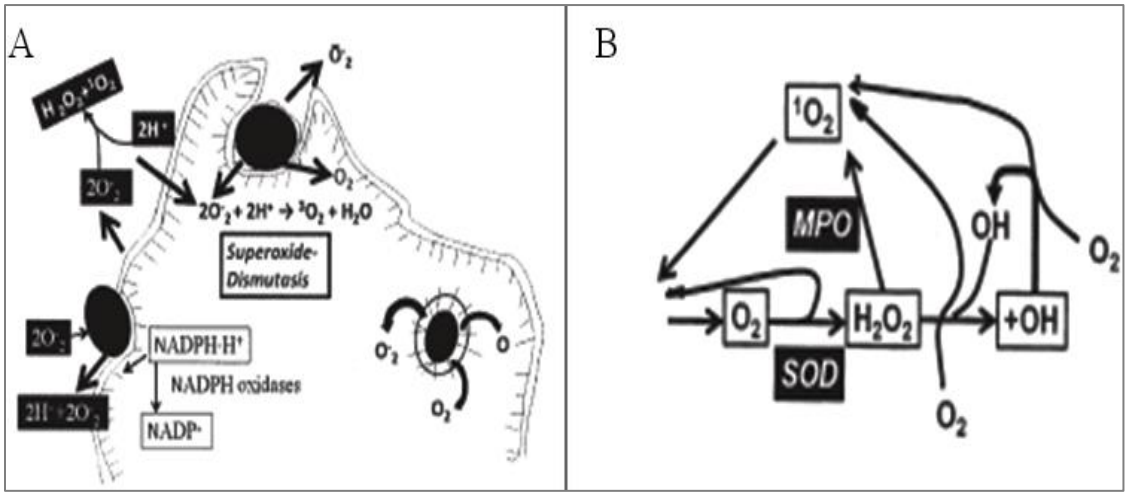


Figure I.1.7. A. Phagocytosis process: microorganisms adherence to the phagocytes → NADPH-oxidase activation → invagination through the pseudopods.
B. The interactions between the activated forms of oxygen. We can notice the intervention of the superoxide-dismutase (SOD) and of the myeloperoxidase (MPO).

In the figure I.1.8. we noticed that phagocytes are able to catalyze the decomposition of the excess of H_2O_2 by three enzymatic systems: peroxide-glutathione peroxidase, catalase and myeloperoxidase (67, 76). The antibacterial systems based on the hydrogen peroxide were detected in some cells. The main products of their activity are the free radicals of oxygen, although additional molecules, including the cyanides, are released. The cyanides are cytotoxic factors, explained by the large amount of energy from the radicals.

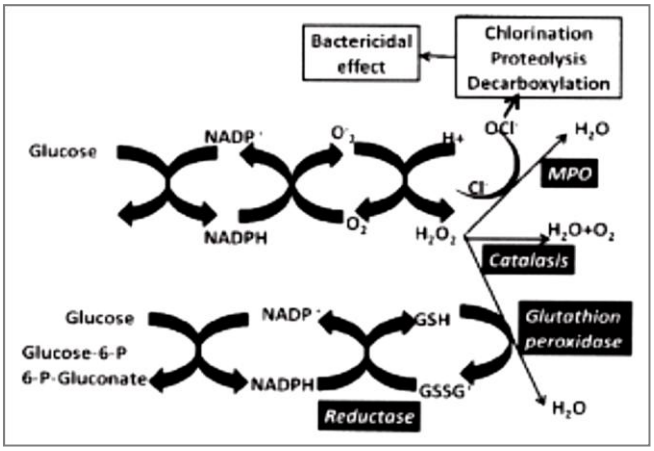


Figure I.1.8. The synthesis and decomposition of hydrogen peroxide (H_2O_2). The hydrogen peroxide is formed of the action of the superoxide-dismutase or through the pentose- phosphates shunt and is used for the catalyzed reaction of myeloperoxidase (MPO). The excess of H_2O_2 is decomposed through the action of the catalase and of the glutathione-peroxidase

In conclusion, the largest part of the oxygen of human metabolism is combined with the hydrogen, resulting water, in normal conditions. However, 4-5 % of the oxygen is transformedinto superoxide anion and hydrogen peroxide, following an enzyme catalysis process.

The free radicals are active molecules produced during the oxygenation reactions of the basic activity of the human body. The chemical structure of free radicals is a superoxide-anion and hydrogen peroxide. The functions of free radicals are to regularize the cells growth, to transmit the biological signals and to suppress the viruses or bacteria. The excess of free radicals in our body, attack the cellular and the mitochondrial membranes, reacting with the unsaturated fatty acids from the membranes and intensifying lipid oxidation. Resulting of the lipid oxidation, free radicals are generating a chain reaction, follow-on by the alteration of the structural integrity of the membranes and cell injuries, related to inflammatory diseases and accelerated aging.

I.1.5. Cardiovascular risk in chronic inflammatory rheumatic diseases

A. Background

Interests in comorbidities in rheumatic conditions had increased significantly in recent years, not only in research but also in daily clinical care. Rheumatologists are facing an aging population with multiple chronic conditions in one patient. For instance, patients with rheumatoid arthritis (RA) have 1.6 additional conditions, increasing with age, disease activity, and also disease duration (77).

Comorbidity and multimorbidity are two concepts related with patients who are affected by more than one disease at the same time. The concept of comorbidity can be defined as *the existence or occurrence of any distinct additional entity during the clinical course of a patient who has the index disease under study* (78). Comorbidities are consequences of the index disease itself or its treatment, for example, in the case of a patient with RA, cardiovascular disease (CVD), and peptic ulcer, RA would be the index disease, CVD could be regarded as a consequence of the chronic inflammatory process of RA, and a peptic ulcer could occur as a consequence of nonsteroidal anti-inflammatory drug (NSAID) treatment of pain related to RA. On the other hand, multimorbidity is defined as *co-existence of two or more chronic diseases in the same individual* (79), the concept being more intricate, placing the patient in the center of interest. Coexisting conditions are seen as equal importance with assumed interaction among each other.

B. Published papers in this field

Patients with chronic inflammatory rheumatic diseases (CIRD) are at increased risk of cardiovascular disease (CVD), attribute not only to classical risk factors, but also to the presence of chronic systemic inflammatory response (80). This was the main reasons I focused on the study of the relationship between cardiovascular disease and the inflammatory rheumatic diseases, the results of my studies has been published in two literature review.

1. Rezuș E., Floria M., Grigoriu A., Tamba B.I., Rezuș C., *Cardiovascular Risk Factors in Chronic Inflammatory Rheumatic Diseases: Modern Assessment and Diagnosis*, Current Vascular Pharmacology, 13, 2015, 1-8.

Atherogenesis is a dynamic inflammatory process that occurs during all the stages of atheromatous plaque formation and the resulting complications (81). Systemic inflammation, which is associated with all chronic inflammatory rheumatic diseases (CIRD), accelerates atherogenesis (82). This concept is supported by the high cardiovascular (CV) morbidity and mortality rates in CIRD patients, the high prevalence of all atherosclerosis (ATS) stages and the resulting complications (endothelial dysfunction, carotid atherosclerotic plaques, fatal/non-fatal acute myocardial infarction (AMI) and stroke), and the recurrence of CV events after traditional CIRD risk factors have been corrected.

The pathogenic mechanisms leading to accelerated ATS in CIRD are complex and have not been fully elucidated. Both an increase in the prevalence of some of the traditional risk factors, as well as the appearance of new 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 rheumatoid arthritis (RA), suggests that joint inflammation, together with the immunologic and metabolic disorders specific to these conditions, are independent CV risk factors (83). The relationship between ATS and chronic inflammation has been studied, especially in rheumatoid polyarthritis. Clinical evidence has shown that CV mortality and morbidity rates are high in this chronic inflammatory pathology because of an accelerated coronary and non-coronary ATS process (84).

Demographic factors

Regarding the demographic factors there is a relatively high risk of CV events for patients with RA in the young age group making prevention strategies important. Women with RA run a risk of AMI that is 2 times higher than women not suffering from RA (83).

Traditional cardiovascular risk factors

Eleven traditional cardiovascular risk factors are listed in table I.1.1, from which hypertension, dyslipidemia, diabetes mellitus, smoking and sedentary lifestyle are among the most unfavorable.

The prevalence of *hypertension* (HTN**)** is 3.8-73% in RA. Despite its high prevalence, HTN is often both underdiagnosed and undertreated in RA patients (85). In addition, issues such as systemic inflammation, sedentary lifestyle, obesity, and medication (non-steroid anti-inflammatory drugs [NSAIDs], glucocorticoids, and disease modifying anti-rheumatic drugs [DMARDs] like leflunomide or cyclosporine) prevent adequate blood pressure control in patients with CIRD (86).

Non-traditional (inflammation-related) CV risk factors

CIRD and vascular ATS share similar pathophysiological mechanisms that include pro-inflammatory cytokines, TNFα, 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) (87).

Table I.1.1. Cardiovascular risk factors in CIRD

Demographic risk factors	**Traditional risk factors**	**Non-traditional risk factors**	**Medication for non-traditional factors**
Age M > 45 years F > 55 years Male sex Ethnic origin	HTN DM Smoking Obesity Insulin resistance Sedentary life Dyslipidemia (↑TC, ↑LDL C, ↓HDL-C) Framingham score ↑homocysteine Premature menopause Generic risk (premature coronary disease)	Onset age Disease duration Activity and osteoarticular destruction (radiological score) Inflammatory markers: CRP, Fg, CK, IL-6, TNF, IL-1, CD40/CD40L Immune factors: antibodies antiPL (aCL, aβ2GPi), anti-oxLDL, anti-ox LDL/β2GPi, anti-HSP, anti-CEA Inflammation and endothelial dysfunction Coagulation abnormalities: Fg, PAI-1, homocysteine Metabolic factor: pro-atherogenic lipid profile Genetic predisposition (HLA) Chronic kidney condition	Glucocorticoids - pro-atherogenic effect: cumulated high dose > 10 mg/day - anti-atherogenic effect: dose ↓ NSAIDs MTX HCQ anti-TNFa

aCL = anti cardiolipin, aβ2GPi = anti beta2-glycoprotein I, anti-ox LDL = anti–oxidized LDL, CD40/CD40L = co-stimulatory proteins found on antigen presenting cells, CEA = carcinoembryonic antigen, CIRD = chronic inflammatory rheumatic disease, CK = creatine kinase, CRP = C reactive protein, DM = diabetes mellitus, F = female, Fg = fibrinogen, HCQ = hydroxychloroquine, HDL-C = high density lipoprotein cholesterol, HLA = human leukocyte antigen, HTN = hypertension, HSP = anti-heat shock protein, IL-1 = interleukin 1, IL-6 = interleukin 6, LDL-C = low density lipoprotein cholesterol, M = male, MTX = methotrexate, NSAIDs = non-steroid anti-inflammatory disease, anti-PL = anti-threonyl tRNA synthetase, PAI-1 = plasminogen activator inhibitor-1, TC = total cholesterol, TNF = tumor necrosis factor.

Regarding *dyslipidemia*, there is an altered lipid profile, called a proatherogenic lipid profile (decreased high-density lipoprotein [HDL] cholesterol, increased low-density lipoprotein [LDL] cholesterol, and increased triglycerides), in RA, systemic lupus erythematosus (SLE), and Sjogren's Syndrome (87, 88).

The prevalence of *diabetes mellitus* in CIRD is controversial. Research conducted as early as the 1980's revealed a positive association between CIRD and insulin resistance from systemic inflammation or glucocorticoid therapy. Control of systemic inflammation using DMARDs and an adequate diet improves insulin resistance (89).

Smoking is a known risk factor for RA and is associated with disease activity and severity (it is associated with seropositive RA). It is also associated with subclinical ATS, which suggests that the CV impact of smoking in RA patients is much greater than in the general population (90).

Patients with CIRD are prone to a sedentary lifestyle because of their chronic musculoskeletal condition (pain, joint stiffness, ankyloses, misalignment, tendon retraction) (91).

Inflammation markers

Inflammation markers (CRP and ESR) are important indicators of the activity and severity of the disease, as well as CV mortality predictors in patients with CIRD. CRP, the most studied inflammatory marker over the last few years, is produced in the liver in response to an inflammatory cytokine stimulus (IL-6). It is a coronary risk identification factor in asymptomatic patients (92). CRP, the most studied inflammatory marker over the last few years, is produced in the liver in response to an inflammatory cytokine stimulus (IL-6). It is a coronary risk identification factor in asymptomatic patients (93). Another measure for CV risk could be ESR, which was found to be higher immediately after heart failure onset in research conducted on patients with RA (94). Additional inflammatory markers that could act as CV risk predictors are shown in table I.1.2.

Table I.1.2. Predictive markers for inflammatory cardiovascular risk

Predictive markers for inflammatory cardiovascular risk
Adhesion molecules (vascular cellular adhesion, inter-cellular adhesion, and leukocyte-endothelium adhesion)
Cytokines
Acute phase reactants
• Fibrinogen
• Serum amyloid A
• CRP
• ESR Leucocyte count

CRP = C reactive protein, ESR = erythrocyte sedimentation rate

Antibodies

Some autoantibodies specific to systemic autoimmune diseases are correlated with endothelial activation and dysfunction, which is conducive to premature ATS development. The following 5 processes characterize endothelial activation:

- loss of vascular integrity;
- elevated leukocyte adhesion molecule expression;
- conversion of an antithrombotic phenotype into a prothrombotic phenotype;
- cytokine and chemokine production;
- human leukocyte antigen upregulation (95).

Cells

Various cells involved in the pathogenesis of CIRD are linked to ATS and CV disease. A decreased number of circulating endothelial progenitor cells (EPC), which are

essential to endothelial repair and revascularization, has been found in RA patients. Under the action of proinflammatory cytokines that stimulate endothelial growth factors, EPCs migrate towards the synovial membrane of the joint where they accumulate and contribute to intrasynovial neoangiogenesis (83).

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-a, IL-1 and matrix metalloproteinases (96, 97). The increased inflammation levels found in the population without RA, which is reflected in the elevated CRP levels, increases individual myocardial infarction risk considerably (98).

Osteoprotegerine is a protein that belongs to the TNFa receptor family, which is involved in bone metabolism and is linked to coronary artery calcifications in RA patients (99).

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 (100). *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 (101).

Table I.1.3. Practical recommendations for CIRD therapy and cardiovascular risk management

Therapeutic agent	Recommendations
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.* gastro- intestinal 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

In conclusion, the pathogenic mechanisms involved in accelerated ATS and the resulting CIRD complications are complex and rely on several factors. 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.

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

Since we have synthesized in the previous review the main cardiovascular risk factors in chronic inflammatory rheumatic diseases, in this review we intended to present in more detail the connection between the most common rheumatic inflammatory diseases and myocardial ischemia.

Regarding cardiovascular events, these can be more important or clinically silent but they are characterized by substantial morbidity and mortality. The most important risk factor for the development of cardiac pathology, namely myocardial ischemia, is considered to be accelerated atherosclerosis. Chronic inflammation and immune as well as endothelial dysfunction participate in the formation of vascular atherosclerotic plaque (102, 103).

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.

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 (figure I.1.9) (104, 105, 106).

Systemic lupus erythematosus is a complex disease associated with premature atherosclerosis and early deaths due to cardiovascular events or severe infections (107). In the pathogenesis of SLE a very important role is played by type 1 interferon which is considered to determine the instability of atherosclerotic lesions, aortic stiffness or dysfunction of the endothelium (108). Studies demonstrated that 40% of patients have myocardial perfusion defects (109) and 50% of patients have impaired endothelial vasodilatation (110).

Rheumatoid arthritis, characterized by chronic inflammation, shows premature atherosclerosis even without common risk factors as well as a high prevalence of ischemic heart disease, namely myocardial infarction (111, 112, 113). Moreover, events such as sudden cardiac death and silent myocardial infarction are more frequent among these patients (114). Figure I.1.10. illustrates the main factors that increase the cardiovascular risk in patients with RA (115, 116, 117, 118).

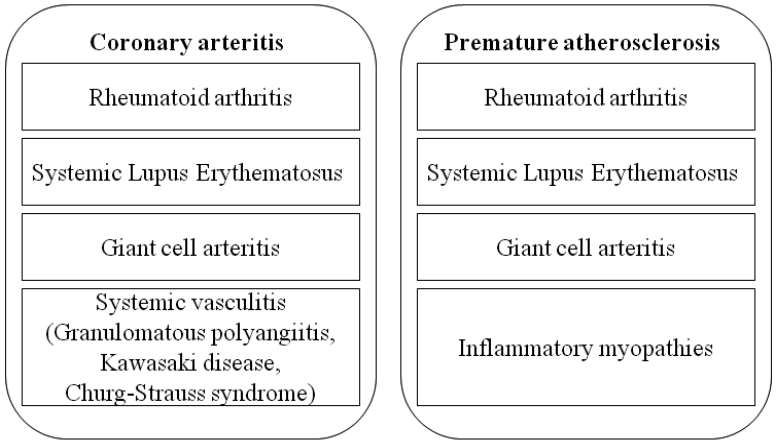


Figure I.1.9. Inflammatory rheumatic diseases and coronary involvement

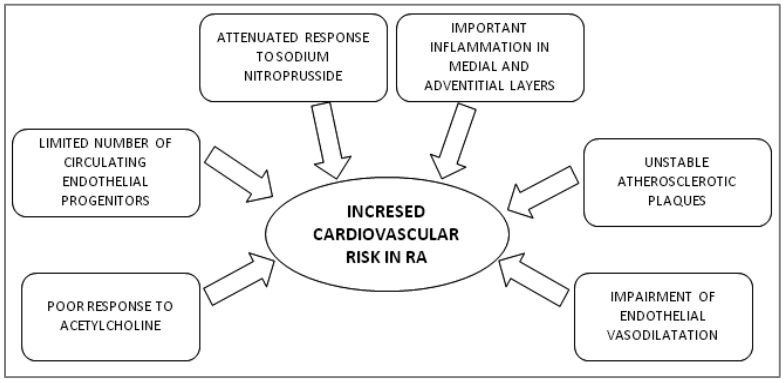


Figure I.1.10. Factors that increase the cardiovascular risk in RA patients.

Systemic sclerosis is also part of the umbrella group of inflammatory rheumatic diseases, being characterized by an increased risk of premature atherosclerosis. 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 (119).

Concerning *Spondylarthropaties*, especially *Ankylosing Spondylitis (SA)* and *Psoriatic Arthritis (PsA*), systemic inflammation remains the most important cardiovascular risk factor for developing a cardiac event. In AS patients, aortic regurgitation and aortic disease, was found but could not be correlated with the progression of AS (120). The genetic background represented by the HLA-B27 antigen increases the risk of a first-degree atrioventricular block (121). 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 (122, 123).

Related to the treatment of rheumatic diseases, the use of synthetic modifying anti rheumatic drugs, mainly Methotrexate, can reduce mortality and cardiovascular risk (124). It decreases carotid thickening (intima media thickness) and improves endothelial vasodilatation

after one year of treatment (125). Concerning biological therapy, particularly anti tumor necrosis factor α (TNFα) antibodies, clinical trials revealed that their use reduces aortic stiffness (126), improves endothelial function (127) decreased the risk of stroke, myocardial infarction and heart failure (128).

In conclusion, we strongly support that inflamatory rheumatic diseases have an important role in the development of atherosclerotic cardiovascular disorders and ischemic events due to the presence of systemic inflammation and immune hyperactivity. The rheumatologic patient should be considered as having an elevated cardiovascular risk which requires a well rounded approach in the management of the disease; the treatment pertaining to the patient's comorbidities. A proper and effective collaboration between a cardiologist and a rheumatologist is necessary for optimal treatment of these patients.

I.1.6. Tuberculosis Infection in Rheumatic Diseases

A. Background

Biologic agents, such as tumor necrosis factor (TNF) antagonist and interleukin-1 (IL-1) receptor antagonist, have become invaluable treatments against chronic inflammatory diseases, such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), and psoriasis. Studies have shown that following TNF antagonist therapy, the related risk for activation of latent tuberculosis (TB) is increased up to 25 times, depending on the clinical setting and the TNF antagonist used. Also it has been reported the activation of latent tuberculosis following IL-1 receptor antagonist therapy. For this reason, latent tuberculosis infection (LTBI) screening and preemptive anti tuberculosis treatment are recommended prior to biologics treatment in patients with latent tuberculosis infection. The diagnosis of LTBI is traditionally based on tuberculin skin test (TST) positivity in the absence of active tuberculosis. The test mentioned above has a low sensitivity in patients with rheumatoid diseases and has a low specificity in patients with prior *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) vaccination. The new gamma interferon release assays (IGRAs) have been introduced to compensate for the drawback of TST in detecting LTBI (129, 130).

B. Published paper in this field

Filipescu I. et al., *The assessment of tuberculosis in patients with inflammatory rheumatic diseases treated with blockers of the tumoral necrosis alpha factor: a retrospective observational multicentre study*, Romanian Journal of Rheumatology, XXIV (3), 2015, 166-172.

I had participated together with other twenty six rheumatologists from 11 Romanian medical center, at a study which had the aim to achieve extensive information (regional) in

relation with the tuberculosis identified in current clinical practice in patients with inflammatory rheumatic diseases treated with biological agents.

We had provided data related to patients with rheumatoid arthritis (RA), ankylosing spondylitis (AS) and psoriatic arthropathy (PsA) in relation with anti-TNFα agent. The patients had been hospitalized between January 1999 and June 2011.

Methods

This observational research included 693 patients (RA n=492, SA n=137, AP n= 64). All patients were screen for latent Mycobacterium tuberculosis infection (LTBI) before they start anti-TNFα treatment (infliximab – IFX, etanercept – ETA or adalimumab – ADA). Chemoprophylaxis with isoniazid before anti-TNFα therapy is recommended if the diameter of tuberculin skin test reaction is more than 5 mm (before 2005 only if indurations was more than 10 mm). We recorded the demographic characteristics, and complex information about disease, treatment, tuberculosis diagnosis and the comorbidities.

Statistical analysis

Based on these report forms, we created afterwards a database to be used for further processing of the items. Quantitative variables were summarized by median and interquartile range (first and third quartiles) for variables that have been shown not to be normally distributed, or as mean and standard deviation for normally distributed data. Qualitative variables were summarized by absolute and relative frequencies (%) with associated 95% confidence interval calculated under assumption of binomial distribution (131).

Incidence rate of TB are presented as events ‰ person-years with associated 95% confidence interval. This index was calculated for each anti-TNFα agent, for each of the three diseases. The identifying of risk factors was tested using logistic regression. Data analysis was conducted with Statistic program (v. 8.0) at a significance level of 5%.

Results

The main characteristics of the 693 patients who met the criteria for inclusion in the study are shown in table I.1.4.

Out of the total 693 patients included in the study 66.8% were women. Analyzing the gender distribution in the three groups, it could be noticed that women were the majority among patients diagnosed with RA and PSA, while 85,4% of those with AS (n=137) were men.

In this research 15 patients were diagnosed with TB. The general characteristics of there cases are presented in Table I.1.5. Two patients had concomitant renal disease and other four subjects used corticotherapy ≥ 10 mg daily, for more than one year.

Related to the history of latent TB, tuberculin skin reaction diameter was > 10 mm in 3 patients (20%), between 5 and 10 mm in 4 cases (26.67%) and < 5 mm in 8 patients (53.33 %).

Lung scans performed prior to initiating biological therapy were normal in all patients who subsequently developed TB. None of the subjects had a personal history of TB, most (60%) were born in rural areas and 40% in areas with higher incidence (≥80%) of TB.

Table I.1.4. The main characteristics of the study patients

	Rheumatoid arthritis (n = 492)	Ankylosing spondylitis (n = 137)	Psoriatic arthritis (n = 64)
Age (years)	52 (16-76)	38 (18-56)	42 (20-67)
Female, n (%)	405 (82.3)	20 (14.6)	38 (59.4)
Anti-TNFα agent			
Infliximab, n (%)	276 (56.1)	64 (46.7)	35 (54.7)
Etanercept, n (%)	164 (33.3)	48 (35.0)	23 (35.9)
Adalimumab, n (%)	52 (10.6)	25 (18.2)	6 (9.4)

Table I.1.5. The characteristics of the 15 TB cases

Subject	Gender	Age	Disease	Biological agent	Disease duration (year)	Biological duration (months)	TST (mm)	Prophylaxis	TB Sites
1.	F	60	RA	IFX	3.2	1	0	NO	Pulmonary
2.	M	56	PSA	IFX	9.3	2	3	NO	Pulmonary
3.	M	27	AS	IFX	5.2	3	16	YES	Pulmonary
4.	M	42	RA	IFX	13.1	6	0	NO	Peritoneum
5.	M	57	PSA	IFX	4.6	6	20	YES	Pulmonary
6.	F	65	RA	IFX	14.6	11	3	NO	Pulmonary
7.	M	47	AS	IFX	9.7	12	0	NO	Pulmonary
8.	M	46	AS	ETA	27.2	12	16	YES	lymph node
9.	F	65	RA	IFX	6.8	15	8	NO	lymph node
10.	M	46	PSA	IFX	12.1	19	8	NO	Peritoneum
11.	F	59	RA	ADA	5	20	6	NO	lymph node
12.	M	55	AS	ADA	11.4	26	5	NO	Peritoneum
13.	F	54	RA	IFX	10	36	0	NO	lymph node
14.	F	63	RA	IFX	16	60	0	NO	Peritoneum
15.	F	72	RA	IFX	12.1	120	3	NO	Meninges

The connection between the biological agent, the rheumatic disease and TB

TB incidence was 21.65‰ [CI 95% (11.546; 34.630)]. Most patients with TB (n=14) had been treated with only one anti-TNFα agent. One patient with AS was diagnosed with TB after the initiation of the second TNFα blocker, which was ADA. ETA has been involved in the development of a single case of TB in other patient with AS. In 8 patients TB was diagnosed in the first year, most of them (87.5%) were treated with IFX.

Most TB cases (n=8) occurred in patients with RA, the biological therapy duration and the number of subjects was highest in this group (table I.1.6). The number of TB cases/1000 patient-years was 0.78/4.35/0.23 for the three biological agents involved (IFX, ADA and ETA).

Tabel I.1.6. The distribution of TB cases according tobiological agents and rheumatic disease

Biological agent	**Patient-years***	**Number of TB cases**	**TB/1000 patient-years [95% CI]**
Rheumatoid arthritis			
IFX	14302.82	7	0.49 [0.13; 0.85]
ETA	4171.68	0	0
ADA	382.4	1	2.61 [0.00; 7.74]
Ankylosing spondylitis			
IFX	369.42	2	5.41 [0.00; 12.92]
ETA	89.77	1	11.13 [0.00; 32.97]
ADA	59.73	1	16.74 [0.00; 49.56]
Psoriatic arthritis			
IFX	618.03	3	4.85 [0.00; 10.35]
ETA	80.48	0	0
ADA	17.53	0	0

* Patient-year index = patient number × biological treatment duration (years)

Discussions

Mycobacterium tuberculosis (Mtb) is responsible for the occurrence of infections in 1/3 of population and causes more than 2 million deaths annually (132).

In our study 9 of the 15 cases with TB were from regions with a moderate and higher incidence, results sustained by national data published in 2012 by Cristea C (133). In Romania, the highest incidence of TB is in age range of 45-65 years, most of the patients included in the study being found in the mentioned range (134, 135).

In our group in most of the cases (53.3%) TBC infection was diagnosed in the first year after starting biological treatment. This result is consistent with other reports in which TB occurs in the first year of anti-TNFα treatment, probably secondary to the reactivation of latent disease and not caused by an overgrowth.

Glucocorticosteroids are often part of the common therapeutic scheme used in patients with various systemic rheumatic and chronic pulmonary diseases. The problem comes from the fact that a considerable number of patients require moderate to high doses of glucocorticosteroids for prolonged periods of time. Studies have proved that these patients are exposed to several types of infections due to the suppression of their immune system caused by steroid treatment or by their disease. Data from the literature suggest that the incidence of development of TB is considerable in patients who receive glucocorticosteroids for the treatment of chronic rheumatic or pulmonary diseases and also for those who live in countries with a high incidence of this infection in the general population (136).

Usually, screening for LTBI and active disease is recommended before the initiation of the treatment. Also, it is suggested to consider a careful medical history before starting anti-TNF therapy. All patients should be questioned regarding their demographic details, history of bacillus Calmette Guerin (BCG) vaccination, TB risk factors, like recent close exposure to TB patients, immigration from high TB prevalence countries or recent stay these countries, radiographic evidence of TB sequelae, and last but not the least, current treatments. Diagnosis of LTBI is based mainly on the tuberculin skin test (TST), but unfortunately this test has several limitations. TST may give false negative results even in patients with TB risk factors, due to the high rate of previous longterm immunosuppressive treatments. Innovative blood tests that measure release of interferon–γ (IFN–γ) by T cells stimulated *in vitro* with Mtb-specific antigens represent a key solution for diagnosing TB infection. T-SPOT.TBTM (TS-TB, Oxford Immunotech, Abingdon, UK) and QuantiFERON In Tube (QFT, Cellestis, Carnegie, Australia) are two exemples of innovative blood tests, based on different methodologies, enzyme-linked immunospot assay (ELISPOT) and whole-blood ELISA. Both tests use 2 Mtb-specific antigens, ESAT-6 (early secretory antigen target-6) and CFP-10 (culture filtrate protein-10). IFN–γ release assays are more sensitive in detecting patients with active TB, and their results correlate better with Mtb exposure in people likely affected by latent infection (137).

Munoz et al. published a study intended to determine first the effectiveness of a comprehensive program for the prevention of anti-TNF–associated tuberculosis, and then to evaluate 3 latent tuberculosis infection screening strategies and the need for retesting patients with negative results at baseline. The study included 726 patients screened prior to anti-TNF therapy using 1 of 3 diagnostic strategies over 3 consecutive periods: first, a 2-step tuberculin skin test (TST); second, a 2-step TST plus QuantiFERON-TB Gold InTube test (QFT-GIT) (2-step TST/QFT); and third, a single-step TST plus QFT-GIT (TST/QFT). The patients with confirmed TB have received preventive therapy. The authors have determined differences in the incidence of tuberculosis between anti-TNF exposed and nonexposed patients, and between the 3 study periods. TB evolved during the first year in 2.85 ‰ exposed patient-years (3/1052 patient-years) and 1.77 ‰ non-exposed patient-years (1/566 patient-years). No cases have been recorded beyond the first year of treatment. LTBI diagnoses decreased with the single-step TST/QFT (26.5%) compared with the 2-step TST (42.5%) and 2-step TST/QFT (38.5%); the incidence of tuberculosis among exposed patients did not change significantly across the 3 periods (2.63‰, 3.91‰ and 2.4‰ patient-years, respectively). The conclusion of the study was that even if anti-

TNF–associated tuberculosis can be reduced, some risk remains during the first year of therapy (138).

The aim of another study was to evaluate the risk of serious infections (SIs) in patients with RA treated with anti-TNF therapy with emphasis on the risk across different ages. The study was a prospective observational study, in which was compared the risk of SI between 11798 anti TNF-treated patients and 3598 non-biologic disease modifying anti-rheumatic drugs (nbDMARD)-treated patients. A total of 1808 patients had at least one SI (anti-TNF: 1512; nbDMARD: 296). Incidence rates were: anti-TNF 42‰ patient-years of follow-up (95% CI 40, 44) and nbDMARD 32‰ patient-years of follow-up (95% CI 28, 36). The adjusted hazard ratio for SI in the anti-TNF cohort was 1.2 (95% CI 1.1, 1.5). Comparing three therapeutic agents: adalimumab, etanercept and infliximab the risk did not differ significantly. It was observed that the risk was highest during the first 6 months of therapy. Even if increasing age was an independent risk factor for SI in the two cohorts, there was no difference in relative risk of infection in patients on anti-TNF therapy in the older population. Also, there was no difference in hospital stay for SI between the two groups. Mortality within 30 days of SI was 50% lower in the anti-TNF cohort. The study offered important data add to currently available evidence suggesting that anti-TNF therapy is associated with a small but significant overall risk of SI (139).

In conclusion, the risk of reactivation of a latent TB during biologic therapy is greater in patients with rheumatic inflammatory diseases living in geographical areas with high endemicity of TB infection. Reduced compliance to chemoprophylaxis may be responsible for the occurrence of these cases.

I.2. Researches regarding rheumatoid arthritis

I.2.1. Introduction

Rheumatoid arthritis (RA) is a destructive inflammatory joint disease, that first of all it is affecting synovial joints. If not treated promptly, can have serious consequences, patients being constrained from functioning or working, with long-lasting effects on mental and physical well-being. Accelerated atherosclerosis and cardiovascular morbidity, infection, some cancers including lymphoma, and chronic mental ill-health are only some of the key comorbid conditions adding to the lifetime burden of RA and increasing mortality (140). Regarding the epidemiology of RA, the incidence (defined as the rate of new cases arising in a given period) is 0.1–0.2 per 1000 of the population for males and 0.2–0.4 per 1000 for females. Age and gender are two of the principal factors contributing to disease occurrence. The female sex preponderance implies that hormonal and reproductive factors strongly influence risk (141).

Rheumatoid arthritis is characterized by four distinct stages of progression. The first stage (stage I) involves initial inflammation in the joint capsule and swelling of the synovial tissue and induces clear symptoms of joint pain, swelling and stiffness. The second stage (stage II) is represented by inflammation of the synovial tissue becomes severe enough to cause cartilage lesions, the symptoms of loss of mobility and the range of movements become more common. The third stage (stage III) is represented by severe rheumatoid arthritis. Inflammation in the synovium destroys not only the cartilage of the joint but also the bone. Potential symptoms of this stage include increased pain and swelling and a further decrease in mobility and even in muscle strength. Physical deformities of the joint may begin to develop. In the final stage of rheumatoid arthritis, the inflammatory process stops and the joints stop working altogether. Pain, swelling, stiffness and loss of mobility are still the primary symptoms at this stage (142).

I.2.2. Factors that influence the quality life in rheumatoid arthritis patients

A. Background

For many years, rheumatic arthritis was an illness that was viewed as chronic and not very dangerous for people. But the evolution of medicine overcome this myth and brought arguments that the illness shortened life by 10 years on average. Nowadays, rheumatic arthritis is considered an incurable illness that impacts the body as well as the mental and social domains. The principal problems leading to the negative influence on the quality of life are pain, joint stiffness and the mobility limitations connected to it (143).

The interest in incorporating the concept of quality of life (QoL) in the evaluation of clinical and medical interventions is increasing considerably. QoL is defined by The World Health Organization (WHO) as *a broad ranging concept incorporating in a complex way the person's physical health, psychological state, level of independence, social relationships, person's beliefs and their relationship to salient features of the environment*. QoL can be measured in various ways, and several generic and RA-specific questionnaires can be used (144).

RA affects a wide range of joints, but the most commonly are those of the hands, knees and feet. RA is also associated with pain, fatigue, disability and functional loss, which can substantially decrease a patient's quality of life (QoL) (145).

B. Published article in this field

Szalontay A.S., Dima-Cozma C., Ifteni P., Paraschiv M., Duca D.S., Padurariu, **E. Rezuş**, *Studying the relevance of some factors that influence the quality of life in rheumatoid arthritis patients,* Nobel Med 2014; 10 (3): 12-17.

Patients diagnosed with RA usually have complains of chronic pain, stiffness and decreased mobility with significant impact on individual independency. Given the fact that the individual is limited in overall functioning, the quality of life of these patients is fairly impaired (146). An important goal in non-pharmacologic management is represented by the improvement of quality of life (147).

Regarding the quality of life, it is known that this parameter is worsened in patients with chronic diseases, including RA (148, 149, 150). The factors that influence the quality of life in patients who suffer from RA are not well understood. In the present study, we analysed how different factors dependent or independent of the disease may influence the quality of life, including some demographic factors, duration of the illness and also the presence of depression and anxiety.

Material and method

The patients selected for the present study were recruited from the Department of Rheumatology Clinical Rehabilitation Hospital, Iaşi. 75 patients met the American College of Rheumatology revised criteria (151). All the patients gave informed consent and the study was approved by the local ethic committee.

Demographic data was recorded for each patient including age, gender, provenance, marital status and number of children. Also, data regarding the duration of the illness and number of hospital admissions were (table I.2.1) recorded.

For evaluation of the quality of life (QOL), we used the *Short Form Health Survey (SF-36)*. The SF-36 scale, a reliable scale which is used both in clinical and research purposes and is currently the most recommended instrument for measuring physical function in patients suffering from RA (152), is a short form of Medical Outcome Study (Ware, 1994). The form applied has 11 items and measures 8 important parameters for estimating the quality of life such as physical function, physical role, body pain, general health, vitality, social function, emotional role and mental health. The scores are varying from 0 to 100, with greater scores indicating less limitation or discomfort (153).

For the evaluation of depression in patients with RA we used the Hamilton Rating Scale for *Depression-17 (HAMD-17) scale*. HAMD-17, a 17 item questionnaire used to measure

depression and is a good indicator for intensity of depression, includes items for evaluation of cognitive, behavioural and somatic aspects. Usually, a score above 14 is a sign of depression and an individual could reach a maximum score of 50 (154). In order to assess the presence of anxiety symptoms and the intensity of anxiety we used *Hamilton Anxiety Scale (HAMA).* HAMA is a semi-structured interview that comprises items for anxiety and also for somatic and cognitive symptoms associated with anxiety. The total score varies between 0 and 56, and the intensity of anxiety is indicated by a higher score (155).

Statistical Analysis

All statistical analyses were conducted with the statistical software package *SPSS statistics version 16* (Inc., Chicago, IL, USA). Descriptive statistics were used to describe demographic characteristic of participants at the baseline. The statistical tests used in this study were represented by independent sampl es t test (for continuous variables), ANOVA one way, Pearson correlations and regression. The statistical significance was indicated by a $p<0.05$. Categorical variables were expressed as frequencies.

Results

Demographic data

In the present study were included 75 patients (7 men and 68 women) with RA, age varied from 17 to 68 years. Provenance was registred as a dichotomous variable. 59 % of the subjects had a rural origin.

The duration of the disease was recorded as the time between the diagnosis and the inclusion in the study and also the number of hospital admissions, table I.2.1.

Table I.2.1. Duration of the disease and number of hospital admission in patients included in the study

Duration of the disease	**n (%)**
0-12 months	18 (24%)
1-5 years	30 (40%)
6-10 years	16 (21.3%)
> 10 years	11 (14.7%)
Number of hospital admissions	
1	17 (22.7%)
1-5	27 (36%)
6-10	9 (12%)
> 10	22 (29.3%)

Demographic data and QOL correlation

The correlation between gender and quality of life did not show statistical significance (r=-0.14, p=0.22). Regarding marital status, this parameter did not influence the overall scores of QOL (t=0.06, p=0.94), neither the physical dimension (t=-0.333; p=0.74) nor the mental dimension (t=0.441; p=0.66) of the scale score. The correlation between education and QOL did not show statistical significance [(F(2.74=1.122; p=0.33].

Duration of disease and QOL

The duration of the disease did not influence the total QOL scores (F=0.68; p=0.56), the physic dimension (F=0.841; p=0.476) or mental dimension (F=0.628; p=0.59) of the scale. There was a negative weak correlation between the quality of life and the duration of the disease (r=-0.13, p=0.25). Also, the results showed a tendency of reduction of QOL scores in patients who had been suffering from RA from 1 to 5 years and more than 10 years (figure I.2.1).

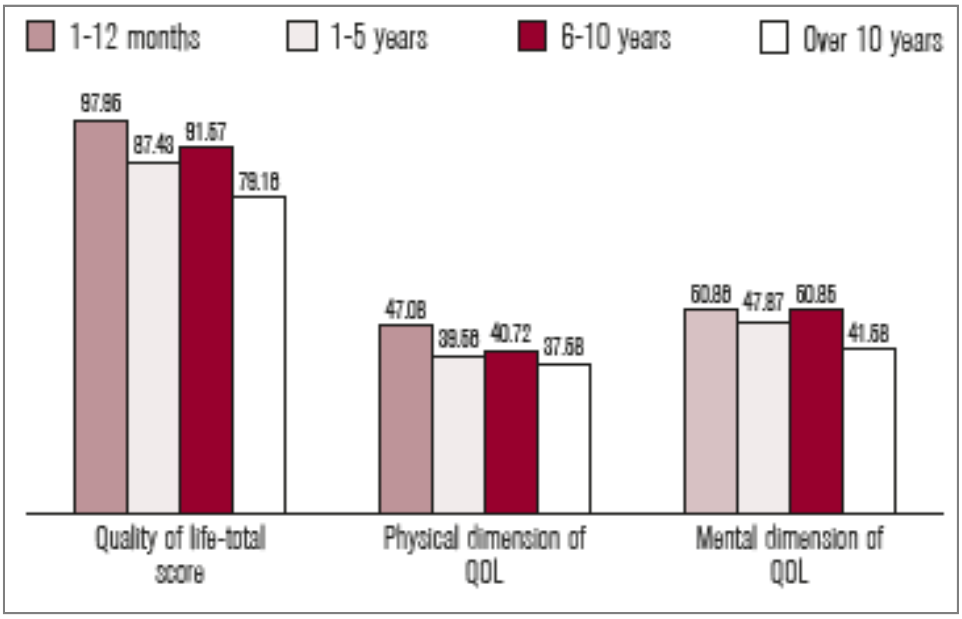


Figure I.2.1. Quality of life scores in RA according to disease duration

Depression and QOL dimensions

We analysed the correlation between QOL scores and depression measured with HAMD scale using Pearson correlation. The results indicated a significant negative correlation between the scores of depression scale and QOL scores for all the domains measured with the QOL scale ($p<0.05$), including physical function, physical role, body pain, general health, vitality, social function, emotional role and mental health (table I.2.2).

Table I.2.2. Correlation between anxiety and depression and QOL domains

Quality of life dimensions	r (Depression)	p (Depression)	r (Anxiety)	p (Anxiety)
Physical function	-0.404	<0.001	-0.41	<0.001
Physical role	-0.379	<0.001	-0.34	0.002
Body pain	-0.394	<0.001	-0.37	0.001
General health	-0.502	<0.001	-0.56	<0.001
Vitality	-0.592	<0.001	-0.47	<0.001
Social function	-0.404	<0.001	-0.5	<0.001
Emotional role	-0.555	<0.001	-0.52	<0.001
Mental health	-0.621	<0.001	-0.52	<0.001

The best models of regression are indicated below:

- The predictor physical function explains a significant percent from depression variance (15%), $F(1.73) = 14.211$; $p<0.001$. $F(1.73) = 14.211$.
- The predictors physical function and physical role explain a significant percent of depression variance (19%), $F(2.72) = 10.09$; $p<0.001$.
- The predictors physical function, physical role, somatic pain and general health explain 26% of the depression variance and the model is statistically significant $F(4.70) = 7.63$; $p<0.001$.
- The predictors physical function, physical role, somatic pain, general health and vitality explain 36% of depression variance $F(5.69) = 9.36$; $p<0.001$). The predictor vitality explains further 10% of variance depression.
- The predictors physical function, physical role, somatic pain, general health, vitality, social function, emotional role and mental health explain almost 50% of depression variance $F(8.66) = 8.841$; $p<0.001$.

Anxiety and QOL dimensions

The correlation between quality of life and anxiety measured with HAMD scale, indicated a significant negative correlation between the scores of depression scale and QOL scores for all the domains measured with the QOL scale ($p<0.05$) including physical function, physical role, body pain, general health, vitality, social function, emotional role and mental health (table I.2.2).

The most powerful models for predicting anxiety variance were:

- The predictors physical function, physical role, somatic pain and general health explain 31% of the anxiety variance and the model has statistical significance $F(4.70) = 9.36$; $p<0.001$).
- The predictors physical function, physical role, somatic pain, general health, vitality, social function, emotional role and mental health explain 50% of anxiety variance. The predictor mental health has the greatest power of prediction regarding the anxiety criteria.

Discussion

The analyses of demographic characteristics in patients with RA demonstrated an increase in the prevalence of RA in female gender group (ratio f:m=10:1). Our results are comparable with the data found by other authors who reported that many rheumatic diseases, including rheumatoid arthrits (RA) are more frequent in females than males. Frequently, RA starts more early in females, meaning that the females in average are exposed to the inflammation longer than males (156).

Even if conditions associated with excess estrogen and progesterone in women often appear joint protective, women are 2 – 4 times more likely than men to develop RA. Studies have shown that women with RA report decreased joint symptoms during the postovulatory phase of the menstrual cycle and during pregnancy, when estradiol and progesterone levels are high. The incidence of RA is also correlated with aging. Among women, the highest RA incidence seems to be between 45 and 49 years of age, suggesting an influence of perimenopausal hormonal changes (157).

Kvien et al. presented a study that had the main objective to examine the female versus male perspective regarding prevalence/incidence, etiological factors, disease severity/outcomes, access to therapy and therapeutic responses. The research group have found that the prevalence of RA is higher in females than males, the incidence being 4–5 times higher below the age of 50, but above 60–70 years the female/male ratio is only about 2. Smoking was a consistent predictor of RA in males, but findings have been more irregular in female. It was confirmed that health status is worse in female than male when corrections were made for different disease duration and for the underlying tendency of healthy females to report worse subjective health status than males. Also, the study has shown that female have less access to health services (156).

The analyses of demographic characteristic of the subjects with RA included in the present study, in relation with the quality of life, indicates that generally these factors did not seem to influence the level of perception of the individuals quality of life. However, a slight difference was seen in how education was associated with the quality of life in patients suffering from RA. When analyzing the duration of the disease in relation to quality of life, we obtained a weak negative correlation between quality of life and duration of disease without statistical significance.

Regarding psychological status analysis, we found that both anxiety and depression symptoms were negatively correlated with the subdomain QOL scores. The results showed that the more depressed or anxious person was the lower the quality of life was. Analysing the

models for predicting depression, we have found that the most powerful predictor for depression was represented by mental health parameter.

Generally, studies show that the main affected areas in RA patients are pain, fatigue and depression (158). Patients with chronic diseases are at a higher risk for psychological distress. Unfortunately, depression is an aggravating factor for the evolution of the illness, symptoms of chronic conditions being deteriorated in the presence of comorbid anxiety or depressive disorders. Psychological symptoms have a substantial negative impact on the quality of life, on the course and outcome of the chronic disorders and also on mortality, morbidity, and service utilization (159).

In the case of patients with RA, major depressive disorder affects between 13% and 17% of subjects, even when careful methods of assessment are applied. In fact, major depressive disorder is two to three times as common in patients with RA as in the general population. Actually, depression associated with RA is often considered to result from the experience of chronic pain and is a factor that worsens the prognosis and increases the risk of mortality (160, 161). Other causes of depression may be chronic inflammation, dysregulation of the hypothalamic-pituitary-adrenal axis, socioeconomic decline, functional and social impairment associated with RA (162).

In our study, anxiety level of the RA patients was measured with HAMA scale. The Pearson correlation showed a significant negative correlation between the HAMA scale scores and the QOL subscale scores. Regarding prediction of anxiety, as in depression, mental health was found to be the most powerful predictor ($r=-0.52$, $p<0.001$).

The challenge of identification and management of depression within the rheumatology clinic should be seriously considered. If depression is not correctly identified or treated, patients may erroneously attribute the source of their symptoms to their rheumatic disease, or there is a risk that patients will underestimate the symptoms of depression. Even in the situation when RA patients recognize the symptoms of depression, they may be circumspect to bring into discussion the topic with the rheumatologist because of time constraints, lack of provider continuity in an academic training center, or because they feel that mental health concerns are best discussed with other providers. Additional problems may occur in the case of ethnic or underserved population, because the patients may encounter supplementary barriers, including language, or the lack of psychotherapy services available in the public clinic setting.

Also, a unique challenge is given by the patient's acceptance of their mental illness. Most rheumatologists do not routinely screen their RA patients for depression, first because of time constraints and second, because of defective referral services, lack of training and confidence in dealing with mental health issues, or because they consider that other healthcare professionals will handle mental health concerns of their patients (163).

In conclusion, in the present we did not find a significant influence of demographic factors on the quality of life in these patients. Also, it seemed that the duration of the disease did not have an important impact on the level of quality of life perceived, either. However, the presences of psychological disturbances, such as anxiety or depression greatly

influenced the QOL scores. Moreover, the QOL dimensions predicted the depression and the anxiety criteria.

I.2.3. Medication used in rheumatoid arthritis patients

A. **Background**

Anti-rheumatic drugs can be classified into three groups:

- nonsteroidal anti-inflammatory drugs (NSAIDs);
- glucocorticoids;
- a spectrum of non-biological and biological immunosuppressive agents.

Non-biological agents are small molecules with a well-defined chemical structure, while biologicals are complex proteins with a defined amino acid sequence but variable glycosylation and three-dimensional structure.

The efficacy of NSAIDs is mostly limited to the control of signs and symptoms, except for a subset of patients with axial spondyloarthritis (SpA) (164). The efficacy of the other two groups enhances beyond the control of symptoms, concealing inflammation and halting progression of disease-specific organ damage.

In RA, the latter effect is comparable to inhibition of radiographic progression and serves as a foundation for a key therapeutic concept termed disease modification. Even if clear evidence that administration of glucocorticoids for a long time, inhibits radiographic progression in RA, the term disease-modifying antirheumatic drugs (DMARDs) is used for a subgroup of immunosuppressive/immunomodulatory agents exerting disease-modifying activity (165).

B. **Published paper in this field**

Chercherita L.E., **Rezus E**., Leon M.M., Stamatin O., Carausu E.M., *Impact of medication with diclofenac sodium Vs. Etoricoxibum in patients with inflammatory rheumatic patology, prosthetic complications and algodysfunctional syndrome*, Rev.Chim. (Bucharest), 65 (8), 2017, 977-981.

In the context of RA as the underlying disease, the temporomandibular joint (TMJ) is affected in 18% of patients (166). The dysfunctional syndrome of the stomatognatic system (DSSS) presents not only a complexetiopathogenesis, but also a complicated symptomatology, in which the signs of the dysfunctional affection of the stomatognatic system are countless, as well as the associated signs which incorrectly conduct the diagnosis to the nearby area (167).

Experimental part

Aim and objective of the study

Affections of TMJ can lead to imbalances and dysfunctions named DSSS. The TMJ affections we will take into consideration in this study, which is, in fact, the frequent pathology at this level, and to which we will measure the pain before and after the administration of the anti-inflammatory therapy. The aim of this study was to investigate the drug treatment of TMJ and the main objective is to realize a compare between I-st generation derivate, Diclofenac sodium and a derivative of II-nd generation, Etoricoxibum.

Material and method

This is a prospective study, based on data obtained from 531 patients with rheumatoid arthritis pathology, 96 (18.07%) of them with TMJ affected and DSSS, hospitalize at the Clinic of Rheumatology, Clinical Rehabilitation Hospital Iasi, between 01.01.2015 and 31.12.2016. The endpoints of interest were pain, physical function an patient global assessment of disease status (PGADS).

The inclusion criteria for the patients in our study were: all patients had to have experienced pain complaints in the temporal-mandibular region for at least 1 month, the presence of muscular tonus and muscular contraction alterations muscular dysfunction (pain at the level of stomatognatic system and cephalic extremity, limitation of mouth opening, and deviation of mandible from the medial line during the opening, fatigue of cephalic extremity muscles and functional alteration of stomatognatic system). Participants admitted or keeping follow-up appointments were also included.

The exclusion criteria of the patients were represented by the presence of joint affliction, of the third molar pathology, osteoarthritis and neoplasm (168, 169). Exclusion criteria were also: refuse of the patient to participate, uncooperative patients or those who did not respect the prescribed treatment. In addition, patients with confirmed congestive heart failure, ischemic heart disease, peripheral arterial disease and cerebrovascular disease were excluded from the group treated with Diclofenac sodium.

In our study, we administered two anti-inflammatories frequently used in the TMJ treatment: Diclofenac [Sodium–(0-(2,6-dichlorophenyl)-amino-phenyl) acetic acid, a phenyl-acetic acid derivative (figure I.2.2.A)], 150 mg/day and a derivative of II generation, (Etoricoxibum), 5-chloro-6'-methyl-3-[4-(methyl-sulfonyl) phenyl]-2,3'-bipyridine in doses of 30 or 60 mg/day for a period of two weeks (figure I.2.2.B).

Diclofenac sodium and Etoricoxib have been used to relieve pain and inflammation of the joints and muscles. For all patients, we used the lowest effective dose and shortest treatment duration to control the symptoms. A database was generated using Microsoft Excel 2010 for Windows and the SPSS statistical software package (version 18.2 for Windows; SPSS, Inc., Chicago, IL, USA) was used in order to perform the statistical processing of data and statistical analysis (170). The obtained data were allowed for the classification of patients with respect to gender distribution, age groups, area or origin, clinical aspects, type of treatment instituted and appreciation of pain.

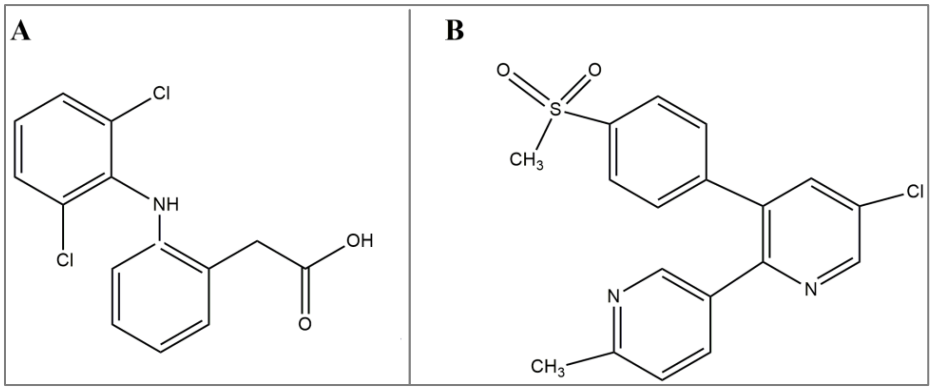


Figure I.2.2. A. Chemical formula for Sodium Diclofenac;
B. Chemical formula for Etoricoxibu

Results

Thereby, we classified the patients included in this study on age groups: 20-29 years– 9 (9.37%) patients, 30-39 years- 15 (15.63%) patients, 40-49 years- 60 (62.50%) patients and 50-59 years-12 (12.50%) patients. A significant statistical difference between the medium age groups, 40-49 years and the other groups ($p<0.001$) may be noticed. Another variable taken into consideration is the gender distribution. Our study included 66 (68.75%) female patients and 30 (31.25%) male patients.

Between the two gender groups studied there was a statistically significant difference ($p<0.001$). The patients were divided depending on the social environment. Thus, in our study there were 56 (58.33%) patients from the rural area and 40 (41.67%) patients from the urban area.

Symptomatology that indicate the DSSS in patients (that were presented in our services, in the Mihail Kogalniceanu Clinical Education Base), like pain, mobility, crackles, vertigo, tinnitus, inflammation, were revealed in figure I.2.3.

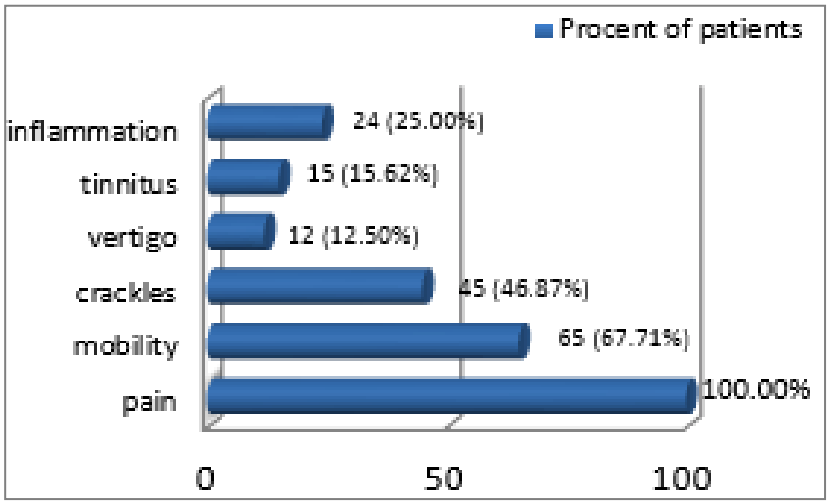


Figure I.2.3. Symptomatology of the patients with DSSS

The articular and muscular affectations

Pain and articular noises affect in a high percentage the studied group, which is due both to edentation grade (complications of it) and the chaotic, uncoordinated, functioning of the stomatognathic muscle system, especially of the external pterigoid (table I.2.3).

Table I.2.3. The articular affection

Clinical sign	Affected patients	
	Number	%
Articular pain	10	30.30
Articular salt	16	16.49
Deviation of the mandible	20	20.61
Subluxation	-	-
Limitation of the mouth opening	18	18.55
Crakles	28	28.86
Articular blockage	-	-

The examination of the muscles was made by inspection and palpation in order to determine the painful points and the irradiation areas and aimed to detect the pain provoked by contraction, symmetric and equal participation in the realization of the mandibular dynamic (table I.2.4).

Another variable taken into consideration is the articular mobility, and the third obvious sign were the crackles produced when using the articulation, which can be heard by the patient and also by the ones around him, but which are not significant in the absence of the pain and articular immobility. From the mobility point of view, in our study articular hypermobility can be found in 41 (42.71%) patients, which is much more frequent that the limitation of the mandible movements, which is present in 24 (25.00%) patients. Between the two parameters there is a statistically significant difference ($p<0.001$).

Modification of the cranial-mandibular relations

Examination of the centric relation had a major impact in establishing the diagnosis of the stomatognathic system's dysfunctions (table I.2.5). Interarcardic reports registered in centric relation will serve to detect the premature contacts (where applicable) in centric relation or at fitting models in the articulator.

Table I.2.4. The muscular affection

Clinical sign	Affected patients	
	Number	%
Muscular pain	10	30.30
Muscular hypertonia	33	100.00
Muscular spasm	33	100.00
Muscular hypertrophy	20	60.60
Muscular fatigue	33	100.00
Limitation of the mandibular movement	28	84.85
Dynamic modification of themandible trajectory	26	78.79

Table I.2.5. The cranial-mandibular relations

Name of the malrelation	Affected patients	
	Number	%
Extrapostural malrelation (I)	16	16.49
Excentric malrelation (II)	7	7.21
Extrapostular-excentric malrelation (III)	10	10.30

In case of dynamic occlusion the testing movement is realized, testing position with contact at the level of all the incisors (normal) and a distal occlusion (Cristhensen sagittal). It is observed the way in which dynamic occlusion is in compliance with mandibular dynamic, articular dynamic or muscular contraction (table I.2.6).

Table I.2.6. Modification of the occlusion

Modifications of the occlusion	Affected patients	
	Number	%
Modifications of the dynamic occlusion	16	16.49
Modifications of the static occlusion	7	7.21

Making proper medical history of the patient revealed a history of bruxism, trauma, local infection, stress and rheumatoid arthritis (figure I.2.4).

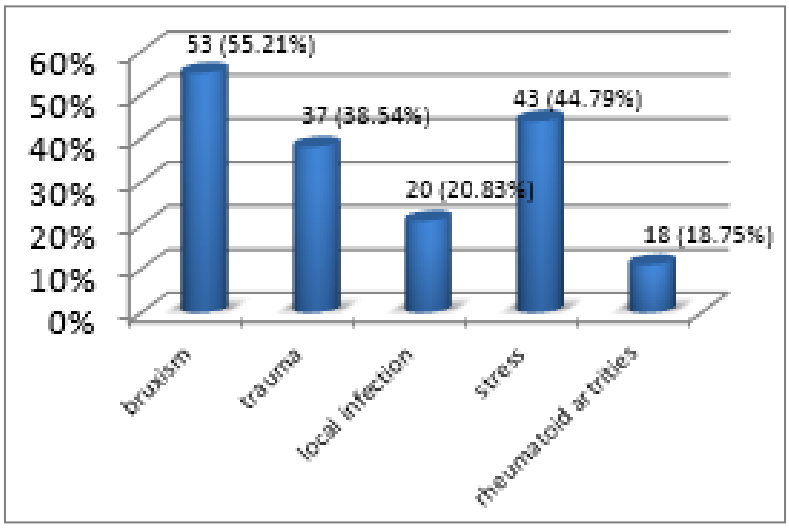


Figure I.2.4. Personal pathological antecedents

Discussions

Acording to literature data, pharmacological treatment of osteoarthritis is a real challenge. The most used drugs for the treatment of the pain are: paracetamol, tramadol, ibuprofen, etoricoxib, diclofenac sodium and so on, some of these having serious side effects. Paracetamol at a dose of > 2 g/day, increases the risk of gastrointestinal complications, especially in patients over 65 years of age, with prior gastrointestinal incidents and in patients > 85 years of age. Tramadol is correlated with a risk of dependence, if is used for more than 1 year, and in patients > 75 years of age the maximum daily dose should be less than 400 mg. Actually, it is a real therapeutic error to introduce tramadol as the first analgesic drug. Topical treatments may represent also a solution, but they have limited indications to use, because some joints (e.g. the hip joint) are poorly accessible through the skin (171).

Etoricoxib is considered a relatively safe and effective analgesic option in patients with osteoarthritis. A comparative study has evaluated treatment with etoricoxib (30 mg/day), ibuprofen (2400 mg/day) and placebo in patients with osteoarthritis. Etoricoxib proved a higher efficacy in the remedy of night pain than ibuprofen and also it had a much lower incidence of gastrointestinal side effects (172).

In our study, for the treatment of the pain it was used Diclofenac at a dose of 150 mg and Etoricoxib, in doses of 30 and 60 mg. Each group included 32 patients each. They were questioned and evaluated immediately after the treatment (4 h), after two days and after two weeks of treatment about the pain threshold according to VAS (Visual Analogue Scale) Etoricoxib (60 mg) demonstrated a significantly greater benefit within 4 h of the first dose (figure I.2.5.A) on the first day of therapy ($p = 0.001$) as evaluated by the percentage of patients with good or excellent responses (values 1-3 VAS). The effect of Diclofenac is moderate at this time and seemed significantly lower than after the administration of Etoricoxib (figure I.2.5.B).

Moreover, the use of the two doses of Etoricoxib highlights a stronger effect on a higher dose, but is not statistically significant.

The second interrogation of the patients took place after two days. It seems that the effects have been similar, no matter what anti-inflammatory was used, the patients declaring that they felt a moderate pain, significantly lower than the one they felt in the initial moment of the administration. Even though, it can be noticed that the patients who were administered Etoricoxib 60 mg were predominant in the group with the lack of pain and minimum pain. The last evaluation was conducted after two weeks of treatment.

Etoricoxib 30 mg determined a moderate reduction (maximum number of patients) of the pain threshold. So, the Etoricoxib demonstrated at least moderate clinical improvements. Diclofenac 150 mg resulted in at least small improvements (figure I.2.6.A). The effects of the anti-inflammatories were preserved throughout the administration, without any significant changes (figure I.2.6.B).

Baraf et al. studied the Etoricoxib gastrointestinal tolerability in contrast with Diclofenac Sodium gastrointestinal tolerability and effectiveness. The diclofenac dose has been similar with the one used in our own study i.e. 150 mg/day, and Etoricoxib dose has been higher than ours i.e. 90 mg/day. For etoricoxib the rate of cumulative discontinuation due to gastrointestinal adverse effects has been 9.4 per 100 patient-years compared with 19.2 per 100 patient-years in the case of diclofenac. For both drugs involved in the study, the risk of thromboembolic cardiovascular events was comparable, more precisely it was 1.25 events per 100 pacient-years for Etoricoxib and 1.15 events per 100 pacient-years for diclofenac. The authors also monitored the risk of treatment discontinuation on account of hypertension and the results indicated a higher risk for etoricoxib (2.3%) than for diclofenac (0.7%). Even if the treatment with etoricoxib involved a higher dose than in our study, the drug was associated with significantly better gastrointestinal tolerability compared to diclofenac in the population of patients with osteoarthritis. As a final remark it was showed that Etoricoxib 90 mg, a dose 50% higher than indicated for osteoarthritis, resulted in more discontinuations due to hypertension-related adverse experiences (173).

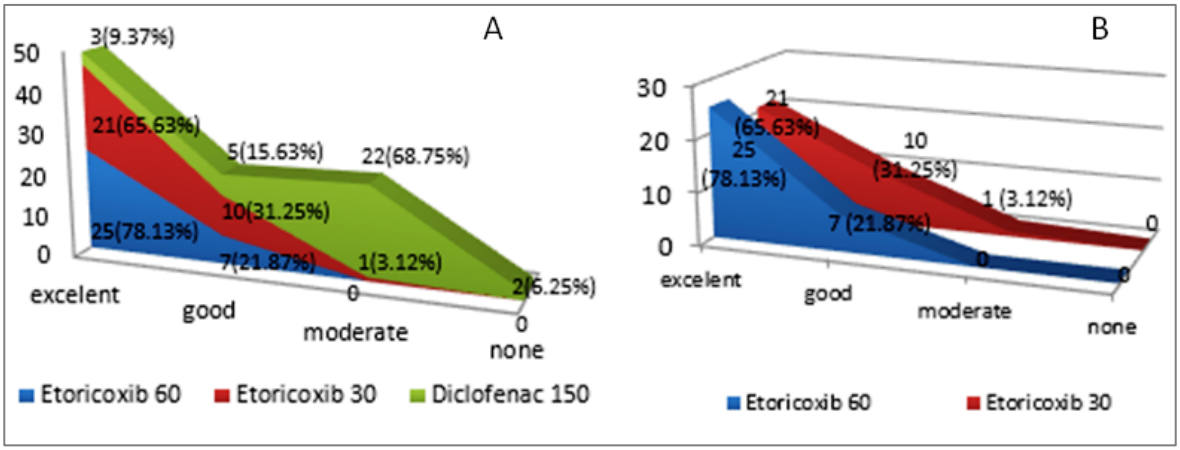


Figure I.2.5. A. Effect of the NSAIDs after 4h; B. Comparison of the effect of Etoricoxib

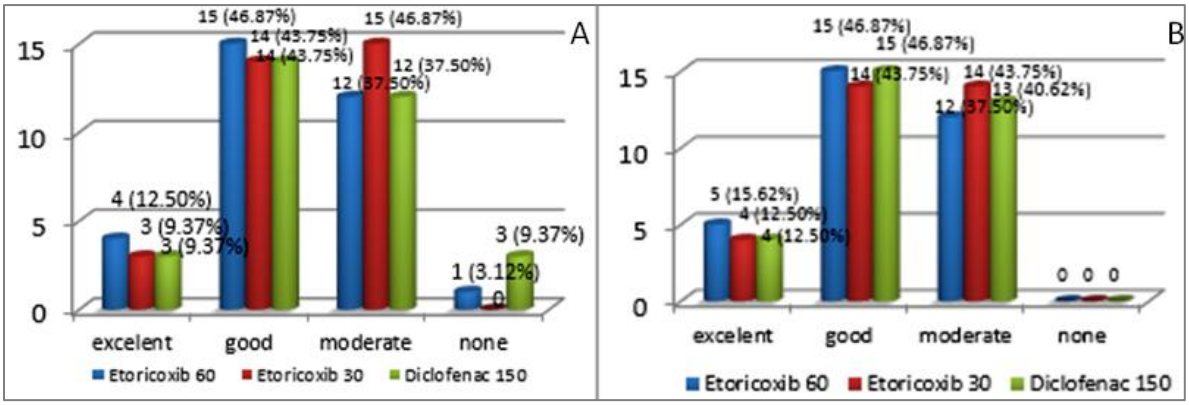


Figure I.2.6. A. Effects of the NSAIDs after 2 days; B. Effects of the NSAIDs after 14 day

The economic evaluation demonstrated that Etoricoxib (60 mg) is an economically superior treatment to that of Diclofenac (150 mg) for both QALY gains and cost savings for a time horizon longer than 5 years (174).

Patients have been applied, simultaneously, different fix prosthetic treatments, mobile, as well as relaxation mouth guards, an improvement of the quality of life of these patients being registered, as it follows: in 75% were applied acrylic prostheses and in the rest, both fixed and mobile, only in 2 cases, representing 2.08%, being used the method of relaxation mouth guards.

To evaluate and compare the efficacy and tolerability of etoricoxib and diclofenac in patients with osteoarthritis of the knee or hip, Zacher et al. have developed a 6-week double-blind study. In the study were involved 516 patients, randomised to receive either etoricoxib 60 mg once daily (n = 256) or diclofenac 50 mg three times daily (n = 260). Western Ontario McMaster osteoarthritis index (WOMAC) pain subscale was the primary study endpoint, together with WOMAC stiffness and physical function subscales, and the Patient's Global Assessment of Response to Therapy (PGART) questionnaire. Early efficacy was evaluated using WOMAC first question (pain walking on a flat surface) and PGART 4 h after the morning dose of each drug on the first and second day of administration. The study aimed to show comparable efficacy between etoricoxib 60 mg/day and diclofenac 150 mg/day. Etoricoxib dose was comparable in efficacy to diclofenac dose on all the above parameters. The treatment effects of both drugs were comparable starting with day 2 and were maintained throughout the 6 weeks of therapy. Both treatments have proved to be generally well tolerated. An important conclusion was the fact that Etoricoxib is clinically effective in the therapy of osteoarthritis providing a magnitude of effect comparable to that of the maximum recommended daily dose of diclofenac (175).

In conclusion, both treatments were generally well tolerated. Etoricoxib is clinically effective in the therapy of TMJ providing a magnitude of effect comparable to that of the maximum recommended daily dose of Diclofenac. The onset of clinical benefit with Etoricoxib on day one is more rapid than that of Diclofenac, both were generally well tolerated. It should also be mentioned that the improved outcome of the patients was not only in terms of pain but also clinically, meaning at the TMJ function.

I.2.4. The impact of proinflammatory cytokines of rheumatoid arthritis in the generalized loss of bone mass

A. Background

The cytokine network in RA is a complex field, with a lot of cytokines showing pleiotropic actions and many different targets. These cytokines can be divided in two groups, the pro-inflammatory and anti-inflammatory cytokines. Controlling the balance between these two groups is considered as an important therapeutic goal.

Two key pro-inflammatory cytokines in RA are IL-1 and TNFα, their regulation being essential in the RA disease. First data of clinical trials showed efficacy, however, revealed also that blockade of these cytokines did not fully control the arthritis in all patients. Recent discoveries of novel cytokines in the pathology of arthritis, such as IL-17, IL-18 and RANK ligand (RANKL) will be a real help for a better understanding of the pathogenesis of chronic arthritis and, very important, may contribute to improvement of current therapies. IL-4 and IL-10 are pleiotropic cytokines, and are considered as promising modulators in the control of RA. It is well established that TNF and IL-1 are key cytokines in the process of chronic joint inflammation and the concomitant erosive changes in cartilage and bone (176).

Bone remodeling is a highly complex process by which old bone is replaced by new bone, The process has three phases: initiation of bone resorption by osteoclasts, the transition (or reversal period) from resorption to new bone formation, and the bone formation by osteoblasts. The coordinated actions of bone cells: osteoclasts, osteoblasts, osteocytes, and bone lining cells, are the key of this process (177).

In the first phase, the maturation and activation of osteoclast precursors are mediated by receptor activator of nuclear factor-kβ ligand (RANKL), which is expressed on the cell membrane of osteoblasts/lining osteocytes and binds to RANK on osteoclasts precursors. Alterations in bone metabolism lead to diseases like osteoporosis. Also, it must be taken into account, the fact that the disequilibrium of bone metabolism is also brought about by immune signals, which consist of immune-competent cells such as macrophages and lymphocytes, the largest and most predominant source of cell-derived regulatory signals in the body. For example, the major source of IL-1 is macrophages; and dendritic cells express multiple co-stimulatory ligands, and through, the immune system has a vast range of effects on many systems, including bone metabolism (178).

B. Published paper in this field

Barzoi R., **Rezus E.,** Badescu C., Al Namat R., Ciocoiu M., *The Impact Of Proinflammatory Cytokynes Of Rheumatoid Polyarthritis On The Generalized Loss Of Bone Mass,* Rev.Chim.(Bucharest), 69 (9), 2018, 2541-2545.

Chronic inflammation is a key mediator for the loss of local and systemic bone mass, especially in patients with rheumatoid arthritis. In these patients, cytokines are present in high

concentrations in both, synovial arthritis and systemic circulation. Some proinflammatory cytokines function as direct or indirect stimulators of osteoclast differentiation, but also of its survival or activity (179).

The observation of the presence of systemic inflammation as a common pathophysiology mechanism for both, rheumatoid arthritis and osteoporosis, has motivated the onset of research in an attempt to gain a better understanding of the causes that lead to the acceleration of bone loss in this category of patients, ensuring thus a better patient management.

Experimental part

Materials and methods

The study was a prospective observational case-control study, conducted between 2017 and 2018, in order to determine the cumulative risk factors underlying the early onset and progression of osteoporosis in patients with RA. In the study were included 93 patients with ages ranging between 20 and 65, diagnosed with rheumatoid arthritis in stage I, II or III and without associate endocrine-related comorbidities.

During the two evaluations, at the initial hospitalization and 6 months after, there were recorded the biological inflammation parameters (VSH, CRP), as well as certain biological parameters that determine possible complications of the basic disease or the side effects of the underlying treatment (hematological, hepatic, renal).

We performed 25-OH vitamin D dosing, in order to determine possible correlations with bone loss and the disease activity. The immunological markers of the disease were also monitorized (the rheumatoid factor, Waaler Rose and Elisa tests, the total anti cyclic citrullinated and antinuclear antibodies). Also, we performed the imaging of mineral-bone density in the hip and lumbar spine, in order to test the degree of bone loss in these patients. In 86 patients, we carried out (initially and after 6 months) dosages for inflammatory cytokines: IL1, TNFα and RANKL, by ELISA tests, in order to track their dynamics over time, correlated with the progression of the inflammatory disease and, at the same time, with the degree of bone loss. Internationally recognized disease scores were carried out: DAS 28, CDAI, SDAI, HAQ.

During this time, patients were treated with classical or biological dMARDS – disease-modifying anti-rheumatic drugs. Most of them received also vitamin D after the initial evaluation, because this study group showed high or moderate vitamin D deficits. The data was processed by using statistical functions from SPSS 18.0 at the significance threshold of 95%.

Results

Out of the 82 patients with RA, reevaluated after 6 months, 28 (34.1%) had systemic osteoporosis. The biochemical parameters monitored upon revaluation after 6 months presented the following characteristics: upon revaluation, the VSH varied from 4 to 115 mm/h, recording a series of homogeneous values ($p = 1.259$); the average level was 34.39±22.25 mm/h, with no significant difference from the initial moment 34.18±22.25 mm/h ($p=0.834$) ($p=0.834$). After treatment, the CRP varied very widely in the range of 0.40-102 mg/dl. The average level was 7.28±8.63 mg/dL, slightly lower compared to the initial moment 7.55±4.06 mg/dl ($p=0.710$).

The series of values for total wseric calcium, after treatment, was homogeneous (p=0.230), with variations in the range of 8.10-10.20 mg/dL and an average level of 9.12±0.40 mg/dL, significantly lower compared to the initial moment 9.20±0.48 mg/dL (p=0.002). The Skewness Test suggests that value series recorded a non-normal distribution for IL1α (value above 2 – p = 3.28, figure I.2.7.A), TNFα (value above 2 – p = 4.26, figure I.2.7.B) and for RANKL (value above 2 – p = 2.05, figure I.2.7.C),

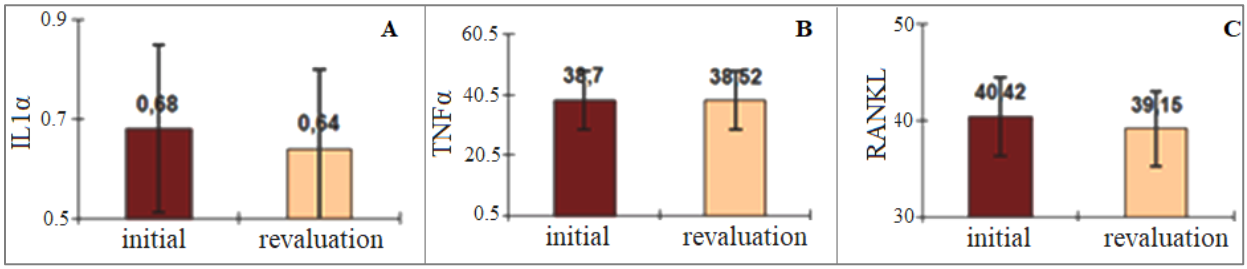


Figure I.2.7. A. Evolution of the average level of IL1α; B. Evolution of the average level of TNFα; C. Evolution of the average level of RANKL;

Initially the correlation between seric calcium and RANKL was direct, reduced in intensity and statistically insignificant (r=+0.132, p=0.781), an aspect which was maintained upon revaluation (r=+0.113; p=0.708). The correlation between vitamin D and RANKL was indirect, and also reduced in intensity and statistically insignificant (r=-0.112, p=0.331), an aspect which was maintained upon revaluation (r=-0.129; p=0.246).

Initially, the correlation between seric calcium and IL1β was indirect, reduced in intensity and statistically insignificant (r=-0.134, p=0.244), an aspect which was maintained upon revaluation (r=-0.104; p=0.353). The correlation between vitamin D and IL1β direct and reduced in intensity at the beginning of the study (r=+0.107, p=0.354), but upon revaluation these parameters seemed to be independent (r=+0.027; p=0.808).

Regarding the correlation between seric calcium and TNFα, initially, it was direct, reduced in intensity and statistically insignificant (r=+0.145; p=0.699), but upon revaluation these parameters seemed to be independent (r=+0.045, p=0.685). Also, vitamin D and TNFα were apparently independent parameters at the onset of the study (r=+0.088; p=0.447) and upon revaluation (r=+0.052; p=0.685).

The RANKL–IL1β correlation (figure I.2.8.A) and IL1β - TNFα correlation (figure I.2.8.C) were both indirect and reduced in intensity at the onset of the study, and upon revaluation. RANKL and TNFα (figure I.2.8.B) were apparently independent parameters at the onset of the study and upon revaluation. Upon revaluation, vitamin D growth was accompanied by a RANKL decrease in 12.9% of patients and approximately the same level of IL1β and TNFα. (figure I.2.8.D).

In the case off patients experiencing osteoporosis during the first evaluation, when reevaluated, the decrease in vitamin D was accompanied by a slight decrease in RANKL, IL1β and TNFα, and a significant decrease in VSH and CRP. Upon revaluation, the lowest average RANKL value was observed in patients treated with biological therapy, but the difference was

not statistically significant (p=0.709). The highest average value of IL1β was found in patients treated with biological therapy associated with classic DMARDs and corticotherapy, but the difference was not statistically significant (p=0.740). The highest average TNFα value was found in patients treated with biological dMARDS associated with classic dMARDS and corticotherapy (p=0.001).

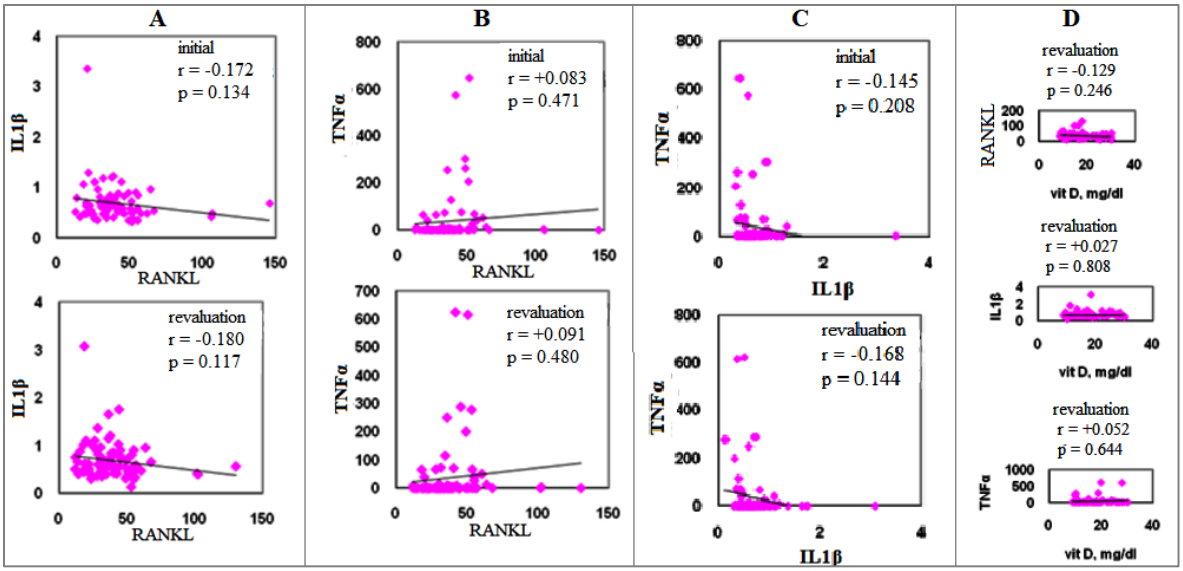


Figure I.2.8. A. RANKL–IL1β correlation; B. RANKL– TNFα correlation C. IL1β–TNFα correlation; D. Correlation of vitamin D with cytokines upon revaluation

The average cytokine level upon revaluation was lower in patients with osteoporosis, but the only significant one from the statistical point of view was the TNFα level: (RANKL: 34.48 vs 40.51; p=0.189; IL1: 0.67 vs 0.68; p=0.871; TNFα: 21.75 vs 43.66; p=0.04). The series of values recorded for IL1a, TNFα and RANKL were non homogeneous, but only the average level of IL1a significantly dropped upon revaluation, with an improvement in the degree of inflammation.

Discussions

Chronic inflammation is considered a risk factor for bone loss. The chronic inflammatory disorders, like RA, ankylosing spondylitis, inflammatory bowel disease and many others, have been linked to an increased fracture risk. Bone loss in RA patients is a frequent and clinically serious event. Thinking of bone remodelling in general, the balance between bone formation and bone resorption determines the net effect. Important gains in knowledge about the role of bone resorption during chronic erosive arthritis have been made and also it has been proved that inflammation itself triggers bone resorption by osteoclasts. Pro-inflammatory cytokines, potent mediators of bone loss, act both directly and indirectly to enhance osteoclastogenesis in the inflamed joint and systemic bone (179).

A direct correlation between inflammatory cytokines and bone loss could not be established in the present study. In the case off patients experiencing osteoporosis during the first evaluation,

when reevaluated, the decrease in vitamin D was accompanied by a slight decrease in RANKL, IL1α and TNFα, and a significant decrease in VSH and CRP. Upon revaluation, the lowest average RANKL value was observed in patients treated with biological therapy, but the difference was not statistically significant.

It was established that, despite the studies showing an important deviation in the bone mineral density in patients with RA, which is more frequent in older conditions, the rate of osteoporosis in the studied group was rather limited.

In conclusion, our study has shown that the early initiation of the therapy with anti-TNF inhibits bone destruction, regardless of the anti-inflammatory activity in patients with RA. In the evaluated cases, the treatment with TNF and IL6 blockers slowed down the progression of bone loss in patients with RA regardless of the duration of disease progression, the degree of activity of the disease, or the administered anti-inflammatory therapy. In these patients, there was no systemic osteoporosis or only a slight decrease in the mineral density of bones, which is within the field of osteopenia. Our increased interest in the pathophysiological mechanisms of bone loss in RA can lead to new therapeutic concepts on RA, including anti-erosive therapies. Despite the progress in osteoimmunology, many studies have shown that even today, a large proportion of patients with RA (up to 80%), develop bone destruction during the progression of the disease. Thus, further research is necessary to completely elucidate the pathophysiology of bone loss directed by osteoclasts in patients with RA.

I.2.5. Cervical spine lesions in rheumatoid arthritis patients

A. Background

The inflammatory processes in rheumatoid arthritis (RA) affect mostly joints of hands and feet, cervical spine being the third most common location of lesions in the course of RA. In 44–86% of patients with RA had been observed pathological lesions in cervical spine structure, the upper cervical spine is usually affected, which is manifested by the instability between C1 and C2 vertebrae (atlanto-axial subluxation – AAS) occurring in 65% of patients with RA. Anterior subluxation occurs most often (around 75% of all AAS). Moreover, about 20% of patients subluxation of the spinal motion segment can occur at the C3 to C7 vertebrae (subaxial subluxation, SAS) (180).

B. Published paper in this field

Macovei L.A., **Rezus E.**, Cervical spine lesions in rheumatoid arthritis patients, Rev. Med. Chir. Soc. Med. Nat., Iaşi, 120 (1), 2016, 70-76.

The present study focused on monitoring clinical and laboratory data of cervical spine lesions in rheumatoid arthritis patients.

Material and method

The study included a group of 107 RA patients who were admitted to the 1st Rheumatology Clinic, Iasi Rehabilitation Hospital between January 2013 and December 2014. T-student and chi-square tests were used for the statistical evaluation of data. They were clinically significant when *p* is less than or equal to 0.05. The database was systemized with Excel (Microsoft Office), and the statistical analysis used the statistical programs MedCalc and Epi Info 2000.

Results

RA incidence and prevalence were reported differently according to the diagnostic criteria used in population studies (table I.2.7). Out of the total number of 20 (18.69%) RA patients with cervical spine involvement admitted during the study period, 13 (12.14%) were women and 7 (6.54%) were men.

Table I.2.7. Incidence and prevalence in the study group

Year	**2013**	**2013**	**Total**
Total number of RA patients	46	61	107
RA with cervical damage – new cases	6	9	15
RA with cervical damage – reappraisal	2	3	5
Incidence of cervical damage in RA patients	13.33%	20.00%	33.33%
Prevalence of cervical damage in RA patients	17.39%	14.75%	32.14%

The main clinical manifestations were pain, swelling, morning stiffness and functional discomfort. In patients with severe cervical damage, extra-articular manifestations included changes in the skin, subcutaneous tissue and juxta-articular tissues, respiratory and cardiovascular systems (table I.2.8).

Tabel I.2.8. Distribution of general signs in RA patients and extra-articular manifestation in RA study group patients

General signs	**%**	**Extra-articular manifestation**	**%**
Asthenia, fatigue	97	Subcutaneous nodules	39.7
Loss of appetite	45	Tenosynovitis	31.0
Transient arthralgia	91	Myopathy	36.3
Raynaud's syndrome, accrocyanosis	49	Cardiomyopathy	6.1
Weight loss	12	Rheumatoid lung	24.3
Irritability	83	Pleurisy	0.93
Depression, insomnia	85	Pulmonary fibrosis	7.5

The values related to the duration (in years) of RA were dispersed, ranging from 0 to 35 years of disease, most patients (64.5%) being in the group with a duration of less than 5 years.

Inflammatory cervicalgia and cervical stiffness was present in all study group patients studied, but the elderly with senile RA, aged 65 years, cervicalgia showed also degenerative changes. Signs suggesting the localization of the rheumatoid process at the cervical level in the study group were found in varying proportions (table I.2.9).

Table I.2.9. Symptoms of cervical damage in RA patients

Clinical signs of cervical damage in RA	**%**
Scalp tenderness	66%
Tenderness of the spinous apophyses, cervical chain	95%
Altered sensitivity of the cervical region and upper limbs	45%
Muscle tenderness	74%
Muscle weakness in regional muscles and upper limb end	54%
Fasciculations	1%
Transient hearing loss	3%
Sensory ataxia	1%
Sensitivity of the carotid sinus	9%

Discussions

The disease causes destructive lesions due to granulomatous infiltration of vertebral structures and medullary sheaths. These lesions lead to damaged discs and instability that produces subluxations and dislocations. The suboccipital region is most affected; in other regions of the spine, high lesions of C4-C5 prevail, where osteolysis damage of spinal apophyses are found. In atlas and axis joints, rheumatoid arthritis causes the inflammation of bursa, synovium and joint capsule and leads to synovial pannus formation. This causes the destruction of cartilage and subchondral bone. Atlantoaxial dislocation is caused by erosive synovitis of atlanto-epistrophic joint, atlanto-odontoid joint and serous bursitis separating the odontoid process from the transverse ligament.

*In conclusion***,** the dominant symptom of cervical spine damage was pain associated with stiffness and limited joint mobility, muscle stiffness, poor posture.

I.2.6. Rheumatoid arthritis and periodontal diseases

A. Background

Oral manifestations are frequent in patients with rheumatic diseases. Oral hyposalivation, xerostomia, temporomandibular joint disorders, periodontal disease, and dysphagia are among the signs and symptoms that may be the first expression of a number of rheumatic diseases.

Periodontal disease is a chronic inflammatory condition caused and perpetuated by dysbiosis of the commensal microbiota in dental plaque. It is quite dangerous disease because can destroy the gingiva and tooth-supporting tissues (bone and periodontal ligaments) and eventually

leads to tooth loss. Chronic inflammation is amplified by the interactions of infectious, environmental and genetical factors and the host immune. Periodontal disease has been frequently described in patients with RA, a chronic autoimmune polyarthritis with a prevalence of 0.5% to 1.0% in the adult population and a female:male ratio of 3:1. In a first stage it affects joints, but is also considered to be an inflammatory systemic disease (181).

B. Published papers in this field

As mentioned above, many studies have shown that there is a close link between two of the most common chronic inflammatory diseases: RA and periodontal disease. Together with colleagues from the Faculty of Dental Medicine and other collaborators, I have studied the similarities between the two mentioned disease, and the results have been published in three papers, presented in chronological order from publication.

1. Boatca R.M., Scutariu M.M., Rudnic I., Martu Stefanache M.A., Hurjui L., **Rezus E.,** Martu S., *Evolution of Inflammatory Biochemical Markers Within Periodontal Therapy to Patients with Rheumatoid Arthritis,* Rev. Chim.(Bucharest), 67 (4), 2016, 741-744.

The study evaluated the impact of specific etiological periodontal therapy on systemic inflammatory biochemical markers during of 6 months. Similarities between rheumatoid arthritis and periodontal disease were analyzed. Also was evaluated the specific etiological periodontal therapy pathology (to eliminate the infection at this level) on serological markers of systemic inflammation: C-reactive protein – CRP, erythrocyte sedimentation rate – ESR and alpha-1-acid glycoprotein – AAG, in patients with rheumatoid arthritis and periodontal disease. The effects of therapy were analyzed clinically periodontal and biochemically to validate the effectiveness of periodontal treatment.

CRP, ESR and AAG have been studied in the context of rheumatoid arthritis because these biomarkers have been associated with biological aspects encountered in the context of periodontal disease, and were found to be significantly increased in this condition, compared to systemically healthy patients (182, 183). These markers were studied in the context of rheumatoid arthritis, chronic inflammatory disorder because this has a positive association with periodontal disease and high levels of these serum mediators occur in patients with rheumatoid arthritis.

Materials and method

The study included 19 patients diagnosed with periodontal disease divided into two groups, one in patients with periodontal disease and rheumatoid arthritis (test group), and another group of patients with periodontal disease but without rheumatoid arthritis (control group). Both groups received specific periodontal therapy and were monitored in terms of the development of biochemical markers levels. The results indicated that the majority values of biochemical markers

are improved after the first 3 months and then are maintained after 6 months from moment of therapy initiation.

Serum samples were collected by venipuncture initially at 3 and 6 months after the completion of treatment. Serum levels CRP were analyzed by specific kits and evaluated by an automated high-sensitivity immunoturbidimetric device (Greiner Diagnostic, Gmbh, the detection limit is less than 0.25 mg/L). Normal values are generally below 0.5 mg/dL. ESR was assessed using Westergren manual method. The normal values are less than 10 mm/1h for men and less than 20 mm/1h for women. AAG concentration was determined by kinetic nephelometry. The normal value is between 55-140 mg/dl.

Laboratory parameters were statistically analyzed at baseline (T0), at 3 (T1) and at 6 (T2) months post-treatment. Mann-Whitney test was performed to compare the differences between groups, and Friedman ANOVA and Wilcoxon tests were conducted to compare the differences between the examination periods (baseline, 3 and 6 months) in each group. Spearman's rank correlation testwas used to analyze the association between variables. All statistical analyzes were considered significant level (p) of 5%.

Results

Results were obtained by conducting comparative clinical - biological studies on basis of personal data concerning health and periodontal damage to susceptible or affected by chronic periodontal disease with or without rheumatic disease. CRP has the highest values in the group of patients with chronic periodontitis and rheumatoid arthritis (table I.2.10).

Table I.1.2.10. Average value of CRP (mg %), ESR (mm/h) and AAg (mg %) in both groups

Analyze (average value)	Patients	T0 initial	T1 after 3 months	T2 after 6 months	Friedman	T1-T10 Wilcoxon	T2-T1 Wilcoxon	T2-T0 Wilcoxon
CRP (mg %)	TEST group	0.90 (1.06)	0.86 (1.11)	0.63 (0.54)	P<0.4203	-	-	-
	CONTROL group	2.07 (1.26)	1.23 (0.77)	0.63 (0.31)	P<0.0000	P=0.0024	P=0.0022	P=0.0015
ESR (mm/h)	TEST group	24.53 (13.13)	24.07 (13.51)	22.40 (13.13)	P<0.6449	-	-	-
	CONTROL group	37.47 (7.36)	34.67 (9.59)	27.67 (9.60)	P<0.0000	P=0.0106	P=0.0007	P=0.0007
AAg (mg %)	TEST group	91.57 (24.93)	92.11 (27.54)	88.67 (23.95)	P<0.9355	-	-	-
	CONTROL group	97.67 (10.87)	98.20 (10.56)	78.27 (12.39)	P<0.0000	P=0.0031	P=0.0012	P=0.0018

*It must be mentioned that the first value is the average and median value in parentheses represents the value from the middle of the range of values, we therefore mean (standard deviation) and median. It is used when data are not symmetrical (unevenly distributed); Mann-Whitney test (U) is used two independent groups and in this case, p = 0.0136; Friedman test (Fr) reveals the extent to which repeated evaluations ranks really different (statistically

significant) together; Wilcoxon signed rank test is used to rank the difference of the two samples pairs that is, when the same subjects are evaluated twice.

On this line, CRP is generally considered as the most sensitive response marker of infectious acute phase of tasks and/or of inflammation. For laboratory results of markers of an acute phase, in test group, the values of ESR, PCR and AAG showed no statistical differences after 6 months (0-3, 0-6 and 3-6), but at three months were observed statistically significant difference. Systemic markers in the control group, CRP ($p < 0.0000$), ESR ($p < 0.0000$) AAG ($p < 0.0001$) showed statistically significant reductions. These differences appeared in the examination periods of 0-3, 0-6, 3-6 months.

CRP, ESR and AAG have been studied in the context of rheumatoid arthritis because these biomarkers have been associated with biological aspects encountered in the context of periodontal disease, and were found to be significantly increased in this condition, compared to systemically healthy patients (184).

Discussions

About 3% of the population suffer from a chronic inflammatory rheumatic disease and many of these patients experience oral manifestations. These kind of manifestations may be the first clinical sign or symptom of a systemic disease. Oral aphthosis is a non-specific and very frequent manifestation. Oral aphthosis presence in association with typical clinical manifestations and disease biomarkers give rise to clinical suspicion: oral aphthous ulcers have a prevalence of up to 50% of patients with systemic lupus erythematosus (SLE), whereas xerostomia and hyposalivation are respectively a symptom and sign reported by 90% of patients with Sjögren's syndrome. Some oral manifestations are quite rare but typical, e.g. strawberry-like gum disease in patients with granulomatosis with polyangiitis (181).

Kasser et al. started a study in order to quantify periodontal disease in rheumatoid arthritis (RA) patients and controls. Also, the authors aimed to correlate the degree of destruction from periodontal disease and from RA. Fifty RA patients were matched for age, sex, smoking status, and oral hygiene with 101 controls. There were determined correlations between indices of chronic destruction in periodontal disease (gingival attachment loss) and in RA (Larsen radiographic score). The study revealed that patients with long-term active RA who were receiving treatment with disease-modifying antirheumatic drugs ($n = 46$), corticosteroids ($n = 38$), or nonsteroidal antiinflammatory drugs ($n = 43$) had a higher rate of gingival bleeding (increased by 50%), greater probing depth (increased by 26%), greater attachment loss (increased by 173%), and higher number of missing teeth (increased by 29%) compared with controls. No correlation was found between the Larsen radiographic score and gingival attachment. It seems that patients with long-term active RA have a substantially increased frequency of periodontal disease, antiinflammatory treatment interfering with periodontal disease and having concealed a possible correlation between the indices of chronic destruction in RA and periodontal disease (182).

Improve periodontal status affected laboratory results (protein in the acute phase) which was found easier to control group, which means that RA is a multifactorial disease, which makes difficult to carry out other inflammatory processes in the body. The results indicated that the majority of clinical improvements occur within the first 3 months maintaining the 6 month study period, suggesting that there is a future scientific support of the clinical utility of biomarkers for evaluating periodontal disease and requires additional studies to delineate the impact of inflammatory systemic to associated diseases in evaluating profile of biomarkers that could be used to monitor the healthy and/or defining unusual about the stages of disease.

In conclusion, the study emphasized that the biomarkers levels ESR, CRP, AAG, are obviously influenced by the local periodontal environment and selective influenced by systemic inflammatory condition like rheumatoid arthritis.

2. Martu M.A., Solomon S.M., Sufaru I.G., Jelihovschi I., Martu S., **Rezus E.,** Surdu A.E., Onea R.M., Grecu G.P., Foia L., *Study on the Prevalence of Periodontopathogenic Bacteria in Serum and Subgingival Bacterial Plaque in Patients with Rheumatoid Arthritis,* Rev. Chim. (Bucharest), 68 (8), 2017, 1946-1949.

In the subgingival plaque, over 500 species of bacteria have been identified as a complex ecological niche. Under the influence of local or systemic factors, some bacteria in the subgingival dental biofilm become the primary etiologic agents of periodontal disease. These polymicrobial infections in most cases involve gram-negative anaerobic periodontal pathogens that act synergistically (185). The most commonly involved bacteria are: *Aggregati bacter actinomycetem comitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Treponema denticola, Peptostreptococcus micros, Fusobacterium nucleatum, Eikenella corrodens, Campylobacter rectus, Eubacterium nodatum, Capnocytophaga spp.*

Periodontopathogens can produce many virulence factors, leading to the destruction of periodontal tissues (186). Clinical studies have associated periodontopathic bacteria with some systemic disorders such as myocardial infarction (187), premature birth, atherosclerosis (188, 189), chronic kidney disease (190) and cerebral vascular accident and rheumatoid arthritis (191).

The purpose of this study was to detect bacterial periodontal DNA in the subgingival dental plaque and serum in patients affected by rheumatoid arthritis and periodontitis.

Materials and methods

The study group included 19 patients with periodontitis and refractory rheumatoid arthritis despite intensive treatment with anti-rheumatic diseases (DMARD) (methotrexate, sulfasalazine, leflunomide and chloroquine). The patients completed a health questionnaire that included information on systemic health and oral diseases. Written and informed consent from patients

was obtained prior to clinical examination in accordance with the ethical principles of the Helsinki Declaration.

The patients included in this study were males and females over 18 years of age who had persistent rheumatoid arthritis with synovial fluid effusion on their knees without other systemic diseases and affected by periodontitis.

The diagnosis of periodontitis was determined by measuring the depth of the periodontal pocket and the clinical index of loss of attachment. These indices were obtained using a periodontal probe, the Merrit B (Hu-Friedy) probe graduated in millimetres (0-10 mm). Ten millilitres of peripheral blood from the cubital vein were collected from each patient and placed inside vacuum tubes and citrate medium. The subgingival dental plaque has always been collected after obtaining blood samples to avoid transient bacteraemia that may influence the presence of different bacterial species in the serum.

The subgingival plate and peripheral blood were transported with ice and stored at -40°C until polymerase chain reaction (PCR) and microbiological assessments were performed. All samples were processed under aseptic requirements to prevent contamination of both the environment and the DNA extraction method for PCR assays.

Positive controls were included in each PCR set using DNA from the following bacterial strains: *P. gingivalis* (ATCC 33277 and HG1691), *T. forsythia* (ATCC 43037), *Prevotella intermedia* (ATCC 25611), *Aggregatibacter actinomycetem comitans* (ATCC 29523 and HK1651), P. nigrescens (ATCC 25261) and T. denticola (ATCC 35405).

A control (negative) test sample was also included in each PCR set containing a sample only with deionized water (instead of a patient sample) to know if non-specific products were amplified. For the statistical analysis, the ESPE SS 12 package was used, and the α value was set to 0.05. Normally distributed variables were reported as standard deviations. The Chi Square and Fisher tests with Anova were used to compare the data.

To determine the statistical differences in the periodontal bacterial DNA detection near the dental plaque and serum, Fisher's exact test was also used and the statistical significance was established at $p < 0.05$.

Results

Subjects presented 63.8% of teeth present in the arcade; lower molars were the most frequently absent teeth (46%) (table I.2.11). The DNA of periodontopathogenic bacteria was detected in 100% of the subgingival plate samples, and in serum samples it was identified in 84.2% of cases. Regarding the number of identified bacteria species, 4.05 different bacterial species were detected in subgingival samples and 1.19 species were detected in serum samples. The found species and their frequency for the two types of biological samples are listed in table I.2.12.

Table I.2.11. Dental parameters on number of patients

	Mean value	Standard Deviation (SD)	Interval
Pocket depth (mm)	3.9	0.81	2.6-2.7
Present teeth (n)	17.89 (63.8)	8.93	3-27
Superior anterior teeth (6)	4.10 (63.8)	2.33	0-6
Inferior anterior teeth (6)	4.52 (75.3)	1.92	1-6
Superior premolars (4)	2.36 (59)	1.60	0-4
Inferior premolars (4)	2.94 (73.5)	1.31	0-4
Superior molars (4)	2.10 (52.5)	1.37	0-4
Inferior molars (4)	1.84 (46)	1.37	0-4

Both rheumatoid arthritis and periodontal disease share similar immunopathological mechanisms, because the virulence factors produced by periodontal bacteria produce an immune response that is mediated by neutrophils, monocytes, B and T lymphocytes which lead to an increase in the release level of prostaglandins that stimulate osteoclastic activity and lead to bone erosion, similar to the mechanism involved in rheumatoid arthritis (192).

Table I.2.12. Subgingival and serum periodontal bacterial DNA

Bacteria	Dental plaque		Serum		p-value
	Frequency	%	Frequency	%	
Prevotella intermedia	19	100	14	73.6	0.0453
Tannerella forsythia	10	52.6	6	31.5	0.0203
Prevotella Nigrescens	13	68.4	0	0	<0.0001
Aggregatibacter	4	21	0	0	0.1204
Porphyromonas gingivalis	15	78.9	8	42.1	0.0674
Treponema denticola	16	84.2	4	21	0.0004

The most commonly found species in all types of serum samples were *P. intermedia* and P. *gingivalis* (63.1% and 36.8%, respectively). There was no negative topic for *P. intermedia* (table I.2.13). The most common species absent in all samples was *A. actinomycetemcomitans* (78.9%).

Table I.2.13. Periodontal bacterial DNA detected in different combinations

Bacteria	Negative subgingival and serum samples (absence of bacterial DNA) (no. of samples/precentage values)	Positive subgingival and serum samples (presence of bacterial DNA) (no. of samples/precentage values)	Positive subgingival samples (presence of bacterial DNA) (no. of samples/precentage values)
Prevotella intermedia	0 (0)	2 (10.5)	0 (0)
Tannerella forsythia	9 (47.3)	4 (21.0)	4 (21.0)
Prevotella Nigrescens	5 (26.3)	0 (0)	9 (47.3)
Aggregatibacter	15 (78.9)	0 (0)	1 (5.2)
Porphyromonas gingivalis	4 (21.0)	1 (5.2)	3 (15.78)
Treponema denticola	3 (15.78)	1 (5.2)	9 (47.3)

Discussions

Periodontitis, an inflammatory disease caused by specific bacteria located in the oral cavity, has been classified in aggressive periodontitis (Ag-P) and chronic periodontitis (Cr-P), with age of onset for Ag-P being much lower than that for Cr-P. Neutrophil dysfunction, singular nucleotide polimorfism, and specific imun response to infectious agent have been associated with Ag-P. Former studies have investigated the neutrophil disfunction, in order to decode chemotaxic mechanism. Periodontitis include: diacilglicerol accumulation, reduction in action for DAG kinase, reduction in activity for protein-kinase, rise in nitric oxide synthesis, rise in super-oxide production and reduction in calcium influx. Several mechanisms have been proposed to explain this pathology and also many studies conducted on the influence of different parameters in Periodontal Disease (185).

In the present study, periodontal bacterial DNA was detected in 100% of subgingival plaque samples and 84.2% in serum samples. Regarding the number of bacterial species detected, a large number (4.05) of bacterial species was identified in the subgingival plate, followed by serum (1.19). The fact that there is a lower presence of serum bacterial DNA can be explained by its dilution from the blood stream by renal filtration. This data is consistent with other studies where bacterial DNA has been detected by DNA-DNA chess hybridization resulting in 100% positive serum samples. Several species commonly identified in serum were P. intermedia, *P. gingivalis* and *T. denticola*; two of them belong to the red complex, which is associated with destructive diseases. On the other hand, *A. actinomycetemcomitans*, mainly responsible for

aggressive periodontitis, was less frequently detected. The reason could be that only one patient was affected by aggressive periodontitis. These data are consistent with previous reports.

Moen et al. investigated the presence of oral bacterial DNAs in serum and synovial fluid (SF) of patients with active RA and psoriatic arthritis – PsA. In the study were included 16 RA patients, 14 PsA patients, and 9 osteoarthritis (controls) patients. Serum and SF samples from all 39 were extracted for oral bacterial DNA. The extracted DNA was used in a checkerboard DNA-DNA-hybridization set up, to identify 40 different bacteria. Mean number ± standard deviation (SD) of oral bacterial species in sera were 6.2 (3.2) in the RA group ($p = 0.004$) and 5.4 (2.7) in the PsA group ($p = 0.009$) compared to 2.1 (1.7) in the controls. *Porphyromonas gingivalis* and *Prevotella nigrescen*s were exclusively detected in RA and PsA. Mean number (± SD) of oral bacterial species in SF were 14.0 (6.8) in the RA ($p = 0.001$) and 19.4 (7.1) in the PsA group ($p < 0.001$) compared to 4.0 (1.7) in controls. *P. gingivalis, Tannerella forsythensis* and *Prevotella intermedia* were exclusively identified in RA and PsA SF. Higher means of DNAs were found in RA SF correlated to RA serum ($p < 0.001$) contained in PsA SF compared to PsA serum ($p < 0.001$). Higher concentrations of bacterial DNAs were observed in RA and PsA compared to controls. Also, higher variety and concentrations of oral bacterial DNAs were found in SF compared to serum of RA and PsA patients. As a final remark, synovial inflammation in RA and PsA may favor trapping of oral bacterial DNAs, suggesting a perpetuating effect of oral pathogens in joint disease (191).

In conclusion, periodontal bacterial DNA was detected in the subgingival plaque of patients with rheumatoid arthritis. It is therefore suggested that periodontal bacterial DNA plays a major pathological role in the severity of rheumatoid arthritis. *P. intermedia, T. forsythia,* and *P. gingivalis* were the most commonly found species in the subgingival dental plaque, the corresponding and predominant bacteria in the red complex, which is involved in the destruction of the periodontal bone. The data obtained in this study provides evidence to demonstrate the existence of a link between rheumatoid arthritis and periodontal disease.

3. Martu A., **Rezus E.,** Sufaru I., Banu C., Martu S., Foia L., *Study on the clinical changes in general and oral status in patients with rheumatoid arthritis, Romanian Journal of Oral Rehabilitation,* 10 (3), 2018, 188-198.

This study proposes an examination of the local inflammatory status by evaluating periodontal indexes Quigley Hein, GI Lõe and Silness, papillary bleeding (PBI) and CPITN, accompanied by a detailed assessment of systemic status in patients with rheumatoid arthritis.

Materials and method

In the retrospective study, 220 patients were admitted to the Clinical Recovery Hospital in Iasi with a definite diagnosis of rheumatoid arthritis. Patients with periodontal therapy or antibiotics in the last 6 months were excluded from the study. In each case, the onset of

symptoms, including some associated symptoms, general condition, clinical signs at admission, paraclinical parameters, associated conditions, and treatment were evaluated.

In terms of dental-periodontal health indices, we used the following indices: Quigley Hein, GI Lõe and Silness, papillary bleeding (PBI) and CPITN. The data were uploaded and processed using statistical functions in SPSS 18.0 at the significance threshold of 95%. Primary processing, systematization of data by centralization and grouping, led to the achievement of primary indicators, which are presented as absolute measures.

Based on primary indicators, in means of different statistical methods of comparison, abstraction and generalization, derived indicators were obtained, which have the role of highlighting the qualitative aspects of an assembly, addressing the relationship between different parts of a patient population or different characteristics, interdependence relationships between variables. The following derived indicators, described by the ANOVA test: average values (mean, median, module, minimum and maximum values, etc.) and dispersion indicators (standard deviation, standard error, variance coefficient) were used. Skewness or Kurtosis tests ($-2<p<2$) measure the normality of the set of values to determine whether the variables are continuous or not.

Results

The DAS28 score (disease activity score) is a measure of disease activity in rheumatoid arthritis, and number 28 refers to the 28 examined joints. There is a wide range of disease activity measures taken into consideration in this score, including: joint examination for swelling and tenderness, global pain scores and general condition, blood markers of inflammation (eg VSH and CRP), questionnaires (eg HAQ which evaluates the function), X-ray and newer imaging techniques, such as ultrasound and MRI. In terms of dental-periodontal health indices, we used the following indices: Quigley Hein, GI Lõe and Silness, papillary bleeding index (PBI) and CPITN, the results being displayed in table I.2.14.

Depending on the clinical stage, the following aspects were found ($p = 0.029$):

- ✓ the mean number of painful joints correlated with stage II polyarthritis (7 ± 5.4);
- ✓ stage III and IV patients had on average about 5 painful joints.

The study was observational, retrospective, and attempted to establish the cumulative risk factors underlying the evolution of oral pathology in patients with rheumatoid arthritis.

In 20.3% of patients, the higher Quigley Hein plate index has been significantly correlated with a higher VAS and in approximately 20% of patients, the higher Quigley Hein index has been significantly correlated with a higher number of painful joints, and in 27.5% of patients with swollen joint counts.

Table I.2.14. Descriptive Indicators of: The Quigley Hein Plaque Index, Löe and Silnes Gingival Index (GI) and the papillary bleeding index (PBI) Index, depending on Staging of RA

	RA Stage	N	Mean	Standard Deviation	Standard Error	Confidence Interval 95%		Min	Max	F ANOVA test
						- 95% CI	+ 95% CI			
Indicators of the Quigley Hein Plaque Index	I	2	3.10	0.00	0.00	3.10	3.10	3.10	3.10	0.654
	II	24	2.81	0.98	0.20	2.40	3.22	1.30	3.90	
	III	104	2.83	0.79	0.08	2.68	2.99	0.80	4.10	
	IV	78	2.69	0.86	0.10	2.50	2.89	1.30	4.50	
	Total	208	2.78	0.83	0.06	2.67	2.89	0.80	4.50	
Descriptive Indicators of Löe and Silnes Gingival Index (GI)	I	2	2.10	0.00	0.00	2.10	2.10	2.10	2.10	0.654
	II	24	1.65	0.62	0.13	1.39	1.91	0.70	2.60	
	III	104	1.97	0.57	0.06	1.86	2.08	0.50	3.00	
	IV	78	1.90	0.56	0.06	1.78	2.03	0.60	2.80	
	Total	208	1.91	0.57	0.04	1.83	1.99	0.05	3.00	
Indicators of the PBI Index	I	2	2.90	0.00	0.00	2.90	2.90	2.90	2.90	0.007
	II	24	1.75	0.75	0.15	1.43	2.07	0.20	2.90	
	III	104	2.32	0.76	0.07	2.17	2.47	0.30	3.80	
	IV	78	2.20	0.78	0.09	2.03	2.38	0.90	3.80	
	Total	208	2.22	0.78	0.05	2.11	2.32	0.20	3.80	
CPITN Index Depending	I	2	3.00	0.00	0.00	3.00	3.00	3.00	3.00	0.679
	II	24	2.99	1.04	0.21	2.55	3.43	1.00	4.00	
	III	104	3.13	0.06	0.06	3.01	3.24	1.60	4.00	
	IV	78	3.02	0.66	0.07	2.87	3.17	1.30	4.00	
	Total	208	3.07	0.68	0.05	2.98	3.16	1.00	4.00	

Discussions

VAS (Analog Visual Scale) is a measuring instrument, often used in epidemiological and clinical research to measure the intensity or frequency of different symptoms. For example, the amount of pain a patient feels varies along a zero continuum to an extreme amount of pain. Using a ruler, the score is determined by measuring the distance (mm) on the 10 cm line of the "no pain" point and the patient's mark, providing a score range of 0-100. The VAS pain assessment score varied between 10-90, averaging 36.55±21.17, which indicated the study group as a moderate pain perception.

Approximately 19% of patients associated higher individual values of the Quigley Hein plaque index with higher scores of DAS28. In 41% of patients, the Lõe and Silnes GI index increased significantly with a higher VAS level. In 25.8% of patients, the Lõe and Silnes GI index increased significantly with a higher number of painful joints and 30.5% of patients with swollen joint counts. A significant number of patients experienced rheumatoid factor and antiCCP antibodies, which could have significant effects on periodontal status. Also, in a significant number of patients, the individual VSH and CRP values exceeded the maximum reference limit, the mean level being significantly higher in the advanced stages of rheumatic disease, values that may exert adverse effects on periodontal health but also increase the overall inflammatory burden in the body.

In conclusion, periodontal parameters (hygienic index, index of gingival inflammation, papillary bleeding index and CPITN) correlated significantly with an increased number of painful and swollen joints; this provides indications of clinical correlation of periodontal inflammatory status with exacerbated joint impairment.

I.3. Researches regarding ankylosing spondylitis

I.3.1. Introduction

The spondyloarthritis (SpA) diseases comprise different diseases, which have in common similar genetic risk factors and similar clinical symptoms such as inflammatory back pain or asymmetric peripheral arthritis. The SpA diseases are: ankylosing spondylitis (AS), reactive arthritis (ReA), arthritis/spondylitis with inflammatory bowel disease (IBD), and arthritis/spondylitis with psoriasis. The main links between each of these is the association with human leukocyte antigen (HLA)-B27, similar clinical symptoms such as inflammatory back pain, and similar patterns of peripheral joint involvement with an asymmetric arthritis predominantly of the lower limbs. Ankylosing spondylitis (AS), the SpA disease with the most severe outcome, starts in the second decade of life.is the prototypic form of SpA, and is characterized by axial inflammation and new bone formation (193).

AS affects young people, around 26 years of age. Men are more often affected than are women, with a ratio of roughly 2:1. About 80% of patients develop the first symptoms at an age younger than 30 years, and less than 5% of patients present at older than 45 years (194).

Overall, autoimmune diseases develop from a complex interplay of genetic risk and environmental triggers. As respects rheumatologic conditions, AS is one of the most genetic diseases. High monozygotic twin concordance (63 %) and familial aggregation studies indicate a heritability of over 90 %. Presence of the major histocompatibility complex (MHC) class I allele human leukocyte antigen B27 (HLA-B27) accounts for the most of genetic risk (195).

I.3.2. Molecular biology techniques for ankylosing spondylitis diagnosis

A. Background

As mentioned before, AS is a common genetic disease and previous studies have indicated that HLA-B27 is closely associated with AS. Many cytokines such as tumor necrosis factor-alpha (TNFα), IL-6 were reported to be involved in AS. Several types of T cells including peripheral Th2 lymphocytes, Th17 lymphocytes, CD4 + T cells and CD8 + T cells play important roles in the pathology of AS. Despite intensive research, the pathogenesis of AS remains elusive (196). Human leukocyte antigens-B27 is a class I surface antigen encoded by B locus in the major histocompatibility complex (MHC) on the short (p) arm of chromosome number 6 at position 21.3 from base pair 31,429,845 to base pair 31,432 923 and presents microbial antigen to T cells. The relationship of HLA-B27 with AS is the strongest for any HLA locus. Almost all nucleated cells in the body, except neurons and striated muscle cells contains class I HLA molecules on it. The most important evidence of the involvement of HLA-B27 in AS came from studies done with mice and rats that had been given HLA-B27 as a transgene (197).

The right diagnosis of AS can be difficult. Most patients are diagnosed based on the severity and appearance of symptoms such as chronic lower back pain fatigue, and lack of mobility judged against standards such as the Assessment of SpondyloArthritis International Society (ASAS) Criteria for Spondyloarthropathy. According to the ASAS Criteria, patients

should be considered for AS if they are under the age of 45 with chronic back pain or radiographic sacroiliitis. Other associated factors that have to be taken into consideration include uveitis, arthritis, psoriasis, a family history of AS, or increased C Reactive Protein levels. The presence of HLA-B27 is the most useful factor in diagnosis of AS, being the strongest known indicator of susceptibility to AS (198).

Flow cytometry is a current available laboratory method to study cells in suspension from a variety of human sources. Application of this technology as a clinical laboratory method has progressed from the identification of cell-surface proteins to characterizing intracellular proteins and even providing multiple different techniques to assess specific features of adaptive and innate immune function. This expanded area of flow cytometric testing approaches has increased the utility of this platform in characterizing and diagnosing disorders of immune function (199).

B. Published paper in this field

Cianga P., Zlei M., **Rezus E.,** Cianga C., *The flow cytometric labeling pattern in HLA-B27 detection may suggest cross reactivities,* Revista Română de Medicină de Laborator, 19 (2/4), 2011, 185-191.

The detection of HLA-B27 expression is one of the most powerful diagnostic tools in ankylosing spondylitis and other spondylarthropaties, hence the importance of a reliable and accurate detection method. Flow cytometry is often used as an alternative HLA-B27 typing technique, mainly because it is a rapid test. However, even though it is based on various monoclonal antibodies, their ability to bind to cross-reactive HLA-B molecules was demonstrated by various groups.

Materials and methods

The patients were referred to our lab with a presumptive diagnostic of AS, based on clinical symptoms and paraclinical investigations (X-ray, Magnetic Nuclear Resonance).

The HLA-B27 typing was performed by flow cytometry using the BD^{TM} HLA-B27 System, and by the HLA-SSP and HLA-SSO typing techniques**.** The BD^{TM} HLA-B27 System provides a mixture of two monoclonal antibodies. The anti-HLA-B27 antibody (clone GS145.2, IgG1, kappa) is conjugated with fluorescein isothiocyanate (FITC), while the anti-CD3 antibody (clone SK7, IgG1, kappa) is conjugated with phycoerithrine (PE). The labelings were performed under standard conditions, according to the manufacturer's indications, using fresh blood harvested in BD EDTA vacutainers. The acquisition of 5000 to 10000 events was performed immediately afterwards using a BD FACSCalibur and the CellQuest software. The kit internal control and/or previously characterized cells were used as positive controls as well as for assessing the acquisition settings and fluorescence intensity, in order to achieve the appropriate discrimination between negative and positive cells.

Molecular biology HLA typing

DNA extraction – The DNA was extracted from whole blood, using a Qiagen kit (QIAamp Mini, Qiagen, Germany), following accurately the manufacturer's instructions.

HLA-SSP (sequence specific primer) – The typing was performed using BAG (Germany) and Olerup (Sweden) kits, following accurately the manufacturer's instructions. The amplicons' pattern (figure I.3.1) was interpreted using the Score software.

HLA-SSO (sequence specific oligonucleotides) – the typing was performed with a InnoLipa HLA-B Update Plus kit (Innogenetics, Belgium). In the last steps, the DNA was initially amplified by PCR according to the manufacturer's indication. The mismatched DNA is removed by stringent washings at 56°C and the bound DNA is evidenced enzymatically by adding alkaline phosphatase coupled with streptavidin and then a BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate p-toluidine and nitroblue tet- razolium in dimethylformamide) substrate, generating a brown precipitate.

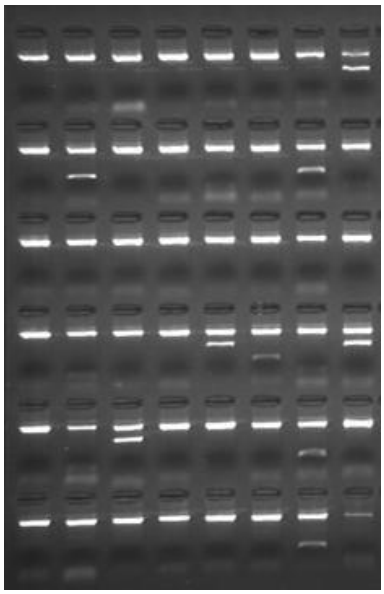


Figure I.3.1. Amplicon pattern in a HLA-SSP typing using a BAG-B low resolution kit that includes 48 pairs of primers

Results

The BD^{TM} HLA-B27 System flow cytometry kit, containing a mixture of two monoclonal antibodies, is designed to allow the easy identification of HLA-B 27 positive T cells in the upper right quadrant, while HLA-B27 negative T cells are projected in the lower right quadrant (in our setting). The typical dot-plots of the HLAB27 positive samples display no events in the lower right quadrant, as shown in figure I.3.2.A. Instead, the negative HLA-B27 samples display all the T cells in the lower right quadrant.

Besides these two distinctive labeling patterns, we also came across a different labeling pattern in which the dots were distributed in both right quadrants, with percentages ranging from 3 to 47% in the lower right quadrant, as shown in figure I.3.2.B.

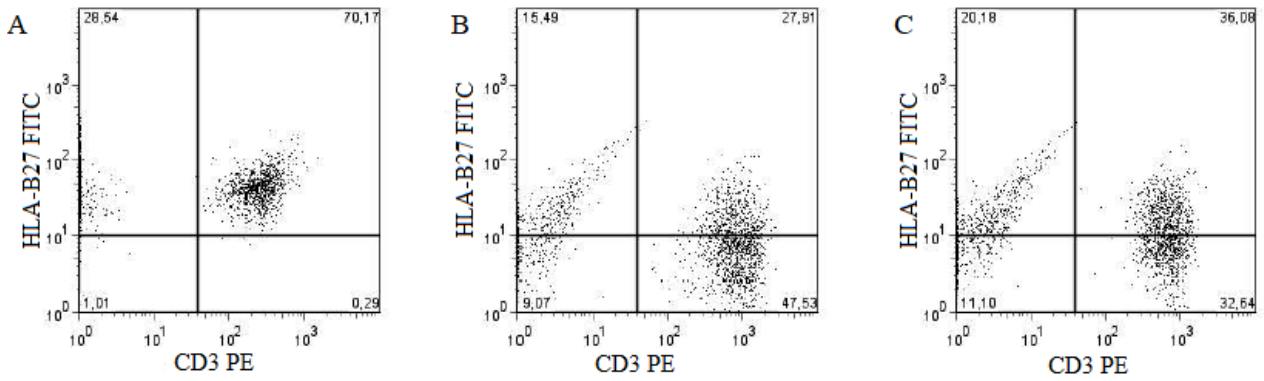


Figure I.3.2. A. Typical dot-plots of the HLA-B27 positive samples; B. Different labeling pattern for HLA-B27 in which the dots are distributed in both right quadrants; C. Labeling pattern produced by HLA-B07.

A classification that takes into account the serologic reactivity of human alloantisera includes the HLA molecules into several CREG (cross reactive groups) groups. HLA-B27 belongs to the CREG 7 group, together with molecules like HLA-B7, HLA-B13, HLA-B22, HLA-B40, HLA-B41, HLA-B42, HLA-B47 and HLA-B48.

Studies from the literature have revealed several strong cross-reactivities for the antibodies investigated in flow cytometry (HLA-B7, B22, B37, B42) as well as some weak cross-reactivities (HLA-B12 (B44, B45-CREG12), B13, B16 (B38, B39 – CREG8), B17 (B57, B58 - CREG5), B40, B41, B47, B48. It was expected the fact that, with the exception of B37, practically all strong cross-reactivities are generated by HLA molecules part of the CREG 7 group as HLA-B07, B22 and B42. Other molecules of the CREG 7 group like B40, B41, B47 and B48 generated only weak cross-reactivities. Even more interestingly, the study demonstrated that the antibodies were also able to generate cross-reactivities with HLA molecules that are included in different CREG groups: B44, B45 (CREG 12), B13, B38, B39 (CREG 8), B57, B58 (CREG 5) (200, 201). Taking into consideration these literature data, we have decided to further investigate by molecular biology typing techniques all the samples yielding unclear results, since this labeling pattern was rather suggesting cross reactivities than clear cut positive or negative results.

The HLA-SSP or HLA-SSO typing techniques confirmed that indeed the dot plots showing events distributed in both upper right and lower right quadrants were generated not by HLA-B27 labeling but by various cross-reactive molecules. In 8 of the 17 samples we have investigated, this type labeling pattern was produced by HLA-B07 (figure I.3.2.C).

Besides HLA-B*07 generated cross-reactivities, we were also able to confirm cross-reactiv- ities determined by HLA-B*37, HLA-B*38, and HLA-B*39 (figure I.3.3.A), as previously reported. A SSP HLA-B*40 confirmed sample, associated with the non-crossreactive HLA-B*51, did not yield the characteristic labeling pattern in flow cytometry, but only the lowest percentage of cells (8%) in the HLA- B27 positive upper left quadrant (figure I.3.3.B). In figure I.3.3.D can be seen that a labeling pattern given by HLA-B07 can mask the presence of HLA-B27.

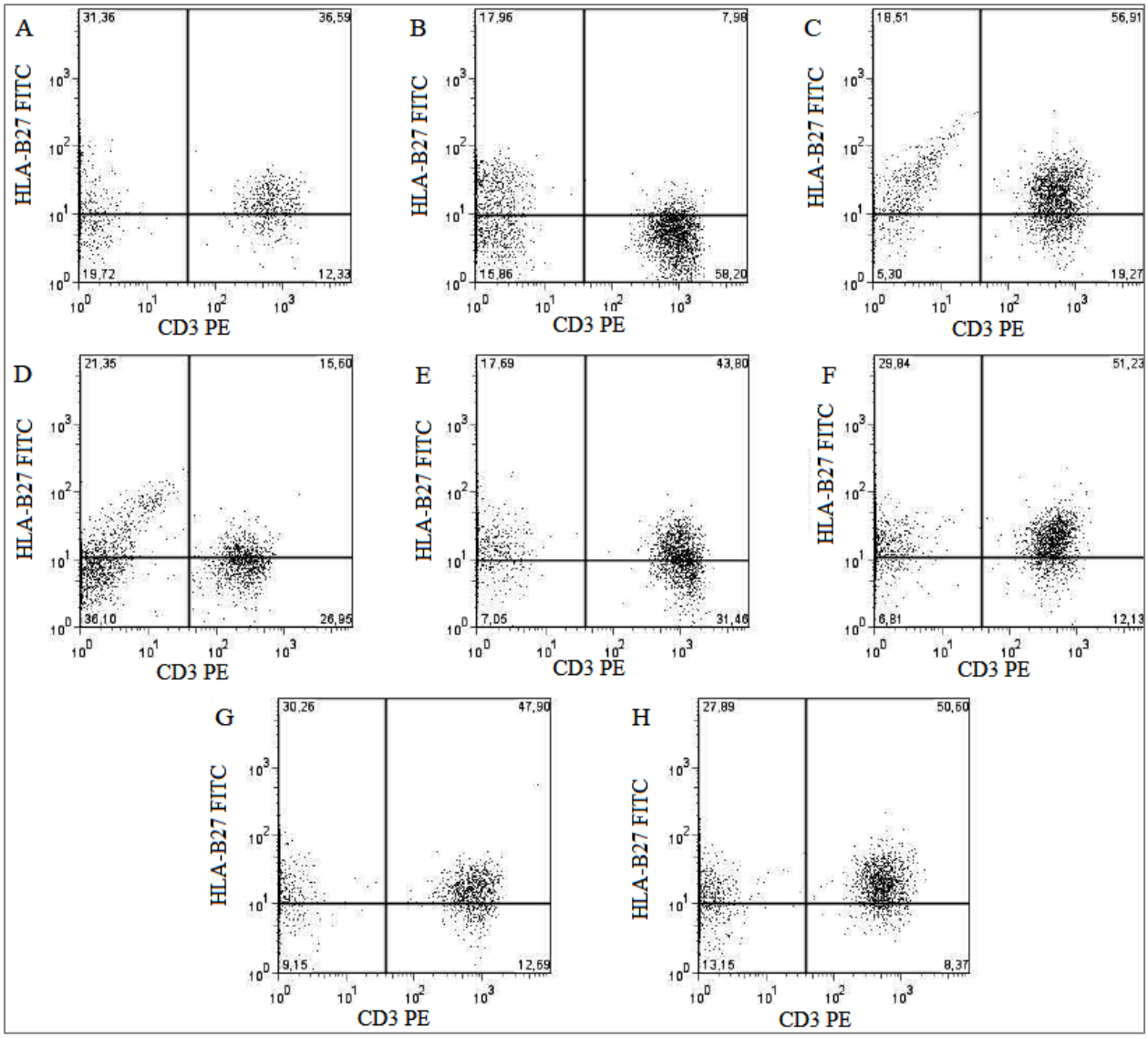


Figure I.3.3. A. Cross-reactivities with HLA-B27, determined by HLAB*37, HLA-B*38, and HLA-B*39; B. Flow cytometric labeling pattern of a HLA-B40/HLA-B51 patient; C. Flow cytometric labeling pattern of a HLA-B07/HLA-B27 patient; D. Flow cytometric labeling pattern of a homozygous HLA-B1 patient; E. Flow cytometric labeling pattern of a HLA-B07/HLA-B18 patient; F. Flow cytometric labeling pattern of a HLA-B35/HLA-B37 patient; G. Flow cytometric labeling pattern of a HLA-B35/HLA-B14 patient, H. Flow cytometric labeling pattern of a HLA-B14/HLA-B07 patient.

The anti-HLA-B07 antibody is responsible for a very strong cross-reactivity with HLA- B27, capable of masking not only HLA-B27 but also many other weaker cross-reactive HLA-B molecules, like for instance HLA-B18 (figure I.3.3.D). HLA-B35 could also behave as a cross-reactive molecule for the BD antibody since we have obtained characteristic labeling patterns in flow cytometry not only when HLA-B*35 was associated with HLA-B*37 (figure I.3.3.F), but also with HLA-B*14 (figure I.3.3.G). The same weaker cross-reactivity hypothesis can be advanced regarding the HLA-B14 molecule, as in our investigation we only came across a sample where its presence was associated with HLA-B*07 (figure I.3.3.H).

Discussions

Literature data have shown that more than 90 % of patients with AS possess the HLA-B27 allele, but only 1 % of people with HLA-B27 develop the disease (166). In the Caucasian race, the allele is present in 90% of patients with AS, compared with healthy population where is present in 3% to 9% of individuals.

HLA-B27 has 105 currently known subtypes encoded by 132 allelic variants, but not every allelic variant is disease-associated, and the strength of the association is correlated with the type of spondyloarthropathy and other factors. HLA is a type of Major Histocompatibility Complex Class I (MHC Class I) receptor on the plasma membrane of a cell that presents molecules to CD8 + and the T cell receptor of T cells, found on almost all cells in the body. Allele presentation of the HLA-B27 family is related to ethnicity (167). For instance, in the Caucasian race the 27:05 subtype is the most associated allelic variant with AS. In general, association with AS occurrence has been noticed for the following subtypes: B*27:01-05, 27:07, 27:08, 27:10, 27:13-15, 27:19, 27:23, 27:24, 27:25 and 27:49. An interesting fact is that some subtypes are not associated with increased risk of AS (27:09) or may play a protective role (27:06) (202).

Flow cytometry technique has shown some limitations in detection of HLA-B27 molecule. The most frequent is the fact that HLA-B27 screening by flow cytometry demands an extensive validation procedure in each laboratory in order to set up cut-off values, which is correlated with monoclonal antibody specificities, reagent batches and instruments. Unfortunately the cut-off values obtained in a study cannot be extrapolated to HLA-B27 typing with flow cytometry in general, because each laboratory must establish its own range (203).

An increasing demand for HLA-B27 typing as one of the tests used in the diagnosis of ankylosing spondylitis around the middle of two decades ago has led a research group (Reynolds et al.) to develop a rapid, automated, flow cytometric assay using whole blood and an HLA-B27 specific monoclonal antibody FD705. An impressive number of samples has been analyzed (2093), during a 2 year period of routine HLA-B27 typing. The final results have shown that 21.6% were clearly HLA-B27 positive whilst 73.2% were HLA-B27 negative, the remaining 5.2% required further testing before assignment of HLA-B27 status. Further, the research group was carried out on blood samples from individuals positive for the newly described subtype HLA-B2708 (204).

Three hundred patients with suspected spondyloarthropathy have been included in a study performed by Skalska et al. For each of them, expression of HLA-B27 on the T cell surface was analysed by flow cytometry assay using GS145.2 monoclonal antibody specific for HLA-B27. In brief, DNA was isolated from the whole blood, and afterwards genes coding for HLA-B27, HLA-B40 and HLA-B47:01 were detected by PCR using a specific primer pair. Positive samples were sequenced in order to discriminate allelic variations of the HLA-B27 gene. Of the total number of samples, 25% were HLA-B27-positive on the basis of flow cytometry analysis (76 patients). Moreover, genetic sequence analysis confirmed positivity only of 73 from among 76 samples. From the 76 samples, 53 samples were identified as allelic variation 27:05, 19 samples as allelic variation 27:02, and one sample as allelic variation 27:07, proving that the majority of HLA-B27-

positive samples belong to the 27:05 allelic variation, which is strongly associated with high risk of AS (202).

A recent study, published by Chheda et al. proved once again, that flow cytometry is a very useful technique to determine HLA-B27 molecule. Summarily, a total of 7543 patients with a presumptive clinical diagnosis of AS were referred for screening of HLA-B27. First the samples were tested by flow cytometry, and second based on flow cytometry results, positive samples were analyzed for the presence of HLA-B27 allele by modern microarray technology. The first results have shown 1551 positive cases, meaning 20.56%, and 5556 negative cases, meaning 73.65%; 436 (5.78%) samples were identified within equivocal zone. Of the total number of cases (1560) analyzed by microarray method, 1333 (85.44%) were detected microarray positive and 227 (14.55%) negative. A subset of samples (n = 200) were then tested by DNA sequencing for identification of HLA-B27 subtypes. Of 200 cases, 20 cases (14 of HLA-B*07 and 6 of HLA-B*37) of HLA-B27 cross-reactive subtypes have been identified (205).

In conclusion, flow cytometry is a very useful test in screening for the presence of the HLA-B27 molecule, and cross-reactivities can be readily identified by a distinctive labeling pattern. However, we advocate that all the unclear situations should be solved by molecular HLA typing techniques.

I.3.3. Inflammatory bowel diseases and ankylosing spondylitis

A. Background

Inflammatory bowel diseases (IBD) can be considered to be systemic diseases since they are often associated with extraintestinal manifestations, complications, and other autoimmune disorders. Actually, physicians who care for patients with ulcerative colitis (UC) and Crohn's disease (CD), the two major forms of IBD, face a new clinical challenge daily, aggravated by the frequent rate of extraintestinal complications. Almost every system can be involved, eyes, skin, joints, kidneys, liver and biliary tracts, and vascular system being the most common sites of systemic IBD and their involvement is dependent on different mechanisms.The major extraintestinal immune-related manifestation (EIM) of IBD are: arthritis, erythema nodosum, pyoderma gangrenosum, aphthous stomatitis, iritis/uveitis. Some of the most frequent autoimmune disorders associated to IBD are: alopecia areata, ankylosing spondylitis, bronchiolitis obliterans, cold urticaria, hemolytic anemia, pancreatitis, primary sclerosing cholangitis, Raynaud phenomenon, rheumatoid arthristis, Sjogren syndrome and so on (206).

Inflammatory arthropathies are the most common extraintestinal manifestations in IBD patients with a prevalence ranging between 7% and 25%. Articular and musculoskeletal manifestations are included in the spondyloarthropathies (SpAs).

IBD affect an estimated 1.5 million individuals in USA, 2.2 million individuals in Europe , and several thousands more worldwide.

The therapeutic goal has evolved from relief of IBD-related symptoms to the more ambitious goal of mucosal healing, though considerable debate exists about the optimal definition

for this endpoint. In the past two decades have observed an important expansion in the number of treatment options available for CD and UC.

Initially the treatment schedule included monoclonal antibodies against tumor necrosis factor anti-TNFα: infliximab, adalimumab, certolizumab pegol, golimumab, and subsequently anti-integrin: natalizumab, vedolizumab therapies.

Also, there is recognition that use of these biologics in combination with a conventional immunosuppressive: azathioprine, 6-mercaptopurine, methotrexate) may yield superior outcomes and improve durability of therapy (207).

B. Published papers in this field

I have studied together with professors with a remarkable experience in gastroenterology and other rheumatologist colleagues, the correlation between IBD and AS, and the results of our work have been reproduced in three articles.

First we have published a review in which we have displayed the impact of IBD and AS on patients' functionality and quality of life.

Second we have published a study in which we have synthesized the results of a case-control retrospective study. The third article is a complex study, that actually brings supplement information about the patients included in the study presented in the second article.

1. Cardoneanu A., **Rezus E.**, Mihai C., Drug V., Cijevschi-Prelipcean C., *The impact of inflammatory bowel diseases and ankylosing spondylitis on patients' functionality and quality of life - a point of view,* 49th Annual Scientific Meeting of the ESCI (Cluj-Napoca, Romania), 2015, 89-92.

IBD and AS are chronic inflammatory and relapsing diseases which have a negative impact on patients functionality and quality of life. Inflammation of the gut mucosa is usually associated with a lot of extraintestinal manifestations among which musculoskeletal impairment is the most common, being detected in more than 30% of the patients (208).

Arthropathy associated with IBD can have an axial or peripheral involvement, being included in the big group of Spondylarthropaties (SpA). The association between AS and IBD has some particular features:

- the antigen HLA B27 is less common (78% in IBD patients with AS in comparison with 90% in idiopathic AS) (209, 210);
- the masculine gender is less affected than in AS;
- there is no correlation between the activity of the bowel disease and the axial manifestations.

A lot of clinical trials demonstrated the fact that both IBD or AS separately, or the association between this disorders, have a big socio-economic impact for the patient and the society. When we speak about disability we refer mainly at the degree of inflammatory activity

which determine stiffness, pain, sleeping disorders and fatigue. Considering AS, bony ankylosis is a very important trigger for a decreased quality of life due to the progressive loss of spinal mobility. These items have a significant impact on the quality of life and health status of patients and cause issues in physical and psychological wellbeing as well as in social life.

Inflammatory bowel diseases and health-related quality of life

Regarding IBD and Health-related Quality of Life (HRQoL), a big survey made on an European cohort of patients 10 years after the diagnosis of IBD brought important data on this issue. The study included 769 patients, 517 with UC, 252 with CD from 9 centers out of 7 countries. All the patients were evaluated clinically and the quality of life was assessed using The Short Form Health Survey-36 (SF-36). Short-Form 36 Health Survey (SF-36) is a generic measure of health status which evaluates eight domains of physical and mental health within the previous 4 weeks: physical functioning, physical role, bodily pain, general health, vitality, social functioning, emotional role and mental health.

These questions are summarized in: the Physical Component Summary (PCS) and the Mental Component Summary (MCS). A better quality of life is indicated by a higher score (211). The results showed that a poor quality of life was associated with: an increased age (due to a worse physical functioning and pain), with female gender, with an active disease at the beginning of the study, with sick leave or disablement pension because of the underlying disease (212). Due to the strong correlation between pain and quality of life in patients with IBD, in 2010, Anja Schirbel et al. published the results of a cross sectional analysis on 334 patients. They used some questionnaires like: the standardized short inflammatory bowel disease questionnaire (SIBDQ) (213) and a modified form of the validated German pain questionnaire (214). The activity of the disease was measured using CDAI (Crohn's Disease Activity Index) and CAI (Colitis Activity Index) (215). Final results proved the following: almost 90% of the patients suffered from pain (men and women equally), surgery improved the pain, but not the quality of life in men, HRQoL was correlated with CDAI and CAI and the use of analgesics didn't improved the quality of life (216).

Ankylosing spondylitis and health-related quality of life

Alongside or in combination with IBD, Ankylosing Spondylitis is a chronic inflammatory rheumatic disease which affects mainly the sacroiliac joints and axial spine. The most important clinical features are low back pain, joint stiffness, progressive loss of spinal mobility and peripheral joint involvement. Quality of life in patients with ankylosing spondylitis was the main subject for many studies because the disease has a significant impact concerning daily activities of the patients. Symptoms like stiffness of joints, constant pain and functional impairment have a huge effect on physical, psychological and social status of the suffering people. The main tools to evaluate the activity of the disease and its impact on everyday life of the patients used in a large number of studies are: Bath AS Disease Activity Index (BASDAI), Bath AS Disease Functional Index (BASFI), Bath AS Metrology Index (BASMI), AS Quality of Life Questionnaire (ASQOL), Short-Form 36 Health Survey (SF-36).

Based on the SF-36 health questionnaire, a cross sectional study of 100 AS tunisian patients was made to evaluate the health status related to general population (217). The results revealed: a poor quality of life in patients with AS, a better status in women than in men, mental health more affected than physical health, worse quality of life in the group of low-educated patients, a better physical functioning in employed persons. The same as other studies (218, 219), this survey demonstrated the association between increased impairment of the quality of life with high levels of BASDAI, BASFI, BASMI, the most important influence being attributed to pain, disease activity, mobility limitation and functional impairment.

Another important clinical trial including a remarkable number of patients (962) with AS evaluated quality of life of the subjects appealing to the well known questionnaires. The authors demonstrated that the quality of life was affected in all patients with particular reference to bodily pain, physical role, general health and vitality subscales. They also showed a correlation between ASQOL and BASDAI, BASFI, BASMI, fatigue, duration of the disease, total and night pain, the most important variables which have a negative impact on the quality of life being: fatigue, disease activity, bodily pain and functional status. This study, by the side of others (220, 221), proved the fact that smoking and sedentariness can effect negatively the quality of life of these patients, while an increased level of education can improve it.

Association between inflammatory bowel diseases, ankylosing spondylitis and health-related quality of life

A number of international studies demonstrated that anemia, which is common associated with chronic inflammatory diseases such as IBD and AS, can have a negative impact on patients' quality of life (222).

Anemia, a common complication associated with IBD is unfortunately overlooked in the management of IBD patients, representing one of the major causes of both decreased quality of life and increased hospital admissions. In IBD, anemia is pathogenically complex, several factors contributing to its development. The most common cause of anemia is iron deficiency, other causes being: vitamin B12 and folic acid deficiencies, the effects of pro-inflammatory cytokines, hemolysis, drug therapies, and myelosuppression. Each of these etiological factors needs to be identified and corrected in order to effectively manage anemia in IBD (223).

The etiology of anemia is complex, being determined by the presence of an increased number of pro-inflammatory cytokines (IL-1, IL-6, TNF-α, IFN-α, IFN-γ) or by myelosuppression after the long-term use of specific therapies with immunosuppressive drugs (224, 225, 226).

Looking at patients with IBD (both CD and UC), rheumatic manifestations have been divided into two principal patterns: (1) peripheral arthritis, often asymmetric; and (2) sacroiliitis and spondylitis resembling idiopathic AS. In their turn, peripheral arthritis can be divided into two groups: type 1, a peripheral oligoarthritis (four joints or less) – involves large joints, is acute course independent of the activity of the IBD.

Due to the common inflammatory pathways in bowel and joint inflammation in AS, several common therapies has proven efficiency for both disorders, mainly TNF-α drugs (the use of non-

steroidal anti-inflammatory drugs in AS can lead to a reactivation of IBD, so their use is limited). These monoclonal antibodies (mAb) targeting TNF-α are biological drugs used in the treatment of IBD and other immune-mediated inflammatory diseases. Infliximab, adalimumab, certolizumab pegol, and golimumab are the most used (227). Between these therapeutic options, Infliximab remain the first choice for patients with active AS associated with IBD (228).

A recent study suggests that biosimilars (biological medicinal products similar, but not identical, to an approved biological drug) can be used for the treatment of IBD. These drugs are obtained from biological sources and can be found on many biochemical forms, like vaccines, gene therapies and recombinant proteins. The first infliximab biosimilar for the treatment of IBD was introduced six years ago and today eight anti-TNFα biosimilars, three for infliximab and five for adalimumab, have been approved and licensed by the European Medicines Agency. Their main advantages are cost saving and possible consequential reinvestment in the health care system. As for all biologics, accurate pharmacovigilance should be done also for biosimilars. Before supportive clinical evidence will become available, cross-switching or multiple switching among biosimilars is not recommended. Patients need to be adequately informed about biosimilars in order to improve their awareness of this drug class (227).

In conclusion, IBD and AS separately, or the association of this chronic inflammatory diseases, are a permanent stress factor for the patients, having a negative effect on their quality of life. Health is considered to be a general well-being condition, including physical, mental and social aspects of living. Firstly, quality of life in patients with chronic diseases depends on: form of disorder, activity, location, extension and duration of the disease and secondly on social and psychological aspects. Besides the objective aspects included in the quantification of quality of life, should not be overlooked the subjective ones like personal perceiving and interpreting of health and disease.

2. Cardoneanu A., Cijeveschi Prelipcean C., Danciu M., Mihai C., Dranga M., Gavrilescu O., **Rezus E.,** *Articular involvement in inflammatory bowel disease – the most frequent extraintestinal manifestation,* Romanian Journal of Rheumatology, XXVII (4), 2018, 174-178.

3. Cardoneanu A., Burlui A., Mihai C., Cijeveschi Prelipcean C., Macovei L.A., **Rezus E**., *Looking beyond gut inflammation in inflammatory bowel Disease*, Rom J Morphol Embryol 2018, 59 (4): 1097–1105.

Materials and methods

We performed a case-control retrospective study including 517 patients with intestinal inflammation (CD, UC, or undifferentiated colitis – NC) diagnosed during 1975-2016 in the

Northeastern (NE) region of Romania. All the cases were extracted from the National Database (IBD Prospect).

The inclusion criteria were: age over 18, patient consent and signing an informed consent; certain diagnosis of CD, UC or NC based on the current diagnosis criteria and on histopathological examination. The exclusion criteria were: uncertain diagnosis of CD, UC and NC; patients's refuse to be included in the national database.

Data was centralized into SPSS 22.0 database. In the statistical analysis, both descriptive and analytical methods were used at 95% significance (CI 95%).

All patients included in the study were clinically and paraclinically analyzed. Of the extraintestinal manifestations – EIM, the following ones were considered: articular signs (arthritis, AS), cutaneous manifestations (pyoderma gangrenosum and erythema nodosum), ocular manifestations (uvevitis/episcleritis, primary sclerosing cholangitis), renal manifestations (oxalate kidney lithiasis, renal amiloidosis, multiple urinary tract infections).

Results

The study included 517 patients with IBD of which only 513 had all data required for the statistical analysis (Table I.3.1). UC predominated against CD cases (*n*=368 *vs.* *n*=135). Only 10 patients were diagnosed with CN. Female gender (51.1% *vs.* 48.9%) predominated in the group of CD patients, while, in the UC group, male gender prevailed (60.3% *vs.* 39.7%) (*p*=0.016). UC patients had an older age than the rest of the cases (*p*=0.003) (figure I.3.4, table I.3.2).

Table I.3.1. Demographic and clinical characteristics of the study group with IBD (CD, UC)

	IBD, *n* (%)	CD, *n* (%)	UC, *n* (%)	*p*
No. of patients	517 (100)	135 (26.1)	368 (71.2)	0.001
Males/females	294 (56.9)/223 (43.1)	66 (48.9)/69 (51.1)	221 (60.3)/147 (39.7)	0.016
Average age [years]	48.24	44.52	49.65	0.003
Area of origin: urban/rural	341 (66.7)/172 (34.9)	95 (70.4)/40 (29.6)	240 (65.2)/128 (34.8)	0.536
Smokers/ex-smokers/ non-smokers	77 (15)/164 (31.8)/276 (53.2)	34 (25.2)/31 (23)/70 (51.9)	37 (10.1)/131 (35.6)/200 (54.3)	0.001
Disease activity: mild/moderate/severe	226 (44.2)/242 (47.4)/43 (8.4)	56 (41.5)/73 (54.1)/6 (4.4)	165 (44.8)/166 (45.1)/37 (10.1)	0.062
Form of CD: L1/L2/L3/L4	NA	34 (25.2)/52 (38.5)/45 (33.3)/4 (3)	NA	0.818
Phenotype of CD: B1/B2/B3	NA	84 (62.2)/40 (29.6)/11 (8.1)	NA	0.026
Form of UC: E1/E2/E3	NA	NA	71 (19.3)/203 (55.2)/94 (25.5)	0.012

IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis; n: No. of cases; L1: Ileitis; L2: Colitis; L3: Ileocolitis; L4: Upper gastrointestinal tract; B1: Inflammatory; B2: Stricturing; B3: Penetrating; E1: Proctitis; E2: Left-sided colitis; E3: Pancolitis; NA: Not applicable.

Table I.3.2. Descriptive indicators of age [years] in patients with IBD

Phenotype	*n*	Median	Standard deviation	Standard error	95% Confidence interval (CI)		Min.	Max.	F_{ANOVA} test
					-95% CI	*+95% CI*			
UC	368	49.65	15.08	0.79	48.11	51.2	18	81	0.003
CD	135	44.52	14.58	1.25	42.04	47	20	81	
CN	10	46.7	16.27	5.14	35.06	58.34	25	72	
Total	513	48.24	15.11	0.67	46.93	49.55	18	81	

IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CD: Crohn's disease; CN: Undifferentiated colitis; *n*: No. of cases; ANOVA: Analysis of variance

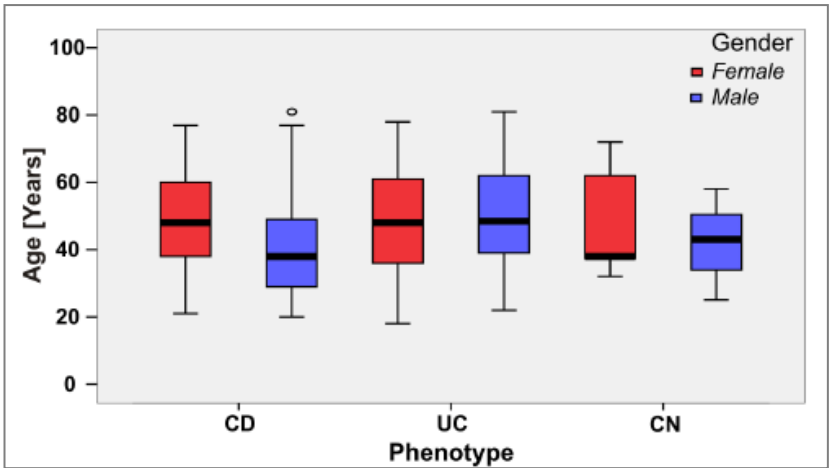


Figure I.3.4. Average age by gender according to the phenotype of the disease. CD: Crohn's disease; UC: Ulcerative colitis; CN: Undifferentiated colitis.

Most of the included patients, both those with CD or UC phenotype, came from urban areas (70.4% *vs.* 65.2%) (p=0.536). The peak incidence of IBD cases was recorded in 2012, with an increasing trend over the next period of time (y=0.5x-1.23), which was maintained at a level of approximately 10 % (figure I.3.5). CD summarized 135 cases. Colonic involvement (L2) (n=52, 38.8%) predominated, followed by ileocolitis (L3) (n=45, 33.6%). Eighty-four (63.4%) of these patients had an inflammatory phenotype (B1) and 40 (28.4%) a stricturing form. The ileal inflammation was identified in 27.4% of patients with a non-stricturing form of CD and in 27.3% of those with a penetrating form of disease. Most commonly, the colonic location of intestinal inflammation was identified in patients with a stricturing disease (42.1%), and the most rare in those with a penetrating form of CD (27.3%). Ileocolitis was most commonly associated with the penetrating phenotype (45.5%). Involvement on the upper gastrointestinal tract was present in 3.6% of patients with non-stricturing disease and in 2.6% of those having a stricturing phenotype.

Patients diagnosed with UC have totalized 368 cases. Among these cases, left-sided disease (n=203, 55.16%), then pancolitis (n=94, 25.5%) predominated. Most patients with UC and those diagnosed with CD had a moderate form of intestinal inflammation (n=242, 47.4%).

All patients underwent colonoscopy with biopsy and pathological examination. Diagnosis of CD, UC or CN was based on biopsy examination, which revealed specific and multiple lesional aspects. Chronic follicular colitis presented as a diffuse and follicular lympho-plasmocytary inflammatory infiltration, edema and congestion in chorion (Figure I.3.6.A). Active UC's pathological examination showed: abundant and polymorph inflammatory infiltration, rich in neutrophils, congestion in chorion; surface epithelium with erosions; deformed crypts, some with a tendency to ramification, elevated from the mucosal muscle, some with decreased mucus secretion, crypt inflammation and crypt abscesses (Figure I.3.6.B). In CD's biopsies were highlighted: predominant transmural lympho-plasmocytary inflammation, edema and congestion, ulcerations, pyloric metaplasia, granulomatous inflammation without central caseification, lymphoid follicles "in rosary" in subserosa (Figures I.3.6.C and I.3.6.D).

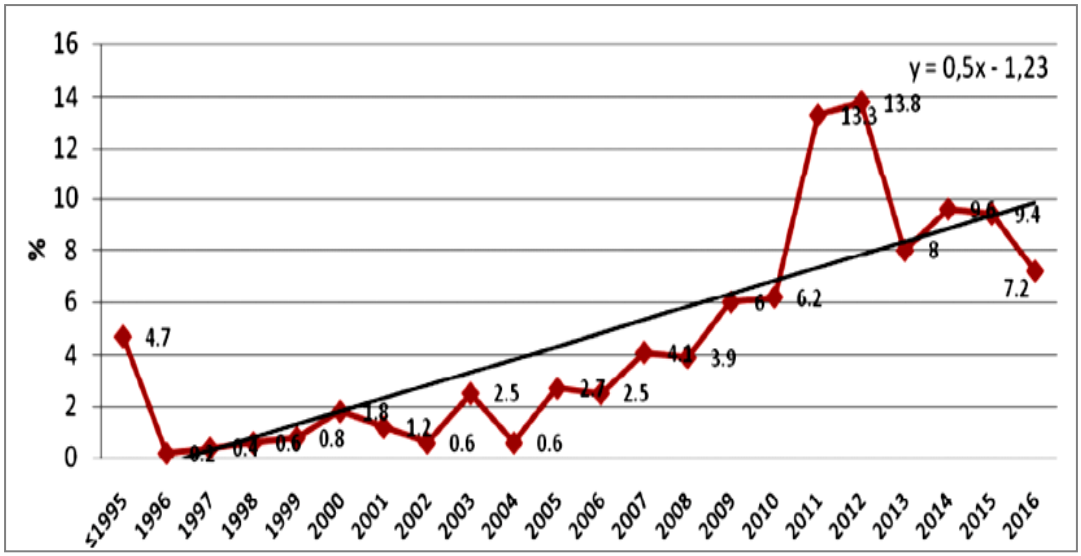


Figure I.3.5. Prevalence of IBD cases per study year. IBD: Inflammatory bowel disease.

Patients diagnosed with UC have totalized 368 cases. Among these cases, left-sided disease (*n*=203, 55.16%), then pancolitis (*n*=94, 25.5%) predominated. Most patients with UC and those diagnosed with CD had a moderate form of intestinal inflammation (*n*=242, 47.4%).

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Over 90% of IBD cases (*n*=484, 93.6%) were on medication at the time of enrollment [Mesalazine – 5- Aminosalicylic Acid (5-ASA), Azathioprine (AZA), Methotrexate, tumor necrosis factor-alpha (TNF-α) blockers – Infliximab (IFX), corticosteroids (CS) and Budesonide, antibiotics – commonly Rifaximine, pro- biotics]. Of these, 223 (46.07%) patients were treated with 5-ASA and 216 (44.62%) had combined therapy, most cases (*n*=136, 62.96%) being treated with 5-ASA and CS.

EIM in the study group

In the study group, 51 cases with IBD and EIM were identified, having a prevalence of 9.9% (Table I.3.3). The most common EIMs were musculoskeletal manifestations (7.4%), followed by renal manifestations (2.2%), cutaneous manifestations (1.2%), ocular (0.6%) and hepatobiliary manifestations (0.2%). EIMs occurred with a higher frequency in patients diagnosed with CD than UC (52.9% *vs.* 47.1%) ($p<0.001$). No patients with CN presented EIM ($p=0.289$). Over 50% of

cases of IBD and EIM belonged to female gender (52.9%, *p*=0.142), higher in the CD group (55.6% *vs.* 50%, *p*=0.692).

Mean age was slightly higher in patients who had EIM (49.31 *vs.* 48.13 years, *p*=0.595). Most patients diagnosed with IBD and EIM came from urban areas (*n*=38, 74.5%, *p*=0.202). The peak years for the occurrence of EIM were in 2011, 2012 and 2015. Ten (19.6%) patients were active smokers, over half – 28 (54.9%) non-smokers and 13 (25.5%) former smokers (*p*=0.465). By logistic regression, it was confirmed that active smokers had a 1.3 times higher risk to develop EIM than non-smokers (OR=1.306, *p*=0.497). Former smokers presented a risk of 0.758 (OR=0.758, *p*=0.431), so smoking status may be a protective factor for the occurrence of EIM.

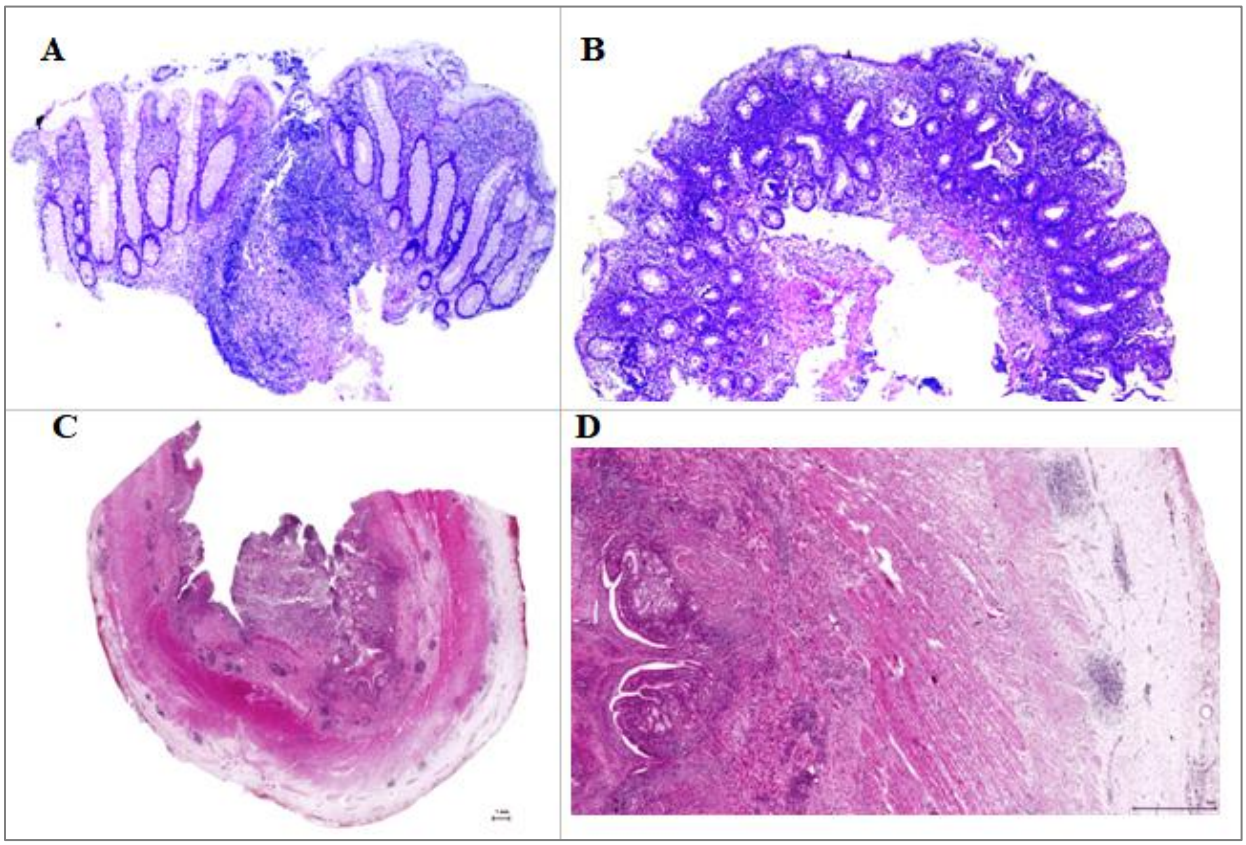

Figure I.3.6. A. Chronic follicular colitis: diffuse and follicular lympho-plasmocytary inflammatory infiltrate,edema and congestion in chorion [Hematoxylin–Eosin (HE) staining, ×40]. B. Active ulcerative colitis: abundant and polymorph inflammatory infiltrate, rich in neutrophils, congestion in chorion; surface epithelium with erosions; deformed crypts, some with a tendency to ramification, elevated from the mucosal muscle, some with decreased mucus secretion, crypt inflammation and crypt abscesses (HE staining, ×40). C. Crohn's disease: predominant transmural lympho-plasmocytary inflammation, edema and congestion, ulcerations, pyloric metaplasia, granulomatous inflammation without central caseification, lymphoid follicles "in rosary" in subserosa (HE staining, ×40). D. Crohn's disease: detail from previous figure (HE staining, ×100).

Uveitis was highlighted in two patients with CD and in an UC case. All cases with ocular signs also showed peripheral articular manifestations – arthritis. *Pyoderma gangrenosum* was more common in CD patients than in UC cases (*n*=3 *vs.* *n*=1) and was associated with articular manifestations. PSC was highlighted in one patient with UC and did not associate other EIM.

Renal manifestations occurred with a higher frequency in CD and were associated with the presence of other EIM. Because the number of these cases was very small, the statistical analysis did not have statistical consistency (Table I.3.3).

Table I.3.3. EIM classification in patients with IBD depending on disease phenotype

EIM	CD (n=27)		UC (n=24)		p	OR	RR	95% CI
	n	%	n	%				
Articular manifestations								
Arthritis	16	59.3	10	41.7	0.209	2.04	1.4_{CD}	0.82–2.39
SI/AS	4	14.8	8	33.3	0.118	0.35	1.63_{UC}	0.94–2.81
Cutaneous manifestations								
Erythema nodosum	1	3.7	1	4.2	0.932	0.89	1.07_{UC}	0.26–4.4
Pyoderma gangrenosum	3	11.1	1	4.2	0.345	2.88	1.47_{CD}	0.78–2.76
Hepatobiliary manifestations								
PSC	0	0	1	4.2	0.216	–	2.17_{UC}	1.61–2.94
Ocular manifestations								
Uveitis	2	7.4	1	4.2	0.619	1.84	1.28_{CD}	0.55–2.98
Renal manifestations								
Oxalate nephrolithiasis	2	7.4	1	4.2	0.619	1.84	1.28_{CD}	0.55–2.98
Multiple urinary infections	5	18.5	3	12.5	0.553	1.59	1.22_{CD}	0.66–2.25

Intestinal complications in the study group

In the study group (n=513), there were 66 (12.9%) cases associated with intestinal complications as follows: abscesses – seven (10.6%) cases, fistula – 11 (16.16%) cases, stenosis – 22 (33.33%) cases, inferior digestive hemorrhage (Hdi) – eight (12.12%) cases, malignancies – six (9.09%) cases, and 12 (18.18%) patients had multiple intestinal complications. Most patients who developed intestinal complications belonged to CD phenotype (n=49, 74.2%) (p<0.001).

Table I.3.4 presents the risk for patients with CD, UC and CN to develop intestinal complications. The association between intestinal complications and EIM was highlighted in 15 (22.7%) patients (p=0.007), being more common in CD cases than in UC (n=12 *vs.* n=3) (Table I.3.5). The risk of developing intestinal complications was found to be 3.35 times higher in patients with EIM as compared to the rest of the cases (p<0.001, OR=3.358, 95% CI 1.72–6.55). Of the intestinal complications, intestinal stenosis and Hdi predominated, while the most common EIM were articular manifestations (with a predominance of peripheral manifestations – arthritis). The stricturing phenotype and ileocolitis in CD apparently favored the association between intestinal complications and EIM (without statistical significance – very few cases).

Table I.3.4. The risk of developing intestinal complications depending on the phenotype of the disease

Disease phenotype	Patients			p	OR	95% CI	
	Without intestinal complications, n (%)	*With intestinal complications, n (%)*	*Total, n (%)*			*Min.*	*Max.*
CD	86 (63.7)	49 (36.3)	135 (100)	<0.001	12.5	3.428	47.829
L1	19 (22.1)	15 (30.6)	34 (25.2)	0.604	–	–	–
L2	36 (41.9)	16 (32.7)	52 (38.5)				
L3	28 (32.6)	17 (34.7)	45 (33.3)				
L4	3 (3.5)	1 (2)	4 (3)				
B1	70 (81.4)	14 (28.6)	84 (62.2)	<0.001	–	–	–
B2	13 (15.1)	27 (55.1)	40 (29.6)	<0.001	10.385	4.325	24.932
B3	3 (3.5)	8 (16.3)	11 (8.1)	<0.001	13.333	3.141	56.595
UC	352 (95.7)	16 (4.3)	368 (100)	<0.001	0.08	0.043	0.147
E1	69 (19.6)	2 (12.5)	71 (19.3)	0.492	–	–	–
E2	195 (55.4)	8 (50)	203 (55.2)				
E3	88 (25)	6 (37.5)	94 (25.5)				
CN	9 (90)	1 (10)	10	0.126	0.195	0.024	1.585

Discussions

This study brings important data on the epidemio-logical and clinical characteristics of patients with IBD in the NE region of Romania. It also highlights the association and correlations between IBD and EIM, as well as those regarding intestinal complications. Most of the obtained results are consistent with the data published in the literature. Our results sustain, once again, the fact that inflammation in IBD is not limited at the level of gastrointestinal tract.

Worldwide, between 5 and 10% of cases of AS are associated with IBD, significantly CD and UC. An increasing genetic and immunological evidence supporting the clinical and histological overlap between gut inflammation in SpA and CD have been shown already by some studies. Gut and joint inflammation are related in SpA, and also gut could have a key pathogenic role. Statistics have highlighted the fact that a much larger percentage of AS patients have subclinical gut inflammation manifested either by endoscopic findings or by histology. At the molecular level, the association with HLA-B27 is less strong in IBD-associated AS than in idiopathic AS, and there is evidence for an association between gut inflammation in AS with the Crohn's disease-related CARD-15 specific mutations. The immunopathology data propose common inflammatory pathways in gut and joint inflammation in AS, and in gut inflammation in AS and IBD (228).

The presence of EIM is certainly validated by the results of numerous worldwide clinical trials published in the literature.

In a study developed by Isene et al. a total of 1145 patients, from Europe and Israel, were followed for 10 years, from 1991 to 2004. The prevalence of first EIM was 16.9% (193 patients). Patients with CD were more likely than UC patients to have immune-mediated (arthritis, eye, skin, and liver) manifestations: 20.1% versus 10.4% ($p < 0.001$). AS has been reported in 3–6% of IBD patients, with asymptomatic sacroiliitis reported in 14–20%. A small group of the present

cohort has been further analyzed and it has been found a cumulative prevalence of AS in 3.6% over 4.2 years of follow up. One important conclusion of the study was that patients with CD are twice as likely as UC patients to experience EIM (229).

In another study conducted over 11 years, 72 CD and 168 UC patients (the total number of patients included in the study was 811, 595 with UC and 216 with CD) suffered from musculoskeletal diseases, ($P < 0.0001$, OR = 0.35, 95%CI: 0.22-0.59). More precisely arthritis was present in 66 CD and in 161 UC (30.5% vs 27.1%) ($P = 0.53$, OR = 0.65, 95%CI: 0.21-2.08) and AS was observed in 5 CD and in 8 UC patients (2.3% vs 1.3%), ($P = 0.53$, OR = 1.52, 95%CI: 0.48-4.8) (230).

In our study the prevalence of UC versus CD cases prevailed. The peak incidence of IBD cases was recorded in 2012, with an increasing trend of prevalence over the next period of time. In our study group, the most frequent EIMs were highlighted at the level of musculoskeletal system. This data is supported by other studies which confirm that the greatest incidence among EIM is owned by articular manifestation. Data on the frequency of developing at least one EIM vary between 6–47%. Perianal CD, colonic involvement and smoking can to increase the susceptibility to EIM. Furthermore, patients who have one EIM are predisposed to an increased risk of developing further extraintestinal symptoms. A common pathogenic pathway is even suggested in some EIM, as they tend to appear simultaneously in affected patients: peripheral arthritis, biliary involvement, erythema nodosum and involvement of the eyes (231).

Table I.3.5. The association between intestinal complications and EIM

IBD				Intestinal complications	EIM
Disease phenotype	*Location of inflammation*	*Form of IBD*	*Severity of disease*		
CD	L1	B2	moderate	intestinal stenosis	arthritis
CD	L3	B2	moderate	intestinal stenosis	multiple urinary tract infections
UC	E2	–	mild	Hdi	multiple urinary tract infections
CD	L3	B2	moderate	intestinal stenosis	arthritis + uveitis
UC	E1	–	mild	Hdi	arthritis
CD	L2	B1	mild	abscesses + fistula	arthritis + uveitis + oxalic nephrolithiasis + multiple urinary tract infections
CD	L3	B1	moderate	Hdi	arthritis
CD	L3	B2	moderate	intestinal stenosis	arthritis
CD	L2	B2	moderate	intestinal stenosis	arthritis + *pyoderma gangrenosum*
CD	L2	B2	severe	intestinal stenosis	arthritis
CD	L3	B1	severe	fistula	multiple urinary tract infections
CD	L1	B2	moderate	abscesses	multiple urinary tract infections
CD	L2	B3	mild	fistula	oxalic nephrolithiasis
UC	E3	–	mild	malignancy	oxalic nephrolithiasis
CD	L3	B1	mild	fistula + Hdi	SI/AS

Following the performed statistical analysis, both patients with CD and UC experienced a greater risk than the rest of patients for developing EIM.

Compared with UC cases, patients with CD had a risk of over three times greater to develop EIM. We cannot sustain that a particular phenotype of CD favors the appearance of EIM.

However, there is a difference between the group of patients with CD and EIM *versus* CD without EIM regarding the location of intestinal inflammation. In patients with CD without EIM predominated the colonic form of the disease. Those having EIM had a more frequent ileo-colonic disease. The study published by Karmiris *et al.* upports the association of EIM (especially articular manifestations) with an extensive CD. Statistical analysis revealed that UC can be considered a protective factor for the occurrence of EIM. Among these patients, the left colonic form of the disease predominated. The obtained results are in contradiction to the literature. The presence of EIM in patients with UC has been mostly correlated with an extensive form of disease (pancolitis) (232). There were 66 cases of intestinal complications, having a prevalence of 12.9% (greater than the incidence of EIM – 9.9%). In a recent study published in 2017 by Hsu *et al.*, the incidence of intestinal complications in a group of 3153 patients was 22.2% (233).

In conclusion, this study focused on highlighting the correlations between EIM and intestinal complications. The obtained results after statistical analysis are promising, even if the number of the analyzed subjects was small. The risk of developing intestinal complications was found to be 3.35 times higher in patients who also had EIM compared to the rest of the patients. The association between EIM – complications was much more common in CD cases. Specialty literature does not currently provide specific data on the association between EIM and intestinal complications. What we know for sure is that CD represents the phenotype of IBD, which has a higher incidence for EIM and intestinal complications.

I.3.4. Neurophysiological abnormalities reported in patients with ankylosing spondylitis

A. Background

Neuro-electric responses to sensory stimuli can be easily recorded using averaging that are non-invasively. The evoked responses (EPs) can be quantified by measuring peak amplitudes and latencies, in the millisecond (ms) domain, and they provide numerical data that are quantitative extensions of the neurological examination. In clinical practice EPs are really useful because they have the ability to demonstrate abnormal sensory system conduction, reveal subclinical involvement of a sensory system, help define the anatomic distribution and give some insight into pathophysiology of a disease process and also monitor changes in a patient's neurological status. Theoretical, almost any sensory modality may be tested, but in routine clinical practice the EPs tested most frequently are: visual evoked potentials (VEPs), short latency somatosensory evoked potentials (SSEPs) and brainstem auditory evoked potentials (BSAEPs) (234).

Neurological complications are uncommon in patients with AS and the frequency of these complications has not been completely examined. Root lesions (cervical and lumbosacral radiculopathies), cauda equina syndrome and compression of the spinal cord (myelopathy) have rarely been reported as extra-articular manifestations of AS. The detailed examination of sensory

and motor pathways of central nervous system has been made possible in clinical practice with the availability of EPs assessment. The abnormalities detected by evoked potentials do not provide a specific diagnosis but point to impaired function in that particular sensory and motor pathway (235).

SSEPs monitoring is reproducible and frequently used to detect changes in electrophysiological conduction in peripheral nerves and central nerve pathways and as a result, to prevent nervous system damage. A significant change in the SSEPs responses is indicated by a decrease in amplitude and/or an increase in latency. Even if the abnormalities detected by SSEP do not provide a specific diagnosis, they point to impaired function in that particular sensory pathway (236).

B. Published papers in this field

1. Stamate I.G., **Rezuș E.,** Trofin D.M., Popescu C.D., *Visual Evoked Potentials Abnormalities In Ankylosing Spondylitis*, Medicine In Evolution, XXI (3), 2015, 402-406.

Neurophysiological abnormalities including visual evoked potentials (VEP) were reported in patients with AS (235, 236). The aim of this study was to determine VEP abnormalities and their correlation with the therapy used in AS.

Materials and methods

All patients have been assessed in Rheumatology Department and in Neurology Department, Clinical Rehabilitation Hospital Of Iasi, Romania. The study has been approved by the institutional review board. Prior to study inclusion, the informed consent was signed by all the patients. The diagnosis of AS was made according to the modified New York criteria (202). After detailed history and physical examination, all patients were assessed by clinical, biological and neurophysiological parameters. The VEP patterns were described by the latencies and the amplitudes of the three waves: N 75, P 100 and N 145. All parameters have been processed and analyzed using the Statistical Package for Social Sciences (SPSS), version 16.0 for Windows. Mann-Whitney U test was used to compare the average measurements. For the correlation test, p value of <0.05 was accepted to be statistical significant.

Results

The mean age of all the 38 patients, 28 males and 10 females, included in this study, was 41.95±11.06 years and the mean duration of disease of the patients was 12.39±8.32 years. For VEP, the recorded response signals had the amplitude up to 20 μV and frequencies between 1-300Hz. The values we recorded for N75, P100 and N 145 are presented in table I.3.6.

It must be mentioned, that 13 patients were treated with TNF-alpha blockers, and this was an aspect to take in consideration for our study. The results for VEP have been recorded in SA

patients without TNF-alpha blokers have been exposed in table I.3.7, while for the patients treated with TNF-alpha blokers we have been shown the results of VEP in table I.3.8.

Table I.3.6. VEP results for all patients included in the study

Indicator	N	X	±S	S	V%	Min	Max
N75 R A1	38	81.26	4.675	28.81	35.463	45.6	177.9
N75 R A2	38	84.38	4.889	30.135	35.714	42.9	199.2
P100 RA1	38	109.58	4.572	28.185	25.721	55.2	210.6
P100 RA2	38	108.11	4.741	29.228	27.035	57.6	210
N145 RA1	38	143.5	5.226	32.216	22.451	70.2	222
N145 RA2	38	140.73	4.987	30.741	21.843	70.5	222
N75 LA1	38	81.51	3.856	23.768	29.162	56.1	174
N75 LA2	38	80.08	4.676	28.823	35.991	45.3	210.3
P100 LA1	38	109.49	4.987	30.743	28.079	74.7	244.2
P100 LA2	38	109.18	4.843	29.856	27.346	75.9	244.2
N145 LA1	38	138.41	5.336	32.892	23.764	87.3	272.4
N145 LA2	38	139.02	5.21	32.117	23.103	91.5	261.3

Table I.3.7. VEP results for patients without TNF-α blocking therapy

Indicator	N	X	±S	S	V%	Min	Max
N75 DRA1	25	88.45	10.158	23.812	30.717	51.6	174.3
N75 DRA2	25	90.62	11.693	21.891	26.981	54	145.2
P100 DRA1	25	118.08	10.711	20.481	19.477	86.4	188.4
P100 DRA2	25	116.22	11.265	20.93	20.145	70.8	185.7
N145 DRA1	25	149.84	11.298	27.153	19.368	11.4	210
N145 DRA2	25	147.39	12.195	21.299	15.516	107.1	198.3
N75 STGA1	25	84.42	8.632	19.486	24.359	56.1	147
N75 STGA2	25	85.55	11.679	19.227	24.891	45.3	128.7
P100 STGA1	25	114.12	11.773	23.205	21.671	81.9	199
P100 STGA2	25	115.02	11.839	20.858	19.652	90.3	189.9
N145 STGA1	25	142.2	12.573	25.072	18.376	108.6	230.4
N145 STGA2	25	145.29	11.522	26.361	19.418	11.8	238.5

Table I.3.8. VEP results for patients with TNF-α blocking therapy

Indicator	N	X	±S	S	V%	Min	Max
N75 DRA1	13	88.45	10.158	36.625	41.405	45.6	177.9
N75 DRA2	13	90.62	11.693	42.16	46.523	42.9	199.2
P100 DRA1	13	118.08	10.711	38.618	32.703	55.2	210.6
P100 DRA2	13	116.22	11.265	40.616	34.949	57.6	210
N145 DRA1	13	149.84	11.298	40.735	27.186	70.2	222
N145 DRA2	13	147.39	12.195	43.97	29.832	70.5	222
N75 STGA1	13	84.42	8.632	31.122	36.867	61.2	174
N75 STGA2	13	85.55	11.679	42.108	49.223	45.9	210.3
P100 STGA1	13	114.12	11.773	42.449	37.198	74.7	244.2
P100 STGA2	13	115.02	11.839	42.685	37.113	75.9	244.2
N145 STGA1	13	142.2	12.573	45.331	31.878	87.3	272.4
N145 STGA2	13	145.29	11.522	41.545	28.594	91.5	261.3

Discussions

Correlation between the two groups, the one with biologic therapy and the one with other therapies showed no significant differences. However, all the waves were delayed in the group with TNF-alpha blocker therapy. Correlation between presence of unilateral or bilateral hip involvement and values of each wave was significant ($p < 0.05$). VEP have revealed cortical and subcortical areas of response to the visual bright stimulation and informs especially about the integrity of visual pathways and less of cortical projection

In conclusion, the physiopathology of these abnormalities is unknown and further studies should be done for interpreting delayed evoked potentials in AS. Demyelinating mechanism induced by TNF alpha blockers may be considered..

2. Stamate I.G., **Rezuș E.,** Trofin D.M., Popescu C.D., *Brainsteam auditory evoked potentials findings in ankylosing spondylitis*, Romanian Journal of Neurology, XIV (3), 2015, 145-149.

The aim of the second study was to investigate the BAEPS and their relation with clinical findings, laboratory tests and pharmacological therapy used in patients with AS.

Materials and methods

In the study were included the 39 patients with AS. A control group was composed of 50 healthy medication-free adults. As in the first study, the patients have been assessed in Rheumatology Department and BAEP were recorded in Neurology Department, from the Hospital mentioned in the first study.

BAEP were performed with Nihno Kohden Neuropack for all patients and for all voluntaries in the control group. We have used 80 dB HL alternating polarity clicks in each ear at a rate of 10/s and a masking white noise of 40 dB was used for the unstimulated ear. The electrodes were placed as following: ground electrode in the midline frontal area, the reference electrode at vertex and the active electrodes (A1, A2) at the ear lobes. We have recorded the latencies of all the waves: I, II, II, IV and V for and the Interval Latencies (IL): I-III, III-V, I-V. Patients were assessed by clinical specific tests, inflammatory laboratory tests, Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and type of pharmacological therapy. As in the previous study, the diagnosis of AS was made according to the modified New York criteria (237). The measured parameters have been processed using the same program, and also for the correlation test, p value of <0.05 was considered statistically significant.

Results

The mean age of all the 39 patients, 26 males and 13 females, included in this study, was 40.65±14.34 years and the mean duration of disease of the patients was 13.31±7.52 years. Most of the patients (n=25) were taking nonsteroidal anti-inflammatory drugs (NSAID) or sulfasalazine (SSZ) or the association between these drugs. 14 patients were treated with TNF-α blockers. BAEP results for all SA patients included in the study may be seen in table I.3.9.

BAEP results for all the control group seen in table I.3.10. The left wave V latency in control group and in patients group had a significant differences in values of I-III left interval ($p<0.05$, CI 95%). For the I-V interval there was a mean difference of 0.29 wich was very significant for $p < 0.001$ and $F > F0.001$, CI 95%. The left wave I latency was in significant correlation ($p < 0.005$, CI 95%) with the duration of the disease.

There were differences of the latencies in the group of SA patients treated with TNFα blockers compared with the group with AINS/DMARDs. Results of BAEP in group treated with TNFα blockers and the group with other pharmacological treatment can be seen in table I.3.11. and table I.3.12.

Table I.3.9. BAEP results for all SA patients included in the study

Wave/Interval	N	$\bar{X}$	$\pm s^x$	s	V%	Min	Max
I *L*	39	1.62	0.062	0.389	24.088	0.77	3.23
I *R*	39	1.64	0.067	0.421	25.627	0.91	3.28
II *L*	39	2.72	0.095	0.59	21.7	1.67	5.31
II *R*	39	2.77	0.131	0.816	29.489	1.87	6.99
III *L*	39	3.79	0.096	0.598	15.781	2.65	6.25
III *R*	39	3.69	0.077	0.483	13.097	2.72	5.43
IV *L*	39	4.83	0.095	0.591	12.234	3.71	7.29
IV *R*	39	4.74	0.078	0.49	10.332	3.72	6.26
V *L*	39	5.83	0.093	0.582	9.988	5.17	8.45
V *R*	39	5.7	0.07	0.44	7.709	4.98	7.2
I-III *L*	39	2.16	0.056	0.351	16.279	1.2	3.11
I-III *R*	39	2.05	0.044	0.274	13.357	1.52	2.66
III-IV *L*	39	2.06	0.054	0.34	16.542	1.44	2.86
III-IV *R*	39	2.01	0.061	0.381	18.927	1.09	3.06
I-V *L*	39	4.21	0.056	0.351	8.342	3.6	5.22
I-V *R*	39	4.06	0.062	0.39	9.601	2.92	5.04

Table I.3.10. BAEP results for the control group

Wave/Interval	N	$\bar{X}$	$\pm s^x$	S	V%	Min	Max
I *L*	50	1.7	0.046	0.328	19.273	1.08	2.4
I *R*	50	1.69	0.048	0.337	19.965	1.2	2.58
II *L*	50	2.7	0.039	0.278	10.322	2.17	3.26
II *R*	50	2.73	0.053	0.373	13.673	1.95	3.63
III *L*	50	3.76	0.034	0.243	6.453	3.19	4.37
III *R*	50	3.71	0.039	0.274	7.387	3.06	4.43
IV *L*	50	4.81	0.045	0.321	6.673	3.87	5.59
IV *R*	50	4.82	0.053	0.371	7.706	4.03	5.69
V *L*	50	5.64	0.03	0.215	3.824	5.2	6.37
V *R*	50	5.66	0.046	0.324	5.716	4.94	6.71
I-III *L*	50	2.06	0.045	0.315	15.266	1.41	2.66
I-III *R*	50	2.01	0.04	0.284	14.108	1.42	2.52
III-IV *L*	50	1.88	0.032	0.227	12.089	1.56	2.63
III-IV *R*	50	1.97	0.028	0.201	10.22	1.67	2.67
I-V *L*	50	3.93	0.058	0.402	10.242	3.35	5.29
I-V *R*	50	3.99	0.058	0.411	10.292	3.09	5.19

Table I.3.11. BAEP results in patients with TNF-α blockers

Wave/Interval	N	$\bar{X}$	$\pm s^x$	S	V%	Min	Max
I *L*	14	1.62	0.082	0.306	18.826	1.12	2.51
I *R*	14	1.64	0.08	0.299	18.26	1.31	2.56
II *L*	14	2.78	0.133	0.497	17.866	2.18	4.04
II *R*	14	2.66	0.078	0.293	11.039	2.21	3.49
III *L*	14	3.88	0.121	0.452	11.676	3.31	4.9
III *R*	14	3.71	0.083	0.31	8.345	3.2	4.39
IV *L*	14	4.96	0.096	0.359	7.236	4.38	5.57
IV *R*	14	4.71	0.083	0.31	6.59	4.14	5.09
V *L*	14	5.89	0.096	0.359	6.104	5.19	6.51
V *R*	14	5.68	0.073	0.275	4.83	5.18	6.17
I-III *L*	14	2.08	0.06	0.223	10.755	1.57	2.37
I-III *R*	14	2.0	0.06	0.223	10.755	1.57	2.37
III-IV *L*	14	2.04	0.087	0.326	16.007	1.44	2.61
III-IV *R*	14	1.97	0.107	0.401	20.35	1.09	2.63
I-V *L*	14	4.27	0.087	0.324	7.596	3.6	4.74
I-V *R*	14	4.05	0.109	0.407	10.064	2.92	4.44

Table I.3.12. BAEP results in patients with AINS/SSZ

Wave/Interval	N	$\bar{X}$	$\pm s^x$	s	V%	Min	Max
I *L*	25	1.61	0.087	0.435	26.989	0.77	3.23
I *R*	25	1.65	0.096	0.482	29.266	0.91	3.28
II *L*	25	2.69	0.129	0.644	23.973	1.67	5.31
II *R*	25	2.83	0.2	0.998	35.308	1.87	6.99
III *L*	25	3.74	0.134	0.67	17.902	2.65	6.25
III *R*	25	3.68	0.113	0.563	15.319	2.72	5.43
IV *L*	25	4.76	0.137	0.685	14.384	3.7	7.29
IV *R*	25	4.76	0.114	0.572	12.014	3.72	6.26
V *L*	25	5.8	0.136	0.681	11.747	5.17	8.45
V *R*	25	5.72	0.103	0.515	9.005	4.98	7.2
I-III *L*	25	2.03	0.06	0.301	14.823	1.52	2.66
I-III *R*	25	2.03	0.06	0.301	14.823	1.52	2.66
III-IV *L*	25	2.07	0.071	0.354	17.112	1.51	2.86
III-IV *R*	25	2.03	0.075	0.375	18.455	1.6	3.06
I-V *L*	25	4.18	0.074	0.369	8.822	3.69	5.22
I-V *R*	25	4.07	0.078	0.388	9.541	3.29	5.04

Discussions

AS, with a prevalence rate of 0.1 – 1% of the worldwide population, is a rare disease and one of the major disabling diseases which starts in early adulthood. Because it is difficult to be treated properly, this disease affects the quality of patients' lives (238). Unfortunately, very few data are available on the prevalence of neurological disease in patients with a rheumatological diagnosis, the neurological complications of AS being barely reported (239).

AS is associated with some comorbidities which contribute significantly to morbidity and mortality. In addition to the already known extra-articular manifestations and increased cardiovascular risk, also, several pulmonary, renal, and neurological complications associated with AS earn equal attention (240). The range of neurological complications of AS are quite variable extending from minor instabilities of a joint to more prominent clinical syndrome (235).

The major problem is the fact that neurological involvement can be associated with significant morbidity in patients with rheumatic diseases, and may indicate heightened disease activity. The main neurological complications in ankylosing spondylitis occur due to axial disease with spinal cord impingement at multiple levels (241). Men have an increased risk for developing neurological complications secondary to AS (235).

CRP seemed to be more valuable than ERS as a possible marker for neurologic subclinical involvement. Axial AS is more likely to be associated with BAEP abnormalities. There were differences of the latencies in the group of SA patients treated with TNF-α blockers compared with the group with AINS/DMARDs. Multiple sclerosis or demyelination due to administration of TNF-α blockers should be excluded in medical practice. Collaboration between neurologists and rheumatologists should be considered in monitoring AS patients.

In a study published by Pillary et al. the authors have been analized visual, brainstem auditory and somatosensory evoked potentials in 30 patients with AS. Abnormalities in somatosensorial evoked potential studies examining the visual pathways were recorded in 18 (60%) patients. SSEps were abnormal in 19 patients, and 9 patients had impaired function on BSAEPs (242).

Recently, Tahmasebi et al. have recorded the VEP in RA patients with hydroxychloroquine treatment. Even if the study have not been accomplished in AS patients, the results of the study provide data for a better understanding of the neurological complications of rheumatic diseases (243).

In conclusion, this study has highlighted some abnormalities of BEAP in patients with AS. The results have shown differences between the control group and the patients with AS in most of the wave latencies.

I.4. Researches regarding other rheumatic disease: systemic scleroderma and gout

I.4.1. Introduction

Autoimmune diseases are a chronic and clinically heterogenous group of diseases affecting 5% of the population worldwide. These kind of diseases have a progressive increase in their incidence and prevalence and may be characterized by share commun immunopathogenic mechanism and risk factor, which explain the fact that one autoimmune disease may coexist with others.

Scleroderma, also named systemic sclerosis (SSc), is a complex disease in which extensive fibrosis, vascular alterations, and autoantibodies against various cellular antigens are among the principal features. It is a chronic connective tissue disease generally classified as one of the autoimmune rheumatic disease.

The word "scleroderma" comes from two Greek words: "sclero" meaning hard, and "derma" meaning skin, in fact, hardening of the skin is one of the most visible manifestations of the disease. There are two major subgroups in the commonly accepted classification of scleroderma: limited cutaneous scleroderma (lcSSc) and diffuse cutaneous scleroderma (dcSSc). In limited cutaneous scleroderma, fibrosis is mainly restricted to the hands, arms, and face. Raynaud's phenomenon is present for several years before fibrosis appears, pulmonary hypertension is frequent, and anti-centromere antibodies occur in 50 to 90% of patients. Diffuse cutaneous scleroderma is a rapidly progressing disorder that affects a large area of the skin and compromises one or more internal organs (244, 245).

SSc is subclassified into diffuse cutaneous SSc (dcSSc) or limited cutaneous SSc (lcSSc) based on the extent of skin involvement. The modified Rodnan skin score (mRSS) is a measure of skin thickness and is used as a primary or secondary outcome measure in clinical trials of systemic sclerosis (SSc, scleroderma) (246).

Even if *gout* is one of the most common rheumatic diseases, it had received little research attention for many years, partly due to a common perception that it is a well-understood disease, easily diagnosed and treated, and often resulting from dietary and lifestyle excesses. But, the increasing prevalence of the disease, along with evidence of poor outcomes in patients with gout, has led to the need to improve this disease management.

Since 2009, four novel pharmacologic agents have been approved for the management of gout (i.e., hyperuricemia of gout or gout attacks), in the USA (247). Unlike osteoarthritis and rheumatoid arthritis, gout is typically an episodic arthritis. The intervals between attacks can be very long, even decades with complete absence of symptoms between attacks. These factors, and also some errors in diagnosis, intrinsically complicate assessment of gout epidemiology (248).

I.4.2. Researches regarding scleroderma

Published papers in this field

1. Burlui A., Graur M., Constantinescu D., Cardoneanu A., Macovei L.A., **Rezuș E.**, *Nutritional Decline in Scleroderma Patients. Data from a single Romanian center*. Rev. Chim. (Bucharest), 69 (5), 2018, 1279-1282.

Systemic sclerosis (SSc), a rare connective tissue disease, is accompanied by multi-system involvement and considerable morbidity and mortality. Disease pathogenic mechanisms involve immune, vascular and neural changes as well as a widespread fibrosis of the skin and visceral organs (249, 250, 251). Food-related behavior (including the ability to procure and process food) is challenged by the severe hand disability resulting from extended skin fibrosis and joint contractures in scleroderma (252). Malnutrition has been known to provide poor survival outcomes in systemic sclerosis (SSc).

Materials and methods

We conducted a cross-sectional study in which we recruited 40 adult patients with systemic sclerosis, 22 (55%) with limited cutaneous involvement and 18 (45%) with the diffuse form of disease. over a period of three months. All participants fulfilled the European League Against Rheumatism (EULAR) 2013 diagnosis criteria for systemic sclerosis and were classified as diffuse cutaneous (dcSSc) or limited cutaneous SSc (lcSSc) according to LeRoy. None of the patients in our study population were under parenteral support. We recorded anthropometric data such as body mass index (BMI), waist circumference and waist/hip ratio as well as the number of daily gastrointestinal (GI) symptoms and unintended weight loss within 6 months prior to the study. We evaluated Malnutrition Universal Screening Tool (MUST), food-related behavior in all patients, as well as circulating albumin and vitamin D. Biological samples (venous blood) were collected in order to investigate circulating vitamin D levels and albumin. We assessed vitamin D values using a solid phase Enzyme Linked Immunosorbent Assay performed on microtiterplates

We measured serum albumin levels through spectrophotometry (bromocresol green reaction in acid environment, BioSystems®). The statistical analysis of the data was performed using Microsoft Office Excel and IBM SPSS Statistics v20 for Windows. The differences between patient subgroups were assessed through student t-test or ANOVA. We established relationships between variables using Pearson R and Spearman's correlation coefficients. Statistical significance was set at $p<0.05$.

Results

In the study were included 34 women and 6 men (F:M = 5.6:1), with ages between 25 and 83 years (54 ± 29 years). Our study population was composed of 22 patients with lcSSc (55%) and 18 patients with dcSSc (45%). The clinical and biochemical characteristics of the study group

are shown in table I.4.1. Serum vitamin D was deficient (<20ng/mL) in 38 patients (95%). Albumin levels were below normal in 4 patients (10%) and low-normal in 9 study participants (22.5%). We identified mRSS score values ≥20 in 9 patients (22.5%). The degree of skin involvement was strongly associated with the diffuse cutaneous form of disease (R=0.66, p<0.001), with a statistically significant difference between lcSSc and dcSSc (p=0.043).

In our study population, 29 patients were eutrophic (72.5%), 4 (10%) were underweight, 2 (5%) overweight and 5 (12.5%) obese. MUST values identified 6 participants at high risk (15%) and 4 patients at medium risk for poor nutrition (10%). Scores were significantly higher in dcSSc patients (p=0.033). The majority of patients (33 - 82.5%) reported theoccurrence of daily GI symptoms. The extent of skininvolvement was strongly correlated with digestive symptoms regardless of disease phenotype (R=0.534, p<0.001). We detected a negative correlation between serum vitamin D and mRSS (R=-0.35, p=0.026) (figure I.4.1.A). Statistical analysis showed no relevant relationships between circulating 25(OH)D levels and other parameters. Biochemical assessment revealed associations between serum albumin and weight (R=0.49, p=0.001), BMI (R=0.53, p<0.001) as well as waist/hip ratio (R=0.37, p=0.019) (figure I.4.1.B).

Tabel I.4.1. Clinical and biochemical characteristics of the study group

	Mean	Minimum	Maximum	Std. Deviation
Age (years)	52.17	25	83	14.27
Mrss	12.90	2	41	9.25
BMI (kg/m^2)	23.70	16.14	33.40	4.23
Waist circumference (cm)	82.85	64	121.00	13.27
Waist/hip ratio	0.81	0.66	1.11	0.08
Weight loss (kg)	2.90	0.00	24.00	5.29
Serum vitamin D (ng/ml)	10.51	2.52	33.89	6.56
Serum albumin (mg/l)	39.38	32.30	54.70	4.20

Data provided by the EPIC-Norfolk Food Frequency Questionnaire showed insufficient energy uptake according to age and gender in 24 patients (60%). The ANOVA testing failed to reveal significant discrepancies in terms of daily caloric intake (p=0.286) or dietary protein (p=0.332), carbohydrates (p=0.172), and fat (p=0.267) in relation with BMI (figure I.4.2). Although normal in the majority of patients, serum albumin levels were lower in the underweight group (p=0.035).

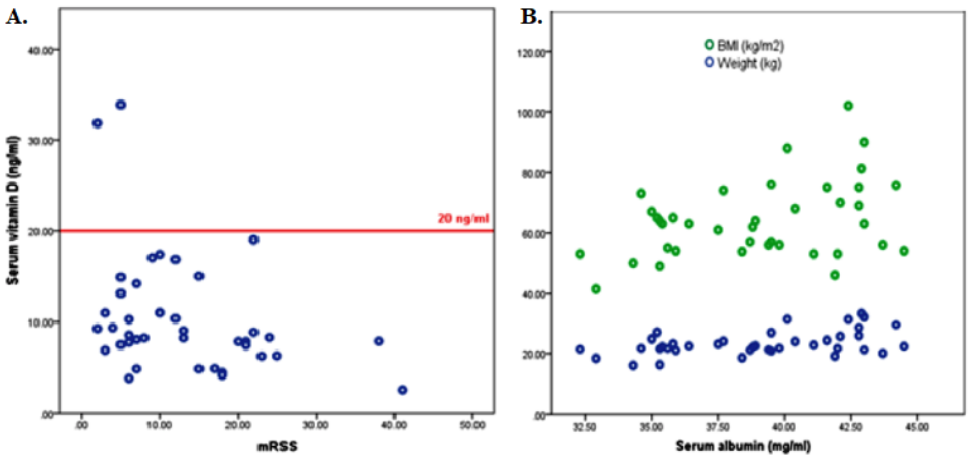

Figure I.4.1. A. Correlation between serum vitamin D levels and mRSS; B. Correlation between serum albumin levels, BMI, and weight

Additionally, we uncovered a greater number of daily gastrointestinal symptoms associated with diets rich in sodium, fat, sugars and snacks, carotene and α-tocopherol equivalents (vitamin E) (table I.4.2).

Malnutrition risk did not correlate with age, macronutrient intake or reported GI involvement. Patients with MUST scores ≥ 2 exhibited more severe weight loss ($p<0.001$), lower BMI and waist circumference ($p=0.006$ and $p=0.021$, respectively), decreased total energy intake ($p=0.006$), lower serum albumin ($p=0.003$), and higher dietary fiber consumption ($p=0.014$) compared to the rest of the group.

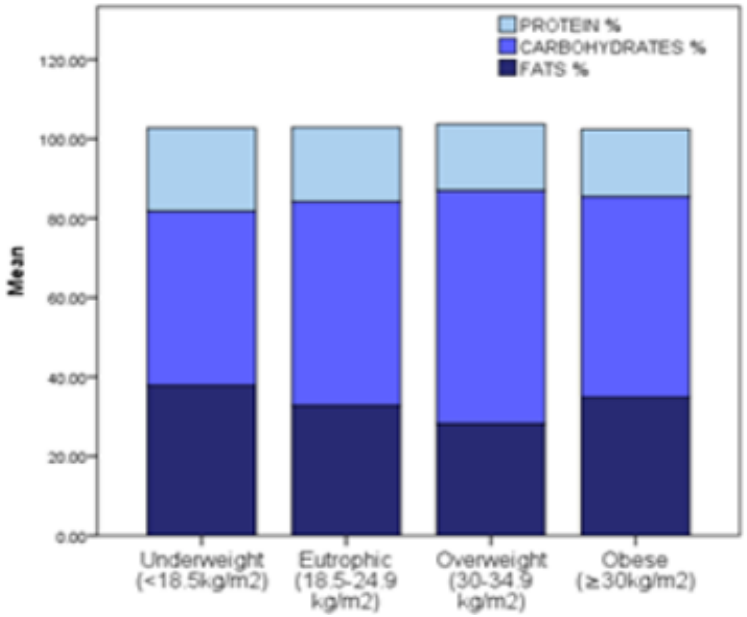

Figure I.4.2. Differences in average daily macronutrient intake according to BMI

Tabel I.4.2. Significant correlations for daily digestive symptoms

	R	**P**
mRSS	0.53	<0.001
Fat (g)	0.43	0.006
Protein (%)	-0.33	0.039
Cholesterol (g/day)	0.33	0.037
Alpha tocopherol equivalents (mg/day)	0.48	0.002
Alcohol (g/day)	-0.35	0.028
Sugars, preserves and snacks (g/day)	0.37	0.033
Carotene equivalents (mcg/day)	0.31	0.049
Iodine (mcg/day)	0.34	0.030
Manganese (mg/day)	0.36	0.024
Sodium (mg/day)	0.35	0.029

Discussions

Systemic sclerosis (SSc) is often characterized by microvascular damage, internal organ involvement, and skin fibrosis, that affects women four times more frequently than men and carries a high morbidity and mortality. It is a multisystem disease characterized by fibrosis of multiple organs including skin, musculoskeletal system, the lungs, kidneys, heart and gastrointestinal tract. Among those organs, gastrointestinal tract is the most commonly affected where up to 90% of SSc patients have GI involvement (253). Being, different organs are affected, including the gastrointestinal tract. Actually, gastrointestinal symptoms are frequently reported by patients with SSc and contribute to the development of protein–energy malnutrition incontinence. Oropharyngeal dysphagia, esophageal dysphagia, gastroesophageal reflux, gastroparesis, pseudo-obstruction, bacterial overgrowth and intestinal malabsorption, constipation, diarrhea and/or fecal are the main gastrointestinal problems that occur in patients with scleroderma. Also, cardiovascular abnormalities, like: pericardial effusion, left ventricular hypertrophy, moderate valvular fibrosis, right ventricular involvement secondary to pulmonary fibrosis and/or pulmonary hypertension, have been reported in patients with SSc (254).

Because malnutrition has been reported in 56% of SSc patients, nutritional status in SSc is beginning to gather attention. Nevertheless, traditional markers of nutritional status including body mass index or serum albumin are not good indicators of malnutrition in SSc. Correlates of malnutrition include length of disease duration, diffuse cutaneous disease, physician global assessment of disease severity, hemoglobin, oral aperture and abdominal distention. In a study that included 258 SSs patients, the risk for malnutrition was identified among 18% of two

hundred fifty-eight SSc patients with poor appetite, bloating and abdominal swelling as the only gastrointestinal predictors of malnutrition using the same screening tool as ours – MUST (255).

The present research have found several risk factors for nutritional decline in patients with scleroderma. Together with an unbalanced diet, disease phenotype and activity, the severity of gastrointestinal involvement may influence systemic sclerosis patients' nutritional status with possible consequences on quality of life and prognosis. To our knowledge, this is the first study to use the EPICNorfolk Food Frequency Questionnaire in scleroderma. More than half of our study population exhibited subnormal daily caloric uptake. In addition, we found an alarming 10% underweight patients. The risk of malnutrition estimated by the MUST score was associated with lower BMI and total energy intake. While the most part of our patients were eutrophic, a number of risk factors for body composition abnormalities were found in all participants.

However, dietary assessment in the present research implied a comparison with normal values recommended for healthy individuals of the same age and gender. We obtained a strong correlation between mRSS values and gastrointestinal symptoms in our study group. Moreover, a relationship between circulating 25(OH)D and the degree of skin involvement was detected in both lcSSc and dcSSc. Vitamin D values were low in 95% of our study population regardless of dietary and supplement intake.

Serum albumin levels were found to be normal in the majority of patients, but lower in the underweight group. Additionally, they correlated with MUST scores, contrary to results obtained by other research teams.

In conclusion, scleroderma patients might benefit greatly from nutritional counseling in order to follow a diet tailored to their specific needs. Bearing in mind the potentially severe disease-related gastrointestinal involvement and subsequent high risk for malnutrition, the need for defining the characteristics of a balanced diet in systemic sclerosis, the implementation of dietary interventions as well as routine screening for malabsorption are of major importance.

2. Burlui A., Cardoneanu A., Macovei L.A., Arhire L., Graur M., **Rezuș E**., *Is There A Place For Anti-Nucleosome Antibody Assessment In Scleroderma?* Romanian Journal of Rheumatology, XXVII (4), 2018.

The hallmarks of systemic sclerosis (SSc) include microangiopathy, autonomic dysfunction, as well as immune disturbance and the widespread fibrosis of the skin and visceral organs. While the significance of SSc-specific autoantibodies such as anti-centromere and anti-topoisomerase it has long been demonstrated, the clinical relevance of non-specific autoantibodies remains a matter of debate. Our primary objective was to assess the relationships between non-SSc-specific antibody titers and the clinical characteristics of scleroderma patients. Secondary objectives included a comparison between SSc, systemic lupus erythematosus – SLE

and healthy controls (HC) with respect to autoantibody values, as well as the analysis of the immune disturbance in elderly individuals in the 3 groups.

Material and method

We conducted a cross-sectional study in which we recruited 67 adult patients with SSc hospitalized in our center between September 2014 and September 2017, 67 age and gender-matched individuals with SLE and healthy controls (HC). For the SSc group, the inclusion criteria were the following: age ≥ 18 years and confirmed diagnosis of systemic sclerosis.

Biological samples (venous blood) were collected in order to determine the levels of anti-SSA/Ro, anti-SSB/La, anti-U1RNP and anti-nucleosome antibodies. All autoantibodies were assessed by enzyme-linked immunoassay (ELISA). We recorded the presence of digital ulcers (DUs), interstitial lung diseases (ILD, by thoracic X-rays), and pulmonary arterial hypertension (PAH, by Doppler echocardiography. The analysis of the data was performed using Microsoft Office Excel and IBM SPSS Statistics v20 for Windows. We used either parametric or non-parametric tests depending on variable.

Results

Our cohort consisted of 67 patients with scleroderma, of which 32 were classified as dcSSc (47.8%) while 35 persons presented with lcSSc (52.2%). The group was composed of 56 women (83.6%) and 11 men (16.4%). We identified the presence of ILD in 24 patients (35.8%), PAH in 5 patients (7.5%) and DUs in 29 patients (43.3%).

Male participants were more likely to present with digital trophic lesions than females (72.7% versus 37.5%, p=0.031), while elderly patients (≥ 60 years of age, 19 patients) demonstrated an increased risk of ILD by 113.8% (RR = 2.138, 95% CI: 1.171-3.903) and 910,5% for PAH (RR = 10.105, 95% CI: 1.206-84.690).

The values of the four autoantibodies tested are illustrated in table I.4.3. With regard to disease phenotype, we have found statistically significant discrepancies between the dcSSc and lcSSc patients in relation to the serum levels of anti-SSA antibodies (student t-test, p=0.014). Furthermore, patients with dcSSc were more likely to exhibit anti-SSA positivity (34.4% versus 5.7%, p=0.003).

We have found higher circulating anti-nucleosome antibodies in persons with PAH (186.60 ± 55.66 U/ml versus 102.92 ± 96.57 U/ml; Mann-Whitney, p=0.030) (figure I.4.3).

We did not identify a notable relationship between age and specific autoantibody titers in the SSc group, with similar values in patients over and under 60 years of age (table I.4.4).

Compared to the SLE group, patients with scleroderma exhibited lower anti-SSA, U1RNP and anti-nucleosome antibodies, while anti-SSB titers did not differ significantly. Moreover, all antibody titers were higher in SSc compared to HC (table I.4.5).

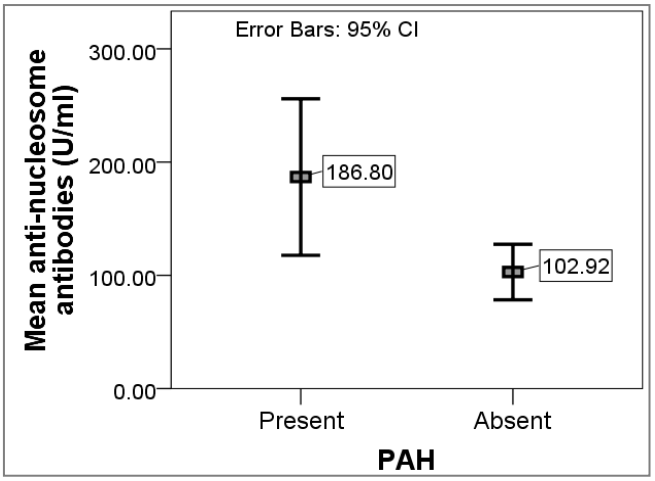

Figure I.4.3. Anti nucleosome antibodies in scleroderma patients with/without PAH

Table I.4.3. Autoantibody values in patients with scleroderma

Autoantibodies (U/ml)	Range	Mean	SD (standard deviation)	P (student t-test)
Anti-SSA	0.00-932.00	112.42	227.83	0.014
dcSSc	0.00-905.00	182.84	270.46	
lcSSc	0.00-932.00	48.03	158.53	
Anti-SSB	0.00-938.00	59.34	182.94	0.125
dcSSc	0.00-938.00	95.47	238.77	
lcSSc	0.00-614.00	26.69	103.22	
Anti-nucleosome	0.00-367.00	109.18	96.44	0.904
dcSSc	0.00-367.00	110.69	102.69	
lcSSc	0.00-349.00	107.80	91.83	
Anti-RNP/Sm	0.00-126.00	21.40	36.37	0.493
dcSSc	0.00-126.00	23.72	24.14	
lcSSc	0.00-114.00	19.28	28.44	

Anti-U1 RNP antibodies, SSA and SSB were significantly more frequent in the SLE group compared to SSc (figure I.4.4). However, anti-nucleosome antibodies demonstrated similar frequencies in the the two aforementioned groups (chi^2 test, p=0.547). Elderly patients in the entire cohort (patients with SSc, SLE and HC) did not demonstrate significantly lower autoantibody titers (student t-test, SSA: p=0.058, SSB: p=0.258, nucleosome: p=0.118, U1RNP: p=0.851). Anti-SSA antibodies approached statistical significance and were found to be higher in persons under 60 years of age (146.28 ± 249.96 U/ml versus 76.77 ± 182.54 U/ml).

Table I.4.4. Autoantibody titers in scleroderma patients over and under 60 years of age

Autoantibody titer (U/ml)		Range	Mean	SD	P (student t-test)
Anti-SSA	≥ 60 years	0.00-932.00	72.47	211.88	0.352
	< 60 years	0.00-905.00	128.23	234.09	
Anti-SSB	≥ 60 years	0.00-614.00	48.79	144.43	0.733
	< 60 years	0.00-938.00	63.79	197.33	
Anti-Nucleosome	≥ 60 years	0.00-349.00	133.32	104.19	0.770
	< 60 years	0.00-126.00	99.63	92.59	
Anti-U1RNP	≥ 60 years	0.00-114.00	19.74	30.49	0.228
	< 60 years	0.00-367.00	22.06	24.88	

Table I.4.5. Autoantibody titers in patients with scleroderma compared to SLE and HC

	Minimum	Maximum	Mean	SD	P (student t-test)
Age (years)					
SSc	33.00	80.00	51.51	11.54	-
SLE	31.00	77.00	51.30	11.50	0.917
HC	33.00	79.00	51.21	11.28	0.88
Autoantibodies (U/ml)					
Anti-SSA					
SSc	0.00	932.00	112.42	227.83	-
SLE	0.00	977.00	252.61	292.42	**0.002**
HC	0.00	62.00	14.69	13.42	**0.001**
Anti-SSB					
SSc	0.00	938.00	59.34	182.94	-
SLE	0.00	1061.00	74.64	183.06	0.634
HC	0.00	83.00	8.19	14.92	**0.025**
Anti-nucleosome					
SSc	0.00	367.00	109.18	96.44	-
SLE	0.00	1654.00	233.21	403.91	**0.017**
HC	0.00	92.00	13.39	21.83	**<0.001**
Anti-U1RNP					
SSc	0.00	126.00	21.4	36.37	-
SLE	0.00	867.00	154.19	209.95	**<0.001**
HC	0.00	106.00	12.7	18.89	**0.03**

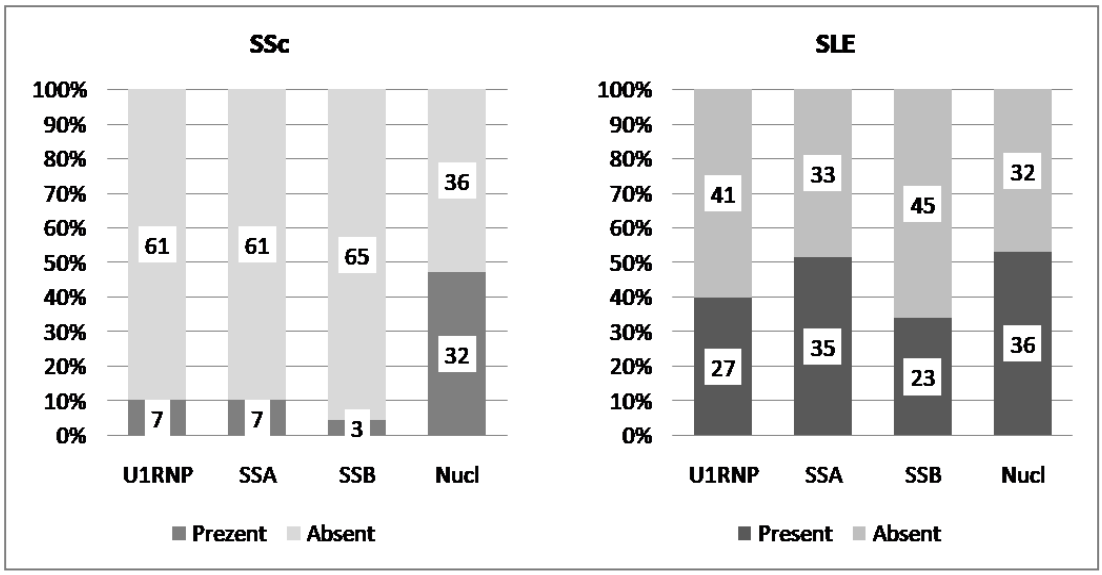

Figure I.4.4. The frequency of autoantibody positivity in patients with SSc versus SLE

Discussions

Our study aimed to analyze the relationship between non-specific antibodies and clinical presentation in SSc patients. Recently, the issue of finding new biomarkers in scleroderma has been gathering attention from the scientific community (256, 257). We recorded a higher prevalence of anti-U1RNP antibodies in our scleroderma group compared to other studies (258).

According to other literature data, PAH is a leading cause of morbidity and mortality in patients with CTD. SSc is the CTD with the highest prevalence of PAH, around 10%, and the worst prognosis, as a recent metaanalysis estimated the 3-year overall survival rate at 56% for patients with SSc-associated PAH. Compared with other CTDs such as SLE or mixed connective tissue diseases (MCTDs), there are less evident data on the prevalence of PAH, but it is very probably lower than in SSc. The prognosis of SLE/ MCTD-associated PAH is also better than in SSc-associated PAH, with a 3-year overall survival rate of 74– 88% in SLE-associated PAH and 63–64% in MCTD-associated PAH. There is no clear explanation for this difference in survival between SSc-, SLE-, and MCTD-associated PAH. Among prognostic factors in SSc-associated PAH, hemodynamics and exercise tolerance gained considerable attention. Concerning the potential of autoantibodies – ANA as prognostic factors in SSc-associated PAH, data were limited, in the few studies assessing their role, anticentromere or antitopoisomerase antibody positivity did not influence outcome. Anti–U1 RNP seems to be another important candidate prognostic factor in SSc- and CTD-related PAH (259). The link between anti-U1RNP antibodies in CTD-related PAH (including SSc) has been described in previously published research (260, 261).

Furthermore, the enhanced expression of anti-nucleosome antibodies in SSc patients exceeded our expectations. Although the mean anti-nucleosome values were found to be lower in scleroderma patients compared to SLE in our study population, around 46% of the SSc group

were positive for these antibodies, similar to the SLE cohort. Whereas their importance in lupus has long been demonstrated, studies describing their high specificity for SLE, as well as their notable nephritogenic potential, the significance of anti-nucleosome antibody positivity in SSc is yet to be fully elucidated (262).

SSc and systemic lupus erythematosus – SLE, another multisystem autoimmune disease, have various similarities, such as autoantibodies directed against nuclear antigens, and in some patients, overlapping clinical features. More precisely, at the gene level, there is emerging evidence that SSc and SLE share common genetic associations, such as *IRF5* and *PTPN22*. It is possible that SSc and SLE belong to the same spectrum of interferon mediated diseases. The classification of SSc based on the presence of the interferon can provide opportunities for better understanding of its pathogenesis and very important, for development of targeted therapeutic interventions (263).

A major problem that may occur in this disease is autoantibody formation, proved by several studies. These studies pointed out that autoantibodies found in patients with SSc carry considerable value in diagnosis and in predicting various clinical outcomes. The role of antinuclear antibodies (ANA) in the pathogenesis of SSc is unclear. The majority of patients with SSc have circulating ANA (90–95%), a small percentage of patients are ANA negative (5–10%).

A study published by Salazar et al. included 3249 patients, of whom 208 were ANA negative. These patients experienced less vasculopathic manifestations of SSc. A large set of medical parameters were carefully monitored in patients. E.g. the percent predicted diffusing capacity of carbon monoxide was higher in ANA negative patients and also, pulmonary arterial hypertension (PAH)/right heart catheterization was less common in the ANA negative group. Additionally, patients with negative ANA had a lower prevalence of telangiectasias and digital ulcers/pits. Even if diffuse cutaneous involvement was more common, the modified Rodnan Skin Score (mRSS) was lower in the ANA negative patients. More than that, at the gastrointestinal level, they experienced more malabsorption, than ANA positive patients. The results of this complex study suggest that SSc patients who are ANA negative represent a distinct subset of SSc with less vasculopathy and possibly, more frequent lower gastrointestinal involvement (264).

In conclusion, recent research provides new insights on the pathogenic processes of autoimmune rheumatic diseases, in an attempt to identify potential risk factors for organ involvement. Our study confirms the link between anti-nucleosome antibodies and cardiopulmonary involvement in the SSc population. Moreover, the impact of immunosenescence on the dynamics of autoantibody production in connective tissue diseases remains in need of further investigation.

I.4.3. Gout and Metabolic syndrome

A. Background

Metabolic syndrome (MetS) is a cluster of metabolic and cardio-vascular (CV) risk factors including obesity and visceral adiposity, insulin resistance (IR), dyslipidemia and hypertension. These factors contribute to CV mortality (265).

Patients with inflammatory diseases such as gout and those with autoimmune rheumatic diseases (ARD), such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), antiphospholipid syndrome (APS), ankylosing spondylitis (AS) and vasculitis among others, have increased prevalence of MetS.

Gout it is the most common inflammatory arthritis in adults and is estimated to affect 8.3 million Americans. This disease has been traditionally associated with other comorbidities like obesity, arterial hypertension, and abnormal lipid and glucose homeostasis, included in MetS. The prevalence of MetS in gout varies between 37% and 51%. Gout is considered one of the oldest diseases would be the first rheumatic disease associated with MetS (266).

B. Published papers in this field

1. Ganceanu Rusu A.R., Mititelu Tartau L., Statescu C., Boanca M., Poroch V., Lupusoru R.V., Dima N., Badescu C., **Rezus E.**, Rezus C., Lupusoru C.E., *Study of Dynamics of Immunobiochemical Parameters and Pharmacological Interferences in the Metabolic Syndrome*, Rev. Chim. (Bucharest), 69 (6), 2018, 1493-1497.

The purpose of the study is 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.

Evaluation of oxidative stress

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.

Determination of the level of glutathione peroxidase – GPX was performed spectrophotometrically. Determination of Malondialdehyde (MDA) values was done also by spectrophotometric measurement using the thiobarbituric acid method.

Statistical data analysis

The data was centralized and statistically processed using the SPSS version 22.0 for Windows 10 and the ANOVA method. In the statistical analysis, both descriptive and analytical metho vds were used at 95% significance (CI 95%). In calculating the significant difference between two media, the t-Student test or F (ANOVA) test was used to compare the average values in three or more groups with normal distributions. P-value values of less than 0.05 were considered to be statistically significant.

Results

Analysis of metabolic syndrome components was performed by evaluating serum titers of total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol. 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 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, LDLcholesterol, triglycerides. Except for Indomethacin, the remaining NSAIDs used in the experiment did not significantly affect the values outlined in figure I.4.5.

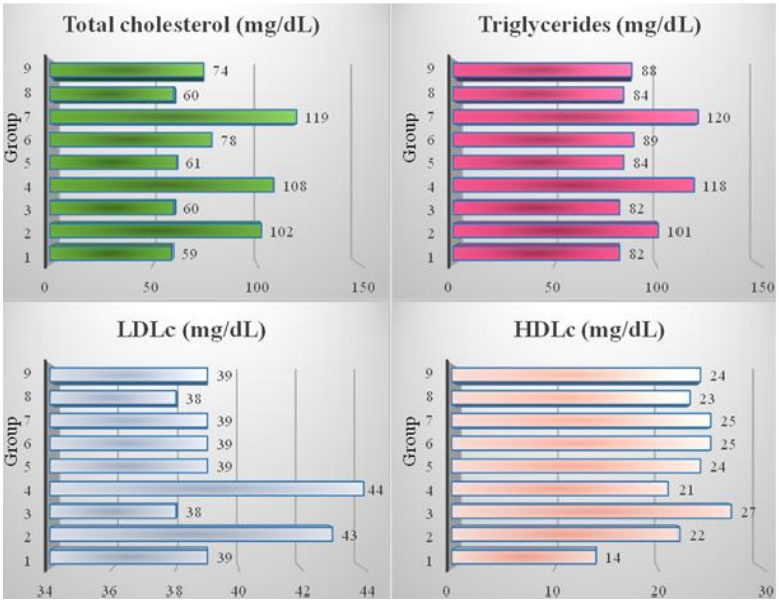

Figure I.4.5. Distribution of average values of lipid metabolism components in rats

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.4.6).

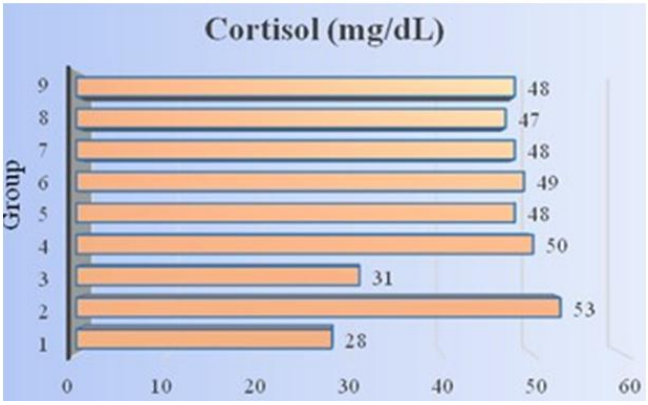

Figure I.4.6. Batch distribution based on serum cortisol value

Compared with cortisol results, the group receiving Enalapril noted the highest values of SOD, compared with data from the control groups. The use of the combination between Enalapril and the NSAIDs studied did not result in significant variations in the determined levels of SOD, as compared to the control cholesterol test group, in the rat exercise test (figure I.4.7).

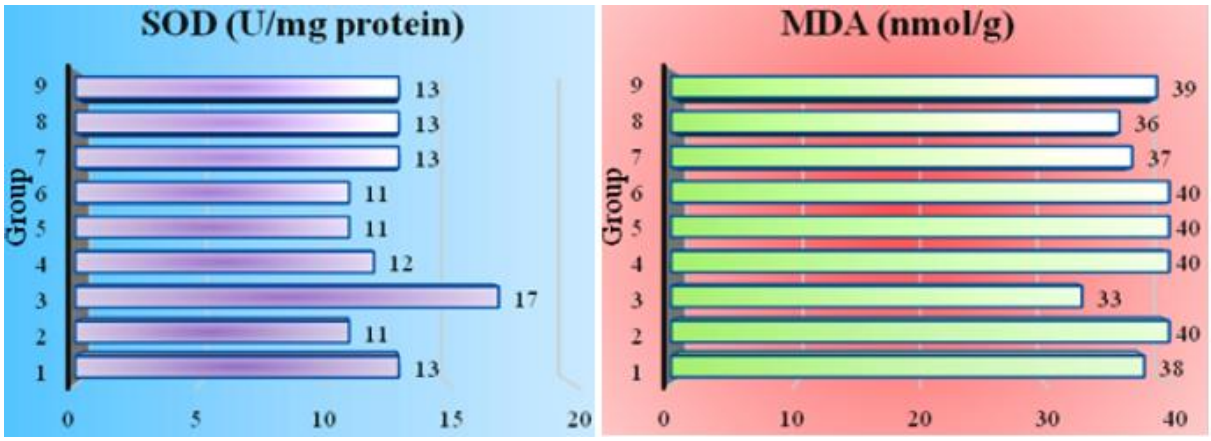

Figure I.4.7. Batch distribution based on superoxide dismutase and malondialdehyde value

The use of Enalapril in exercise-induced cholesterol diet rats was accompanied by a decrease in serum IL-1β but statistically insignificant from the control group with cholesterol diet. It can be appreciated that in the context of experimentally induced MetS rats, administering Enalapril in combination with Indomethacin significantly improves the process of chronic inflammation. The statistical analysis revealed the establishment of weak positive relationships between the control group and the NSAIDs lots; this means that an increase in IL-6 value for one of the two compared lots will associate an increase in the value for IL-6 for the other batch. The only lots with a high IL-6 value were the control + cholesterol diet and Enalapril (figure I.4.8).

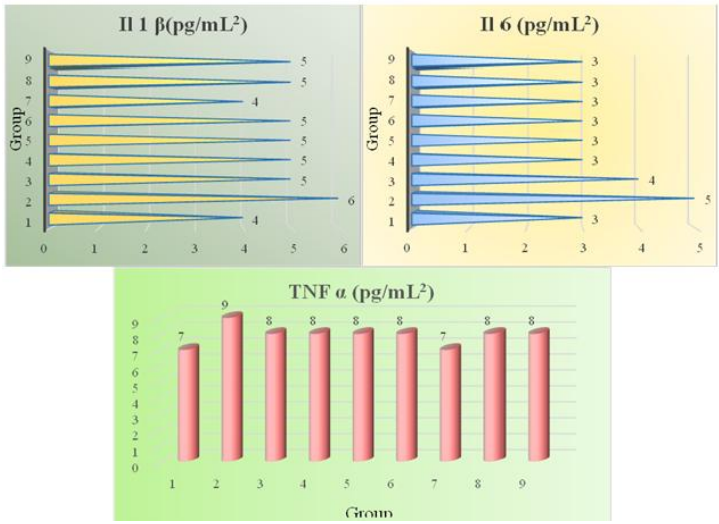

Figure I.4.8. Batch distribution based oninterleukin-1β, interleukin 6 and TNF-α

Cortisol and BP are two related variables; their correlation field is not uniform (it consists of three disjoint surfaces, at the cortisol values 30 and 50), which is why the link between them occurs after the stratification of the values according to the group they are part of. The variable effects of NSAIDs used in this study are influenced by the selective inhibitory action on either COX-1 or COX-2 (figure I.4.9).

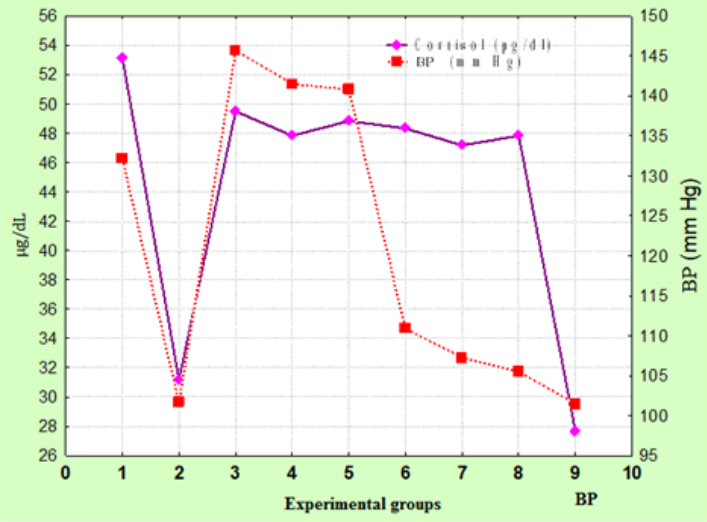

Figure I.4.9. Batch distribution according to BP and cortisol

Discussions

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.

In conclusion, comparing the results obtained in this study with the literature, it was noted that the administration of ACE and / or NSAIDs significantly improves the process of chronic inflammation.

2. **Rezus E.**, Leon Constantin M.M., Rezus C., *Correlations Between Hyperuricemia and Metabolic Syndrome*, Rev. Chim. (Bucharest), 66 (7), 2015, 1015-1018.

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. Individuals that have SU concentrations elevated above saturation levels but have not developed clinical manifestations of gout are considered to have asymptomatic hyperuricemia (270, 271, 272).

The aim of the study was to evaluate the association of asymptomatic hyperuricemia and gout with cardiometabolic risk factors.

Materials and methods

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 the patients who fulfilled at least three of the five criteria defining the metabolic syndrome. We used the criteria recommended by AHA/NHLBI (American Heart Association). Any three criteria of the five listed establish the MS diagnosis (table I.4.6). 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).

Table I.4.6. Diagnostic Criteria for Metabolic Syndrome (According To Aha/Nhlbi)

Clinical criteria	**Normal values of the parameters**
Obesity	$\geq 30 kg/m^2$
Serum triglycerides level (TG)	≥150 mg/dL or with treatment for high levels of TG
High-density lipoprotein cholesterol level (HDL)	<40 mg/dl in men; <50 mg/dL in women or with treatment for low levels of HDL↓
Blood pressure (BP)	≥130 mmHg BP systolic or ≥85 mmHg BP diastolic with treatment for hypertension
Serum glucose level (Glu)	≥100 mg/dL or with treatment for diabetes

Triglycerides are lipid fractions, formed by combining glycerol with three fatty acid molecules. Alcohols have a hydroxyl (HO-) group. 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. There are five subfractions of HDL, types 2a, 2b, 3a, 3b, and 3c. HDL inhibits the atherosclerotic process. Last clinical criteria used to establish the diagnostic of metabolic syndrome is glycaemia. 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.

Statistical analysis

Differences between groups and associations between CV risk variables and titers of serum uric acid were tested with ANOVA (for continuous CV risk factors), adjusted for differences by age and gender.

Results

From the total number of patients diagnosed with MS, there were 60 female (39.2%) patients and 93 male (60.8%) patients. The number of male patients was significantly higher than the number of female patients ($p < 0.01$). 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 parameter taken in the study was hypertension, 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/m^2, without a significant difference ($p = 0.227$). It was present in only 27 of the patients (13 with gout and 14 asymptomatic hyperuricemia).

Discussions

Uric acid (figure I.4.10) is a byproduct of normal purine catabolism is excreted mostly in urine but also through the gastrointestinal tract. Several studies have identified serum urate concentrations >7 mg/dL as an independent, major risk factor for hypertension. Also, a low serum urate concentration is associated with reduction in blood pressure. Unfortunately the mechanism that links hyperuricemia and these adverse clinical outcomes has not been elucidated (273).

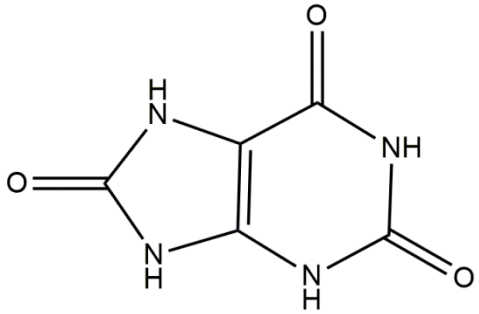

Figure I.4.10. Chemical structure of uric acid

MetS is a cluster of metabolic and cardiovascular (CV) risk factors including obesity and visceral adiposity, insulin resistance (IR), dyslipidemia and hypertension. Recently, the interface between the metabolic and immune systems has gained significant interest. These interactions are regulated through genetics, nutritional status, and the intestinal microbiome. Adipokines, pleiotropic molecules produced by white adipose tissue, are involved in the regulation of both

inflammatory processes and autoimmunity which have participation in rheumatic diseases. Patients with autoinflammatory disease such as gout and those with autoimmune rheumatic diseases: SLE, RA, antiphospholipid syndrome (APS), AS and vasculitis, have increased prevalence of MetS. Unfortunately, in the last decades, accelerated atherosclerosis and CV disease have been increased in patients with autoimmune rheumaric diseases. Traditional CV risk factors do not fully explain the augmented risk in this population, which suggest the existence of other factors, e.g. MetS and altered secretion patterns of proinflammatory adipokines could be the link between CVD and ARD (274).

Inflammatory syndrome has been seen in both the metabolic syndrome and rheumatic disease and has been studied in our group. Thus, elevated ESR was found in 97 patients (47 with gout and hyperuricemia 50 with asymptomatic hyperuricemia), and the CRP increased in 40 patients (15 with gout and 25 asymptomatic hyperuricemia). The conclusion is that inflammatory syndrome was more important in patients with gout.

The presence of three or more parameters establishes the diagnosis. It was observed in our study that most patients had three criteria (54%), then four criteria (37%) and 9% five criteria. Increased amount of uric acid was directly proportional to the number of criteria, confirming existing data in the literature. All patients with five criteria showed hyperuricemia, 70.4% of those with four criteria and 51.2% of those with three criteria.

A study done by Fraile et al. include 41 subjects (37 males and 4 women) with a mean age of 58.5 ± 12 years. Twenty one gout patients showed three or even more criteria to establish the diagnosis of MS. The number of patients with none, one or two criteria were 1, 8, and 11, respectively, while the number of patients with three, four, and five criteria were 12, 8, and 1. The MS pathological conditions associated with gout were obesity (21/41), high blood pressure (30/41), dyslipidemia (22/41), and fasting plasma glucose ≥100 mg/dL (30/41). The results of this study have confirmed that the prevalence of MS is significantly high among patients with gout. Hyperuricemia has been related with a decrease of renal uric acid excretion due to enhanced sodium reabsorption involving also two of the most common diseases associated with the metabolic syndrome: obesity and hypertension. Physicians should seriously consider the presence of MS in gout patients, in order to establish a right treatment aimed to reduce the increased overall cardiovascular risk of these patients. The study revealed a serum urate concentration above 7.0 mg/dL in all patients, except in two. Mean serum urate concentration did not differ significantly in gout patients with MS (8.5 mg/dl) and without MS (8.1 mg/dl) (275).

The relationship between elevated serum uric acid level and MS has been debated also by Bonakdaran et al. in a study published five years ago. The aim of this study was to determine the prevalence of hyperuricemia and its association with MS in type 2 diabetes mellitus (DM). Hyperuricemia is defined as uric acid ≥ 7 and ≥ 5.5 mg/dl for men and women respectively. The prevalence of hyperuricemia was 12.7% and of MS was 65.5% respectively. The prevalence of MS significantly increased in the highest quartile of uric acid levels compared with lowest quartile (74.4% vs 55.9%). Positive serum uric acid has been associated with cholesterol, triglyceride, non-HDL cholesterol and a negative association with fasting blood sugar,

glycosylated hemoglobin and HDL cholesterol. Possible independent biochemical predictors of hyperuricamia were cholesterol, triglyceride, creatnine and FBS (276).

The aim of a study performed by Zapolski et al. was to examine the association between renal function, serum uric acid and markers of both pro-inflammatory and prothrombotic state in patients with DM, metabolic syndrome and coronary artery disease. The study included 58 men and 33 women, aged 57.6 ± 10.3 years with metabolic syndrome and type 2 DM, selected from a large group of patients scheduled for routine coronary angiography. Common risk factors for atherosclerosis: smoking, hypertension, DM, family history and hyperlipidaemia were evaluated for all the patients. Laboratory tests included complete blood counts, serum urea and creatinine, aminotransferases, C-reactive protein (CRP), fibrinogen, uric acid, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, fasting glucose, glycated haemoglobin (HbA1c), glomerular filtration rate (GFR) and urinary protein. The authors also measured body mass, height, waist circumference, hip circumference and calculated BMI and waist-to-hip ratio (WHR). The results of the study showed that in the case of patients with ischaemic heart disease, DM and metabolic syndrome, obesity, particularly visceral obesity, is associated with renal dysfunction and elevated markers of pro-inflammatory state. Renal dysfunction co-exists with elevated serum uric acid, while elevated serum uric acid is associated with markers of pro-inflammatory state. In their turn, markers of pro-inflammatory state were correlated with prothrombotic markers such as serum fibrinogen and platelet count. As a final remark, uric acid should be taken into consideration as a link between renal dysfunction and both pro-inflammatory and prothrombotic state in patients with MS and coronary artery disease (277).

In the present study, we confirmed the previously reported sex-specific differences and the strong associations between serum UA concentration and age, obesity, hypertension, serum triglygerides levels, serum cholesterol levels, links between increased serum UA of metabolic syndrome.

In conclusion, we demonstrated the following findings: the serum UA concentration was positively correlated with the number of MS criteria (obesity, hypertension, serum triglycerides levels, serum cholesterol levels) that were met, the association between UA and MS components, the relationship between serum uric acid and markers of systemic inflammation (ESR, CRP)

I.5. Researches regarding the relationship between plantar pressures of the elderly

I.5.1. Introduction

Foot involvement is a really important problem for people with rheumatoid arthritis. The duration and severity of the disease influence the progression of the foot-related symptoms. Pain, increased plantar pressure, and decreased functional capacity are the most frequent problems in the foot. These problems have a negative impact on quality of life and increase the risk of falling. The forefoot is commonly affected, especially the metatarsophalangeal joints. Common structural problems include hallux valgus deformity, deformities of the lesser toes, subluxation of the metatarsophalangeal joints, and displacement of the plantar fat-pad. One of the most prevalent foot deformities in rheumatoid arthritis patients is rear foot valgus, which is associated with the presence of other deformities (278).

Some studies have shown that pressures under the forefoot are increased in older people with forefoot pain. Waldecker et al, reported higher peak pressure and pressure time integrals under the lateral forefoot in those with forefoot pain, in a study including 100 people with hallux valgus (279). Also Menz et al (280) reported that older people with forefoot pain exhibited significantly greater peak pressure under the 3rd to 5th metatarsal heads compared to those without forefoot symptoms (281).

1.5.2. Published papers in this filed

Given that most of my patients with arthritis are women which faces the issues mentioned above, to improve their condition I have studied together with a group of researchers from Gheorghe Asachi Technical University of Iasi the relationship between plantar pressures of the elderly women. Two of the studies including the results of the studies are presented below.

1. Costea M., Sarghie B., Mihai A., **Rezus E**., *Classification of the Ederly Foot Types Based on Plantar Footprints*. Procedia Engineering 181 (2017) 36-43.

Foot type is a general term used to describe a number of architectural features of the foot in order to obtain clues of its dynamic functioning (282). Foot dynamics is perceived as being linked to a variety of musculoskeletal symptoms, including personal injury from running and plantar pain, especially on the heel (283). The biomechanical studies have progressed by comparing the presence, absence or size of the foot typological parameters between groups with or without pathologies under investigation (284). Despite the widespread use of these parameters, it was recognized that the objective and quantitative analysis of foot typologies remains elusive (285). The absence of an absolute dimension of the foot type has resulted in considerable variation in choosing the measurements to determine the foot typology (286, 287). This is in opposition to the suggestion that in order to identify the relations between foot typology and

pathology is necessary to use unique classification system to allow accurate recognition (288). The article presents the methodology and the results of the studies conducted in order to develop a rational classification technique of the elderly foot typology, based on parameters derived from the plantar footprint.

Materials and methods

The plantar footprints (figure I.5.1) were taken from 67 women, aged between 52-84 years old with RScan pressure plate (289) and the associated system, Foot scan 7 Gait, 2nd Generation, 0.5 Gait Scientific System. To classify the foot typology, Chippaux-Simark Index and Hallux-Valgus Angle have been used.

Figure I.5.1. Taking plantar footprints with RSscan

Both statistical and dynamic measurements were achieved. The recorded images were automatically linked in all phases of walking using a software, and were exported to scale 1:1 and 5 dimensions were measured in order to determine the main parameters of the footprint (290, 291).

Results

Each plantar footprint has been manually processed by constructing the reference lines (292) as can be seen in figure I.5.2.

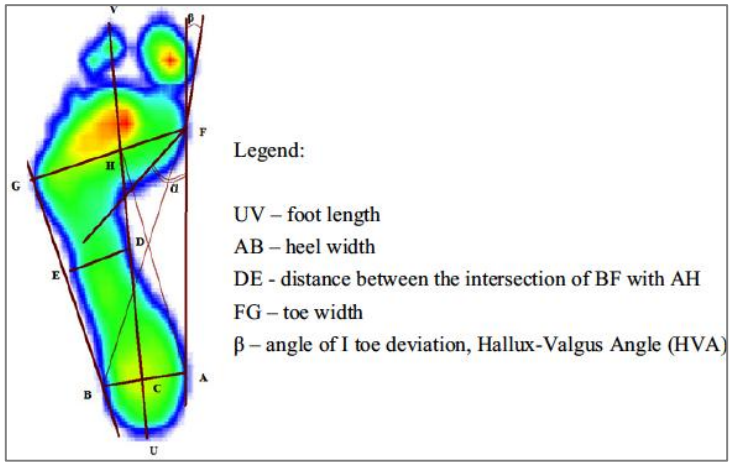

Figure I.5.2. Plantar footprints – reference line

Foot types based on Chippaux-Simark Index (CSI)

Following the analysis CSI, the results show that 22% of the subjects have high arched foot, 11% have a tendency of flattening, 11% have flat foot and the rest of 56% have normal and intermediary foot, table I.5.1.

Table I.5.1. Chippaux-Simark Index (CSI)

		The entire group		Group 1, 52-59 years old		Group 2, 60-64 years old		Group 3, 65-84 years old	
Foot type	Index values	Left	Right	Left	Right	Left	Right	Left	Right
High arched	[0,20)	15	19	10	11	3	5	2	3
Normal	[20,30)	13	15	5	8	6	4	2	3
Intermediary	[30,40)	25	17	9	6	8	7	8	4
Low arch	[40,45)	7	11	3	3	0	2	4	6
Flat	>45	7	5	2	1	2	1	3	3

Foot types based on Hallux-Valgus Angle (HVA)

HVA is used to detect the presence on the foot of Hallux-Valgus (interior deviation of first toe) and Hallux-Varus (exterior deviation of first toe). Following the results of β angle, the results show that 28% of women have Hallux-Valgus, 17% have the imminent risk to develop it, 30% have normal foot and 25% have Hallux-Varus condition, the results being shown in table I.5.2.

Table I.5.2. Hallux-Valgus Angle (HVA)

		The entire group		Group 1, 52-59 years old		Group 2, 60-64 years old		Group 3, 65-84 years old	
Foot type	Index values	Left	Right	Left	Right	Left	Right	Left	Right
Hallux-Valgus	>15	19	12	5	4	5	2	9	6
Outward movement	[10, 15)	11	9	7	6	3	3	1	0
Normal	[0, 10)	20	30	11	14	6	7	3	9
Hallux-Varus	<0	17	16	6	5	5	7	6	4

Table I.5.3. Independent Samples Test of Chippaux-Simark, comparing age groups

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	p	t	df	p (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
CSI-left-comparing group 1to group 2	Equal variances assumed	2.661	0.110	-0.959	46	0.343	-4.16171	4.34162	-12.90093	4.57752
	Equal variances not assumed			-0.999	43.523	0.323	-4.16171	4.16649	-12.56133	4.23791
CSI-right-comparing group 1to group 2	Equal variances assumed	0.124	0.727	-1.059	46	0.295	-4.60091	4.34306	-13.34304	4.14122
	Equal variances not assumed			-1.066	39.461	0.293	-4.60091	4.31588	-13.32735	4.12553
CSI-left-comparing group 1 to group 3	Equal variances assumed	3.406	0.071	-2.340	46	**0.024**	-9.91434	4.23684	-18.44266	-1.38602
	Equal variances not assumed			-2.481	44.975	0.017	-9.91434	3.99649	-17.96380	-1.86487
CSI-right-comparing group 1 to group 3	Equal variances assumed	1.009	0.320	-2.812	46	**0.007**	-11.59038	4.12161	-19.88674	-3.29402
	Equal variances not assumed			-2.922	43.243	0.006	-11.59038	3.96643	-19.58815	-3.59261
CSI-left-comparing group 2 to group 3	Equal variances assumed	0.008	0.928	-1.427	36	0.162	-5.75263	4.02997	-13.92578	2.42052
	Equal variances not assumed			-1.427	35.739	0.162	-5.75263	4.02997	-13.92785	2.42259
CSI-right-comparing group 2 to group 3	Equal variances assumed	0.322	0.574	-1.600	36	0.118	-6.98947	4.36857	-15.84934	1.87039
	Equal variances not assumed			-1.600	35.191	0.119	-6.98947	4.36857	-15.85642	1.87747

In table I.5.4. are presented Levene's and T tests for HVI left and right foot, following the same comparisons as those for CSI.

Table I.5.4. Independent Samples Test of Hallux Valgus Angle, comparing age groups

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	p	t	df	p (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
HVA-left-comparing group 1to group 2	Equal variances assumed	0.104	0.748	0.865	46	0.391	2.79492	3.23088	-3.70850	9.29834
	Equal variances not assumed			0.872	39.736	0.388	2.79492	3.20376	-3.68147	9.27131
HVA -right-comparing group 1to group 2	Equal variances assumed	0.034	0.855	1.260	46	0.214	3.80399	3.02019	-2.27534	9.88332
	Equal variances not assumed			1.312	43.486	0.196	3.80399	2.89945	-2.04141	9.64940
HVA -left-comparing group 1 to group 3	Equal variances assumed	7.020	**0.011**	-0.040	46	0.969	-0.15245	3.85782	-7.91784	7.61294
	Equal variances not assumed			**-0.037**	**29.790**	**0.971**	**-0.15245**	**4.13835**	**-8.60658**	**8.30168**
HVA -right-comparing group 1to group 3	Equal variances assumed	3.061	0.087	0.246	46	0.807	0.90926	3.69870	-6.53585	8.35436
	Equal variances not assumed			0.231	30.895	0.819	0.90926	3.93009	-7.10732	8.92583
HVA -left-comparing group 2 to group 3	Equal variances assumed	5.978	**0.020**	-0.678	36	0.502	-2.94737	4.34422	-11.75786	5.86312
	Equal variances not assumed			**-0.678**	**31.797**	**0.502**	**-2.94737**	**4.34422**	**-11.79848**	**5.90374**
HVA -right-comparing group 2 to group 3	Equal variances assumed	3.978	0.054	-0.733	36	0.468	-2.89474	3.94854	-10.90274	5.11327
	Equal variances not assumed			-0.733	29.905	0.469	-2.89474	3.94854	-10.95980	5.17033

Discussions

According to the obtained results, the subjects were divided into five categories, subjects with Normal Foot, High Arched Foot, Flat Foot, Hallux-Valgus Foot and Hallux Varus Foot. The authors have developed a method of determining the middle area of a plantar footprint for a more accurate measurement, being able to register it in the same way, for all subjects. Using statistical analysis, differences between age groups have been found, demonstrating the necessity of modeling and designing customized shoe lasts, footwear and prophylactic components according to the age of the subject. A good fit of the shoe on the elderly foot involves modifying the shoe last, but this can only be achieved under certain standards. Having biomechanical data of the foot and a clear classification of it, the customization of the product is simplified, reducing the number of tests and increasing the footwear technological process efficiency.

Differences in foot structure are presumed to be associated with differences in foot function during static posture or dynamic movement. A considerable number of foot pathologies are biomechanical in origin and often associated with foot type. Foot type is a clinical concept that have the purpose to simplify the anatomical complexities of the human foot: 28 bones, 33 joints, 112 ligaments, controlled by 13 extrinsic and 21 intrinsic muscles. Foot type categorizes feet as planus – defined as low arched with a valgus hindfoot and/or varus forefoot, rectus – well aligned hindfoot and forefoot and cavus – defined as a high arched with a varus hindfoot and/or valgus forefoot. Planus feet, associated with hallux valgus, hallux limitus and rigidus, and posterior tibial tendon dysfunction are considered a risk factor in the development of overuse injuries. On the other hand, cavus feet are associated with hammertoes and claw toe deformities, while rectus feet have not been directly associated with pathology or injury in the literature. Certain foot pathologies are associated with specific foot types or why some individuals with non-rectus foot types are asymptomatic (293).

According to the pattern of joint involvement in RA, various types of foot deformities can result. Within the foot, the most frequently involved joints are the subtalar, mid-tarsal joints and the ankle joint. The subtalar and talonavicular joints are commonly affected in RA. Valgus deformity increases with progressive flattening of the longitudinal arch, as cartilage loss and bone erosion develop. Forefoot deformity starts with synovitis of the metatarsophalangeal-joints, followed by the involvement of the flexor tendons. Weight bearing in the active stage of RA, is sometimes followed by hallux valgus, hallux rigidus, mallet toe, claw toe and splay toe deformities. Unfortunately, although patients with RA complain of foot pain and disability due to foot problems, physicians generally overlook or neglect the feet in routine clinical examination (294).

A study published by Williams et al. demonstrates that poor foot health and foot pain is highly prevalent in patients with rheumatic diseases. The impact of these problems has consequences in various levels of functional limitation and disability in patients with both acute and chronic disease. Patients with autoimmune disorders, or those taking medication that compromises the immune system should be considered at risk of infection and foot ulceration, and as a consequence, they should receive priority for foot care specialist. Also patients with micro-vascular or large vessel disease, foot deformity and poor footwear are also at risk of foot

trauma, ulceration and subsequent infection. Another category of patients who should be monitored by foot care specialist are those diagnosed with rheumatoid arthritis. In their case foot orthoses should be considered, because this intervention has been demonstrated to reduce pain and the effects of abnormal joint function in the foot. Rheumatology teams and podiatry services should collaborate and aim to improve the foot health service to patients with these disabling foot problems. Measurement of plantar foot pressures may have been a useful addition in the assessment of patients with different diseases (295).

2. Sarghie B., Mihai A., Costea M., **Rezus E.**, *Comparative Anthropometric Study Regarding the Foot of the Ederly Female Population*, Procedia Engineering 181 (2017) 182-186.

To design and manufacture proper footwear that satisfies the need of the elderly female population the anthropometrical characteristics, and particularities of the foot are necessary to be evaluated, which represents the main topic of the research presented in this paper.

Materials and methods

The 3D shape acquisition of the feet was made using the INFOOT-USB scanning system (figure I.5.3.A), which consist of a 3D scanner and a dedicated software program, Measure 2.8, optimized for recognizing foot shapes.

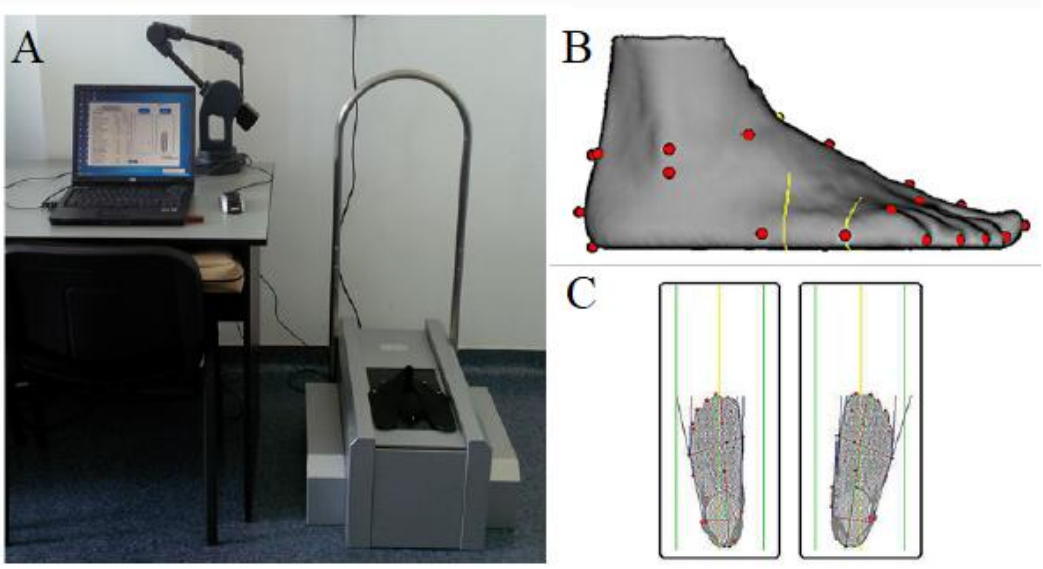

Figure I.5.3. A. Infoot USB 3D Scanning system; B. Position of the anatomical points; C. Anthropometric data sheet.

After the scanning process, up to 20 anatomical points are manually placed on the 3D shape of the foot to measure the scanned feet anthropometric dimensions (figure I.5.3.B). Based on the position of the anatomical points, the software will automatically generate an anthropometric data sheet (figure I.5.3.C).

The collected data are used for establishing the morphological and structural characteristics of subject's feet (296, 297). The anthropometric measurements were statistically analyzed using

the SPSS software program, which is dedicated to the statistical processing of data, providing fast results (298).

Fit is one of the most important functional aspects in footwear comfort and one of the most important considerations for users when purchasing new footwear, especially in the case of patients with medical problems. Footwear customization systems have been developed by diffferent specialized companies. The problem is the fact that footwear customization is a costly option, with aesthetic limitations, which means that it is available only to a limited number of users (299).

Results

In this study have participated 92 women, aged between 55 and 80 years old, which do not have any impairment movements. After preliminary analysis, 9 subjects were excluded. The identified differences between the anthropometric parameters of the left and right foot reflect various anatomical anomalies which will be further investigated. Three groups have been made, according to the subjects' age. The main anthropometric parameters that that were analyzed in this study are the following: Foot length, Ball Girth, Foot breadth and Instep Circumference. The statistical indicators determined were: Average, Median, Standard Deviation (SD) and Coefficient of Variation (CV). The indicators that characterize each anthropometric parameter are presented in table I.5.5.

Based on the determined indicators, a comparative analysis between groups was conducted, as well a comparative analysis between the entire group of elderly female subjects and the general female population from Romania. For all four analyzed anthropometric parameters, the highest values for all groups can be observed in the case of the second group.

Table I.5.5. Indicators that characterize each anthropometric parameter

	Foot Length				Ball Girth				Foot Breadth				Instep Circumference			
Statistical	Age group (years)				Age group (years)				Age group (years)				Age group (years)			
indicator	55-59	60-64	65+	55- 65+	55-59	60-64	65+	55- 65+	55-59	60-64	65+	55- 65+	55-59	60-64	65+	55- 65+
Average	244.05	249.56	240.61	244.28	242.66	253.67	244.68	246.13	99.88	104.99	101.95	101.87	237.88	248.25	241.53	241.73
Median	243.05	252.15	242.78	244.30	242.55	256.10	244.18	245.75	100.25	105.20	102.90	102.85	238.35	248.21	241.87	241.80
SD	8.92	9.81	12.57	10.92	14.41	10.98	11.36	13.26	5.93	5.75	5.64	6.07	12.76	11.58	12.09	12.78
CV	3.66	3.93	5.22	4.47	5.94	4.33	4.64	5.39	5.94	5.48	5.53	5.96	5.36	4.67	5.00	5.29

The lowest values for Ball Girth, Foot Breadth and Instep Circumference can be observed in the case of the first group, age between 55 and 59 years old. Average values for the four parameters are presented in figure I.5.4.

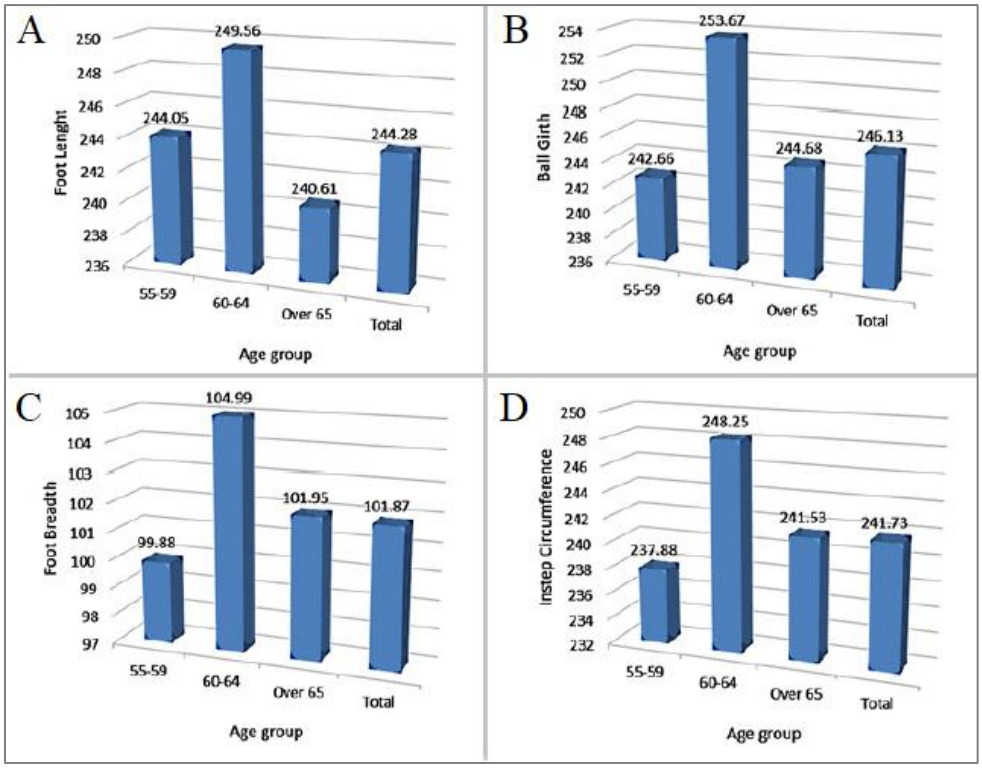

Figure I.5.4. Average values for A. Foot Length; B. Ball Girth; C. Foot Breadth; D. Instep Circumference.

Discussions

Ageing process are associated with changes to the aspect, biomechanics, structure and function of the foot. This process can be related with a marked presence of foot conditions, pain, disability and other overall health problems that constitute a major health problem worldwide. Foot problems, defined as a person's inability to maintain basic foot hygiene or dificulty on putting adequate footwear, are risk factors for the development of many considerable complications of multiple systemic diseases such as depression, diabetes mellitus, high blood pressure, inflammatory arthritis, obesity, osteoporosis, stress and vascular alterations. Foot problems increase with age and are a frequent cause of medical and foot care.

Literature data suggest that such problems currently affect between 71 and 87% of older patients and are a frequent cause of medical and foot care. The most common foot problems are lesser toe deformities, hallux valgus, tailor's bunions, abnormal medial arch structure, prominent metatarsal heads, pain, swelling, infections and some circulatory disease. All these have a significant impact on older people and are correlated with poor quality of life, balance impairment, increase the risk of falls, fractures, restrict mobility and performance of activities of daily living that turn can produce serious physical, mental and social consequences in the older persons (300, 301).

Foot problems and their impact on elderly people have led to the need of a treatment optimization in order to enhance mobility and improve the quality of life in this age group. Other aspects correlated with the aged foot are its general tendency to exhibit increased soft-tissue

stiffness, a decreased range of motion, decreased strength and a more pronated posture, functioning in a more pronated position with a reduced range of motion and less efficient propulsion when walking.

Plantar pressure measurement offers important insights into the underlying mechanisms that may be responsible for the development of foot disorders in older people. Also this measurement has considerable potential to assist in optimising the design of mechanical interventions such as footwear and orthoses to alleviate foot pain in this age group (302).

In conclusion, the feet of the elderly females from Romania differ from those of the general female population, which justifies the need to develop and manufacture footwear that specially addresses to the needs of the elderly females.

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

1. Perspectives in the professional activity

Our department treats around 390 patients per month, demonstrating high efficacy and efficiency with respect to the assessment and treatment of both inflammatory and non-inflammatory rheumatology patients. As the Head of the 1st Rheumatology Clinic, I have seeked to establish a team of highly qualified professionals in order to optimize patient care. By setting high standards, careful monitoring, and establishing clear and attainable goals, I believe that I have provided means for the medical staff to improve their knowledge and skills. Nonetheless, I admit that competition in the clinical setting is vital for the improvement of health care quality.

Given the complexity of the management of rheumatic conditions, as well as that of the relationship between patients and the medical staff, I believe that the issue of improving the quality of care in hospital settings requires a multifaceted response. As yet, I have identified several achievable goals in terms of professional activity:

- First and foremost, I wish to continue to update my knowledge on the management of rheumatic conditions through a constant and thorough research of the currently available literature (recent protocols, research articles, case reports of orphan diseases or infrequent clinical manifestations);
- Also, I intend to promote the betterment of the medical staff's (doctors and/or nurses) knowledge and skills by allowing and facilitating their participation in ongoing projects or workshops, while also encouraging professionals to register for courses on specific aspects of patient assessment and/or care (ultrasonography, capillaroscopy, LASER speckle imaging, intraarticular injections, scleroderma digital ulcer care, scoring tools, immunosuppressant drugs, biologicals);
- Knowing that effective doctor-patient communication is crucial for the development of personalized care, I intend to improve my team's relationship with patients by allowing and encouraging feedback from both sides;
- In order to increase our patients' knowledge of disease pathogenesis, risk factors, evolution/prognosis, treatment options, and possible side effects, I intend to organize doctor-patient discussion groups on specific subjects/diseases, as well as supply them with informative material (in both printed form as well as on-line);
- I wish to encourage Romanian patients to join national or international organizations and participate in the patients' section of scientific congresses by providing details on the subject (contact details and upcoming congresses);
- Moreover, I wish to establish and cultivate a relationship with patient organizations, as well as to form a doctor-patient partnership in order to set common goals (requesting new treatment options or funding for disease-specific rehabilitation plans and orthoses, aids or devices required by disabled patients in their daily activities, patient congresses);
- From a biopsychosocial perspective, it has been shown that psychological characteristics together with the degree of social support share a notable connection with the perceived

pain levels (a symptom which is considered to be virtually ubiquitous in rheumatic diseases), coping skills, and the self-reported quality of life of rheumatology patients; Therefore, I wish to encourage the psychological assessment (and therapy, where needed) of our patients, as well as to help create support groups;

- Admitting that early diagnosis and correct therapy are of paramount importance in inflammatory rheumatic conditions, I intend to organize workshops with general practitioners, focusing on new diagnosis criteria, available treatment options and side-effects, possible complications monitoring and "red flags";
- The management of immune-inflammatory rheumatic diseases requires the oint effort of a team of specialists (rheumatologist, ophthalmologist, nephrologist, gastroenterologist, radiologist, cardiologist, neurologist, and many others); I would like to continue to cultivate the relationship between our center and other medical specialties.

2. Perspectives in the academic activity

Heretofore, I have held rheumatology lectures for medical students in 3 languages (Romanian, English and French), as well as lectures on medical rehabilitation. My interest in providing quality information for medical students and rheumatology trainees is supported by a strong belief that remarkable teaching can lead to a more profound understanding of rheumatic conditions and their management. I have ensured that both students and trainees have access to relevant material regarding the pathomechanisms, assessment, and treatment of rheumatic diseases. In 2014, the release of the book "Reumatologie" marked the beginning of my endeavor to supply the local student bodies with updated textbooks. Two other books followed, written in English ("Essential Rheumatology for Trainees and Medical Students", released in 2016) and French ("Rhumatologie", released in 2017). Based on my personal experience as well as on foreign students' feedback, the availability of teaching material in the sholars' chosen study language remains crucial for their performance.

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 rheumatic disease management (evaluation, diagnosis, treatment, protocols);
- Releasing new and updated editions of the rheumatology textbooks in the 3 study languages mentioned above;
- Acquiring anatomical teaching models (flexible spine models, knee injection models, muscular limb models with removable elements, flexible hand model, shoulder joint,

human skeleton, ultrasound training models), charts (human skeleton charts, muscle charts, bone structure charts, joint injection charts) and anatomical software;

- The introduction of rheumatology and immunology flash cards (questions and answers) and infographics on specific topics (clinical evaluation, overview of certain rheumatic conditions, conventional and biological DMARDs, 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. "eye involvement in rheumatology") and clinical case report presentations with students and trainee doctors;
- Encouraging students and trainee doctors to present their work in congresses;
- Assisting 6th year medical students in writing their final thesis;
- Inspiring and supporting rheumatology trainees to participate in the annual sessions of the Rheumatology Summer School.

3. Perspectives in the scientific activity

So far, my scientific work focused both on inflammatory, as well as non-inflammatory rheumatic conditions. I have created a small but effective research team in my center, while also cultivating relationships and participating in projects with other medical specialties (gastroenterology, diabetes and nutrition, immunology, cardiology, internal medicine). Our center was also involved in several clinical studies, in an effort to bring the newest available treatment options to the local patients 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 rheumatic conditions (capillaroscopes, ultrasonographs, LASER 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 Clinical Rehabilitation Hospital, as well as by accessing further funding (grants, projects). Furthermore, the future perspectives of my scientific work include the following objectives and activities:

- Conducting further research in the field of autoimmunity (clinical and immunological aspects in systemic lupus erythematosus, systemic sclerosis, inflammatory myopathies, mixed connective tissue disease);
- Additional research on osteoporosis in rheumatoid arthritis patients;
- Further studying the relationship between spondyloarthropathies and bowel inflammation and microbiota;

- Further analyzing the significance and clinical correlates of nutritional decline in patients with inflammatory rheumatic conditions;
- Conducting studies on the clinical correlates of inflammaging and immunosenescence;
- Carrying out research on pain perception and optimal management, pain medication consumption among rheumatology patients, fibromyalgia;
- Additional research on the use of biologicals in rheumatology (including the clinical relevance of anti-drug antibodies);
- Collection and analysis of scientific data related to health-related quality of life of scleroderma patients treated with bosentan;
- Testing the biopsychosocial approach to the assessment of patients with either inflammatory or non-inflammatory rheumatic conditions;
- Contributing to and accessing the EUSTAR database;
- 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 further clinical studies in our center and supporting the development of the ongoing trials;
- 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 approach to our research in order to secure scientific integrity.

SECTION III
REFERENCES

1. Raychaudhuri S, Sandor C, Stahl EA, et al. Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthriti. *Nat Genet*, 2012; 44 (3): 291-296.
2. Ionescu R. Esențialul în Reumatologie. *Ed. Medicală Amaltea*. Bucuresti; 2006.
3. Calle E, Gomez-Puerta JA. The Spectrum of Rheumatic Disease, In: *Surgery in Rheumatic and Musculoskeletal Diseas*. Ed: Atzeni F, Masala IF, Aletaha D, Lee M, Baraliakos X, 2018; 15: 1-13.
4. Goldblatt F, O'Neill SG. Clinical aspects of autoimmune rheumatic diseases. *Lancet* 2013; 382 (9894): 797-808.
5. Abrão AL, Santana CM, Bezerra AC, Amorim RF, Silva MB, Mota LM, Falcão DP. What rheumatologists should know about orofacial manifestations of autoimmune rheumatic diseases, *Rev Bras Reumatol* 2016; 56 (5): 441–450.
6. Schirmer M, Dejaco C, Duftner C. Advances in the Evaluation and Classification of Chronic Inflammatory Rheumatic Diseases. *Discov Med* 2012; 13 (71): 299-304.
7. Gallo J, Raska M, Kriegova E, Goodman SB. Inflammation and its resolution and the musculoskeletal system. *J Orthop Translat* 2017; 10: 52-67.
8. Full LE, Ruisanchez C, Monaco C. The inextricable link between atherosclerosis and prototypical inflammatory diseases rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Res Ther* 2009; 11: 217.
9. McPhee SJ, Hammer GD. Pathophysiology of Disease: an Introduction to Clinical Medicine. 6th *Ed. New York: McGraw-Hill Medical*; 2009.
10. Parnes O. Inflammation. *Lancet* 2008, 372 (9639): 621.
11. Ley K. History of Inflammation Research. In: Physiology of Inflammation, Ed: Ley K, *Springer*, New York, 2001; 1-10.
12. Medzhitov R. Inflammation: New Adventures of an Old Flame. *Cell* 2010; 37 (6): 771-776.
13. Furie MB. An Overview of Inflammation. In: Reference Module in Biomedical Sciences. Pathobiology of Human Disease, Ed: Caplan MJ, Amsterdam: *Elsevier* 2014; 226-230.
14. Greene MA, Loeser RF. Aging-related inflammation in osteoarthritis. *Osteoarthritis Cartilage*. 2015; 23(11): 1966-71.
15. Sophia Fox AJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. *Sports Health*. 2009; 1(6): 461-8.
16. Bucci L, Ostanj R, Capri S, Salvioli S, Cevenini E, Celani L, Monti D, Franceschi, C. *Inflamm-Aging: Handbook on Immunosenesce—basic Understanding and Clinical Applications 1*; Fulop T, Franceschi C, Hirokawa K, Pawelec G, Eds.; Springer Science Business Media, B.V.: Dordrecht, The Netherlands, 2009; 893–918.
17. Rezus E, Floria M, Grigoriu A, Tamba B, Rezus C. Cardiovascular Risk Factors in Chronic Inflammatory Rheumatic Diseases: Modern Assessment and Diagnosis. *Curr. Vasc. Pharmacol.* 2015; 13: 716–724.

18. Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G. Inflamm-aging: An evolutionary perspective on immunosenescence. *Ann. N. Y. Acad. Sci.* 2000; 908: 244–254.
19. Brandl A, Hartmann A, Bechmann V, Graf B, Nerlich M, Angele P. Oxidative stress induces senescence in chondrocytes. *J. Orthop. Res*. 2011; 29: 111–1120.
20. Findlay DM. Vascular pathology and osteoarthritis. *Rheumatology* 2007; 46: 1763–1768.
21. Altindag O, Erel O, Aksoy N, Selek S, Celik H, Karaoglanoglu M. Increased oxidative stress and its relation with collagen metabolism in knee osteoarthritis. Rheumatol. Int. 2007, 27, 339–344.
22. Lepetsos P, Papavassiliou AG. ROS/oxidative stress signaling in osteoarthritis. *Biochim. Biophys. Acta* 2016; 1862: 576–591.
23. Yudoh K, Nguyen T, Nakamura H, Hongo-Masuko K, Kato T, Nishioka K. Potential involvement of oxidative stress in cartilage senescence and development of osteoarthritis: oxidative stress induces chondrocyte telomere instability and downregulation of chondrocyte function. *Arthritis Res Ther*. 2005; 7(2): R380-91.
24. Goldring SR, Goldring MB. Changes in the osteochondral unit during osteoarthritis: Structure, function and cartilage–bone crosstalk. *Nat. Rev. Rheumatol*. 2016; 12: 632.
25. Mobasheri A, Vannucci SJ, Bondy CA, Carter SD, Innes JF, Arteaga MF, Trujillo E, Ferraz I, Shakibaei M, Martín-Vasallo P. Glucose transport and metabolismin chondrocytes: A key to understanding chondrogenesis, skeletal development and cartilage degradation in osteoarthritis. *Histol. Histopathol*. 2002; 17: 1239–1267.
26. Salvioli S, Capri M, Valensin S, et al. Inflamm-aging, cytokines and aging: State of the art, new hypotheses on the role of mitochondria and new perspectives from systems biology. *Curr. Pharm. Des*. 2006; 24: 3161–3171.
27. Maggio M, Guralnik JM, Longo DL, Ferrucci L. Interleukin-6 in aging and chronic disease: A magnificent pathway. *J. Gerontol. Ser. A Biol. Sci. Med.* Sci. 2006; 61: 575–584.
28. De Martinis M, Franceschi C, Monti D, Ginaldi L. Inflamm-ageing and lifelong antigenic load as major determinants of ageing rate and longevity. *FEBS Lett.* 2005; 579: 2035–2039.
29. Di Bona D, Vasto S, Capurso C, et al. Effect of interleukin-6 polymorphisms on human longevity: A systematic review and meta-analysis. *Ageing Res. Rev.* 2009; 8: 36–42.
30. Lio D, Scola L, Crivello A, et al. Inflammation, genetics, and longevity: Further studies on the protective effects in men of IL-10 -1082 promoter SNP and its interaction with TNF-alpha-308 promoter SNP. *J. Med. Genet.* 2003; 40: 296–299.
31. Adams AA, Breathnach CC, Katepalli MP, Kohler K, Horohov DW. Advanced age in horses affects divisional history of T cells and inflammatory cytokine production. *Mech. Ageing Dev*. 2008; 129: 656–664.
32. Cannizzo ES, Clement CC, Sahu R, Follo C, Santambrogio L. Oxidative stress, inflammaging and immunosenescence. *J. Proteom*. 2011; 74: 2313–2323.
33. Alexa AI, Cantemir A, Antioch I, et al. The Dynamics of the Main Oxidative Stress Chemical Markers in the Serum of Rats Stressed by Various Behavioural Tasks. *Rev. Chim.* 2017; 68: 350–353.

34. Stanciu GD, Solcan G. Acute idiopathic polyradiculoneuritis concurrent with acquired myasthenia gravis in a West Highland white terrier dog. *BMC Vet. Res*. 2016; 12: 111.
35. Ginaldi L, De Martinis M, Monti D, Franceschi C. The immune system in the elderly: Activation-induced and damage-induced apoptosis. *Immunol. Res.* 2004; 30: 81–94.
36. Demaria M, Ohtani, N, Youssef, SA, et al. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Dev. Cell* 2014; 31: 722–733.
37. Mosteiro L, Pantoja C, Alcazar N, et al. Tissue damage and senescence provide critical signals for cellular reprogramming *in vivo*. *Science* 2016; 354: 4445.
38. Muñoz-Espín D, Serrano M. Cellular senescence: From physiology to pathology. *Nat. Rev. Mol. Cell Biol*. 2014; 15: 482–496.
39. Balistreri CR, Candore G, Accardi G, Colonna-Romano G, Lio D. NF-κB pathway activators as potential ageing biomarkers: Targets for new therapeutic strategies. Immun. Ageing 2013; 10: 24.
40. Weiskopf D, Weinberger B, Grubeck-Loebenstein B. The aging of the immune system. *Transpl. Int.* 2009; 22: 1041–1050.
41. White E, Lowe SW. Eating to exit: Autophagy-enabled senescence revealed. *Genes Dev*. 2009; 23: 784–787.
42. Trayhurn P, Drevon, CA, Eckel J. Secreted proteins from adipose tissue and skeletal muscle – Adipokines, myokines and adipose/muscle cross-talk. *Arch. Physiol. Biochem*. 2011; 117: 47–56.
43. Salminen A, Kaarniranta K, Kauppinen A. Inflammaging: Disturbed interplay between autophagy and inflammasomes. *Aging* 2012; 4: 166–175.
44. De Heredia FP, Sonia GM, Ascension M. Chronic and degenerative diseases: Obesity, inflammation and the immune system. *Proc. Nutr. Soc*. 2012; 71: 332–338.
45. Kerr JF, Wyllie AH, Currie AR. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* 1972; 26: 239–257.
46. Savill JS, Wyllie AH, Henson JE, et al. Macrophage phagocytosis of aging neutrophils in inflammation. Programmed cell death in the neutrophil leads to its recognition by macrophages. *J. Clin. Investig*. 1989; 83: 865–875.
47. Firestein GS, Nguyen K, Aupperle KR, et al. Apoptosis in rheumatoid arthritis: p53 overexpression in rheumatoid arthritis synovium. *Am. J. Pathol*. 1996; 149: 2143–2151.
48. Hasunuma T, Kayagaki N, Asahara H, et al. Accumulation of soluble Fas in inflamed joints of patients with rheumatoid arthritis. *Arthritis Rheum*. 1997; 40: 80–86.
49. Green D. *Means to an End: Apoptosis and other Cell Death Mechanisms*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA, 2011.
50. Raychaudhuri S. A minimal model of signaling network elucidates cell-to-cell stochastic variability in apoptosis. *PLoS ONE* 2010; 5: e11930.
51. Ventura MT, Casciaro M, Gamgemi S, Buquicchio R. Immunosenescence in aging: Between immune cells depletion and cytokines up-regulation. *Clin. Mol. Allergy* 2017; 15: 21.
52. Chiurchiù V, Leuti A, Maccarrone M. Bioactive Lipids and Chronic Inflammation: Managing the Fire Within. *Frontiers in Immunology* 2018; 9 (38): 1-11.
53. Smallwood MJ, Nissim A, Knight AR, Whiteman M, Haigh R, Winyard PG. Oxidative stress in autoimmune rheumatic diseases. *Free Radic Biol Med* 2018. 125: 3-14.

54. Bala A, Haldar PK. Free radical biology in cellular inflammation related to rheumatoid arthritis. *OA Arthritis* 2013; 1(2):15, 1-6.
55. Rezus E. Reumatologie. *Ed. „Grigore T. Popa" UMF* Iași; 2014.
56. Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative, *Ann Rheum Dis* 2010; 69 (9): 1580-1588.
57. Bălănescu A. Poliartrita reumatoidă. De la patogenie la clinică. *Ed. Medicală Amaltea*. București; 2007.
58. Housecroft CE, Constable EC. Chemistry: An Introduction to Organic, Inorganic and Physical Chemistry. 4th Edition, *Pearson Education*, Edinburgh 2010; 1286-1348.
59. Hussein JS. Cell membrane fatty acids and health. *Int J Pharm Pharm Sci* 2013; 5(3): 38-46.
60. Greabu M, Totan A, Mohora M, Dricu A, Pârvu AE, Foia L, Motoc M. Ghid de Biochimie Medicală, *Ed. Curtea Veche*, București; 2014.
61. Darie V, Grigorescu M, Firu D, Bugă AM, Popescu SO. Biochimie Medicalã. Mic Tratat Vol. II. *Editura SITECH*. Craiova; 2006.
62. Darie V, Grigorescu M, Firu D, Bugă AM, Popescu SO. Biochimie Medicalã. Mic Tratat Vol. I. *Editura Sitech*. Craiova; 2005.
63. Robinson PC, Brown MA. The genetics of ankylosing spondylitis and axial spondyloarthritis. *Rheum Dis Clin North* Am 2012; 38(3): 539-53.
64. Mohora M. Biochimie Medicală. Ediția a II-a. București; 2005.
65. Mcintyre TM, Hazen SL. Lipid Oxidation And Cardiovascular Disease: Introduction To A Review Serie. *Circ Res* 2010; 107(10): 1167-1169.
66. Michele D. Biochimie Clinică. Ed. a II-a, *Ed. Medicală*, București 2010.
67. Staroń A, Mąkosa G, Koter-Michalak M. Oxidative stress in erythrocytes from patients with rheumatoid arthritis. *Rheumatol Int* 2012; 32 (2): 331–334.
68. Dobreanu M. Biochimie clinică, Implicații Practice. Ediția a II-a Editura Medicală; 2010.
69. Laguerre M, Bayrasy C, Lecomte J, Chabi B, Decker EA, Wrutniak-Cabello C, Cabello G, Villeneuve P. How to boost antioxidants by lipophilization? *Biochimie* 2013; 95(1): 20-26.
70. Deponte M. Biochim Biophys Acta (BBA) - General Subjects 2013; 1830(5): 3217–3266.
71. Raducan A, Cantemir A, Puiu M, Oancea D. Kinetics Of Hydrogen Peroxide Decomposition By Catalase: Hydroxylic Solvent Effects. *Bioprocess And Biosystems Engineering* 2012;
72. Kato S, Ueno T, Fukuzumi S, Watanabe Y. Catalase Reaction By Myoglobin Mutants And Native Catalase. *The Journal Of Biological Chemistry* 2004; 279: 52376-52381.
73. Manole G, Gălățescu EM. Analize De Laborator. *Ed. CNI Coresi*; 2007.
74. Lothrop AP, Snider GW, Ruggles EL, Patel AS, Lees WJ, Hondal RJ. Selenium as an electron acceptor during the catalytic mechanism of thioredoxin reductase. *Biochemistry* 2014; 53: 654–663.

75. Ohta Y, Yashiro K, Ohashi K, Imai Y. Disruption of non-enzymatic antioxidant defense systems in the brain of rats with water-immersion restraint stress. *J Clin Biochem Nutr* 2012; 51(12): 136–142.
76. Singer FR, Eyre DR. Using biochemical markers of bone turnover in clinical practice. *Cleve Clin J Med* 2008; 75: 739.
77. Radner H. Comorbidities in Patients With Rheumatic Disease. In: *Surgery in Rheumatic and Musculoskeletal Disease*, Vol. 15, Ed: Atzeni F, Masala IF, Aletaha D, Lee M, Baraliakos X. 2018; 15-37.
78. Feinstein AR. The pre-therapeutic classification of co-morbidity in chronic disease. *Chronic Dis* 1970; 23: 455-468.
79. Van Den Akker M. Comorbidity or multimorbidity: what's in a name? *Eur J Gen Pract* 1996; 65-70.
80. Arida A, Protogerou AD, Kitas GD, Sfikakis PP. Systemic Inflammatory Response and Atherosclerosis: The Paradigm of Chronic Inflammatory Rheumatic Diseases. *Int J Mol Sci* 2018; 19(7): 1-27.
81. Ross R. Atherosclerosis is an inflammatory disease. *Am Heart J* 1999; 138(5 supply. 2): 419-420.
82. Sewell KL, Trentham DE. Pathogenesis of rheumatoid arthritis. *Lancet* 1993; 341: 283-286.
83. Kaplan MJ. Cardiovascular disease in rheumatoid arthritis. *Curr Opin Rheumatol* 2006; 18(3): 289-297.
84. Chung CP, Avalos I, Raggi P, et al. Atherosclerosis and inflammation: insights from rheumatoid arthritis. *Clin Rheumatol* 2007; 26(8): 1228-1233.
85. Panoulas VF, Metsios GS, Pace AV. et al. Hypertension in rheumatoid arthritis. *Rheumatology* 2008; 47: 1286-98.
86. Haraoui B, Liu PP, Papp KA. Managing cardiovascular risk in patients with chronic inflammatory diseases. *Clin Rheumatol* 2012; 31: 585-594.
87. Van Leuven SI, Franssen R, Kastelein JJ, et al. Systemic inflammation as a risk factor for atherothrombosis. *Rheumatology* 2008; 47: 3-7.
88. Khovidhunkit W, Memon RA, Feingold KR, et al. Infection and inflammation induced proatherogenic changes of lipoproteins. *J Infect Dis* 2000; 181: 462-472.
89. Doran M. Rheumatoid arthritis and diabetes mellitus: evidence for an association? *J Rheumatol* 2007; 34: 460-462.
90. Gerli R, Sherer Y, Bocci EB, et al. Precocious Atherosclerosis in Rheumatoid Arthritis. Role of Traditional and Disease-Related Cardiovascular Risk Factors. *Ann N Y Acad Sci* 2007; 1108: 372-381.
91. John H, Kitas G, Toms T, et al., Cardiovascular co-morbidity in early rheumatoid arthritis. *Best Pract Res Clin Rheumatol* 2009; 23(1): 71-82.
92. Gonzalez-Gay MA, Gonzalez-Juanatey C, Miranda-Filloy JA, et al. Cardiovascular disease in rheumatoid arthritis. *Biomed Pharmacother 2006;* 60(10): 673-677.
93. Libby P. Inflammation in atherosclerosis. *Nature* 2002; 420(6917): 868-874.

94. Maradit-Kremers H, Nicola PJ, Crowson CS, et al. Raised erythrocyte sedimentation rate signal heart failure in patients with rheumatoid arthritis. *Ann Rheum Dis* 2007; 66: 76-80.
95. Bijl M. Endothelial activation, endothelial dysfunction and premature atherosclerosis in systemic autoimmune diseases. *Neth J Med* 2003; 61(9): 273-277.
96. Kato H, Yamakawa M, Ogino T. Complement mediated vascular endothelial injury in rheumatoid nodules: a histopathological and immunohistochemical study. *J Rheumatol* 2000; 27: 1839-1847.
97. Ridker PM, Hennekens CH, Buring JE, et al. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000*;* 342: 832-834.
98. Vaudo G, Marchesi S, Gerli R, et al. Endothelial dysfunction in young patients with rheumatoid arthritis and low-disease activity. *Ann Rheum Dis* 2004; 63: 31-35
99. Pearson TA, Mensah GA, Alexander RW, et al. Centers for Disease Control and Prevention; American Heart Association. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2013; 107: 499-511.
100. Moller DE. Potential role of TNF-alpha in the pathogenesis of insulin resistance and type-2 diabetes. *Trends Endocrinol Metab* 2000, 6: 212-217.
101. Arnett DK, Evans GW, Riley WA. Arterial stiffness: a new cardiovascular risk factor? *Am J Epidemiol* 1994; 140: 669-682.
102. Full LE, Ruisanchez C, Monaco C. The inextricable link between atherosclerosis and prototypical infl ammatory diseases rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Res Ther 2009*; 11: 217.
103. Şerban M, Jurcuţ R, Cosmin M, Bucşă A, Jurcuţ C, Ginghină C. Afectare aterosclerotică la o pacientă cu poliartrită reumatoidă - Inflamaţia şi riscul cardiovascular, Revista Română de Cardiologie 2008 XXIII (2): 157-161.
104. Dregan A, Charlton J, Chowienczyk P, Gulliford MC. Chronic inflammatory disorders and risk of type 2 diabetes mellitus, coronary heart disease, and stroke: a population-based cohort study. *Circulation* 2014; 130: 837–844.
105. Soto ME, Melendez-Ramirez G, Kimura-Hayama E, Meave-Gonzalez A, Achenbach S, Herrera MC, Guering EL, Alexanderson-Rosas E, Reyes PA. Coronary CT angiography in Takayasu arteritis. *JACC Cardiovasc Imaging* 2011; 4: 958–966.
106. Tervaert JWC. Cardiovascular disease due to accelerated atherosclerosis in systemic vasculitides. *Best Prac Res Clin Rheum* 2013; 27: 33–44.
107. Nossent J, Cikes N, Kiss E, Marchesoni A, Nassonova V, Mosca M, Olesinska M, Pokorny G, Rozman B, Schneider M, Vlachoyiannopoulos PG, Swaak A. Current causes of death in systemic lupus erythematosus in Europe, 2000–2004: relation to disease activity and damage accrual. *Lupus* 2007; 16: 309–317.
108. Kahlenberg JM, Kaplan MJ. Mechanisms of premature atherosclerosis in rheumatoid arthritis and lupus. *Annu Rev Med* 2013; 64: 249–263.

109. Bruce IN, Burns RJ, Gladman DD, Urowitz MB. Single photon emission computed tomography dual isotope myocardial perfusion imaging in women with systemic lupus erythematosus. I. Prevalence and distribution of abnormalities. *J Rheumatol* 2000; 27: 2372–2377.
110. El-Magadmi M, Bodill H, Ahmad Y, Durrington PN, Mackness M, Walker M, Bernstein RM, Bruce IN. Systemic lupus erythematosus: an independent risk factor for endothelial dysfunction in women. *Circulation* 2004; 110, 399–404.
111. Van Doornum S, McColl G, Wicks IP. Accelerated atherosclerosis: an extraarticular feature of rheumatoid arthritis? *Arthritis Rheum*. 2002; 46: 862–873.
112. Michaud K, Vera-Llonch M, Oster G. Mortality risk by functional status and health-related quality of life in patients with rheumatoid arthritis. *J. Rheumatol*. 2012; 39: 54–59.
113. del Rincon ID, Williams K, Stern MP, Freeman GL, Escalante A. High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. *Arthritis Rheum*. 2001; 44: 2737–2745.
114. Maradit-Kremers H, et al. Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study. *Arthritis Rheum*. 2005; 52: 402–411.
115. Herbrig K, Haensel S, Oelschlaegel U, Pistrosch F, Foerster S, Passauer J. Endothelial dysfunction in patients with rheumatoid arthritis is associated with a reduced number and impaired function of endothelial progenitor cells. *Ann Rheum Dis* 2006; 65: 157–163.
116. Bergholm R, Leirisalo-Repo M, Vehkavaara S, Makimattila S, Taskinen MR, Yki-Jarvinen H. Impaired responsiveness to NO in newly diagnosed patients with rheumatoid arthritis. *Arterioscler Thromb Vasc Biol* 2002; 22: 1637–1641.
117. Semb AG, Rollefstad S, Provan SA, Kvien TK, Stranden E, Olsen IC, Hisdal J. Carotid plaque characteristics and disease activity in rheumatoid arthritis. *J Rheumatol* 2013; 40: 359–368.
118. Tanasescu C, Jurcut C, Jurcut R, Ginghina C. Vascular disease in rheumatoid arthritis: from subclinical lesions to cardiovascular risk. *Eur J Intern Med* 2009; 20(4): 348-54.
119. Shoenfeld Y, et al. Accelerated atherosclerosis in autoimmune rheumatic diseases. *Circulation* 2005; 112: 3337–3347.
120. Roldan CA, Chavez J, Wiest PW, Qualls CR, Crawford MH. Aortic root disease and valve disease associated with ankylosing spondylitis. *J Am Coll Cardiol* 1998; 32: 1397–1404.
121. Bergfeldt L, Vallin H, Edhag O. Complete heart block in HLA B27 associated disease. Electrophysiological and clinical characteristics. *Br Heart J* 1984; 51: 184–188.
122. Park S, Lakatta EG. Role of infl ammation in the pathogenesis of arterial stiffness. *Yonsei Med J* 2012; 53: 258–61.
123. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005; 352: 1685–1695.
124. Micha R, Imamura F, Wyler von Ballmoos M, Solomon DH, Hernan MA, Ridker PM, Mozaffarian D. Systematic review and meta-analysis of methotrexate use and risk of cardiovascular disease. *Am J Cardiol* 2011; 108: 1362–1370.

125. Guin A, Chatterjee Adhikari M, Chakraborty S, Sinhamahapatra P, Ghosh A. Effects of disease modifying anti-rheumatic drugs on subclinical atherosclerosis and endothelial dysfunction which has been detected in early rheumatoid arthritis: 1-year follow-up study. Semin. *Arthritis Rheum* 2013; 43: 48–54
126. Angel K, Provan SA, Fagerhol MK, Mowinckel P, Kvien TK, Atar D. Effect of 1-year anti-TNF-alpha therapy on aortic stiffness, carotid atherosclerosis, and calprotectin in infl ammatory arthropathies: a controlled study. *Am J Hypertens* 2012; 25: 644–650.
127. Hurlimann D, Forster A, Noll G, Enseleit F, Chenevard R, Distler O, Bechir M, Spieker LE, Neidhart M, Michel BA, Gay RE, Luscher TF, Gay S, Ruschitzka F. Antitumor necrosis factor-alpha treatment improves endothelial function in patients with rheumatoid arthritis. *Circulation* 2002; 106: 2184–2187.
128. Barnabe C, Martin BJ, GhaliWA. Systematic review and meta-analysis: anti-tumor necrosis factor alpha therapy and cardiovascular events in rheumatoid arthritis. *Arthritis Care Res (Hoboken)* 2011; 63: 522–529.
129. He D, Bai F, Zhang S, et al. High incidence of tuberculosis infection in rheumatic diseases and impact for chemoprophylactic prevention of tuberculosis activation during biologics therapy. *Clin Vaccine Immunol* 2013; 20(6): 842-7.
130. Arkema EV, Jonsson J, Baecklund E, et al. Are patients with rheumatoid arthritis still at an increased risk of tuberculosis and what is the role of biological treatments? *Ann Rheum Dis* 2014; 1–6.
131. Jantschi L, Bolboacă SD, Exact probabilities and confidence limits for binomial Asmples: Applied to the Difference between two proportions. *ScientificWorld Journal* 2010, 10: 865-878.
132. World Health Organization: "Global tuberculosis control: WHO report 2011", Geneva, World Health Organization, 2011, WHO/HTN/TB/2011.6.
133. Cristea C. Evoluția Tuberculozei în anii 1999-2012 în Romania. Available from: http://www.ccss.ro/public_html/sites/default/files//Evoluția Tuberculozei in anii 1999-2012 in Romania.pdf
134. Ibraim E, Cioran N, Cocei H. Tuberculoza, în continuare în atenție. *Pneumologia* 2012; 61(2): 78-83.
135. Simard JF, Arkema EV, Sundstrom A, et al. Ten years with biologics: to whom do data on effectiveness and safety apply? *Rheumatology* 2011; 50(1): 204-213.
136. Falagas ME, Voidonikola PT, Angelousi AG. Tuberculosis in patients with systemic rheumatic or pulmonary diseases treated with glucocorticosteroids and the preventive role of isoniazid: a review of the available evidence. *Int J Antimicrob Agents*, 2007; 30(6): 477–486.
137. Bellofiore B, Matarese A, Balato N, et al. Prevention of tuberculosis in patients taking tumor necrosis factor-alpha blokers. *J Rheumatol* 2009; 83: 76-77.
138. Munoz L, Casas S, Juanola X, et al. Prevention of anti-tumor necrosis factor-associated tuberculosis: a 10-year longitudinal cohort study. *Clin Infect Dis* 2015; 60: 349.

139. Galloway JB, Hyrich KL, Mercer LK, et al, Anti-TNF therapy is associated with an increased risk of serious infections in patients with rheumatoid arthritis especially in the first 6 months of treatment: updated results from the British Society for Rheumatology Biologics Register with special emphasis on risk in the elderly. *Rheumatology* 2010; 31: 1-8.
140. Kiely PDW, Nikiphorou E., Management of rheumatoid arthritis, *Medicine* 2018; 46(4): 216-221.
141. Cope AP, Rheumatoid Arthritis, In: *Clinical Immunology (Fifth Edition),* Ed: Rich RR, Fleisher TA, Shearer WT, Schroeder HW, Frew AJ, Weyand CM. 2019; 705-721.
142. Kumar SS, et al. Indian Medicinal Plants Used for Treatment of Rheumatoid Arthritis, *Research J Pharm and Tech* 2015; 8(5): 597-610.
143. Kaas J, Tóthová V, Quality of life of patients who suffer from rheumatic arthritis, *Kontakt* 2017, 19(3): 159-164.
144. Malm K, Bergman S, Andersson ML, Bremander A, Larsson I, Quality of life in patients with established rheumatoid arthritis: A phenomenographic study, *SAGE Open Med*. 2017, 5, 1-8.
145. Michaud K, Bombardier C, Emery P, Quality of life in patients with rheumatoid arthritis: does abatacept make a difference? *Clin Exp Rheumatol*. 2007; 25 (5 supply. 46): 35-45.
146. Pollard L, Choy EH, Scott DL. The consequences of rheumatoid arthritis: quality of life measures in the individual patient. *Clin Exp Rheumatol,* 2005; 43-52.
147. Salesi M, Mottaghi P, Karimifar M, et al. Intravenous pamidronate for refractory rheumatoid arthritis. *J Res Med Sci* 2012; 17: 422-427.
148. Alonso J, Ferrer M, Gandek B, et al. Health-related quality of life associated with chronic conditions in eight countries: results from the International Quality of Life Assessment (IQOLA) Project. *Qual Life Res* 2004; 13: 283-298.
149. Lam CL, Laudera IJ. The impact of chronic diseases on the health-related quality of life (HRQOL) of Chinese patients in primary care. *Fam Pract* 2000; 17(2): 159-156.
150. Núñez M, Sanchez A, Nuñez E, et al. Patients' perceptions of health related quality of life in rheumatoid arthritis and chronic low back pain. *Qual Life Res* 2006; 15: 93-102
151. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-324.
152. Oude Voshaar MA, ten Klooster PM, Taal E, van de Laar MAFJ. Measurement properties of physical function scales validated for use in patients with rheumatoid arthritis: A systematic review of the literature. *Health and Quality of Life Outcomes* 2011; 9: 99.
153. Ware JE Jr, Kosinski M, et al. Comparison of methods for the scoring and statistical analysis of SF-36 health profile and summary measures: summary of results from the Medical Outcomes Study. *Med Care* 1995; 33: 264-279.
154. Zimmerman M, Martinez JH, Young D, et al. Severity classification on the Hamilton Depression Rating Scale. *J Affect Disord* 2013; 150: 384-388.

155. Koerner N, Antony MM, Dugas MJ. Limitations of the Hamilton Anxiety Rating Scale as a primary outcome measure in randomized, controlled trials of treatments for generalized anxiety disorder. *Am J Psychiatry*. 2010; 167(1): 103-4
156. Kvien TK, Uhlig T, Ødegård S, Heiberg MS. Epidemiological aspects of rheumatoid arthritis: the sex ratio, *Ann N Y Acad Sci* 2006; 1069: 212-222.
157. Costenbader KH, Manson JE. Do Female Hormones Affect the Onset or Severity of Rheumatoid Arthritis? *Arthritis & Rheumatism* 2008; 59(3): 299-301.
158. Ang DC, Choi H, Kroenke K, Wolfe F. Comorbid depression is an independent risk factor for mortality in patients with rheumatoid arthritis. *J Rheumatol* 2005; 32: 1013-1019.
159. Bayat N, Alishiri GH, Salimzadeh A, et al. Symptoms of anxiety and depression: A comparison among patients with different chronic conditions. *J Res Med Sci* 2011; 16: 1441-1447.
160. Dickens C, McGowan L, Clark-Carter D, Creed F. Depression in rheumatoid arthritis: a systematic review of the literature with meta- analysis. *Psychosom Med* 2002; 64: 52-60.
161. Pincus T, Griffith J, Pearce S, Isenberg D. Prevalence of self-reported depression in patients with rheumatoid arthritis. *Br J Rheumatol* 1996; 35: 879-883.
162. Gossec L, Berenbaum F, Chauvin P, et al. Reporting of patient perceived impact of rheumatoid arthritis and axial spondyloarthritis over 10 years: a systematic literature review. *Rheumatology (Oxford)* 2014; 53(7): 1274-1281.
163. Withers MH, Gonzalez LT, Karpouzas GA. Identification and Treatment Optimization of Comorbid Depression in Rheumatoid Arthritis. *Rheumatol Ther*. 2017; 4(2): 281-291.
164. Padjen I. Drugs Used in Rheumatic Disease, In*: Surgery in Rheumatic and Musculoskeletal Disease,* Vol. 15, Ed: F. Atzeni, I. F. Masala, D. Aletaha, M. Lee, X. Baraliakos. 2018; 39-76.
165. Bijlsma JW. Disease control with glucocorticoid therapy in rheumatoid arthritis. *Rheumatology (Oxf)* 2012; 51(4): 9-13.
166. Checherita LE, Brezulianu C, Forna D, Stamatin O, Ioanid N, Foia L. Neuromuscular disabilities in patients diagnosed with algo-dysfunctional syndrome, *Romanian Journal Of Oral Rehabilitation (Rjor)* 2012; 4(2): 60-66.
167. Checherita LE, Forna NC, Surdu Macovei A, et al. Influence of chemical therapeutical methods on manducatory muscles, *Rev Chim (Bucharest)* 2013, 64(11): 1312-1316.
168. Carausu EM, Checherita LE, Stamatin O, Albu A. Study of Serum and Saliva Biochemical Levels for Copper, Zinc and Cooper-Zinc Imbalance in Patients with Oral Cancer and Oral Potentially Malignant Disorders and their Prostetical and DSSS (Disfunctional Syndrome of Stomatognathic System) Treatment. *Rev Chim (Bucharest)* 2016; 67(9): 1832-1836.
169. Carausu EM, Checherita LE, Stamatin O, Manuc D. Study of Biochemical Level for Mg and Ca-Mg Imbalance in Patients with Oral Cancer and Potentially Malignant Disorder and their Prostetical and DSSS Treatment. *Rev Chim (Bucharest)* 2016; 67 (10): 2087-2090.

170. Dascalu CG, Carausu EM, Manuc D. Methods for Data Selection in Medical Databases: The Binary Logistic Regression - Relations with the Calculated Risks *World Academy of Science, Engineering and Technology* 2008; 14: 278-282.
171. Kwiatkowska B, Majdan M, Mastalerz-Migas A, Niewada M, Skrzydło-Radomańska B, Mamcarz A. Status of etoricoxib in the treatment of rheumatic diseases. *Expert panel opinion. Reumatologia*. 2017; 55(6): 290-297.
172. Puopolo A, Boice JA, Fidelholtz JL, Littlejohn TW, Miranda P, Berrocal A, Ko A, Cichanowitz N, Reicin AS. A randomized placebo-controlled trial comparing the efficacy of etoricoxib 30mg and ibuprofen 2400mg for the treatment of patients with osteoarthritis. *Osteoarthritis and Cartilage* 2007; 15(12): 1348-1356.
173. Baraf HS, Fuentealba C, Greenwald M, et al. Gastrointestinal side effects of etoricoxib in patients with osteoarthritis: results of the Etoricoxib versus Diclofenac Sodium Gastrointestinal Tolerability and Effectiveness (EDGE) trial. *J Rheumatol.* 2007; 34: 408–419.
174. Kienzler JL, Gol, M, Nollevaux F. Systemic bioavailability of topical diclofenac sodium gel 1% versus oral diclofenac sodium in healthy volunteers. *J Clin Pharmacol* 2010, 50(1): 50-61.
175. Zacher J, Feldman D, Gerli R. A comparison of the therapeutic efficacy and tolerability of etoricoxib and diclofenacn in patients with osteoarthritis. *Curr Med Res Opin*. 2003;19(8):725-36.
176. Lubberts E, van den Berg WB. Cytokines in the Pathogenesis of Rheumatoid Arthritis and Collagen-Induced Arthritis. In: Madame Curie Bioscience Database [Internet]. Austin (TX): *Landes Bioscience*; 2000-2013.
177. Florencio-Silva R, Sasso GR, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells. *Biomed Res Int* 2015; 2015: 421746.
178. Tanaka Y, Nakayamada S, Okada Y. Osteoblasts and Osteoclasts in Bone Remodeling and Inflammation, *Current Drug Targets - Inflammation & Allergy* 2005, 4: 325-328.
179. Braun T, Zwerina J. Positive regulators of osteoclastogenesis and bone resorption in rheumatoid arthritis. *Arthritis Research & Therapy* 2011; 13(4): 235.
180. Mańczak M, Gasik R. Cervical spine instability in the course of rheumatoid arthritis - imaging methods. *Reumatologia* 2017; 55(4): 201-207.
181. Gualtierotti R, Marzano AV, Spadari F, Cugno M. Main Oral Manifestations in Immune-Mediated and Inflammatory Rheumatic Diseases, *J Clin Med* 2018, 8(1): E21.
182. Kasser UR, Gleissner C, Dehne F, Michel A, Willershausen-Zonnchen B, Bolten WW. Risk for periodontal disease in patients with longstanding rheumatoid arthritis. *Arthritis Rheum* 1997; 40: 2248–2251.
183. Macovei-Surdu A, Rudnic I, Martu I, Solomon S, Pasarin L, Martu S. Studies regarding the bidirectional relationship between the periodontal disease and hyperlipidaemia. *Romanian Journal Of Oral Rehabilitation* 2013; 5(1): 76-81.

184. Macovei-Surdu A, Rudnic I, Martu I, Solomon S, Pasarin L, Martu S. Studies Regarding The Bidirectional Relationship Between The Periodontal Disease And Hyperlipidaemia. *Romanian Journal of Oral Rehabilitation*2013; 5(1): 77-82.
185. Luchian I, Martu I, Martu C, Goriuc A, Beldiman A, Martu S, Changes in Biochemical Parameters Associated with Periodontal Disease. *Rev Chim (Bucharest)* 2016, 67(6): 1073-1075.
186. Solomon SM, Matei NM, Badescu AC, Jelihovschi I, Martu Stefanache A, Teusan A, Martu S, Iancu LS. Evaluation of DNA Extraction Methods from Saliva as a Source of PCR - Amplifiable Genomic DNA. *Rev Chim (Bucharest)* 2016; 66(12): 2101-2103.
187. Doan T, Marquis KA, Rudner DZ. Subcellular localization of a sporulation membrane protein is achieved through a network of interactions along and across the septum. *Mol Microbiol* 2005; 55(6): 1767–1781.
188. Martu S, Nicolaiciuc O, Solomon S, Sufaru I, Scutariu M, Rezus 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.
189. Nicolaiciuc O, Ursărescu IG, Mârţu-Ştefanache A, Păsărin L, Tatarciuc D, Oanţă, C Mârţu S. Study on the risk markers for atherosclerosis in patients with severe periodontitis. *Int J Med Dent* 2015; 5(4): 301-306.
190. Veisa G, Donciu M, Segall L, Hurjui L, Nistor I, Ursarescu I, Martu S, Burlea SL, Solomon SM. Albumin as a Prognostic Factor for Malnutrition and Inflammation in Chronic Kidney Disease. *Rev Chim (Bucharest)* 2016; 67(1): 103-105.
191. Moen K, Brun JG, Valen M, Skartveit L, Eribe Ek, Olsen I, Jonsson R. Synovial inflammation in active rheumatoid arthritis and psoriatic arthritis facilitates trapping of a variety of oral bacterial DNAs. *Clin Exp Rheumatol* 2006; 24(6) 656-663.
192. Boatca RM, Scutariu MM, Rudnic I, Martu Stefanache MA, Hurjui L, Rezus E, Martu S. Evolution of Inflammatory Biochemical Markers Within Periodontal Therapy to Patients with Rheumatoid Arthritis. *Rev Chim (Bucharest)* 2016; 67(4): 741-744.
193. Syrbe U., Sieper J., Spondyloarthritides, In: The Autoimmune Diseases (Fifth Edition), Ed: Rose NE, Mackay IR, 2014, 537-545.
194. Braun J, Sieper J. Ankylosing spondylitis. *Lancet* 2007; 369(9570): 1379–90.
195. Smith JA. Update on Ankylosing Spondylitis: Current Concepts in Pathogenesis, *Curr Allergy Asthma Rep* 2015; 15(489): 1-9.
196. Xu ZY, Zhou C, Zhang KF, Zheng YP. Identification of key genes in Ankylosing spondylitis. *Immunology Letters* 2018; 204: 60–66.
197. Sharma N. et al., Usage of Conventional PCR Technology for the Detection of HLA-B27 Allele: A Significant Molecular Marker of Ankylosing Spondylitis, *Ind J Clin Biochem* 2013, 28(2): 189–192.
198. Dowlearn E. A review of the molecular and genetic foundations of Ankylosing Spondylitis, A Senior Thesis submitted in partial fulfillment of the requirements for graduation in the Honors Program Liberty University 2011, 5-6.

199. Delmonte OM, Fleisher TA. Flow cytometry: Surface markers and beyond. *Journal of Allergy and Clinical Immunology* 2018, article in press.
200. Levering WHBM, Wind H, Sintnicolaas K, Hooijkaas, Gratama JW. Flow cytometric HLA-B27 screening: cross reactivity patterns of commercially available anti-HLA-B-27 monoclonal antibodies with other HLA-B antigens. *Cytometry part B* 2003, 54B: 28-38.
201. Rodey GE. HLA beyond tear, In: *Introduction to Human Histocompatibility*, second edition, chapter 2: Structure and function of the HLA Complex, Ed: Freez P, 2000.
202. Skalska U, Kozakiewicz A, Maśliński W, Jurkowska M. HLA-B27 detection - comparison of genetic sequence-based method and flow cytometry assay, *Reumatologia*. 2015; 53(2): 74-8.
203. Bonnaud G, Aupetit C, Preux PM, Cogne M, Drouet M. Optimisation of HLA-B27 Testing by Association of Flow Cytometry and DNA Typing. *Clin Rheumatol* 1999, 18: 23–27.
204. Reynolds WM, Evans PR, Wilson PJ, Wong WM, Darke C, Smith JL. Automated routine HLA-B27 typing by flow cytometry, *J Immunol Methods*. 1996; 197(1-2): 1-5.
205. Chheda P, Warghade S, Mathias J, Dama T, Matkar S, Shah N, Bendre R. HLA-B27 testing: A journey from flow cytometry to molecular subtyping, *J Clin Lab Anal*. 2018, 32(5): e22382.
206. Danese S. et al. Extraintestinal manifestations in inflammatory bowel disease, *World J Gastroenterol* 2005; 11(46): 7227–7236.
207. Cholapranee A, Hazlewood GS, Kaplan GG, Peyrin-Biroulet L, Ananthakrishnan AN. Systematic review with meta-analysis: comparative efficacy of biologics for induction and maintenance of mucosal healing in Crohn's disease and ulcerative colitis controlled trials, *Aliment Pharmacol Ther* 2017, 45(10): 1291-1302.
208. Lanna CC, Ferrari Mde L, Rocha SL, Nascimento E, de Carvalho MA, da Cunha AS. A cross-sectional study of 130 Brazilian patients with Crohn's disease and ulcerative colitis: analysis of articular and ophthalmologic manifestations. *Clin Rheumatol* 2008; 27(4): 503–509.
209. Brown M, Pile K, Kennedy L, et al. HLA class I associations of ankylosing spondylitis in the white population in the United Kingdom. *Ann Rheum Dis* 1996; 55: 268–70.
210. Vlam K, Mielants H, Cuvelier C, et al. Spondyloarthropathy is underestimated in inflammatory bowel disease: prevalence and HLA association. *J Rheumatol* 2000; 27: 2860–2865.
211. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Medical Care* 1992; 30(6): 473–483.
212. Huppertz-Hauss G, Høivik ML, Langholz E, et al. Health-related quality of life in inflammatory bowel disease in a european-wide population-based cohort 10 years after diagnosis. *Inflamm Bowel Dis* 2015; 21(2): 337-344.
213. Jowett SL, Seal CJ, Barton JR, Welfare MR. The short inflammatory bowel disease questionnaire is reliable and responsive to clinically important change in ulcerative colitis. *Am J Gastroenterol* 2001; 96: 2921-2928.

214. Nagel B, Gerbershagen HU, Lindena G, Pfingsten M. Development and evaluation of the multidimensional German pain questionnaire, *Schmerz* 2002; 16: 263-270.
215. Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomised trial. *BMJ* 1989; 298: 82-86.
216. Schirbel A, Reichert A, Roll S, et al. Impact of pain on health-related quality of life in patients with inflammatory bowel disease. *World J Gastroenterol* 2010; 16(25): 3168-3177.
217. Hamdi W, Azzouz D, Ghannouchi MH, et al. Health-related Quality of Life Assessment on 100 Tunisian Patients with Ankylosing Spondylitis using the SF-36 Survey. *Oman Medical Journal* 2012; 27(6): 455-460.
218. Ariza-Ariza R, Hernandez-Cruz B, Navarro-Sarabia F. Physical function and health-related quality of life of Spanish patients with ankylosing spondylitis. *Arthritis & Rheumatism* 2003; 49(4): 483-487.
219. Ibn Yacoub Y, Amine B, Laatiris A, Abouqal R, Hajjaj-Hassouni N. Healthrelated quality of life in Moroccan patients with ankylosing spondylitis. *Clin Rheumatol* 2011; 30(5): 673-677.
220. Ward MM, Predictor of the progression of functional disability in patients with ankylosing spondylitis. *Journal of Rheumatology* 2002; 29: 1420–1425.
221. Ozgul A, Peker F, Taskaynatan MA, et al. Effect of ankylosing spondylitis on healthrelated quality of life and different aspects of social life in young patients. *Clinical Rheumatology* 2006; 25: 168– 174.
222. Pizzi LT, Weston CM, Goldfarb NI, Moretti D, Cobb N, Howell JB, Infantolino A, Dimarino AJ, Cohen S. Impact of chronic conditions on quality of life in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2006; 12: 47-52.
223. Guagnozzi D, Lucendo JA. Anemia in inflammatory bowel disease: a neglected issue with relevant effects. *World J Gastroenterol* 2014; 20(13): 3542-3551.
224. Reinisch W, Staun M, Bhandari S, Muñoz M. State of the iron: how to diagnose and efficiently treat iron deficiency anemia in inflammatory bowel disease. *J Crohns Colitis* 2013; 7: 429-440 .
225. Means RT. Recent developments in the anemia of chronic disease. *Curr Hematol Rep* 2003; 2: 116-121.
226. Beaugerie L, Brousse N, Bouvier AM, Colombel JF, Lémann M, Cosnes J, Hébuterne X, Cortot A, Bouhnik Y, Gendre JP, Simon T, Maynadié M, Hermine O, Faivre J, Carrat F. Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study. *Lancet* 2009; 374: 1617-1625.
227. Fiorino G,Caprioli F, Daperno M, Mocciaro F, Principi M, Viscido A, Fantini MC, Orlando A, Papi C, Annese V, Danese S, Vecchi M, Rizzello F, Armuzzi A. Use of biosimilars in inflammatory bowel disease: A position update of the Italian Group for the Study of Inflammatory Bowel Disease (IG-IBD). *Digestive and Liver Disease* 2019, in press.
228. Rudwaleit M, Baeten D. Ankylosing spondylitis and bowel disease. *Best Pract Res Clin Rheumatol* 2006; 20(3): 451-471.

229. Isene R. et al. Extraintestinal manifestations in Crohn's disease and ulcerative colitis: results from a prospective, population-based European inception cohort. *Scand J Gastroenterol*. 2015; 50(3): 300-5.
230. Zippi M, Corrado C, Pica R, et al. Extraintestinal manifestations in a large series of Italian inflammatory bowel disease patients. *World J Gastroenterol.* 2014; 20(46): 17463-7.
231. Ott C, Scholmerich J. Extraintestinal manifestations and complications in IBD, *Nat Rev Gastroenterol Hepatol.*2013; 10(10): 585-95.
232. Karmiris K. et al, Prevalence and characteristics of extra-intestinal manifestations in a large cohort of Greek patients with inflammatory bowel disease. *J Crohns Colitis* 2016, 10(4): 429–436.
233. Hsu YC, Wu TC, Lo YC, Wang LS. Gastrointestinal complications and extraintestinal manifestations of inflammatory bowel disease in Taiwan: a population-based study. *J Chin Med Asso*c 2017, 80(2): 56–62.
234. Ramadas AV, Gunesh S, Thomas GA, Williams GT, Hawthorne AB. Natural history of Crohn's disease in a population-based cohort from Cardiff (1986–2003): a study of changes in medical treatment and surgical resection rates. *Gut* 2010, 59(9): 1200–1206.
235. Walsh P, Kane N, Butler S. The Clinical Role Of Evoked Potentials. *Neurol Neurosurg Psychiatry* 2005; 76(II): 16–22.
236. Khedr EM, Rashad SM, Hamed SA, El-Zharaa F, Abdalla AK. Neurological complications of ankylosing spondylitis: neurophysiological assessment. *Rheumatol Int* 2009; 29: 1031–1040.
237. Cidem M, Sahin Z, Aydin T, Aysal F. Somatosensory evoked potential findings in ankylosing spondylitis. *Eurasian J Med* 2014; 46(1): 42-46.
238. Goie The HS, Steven MM, van der Linden SM, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis: a comparison of the Rome, New York and modified New York criteria in patients with a positive clinical history screening test for ankylosing spondylitis. *Br J Rheumatol* 1985; 24(3): 242-249.
239. Ramos-Remus C, Gomez-Vargas A, Guzman-Guzman JL, Jimenes- Gil F, Games-Nava JI, Gonzales-Lopez L, Farrera-Gamboa H et al. Frequency of atlantoaxial subluxation and neurologic involvement in patients with AS. *J Rheumatol* 1995; 22: 2120–2125.
240. Sofat N, Malik O, Higgens CS. Neurological involvement in patients with rheumatic disease, *Q J Med* 2006; 99:69–79.
241. Mercieca C, van der Horst-Bruinsma IE, Borg AA. Pulmonary, renal and neurological comorbidities in patients with ankylosing spondylitis; implications for clinical practice, *Curr Rheumatol Rep* 2014, 16: 434.
242. Pillary N, Hunter T. Delayed evoked potentials in patients with ankylosing spondylitis. *J Rheumatol* 1986; 13: 137–141.
243. Tahmasebi S, Adhami-Moghdam F. Recording of Visual Evoked Potential in Rheumatoid Arthritis Patients with Hydroxychloroquine Treatment, *J Opthalmol Res* 2019; 2(1): 001-005.

244. Gabrielli A, Avvedimento EV, Krieg T. Mechanisms of Disease. Scleroderma. *N Engl J Med* 2009; 360(19): 1989-2003.
245. http://www.scleroderma.org/site/PageNavigator/patients_whatis.html#.XDB_VFwzbDc
246. Khanna D, Furst DE, Clements PJ, et al. Standardization of the modified Rodnan skin score for use in clinical trials of systemic sclerosis. *J Scleroderma Relat Disord* 2017; 2(1): 11-18.
247. Vargas-Santos AB, Taylor WJ, Neogi T. Gout Classification Criteria: Update and implications. *Curr Rheumatol Rep*. 201618(7): 46.
248. Krishnan E. Gout: Epidemiology and Risk Factors, In: *Gout & Other Crystal Arthropathies* Ed: R. Terkeltaub, 2012; 72-84.
249. Harrison E, Herrick AL, Mclaughlin J, Lal S. Malnutrition in systemic sclerosis. *Rheumatology* 2012; 51(10): 1747-1756.
250. Krause L, Becker MO, Brueckner CS, et al. Nutritional status as marker for disease activity and severity predicting mortality in patients with systemic sclerosis. *Ann Rheum Dis* 2010; 69(11): 1951-1957.
251. Cruz-Dominguez MP, Garcia-Collinot G, Saavedra MA, et al. Malnutrition is an independent risk factor for mortality in Mexican patients with systemic sclerosis: a cohort study. *Rheumatol* 2017; 37(7): 1101-1109.
252. McMahan ZH, Paik JJ, Wigley FM, Hummers LK. Determining the Risk Factors and Clinical Features Associated With Severe Gastrointestinal Dysmotility in Systemic Sclerosis. *Arthritis Care Res* 2018; 70(9): 1385-1392.
253. McFarlane IM, Bhamra MS, Kreps A, et al. Gastrointestinal Manifestations of Systemic Sclerosis. *Rheumatology (Sunnyvale).* 2018; 8(1): 235.
254. Rosato E, Gigante A, Gasperini ML, et al. Nutritional status measured by BMI is impaired and correlates with left ventricular mass in patients with systemic sclerosis. *Nutrition* 2014; 30(2): 204-209.
255. Murtaugh MA, Frech TM. Nutritional status and gastrointestinal symptoms in systemic sclerosis patients. *Clinical Nutrition* 2013; 32(1): 130-135.
256. Silva I, Almeida J, Vasconcelos C. A PRISMA-driven systematic review for predictive risk factors of digital ulcers in systemic sclerosis patients. *Autoimmun Rev* 2015; 14(2): 140-152.
257. Liaskos C, Marou E, Simopoulou T, et al. Disease-related autoantibody profile in patients with systemic sclerosis. *Autoimmunity* 2017; 50(7): 414-421.
258. Mahler M, Meroni PL, Bossuyt X, Fritzler MJ. Current concepts and future directions for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *J Immunol Res* 2014; 21(1): 1-19.
259. Sobanski V, Giovannelli J, Lynch BM, et al. Characteristics and Survival of Anti–U1 RNP Antibody–Positive Patients With Connective Tissue Disease–Associated Pulmonary Arterial Hypertension. *Arthritis Rheum.* 2016, 68(2): 484–493.

260. Choi MY, Fritzler MJ. Progress in understanding the diagnostic and pathogenic role of autoantibodies associated with systemic sclerosis. *Curr Opin Rheumatol* 2016; 28(6): 586-594.
261. Lian F, Chen D, Wang Y, et al. Clinical features and independent predictors of pulmonary arterial hypertension in systemic lupus erythematosus. *Rheumatol Int* 2012; 32(6): 1727-1731.
262. Sui M, Lin Q, Xu Z, et al. Simultaneous positivity for anti-DNA, anti-nucleosome and anti-histone antibodies is a marker for more severe lupus nephritis. *J Clin Immunol* 2013; 33(2): 378-87.
263. Assassi S, Mayes MD, Arnett FC, et al. Systemic sclerosis and lupus: points in an interferon-mediated continuum. *Arthritis Rheum*. 2010; 62(2): 589-98.
264. Salazar GA, Assassi S, Wigley F. Antinuclear Antibody Negative Systemic Sclerosis. *Semin Arthritis Rheum*. 2015; 44(6): 680–686.
265. Hess PL, Al-Khalidi HR, Friedman DJ, et al. The metabolic syndrome and risk ofsudden cardiac death: the atherosclerosis risk in communities study. *J Am Heart Assoc* 2017; 63(8): e006103.
266. Medina G. et al. Metabolic syndrome, autoimmunity and rheumatic disease. *Pharmacological Research* 2018; 133: 277–288.
267. Marginean M, Tranca S, Ardelean-Maghiar A, Dirzu D, Hutanu A, Platon O, Dobreanu F. Comparing the Anti-Inflammatory Effects of Simvastatin and Rosuvastatin by Measuring IL-1β, IL-6 and TNF-α Levels Using a Murinic Caecal Ligation And Puncture Induced Sepsis Model. *Rev Med Lab* 2014; 22(4): 439-450.
268. Gilca GE, Diaconescu S, Balan GG, Timofte O, Stefanescu G. Sarcoidosis associated with infliximab therapy in ulcerative colitis: A case report. *Medicine* 2017; 96(10): e6156.
269. Necula LG, Chivu-Economescu M, Stanciulescu EL, Bleotu C, Dima SO, Alexiu I, Dumitru A, Constantinescu G, Popescu I, Diaconu CC. IL-6 and IL-11 as Markers for Tumor Aggressiveness and Prognosis in Gastric denocarcinoma Patients without Mutations in Gp130 Subunits. *J Gastrointestin Liver Dis* 2012; 21(1): 23-29.
270. Singh JA, Strand V. Gout is associated with more comorbidities, poorer health-related quality of life and higher healthcare utilisation in US veterans. *Annals Of The Rheumatic Diseases* 2008; 67: 1310-1316.
271. Robinson PC, Merriman TR, Herbison P, Highton J. Hospital admissions associated with gout and their co-morbidities in New Zealand and England 1999-2009. *Rheumatology* 2013; 52: 118-126.
272. Sari I, Akar S, Pakoz B, Sisman AR, Gurler O, Birlik M, et al. Hyperuricemia and its related factors in an urban population, Izmir, Turkey. *Rheumatol Int* 2009; 29: 869–874.
273. Krishnan E. Interaction of inflammation, hyperuricemia, and the prevalence of hypertension among adults free of metabolic syndrome: NHANES 2009-2010. *J Am Heart Assoc*. 2014; 3(2): e000157.

274. Medina G, Vera-Lastra O, Peralta-Amaro AL, Jiménez-Arellano MP, Saavedra MA, Cruz-Domínguez MP, Jara LJ. Metabolic syndrome, autoimmunity and rheumatic diseases. *Pharmacol Res.* 2018; 133: 277-288
275. Fraile JM, Torres RJ, de Miguel ME, Martínez P, Lundelin KJ, Vázquez JJ, Puig JG. Metabolic syndrome characteristics in gout patients. *Nucleosides Nucleotides Nucleic Acids*. 2010; 29(4-6): 325-9.
276. Bonakdaran S, Kharaqani B. Association of serum uric acid and metabolic syndrome in type 2 diabetes. *Curr Diabetes Rev*. 2014; 10(2): 113.
277. Zapolski T, Waciński P, Kondracki B, Rychta E, Buraczyńska MJ, Wysokiński A. Uric acid as a link between renal dysfunction and both pro-inflammatory and prothrombotic state in patients with metabolic syndrome and coronary artery disease. *Kardiol Pol.* 2011; 69(4): 319-26.
278. Reina-Bueno M, Vázquez-Bautista MDC, Pérez-García S, Rosende-Bautista C, Sáez-Díaz A, Munuera-Martínez PV. Effectiveness of custom-made foot orthoses in patients with rheumatoid arthritis: a randomized controlled trial. *Clin Rehabil*. 2018; 1-9.
279. Waldecker U. Metatarsalgia in hallux valgus deformity: a pedographic analysis. *Journal of Foot and Ankle Surgery* 2002; 41: 300–8.
280. Menz HB, Fotoohabadi MR, Munteanu SE, Zammit GV, Gilheany MF. Plantar pressures and relative lesser metatarsal lengths in older people with and without forefoot pain. *Journal of Orthopaedic Research* 2013; 31: 427–33.
281. Lane TJ, Landorf KB, Bonanno DR, Raspovic A, Menz HB. Effects of shoe sole hardness on plantar pressure and comfort in older people with forefoot pain. *Gait & Posture* 2014; 39: 247–251.
282. Lee H, Grosse R, Ranganath R, Ng AY. Convolutional deep belief networks for scalable unsupervised learning of hierarchical representation, *International Conference on Machine Learning* 2009; 77-85.
283. Sivapalan S, Chen D, Denman S, Sridharan S, Fookes C, Gait energy volumes and frontal gait recognition using depth images, *IEEE International Joint Conference on Biometrics* 2011; 501-506.
284. Zheng S, Huang K, Tan T. Evaluation framework on translation-invariant representation for cumulative foot pressure image. *The 18th IEEE International Conference on Image Processing (ICIP)* 2011; 201-204.
285. Zheng S, Huang K, Tan T, Tao D. A cascade fusion scheme for gait and cumulative foot pressure image recognition. *Pattern Recognition* 2012, 45: 3603-3610.
286. Felzenszwalb PF, Girshick RB, McAllester D, Ramanan D. Object detection with discriminatively trained part-based models. *IEEE Transactions on Pattern Analysis and Machine Intelligence* 2010; 32: 1627-1645.
287. Bouchrika I, Nixon M. Model-based feature extraction for gait analysis and recognition, *International Conference on Computer Vision/Computer Graphics Collaboration Techniques*, 2007; 150-160.

288. Zhang T, Li X, Tao D, Yang J. Multimodal biometrics using geometry preserving projections. *Pattern Recognition* 2008; 41: 805-813
289. http://www.rsscan.co.uk/, accessed at 03/10/2016.
290. Shan C, Gong S, McOwan PW. Fusing gait and face cues for human gender recognition, *Neurocomputing* 2008; 71: 1931-1940.
291. Costea M, Vasilescu AM, Hortal G, Mihai A. Plantar footprints analysis - case study (part 2). *Leather and Footwear Journal* 2014; 14(4): 243-250.
292. Mihai A, Pastina M. Classification of foot types, based on plantar footprint, Proceedings of "The 4th International Conference on Advanced Materials and Systems", *ICAMS, Bucuresti,* 2012; 347-352.
293. Hillstrom HJ, Song J, Kraszewski AP, et al. Foot type biomechanics part 1: structure and function of the asymptomatic foot. *Gait Posture.* 2012; 37(3): 445-51.
294. Borman P, Ayhan F, Tuncay F, Sahin M. Foot problems in a group of patients with rheumatoid arthritis: an unmet need for foot care. Open Rheumatol J. 2012;6:290-5.
295. Williams AE, Bowden AP. Meeting the challenge for foot health in rheumatic diseases. *The Foot* 2004; 14: 154–158.
296. Sarghie B, Costea M, Liute D. Anthropometric study of the foot using 3D scanning method and statistical analysis. *Procceding of ISKA Symposium-Textiles of the Future*, Iaşi, România, 2013; 428-433.
297. www.ibm.com/software/analytics/spss.
298. Malureanu G, Mihai A. Bazele proiectarii incaltamintei, *Performantica Publishing House*, Iasi, 2003.
299. Nácher B, Alemany S, González J, Alcántara E et al. A Footwear Fit Classification Model Based on Anthropometric Data. *SAE Technical Paper* 2006; 2006-01-2356.
300. Leikin JB. Foot disorders in the elderly: A mini-review. *Dis Mon.* 2018; 64(3): 63.
301. Menz H, Lord S. Foot problems, functional impairment, and falls in older people. *J Am Podiatr Med Assoc* 1999; 89(9): 458–467.
302. Menz HB. Biomechanics of the Ageing Foot and Ankle: A Mini-Review. *Gerontology.* 2015; 61(4): 381-8.