GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

RISK FACTORS AND COMORBIDITIES IN CARDIOVASCULAR PATHOLOGY

- Habilitation Thesis -

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CONTENTS

REZUMAT

Teza de abilitare cu titlul **"Factorii de risc cardiovascular şi comorbidităţile în patologia cardiovasculară"** sumarizează cele mai importante realizări ştiinţifice, profesionale şi academice ce au fost finalizate după obţinerea titlului de Doctor în medicină (din 2001 și până în prezent) centrate pe 3 direcții importante de cercetare:

1 Cercetări privind bolile cardiovasculare prin prisma factorilor de risc cardiovasculari.

2. Cercetări privind bolile cardiovasculare prin prisma interrelațiilor cu factori genetici și biochimici.

3. Cercetări privind bolile cardiovasculare prin prisma comorbidităților.

Teza este alcătuită conform recomandărilor Consiliul Naţional de Atestare a Titlurilor, Diplomelor si Certificatelor Universitare (CNATDCU) din **trei secțiuni principale,** începând cu dezvoltarea ştiinţifică, profesională şi academică, subliniind contribuţia personală în domeniul de cercetare al patologiei cardiovasculare, urmată de planurile pentru dezvoltarea profesională şi ştiinţifică viitoare şi lista completă de referinţe bibliografice.

Secțiunea I prezintă realizările științifice din perioada postdoctorală;

Secțiunea II prezintă evoluția și planurile de dezvoltare viitoare;

Secțiunea III este alcătuită din lista referințelor bibliografice utilizate în teză.

Cele 3 secțiuni sunt precedate de o sinteză a întregii mele cariere, cu prezentarea principalelor realizări profesionale, academice și științifice, punând accent pe baza teoretică şi abilităţile practice care îmi conferă capacitatea de a coordona echipe de cercetare, de a organiza şi manageria procesul de predare, de a explica şi facilita procesul de învăţare şi cercetare.

Lucrarea oferă o imagine de ansamblu a activității pe care am desfășurat-o în domeniul cardiologiei și medicinei interne, în cadrul Universității de Medicină și Farmacie "Grigore T. Popa" Iași.

De –a lungul carierei mele profesionale activitatea mea clinică şi academică au interferat și s-au dezvoltat în paralel și pe mai multe planuri.

În dezvoltarea carierei mele medicale am avut drept scop îmbunătăţirea continuă a cunoştinţelor şi abilităţilor practice medicale cât şi perfecționarea pregătirii mele cu ultimile noutăți și progrese din domeniul medicinii. Faptul că dețin 2 specialități (inițial de medicină internă și ulterior de cardiologie) mi-a permis o perspectivă mult mai largă asupra pacientului și o abordare mult mai complexă a patologiei cardiovasculare. Astfel, actualmente sunt medic primar de medicină internă şi cardiologie și dețin competență în ecografia generală.

Deși îmi desfășor activitatea într-un serviciu de Cardiologie, am rămas în continuare legată și de prima specialitate – cea de medicină internă motiv pentru care am încercat în toată cariera mea să fiu la curent cu tot ce este nou și în acest domeniu și să integrez Cardiologia în domeniul larg oferit de medicina internă, fapt care se reflectă și în alegerea titlului și subiectului acestei teze de abilitare.

În cadrul sintezei care precede Secțiunea I am prezentat ideile principale de cercetare care au stat la baza tezei de doctorat cu titlul " **TRATAMENTUL HIPERTENSIUNII ARTERIALE CU INHIBITORI AI**

ENZIMEI DE CONVERSIE A ANGIOTENSINEI.", susținută în anul 2001 și am trecut în revistă granturile și proiectele obținute prin competitie, colaborările științifice și tematica de cercetare aborbată în cadrul unor echipe interdisciplinare.

Au fost trecute în revistă proiectele de educație medicală continuă în care m-am implicat în calitate de coordonator și lector, activitatea de predare la studenți și rezidenți, activitățile desfășurate în cadrul comunității academice și a societăților profesionale din care fac parte, precum și principalele publicații.

A fost subliniată importanța activității de cercetare și a realizării de publicații în decursul întregii mele cariere, dintre care menționez un număr de 29 articole cotate în Thomson ISI Web of Science Core Collection (dintre care 19 ca autor principal, 10 articole coautor) și ISI proceedings indexate în Thomson ISI Web of Science Core Collection (11 rezumate) și articole indexate în alte baze de date internaționale (68 articole, 42 BDI dintre care 33 ca autor principal), 20 cărți ca autor unic, 1 carte coordonator, 13 cărți colectiv de autori, un număr de 37 capitole de carte în calitate de autor sau coautor, în 20 cărți, dintre care 2 capitole în cărți internaționale (coautor) un număr de 106 citări în Thomson ISI Web of Science Core Collection și un Hirsch-index de 6.

La sfârșitul acestei secțiuni au fost prezentate elementele vizibilității naționale și internaționale ale activității mele, sub forma publicării de cărți, capitole de cărți (inclusiv coautor la 2 capitole publicate în tratate internaționale), participării la congrese naționale și internaționale, activității în cadrul granturilor obținute prin competiție (1 ca director și 4 ca membru în echipa proiectelor), implicării în activități de cercetare efectuate în colaborare cu echipe de cercetare din Universitatea de Medicină și Farmacie "Grigore |T. Popa" Iași, publicării de articole în reviste incluse în bazele de date Thomson ISI și în alte baze de date internaționale și citării lor, conducând la un Hirsch-index de 6 în Thomson ISI Web of Science Core Collection.

Următoarele capitole detaliază contribuţia mea originală în domeniul abordării patologiei cardiovasculare de la cercetarea fundamentală la cea clinică, şi furnizează lista de articole ştiinţifice şi abstracte publicate pe care este bazată contribuţia originală.

In prima Secțiune a tezei de abilitare intitulată "**Realizări științifice din perioada postdoctorală**" am ilustrat publicațiile acumulate în perioada postdoctorală în cadrul a 3 teme principale de studiu :

1 Cercetări privind bolile cardiovasculare prin prisma factorilor de risc cardiovasculari. Implicații clinice.

2. Cercetări privind bolile cardiovasculare prin prisma interrelațiilor biochimice / genetice. Implicații clinice.

3. Cercetări privind bolile cardiovasculare prin prisma comorbidităților. Implicații clinice.

În cadrul **primului subcapitol al Secțiunii I** am luat în discuție unii factori de risc cardiovascular, interacțiunile și o parte din implicațiile lor în patologia cardiovasculară.

Al doilea subcapitol al Secțiunii I aduce în discuție abordarea patologiei cardiovasculare în interrelație cu factorii biochimici și genetici implicați în apariția acestei patologii insistând pe implicațiile clinice ale cercetării acestora.

Al treilea subcapitol al Secțiunii I se referă la abordarea patologiei cardiovasculare prin prisma comorbidităților asociate, fapt cu implicații clinice deosebite în abordarea și managementul complex al pacientului cu boală cardiovasculară.

Cea mai mare parte a Secțiunii I este alcătuită din rezultatele publicate din tematica de cercetare în cadrul a 12 lucrări cotate ISI Thomson, 10 lucrări indexate în baze de date internaționale o parte acestea prezentând rezultatele grantului de cercetare obtinut prin competiție.

Secțiunea II a tezei de abilitare descrie planul de dezvoltare în **domeniul profesional, academic și de cercetare** pentru următorii ani, în care mi-am propus să pun accentul pe formarea unei echipe de cercetare multidisciplinare, pe dezvoltarea bazei logistice și pe creșterea gradului de diseminare și vizibilitate a rezultatelor cercetării. Îmi doresc să abordez noi subiecte de cercetare clinică pe care să le pot prezenta în competițiile naționale și internaționale de proiecte științifice, antrenând participarea tinerilor medici și doctoranzi.

Ca proiecte de viitor îmi propun să continui activitatea mea folosind aceleași valori care m-au ghidat de-a lungul întregii mele cariere și care, consider eu sunt esențiale pentru cariera didactică și cea de cercetare – dorința de continuă perfecționare, onestitatea, comunicarea, lucrul în echipă, interesul pentru cercetare și diseminarea de informații.

Activitatea de cercetare se va concentra spre abordarea mai multor subiecte de interes. Astfel, pentru a-mi continua activitatea de cercetare în viitor, intenționez să fac mai eficientă interacțiunea profesională dintre colaborările actuale și viitoare, între capacitatea mea de a coordona cercetările originale și nevoia de a supraveghea tinerii studenți și doctoranzi, respectând normele actuale privind cercetarea clinică și experimentală.

Din punctul de vedere al temelor abordate, voi lua în considerare continuarea principalelor subiecte dezbătute până acum, dar și dezvoltarea de noi teme de cercetare.

• Datorită faptului că domeniile mele de interes sunt foarte extinse (după cum o demonstrează munca depusă și colaborarea până acum), unul dintre obiectivele mele principale este de a continua cercetările începute cu scopul de a identifica noi aspecte și implicit rezultate care ar putea fi bazate pe dezvoltarea de noi lucrări științifice. În acest sens, colaborarea ulterioară prin organizarea de întâlniri periodice cu cei implicați în cercetarea menționată mai sus, și anume Departamentul de Genetică, Laboratorul de Imunologie, Disciplina de Gastroenterologie, sunt esențiale pentru evaluarea stării actuale a cercetării ca început punct pentru cercetări viitoare.

• Mai mult, doresc să valorific rezultatele granturilor de cercetare la care am participat fie ca director, fie ca membru în scopul realizării de lucrări științifice și eventual extinderea colaborării cu specialitățile conexe. O arie de interes pentru cercetarea viitoare este de a colabora cu Cardiologia Intervențională pentru a extinde cercetarea inițiată în Grant pentru a detecta rezistența la clopidogrel la pacienții care au fost stentați și implicit demonstrarea faptului că această rezistență este una din cauzele trombozei precoce intrastent . Extinderea cercetării asupra

pacienților cu ciroză hepatică ar putea avea o implicare practică, ajutând la identificarea pacienților cu risc de sângerare sau tromboză postangioplastie.
Intenționez să extind tema de cercetare a factorilor de risc cardiovascular prin încercarea de a analiza în continuare implicarea acestora în bolile sistemice și, pe de altă parte, să demonstrez relația dintre acești factori și anumite mecanisme genetice, precum și implicarea acestora în diferite comorbidități cum ar fi spre exemplu boala arterială periferică.
O altă direcție de cercetare e reprezentată de noii biomarkeri utilizați în aprecierea prognosticului pacienților cu insuficiență cardiacă care asociază și alte comorbidități mai ales a celor cu boală hepatică în stadiu avansat.
Având certitudinea că munca în echipă este esențială pentru succesul în cercetare și dezvoltarea carierei, doresc să sprijin consolidarea unor echipe de cercetare prin atragerea de tineri entuziaști.
De la începutul carierei mele universitare am fost mereu implicată în procesul de predare și de mentorat al tinerilor medici, atât în activitatea clinică precum şi în cea de cercetare. Cu experiența acumulată anterior ca director de grant, investigator/subinvestigator în diferite studii clinice și cu experiența acumulată de-a lungul a mulţi ani de formare a tinerilor medici, cred cu tărie că în calitate de conducător de doctorat voi avea posibilitatea și capacitatea de a transfera abilitățile mele, cunoștințele și entuziasmul meu celor tineri pentru a-i ajuta să atingă obiectivul final - cel de a obține titlul de Doctor în ştiinţe medicale.
În plus, planul de dezvoltare a carierei viitoare se va baza pe o cât mai eficientă armonizare între cele 3 sectoare de activitate prezentate, urmărind integrarea în eforturile comunității academice de menținere și creștere a prestigiului universității noastre, instituție în care mi-am desfășurat neintrerupt activitatea în ultimii 29 de ani.

Secțiunea III include o listă a referințelor bibliografice utilizate pe parcursul prezentării tezei de abilitare.

ABSTRACT

The Habilitation thesis entitled **"Risk factors and comorbidities in cardiovascular pathology"** summarizes the most important scientific, professional and academic achievements that have been finalized after obtaining the title of Doctor in Medicine (since 2001) centered on 3 important research directions:

1. Researches regarding cardiovascular diseases in terms of risk factors.

2.Researches regarding cardiovascular diseases in terms of biochemical and genetics interrelations data.

3. Researches regarding cardiovascular diseases in terms of comorbidities.

The thesis was elaborated in accordance with the recommendations of The National Council for the Attestation of University Titles, Diplomas and Certificates (CNATDCU) in three main sections starting with scientific, professional and academic development, underlining the personal contribution in the field of cardiovascular pathology, followed by the plans for the professional and scientific development and full list of bibliographic references.

Section I presents the scientific achievements of the post-doctoral period;

Section II is devoted to evolution and future development plans;

Section III is made up of the list of bibliographic references used in the thesis.

The three sections are preceded by a synthesis of my entire career, with the presentation of the main professional, academic and scientific achievements, focusing on theoretical and practical skills that give me the ability to coordinate research teams, organize and manage the teaching process, to explain and facilitate the process of learning and research.

The paper provides an overview of my work in the field of cardiology and internal medicine at the "Grigore T. Popa" University of Medicine and Pharmacy Iasi.

Throughout my professional career, my clinical and academic work has interfered and developed in parallel and on several levels.

In developing my medical career, I was aiming to continuously improve my medical knowledge and skills and to refine my training with the latest advances and progresses in medicine. Having two specialties (originally for internal medicine and subsequently for cardiology) I had a much wider perspective on the patient and a much more complex approach to cardiovascular pathology. Thus, I am currently a primary physician in internal medicine and cardiology and I own a competence in general echography.

Although I'm working in a Cardiology department, I am still linked to the first specialty of internal medicine, which is why I have tried throughout my career to be aware of everything new in this field and to integrate Cardiology into the broad field offered by internal medicine, fact which is also reflected in the choice of the title and subject of this habilitation thesis.

In the overview preceding Section I, I presented the main research ideas underlying the thesis **"TREATMENT OF ARTERIAL HYPERTENSION WITH INHIBITORS OF**

ANGIOTENSYN CONVERTING ENZYMES", sustained in 2001 and I reviewed the grants and projects obtained through competition, the scientific collaborations and the research theme approached within interdisciplinary teams.

In brief, I have reviewed the continuing medical education projects in which I have been involved as a coordinator and lecturer, teaching activity for students and residents, activities carried out within the academic community and their professional societies, also the main publications.

It has been highlighted the importance of research and publishing throughout my career, of which I mention a total of 29 articles listed in the Thomson ISI Web of Science Core Collection (of which 19 as principal author, 10 co-author articles) and ISI proceedings indexed in Thomson ISI Web of Science Core Collection (11 abstracts) and articles indexed in other international databases (68 articles, 42 BDI of which 33 as principal author), 20 books as sole author, 1 coordinator book, 13 collective books authors, a total of 37 chapters as author or coauthor in 20 books, of which 2 chapters in international books (co-author) a number of 106 citations in the Thomson ISI Web of Science Core Collection and a Hirsch index of 6.

At the end of this section, are presented the elements of the national and international visibility of my activity, such as the publication of books, chapters of books (including co-authors on 2 chapters published in international books), participation in national and international congresses, competition grant activities (1 as a director and 4 as a member of the project team), involvement in research activities carried out in collaboration with research teams from the University of Medicine and Pharmacy "Grigore | T. Popa "Iasi, publication of articles in magazines included in Thomson ISI databases and other international databases and their quoting, leading to a Hirsch-index of 6 in the Thomson ISI Web of Science Core Collection.

The following chapters detail my original contribution to cardiovascular pathology from fundamental to clinical research, and provide the list of published scientific and abstract articles on which the original contribution is based.

In **the first section** of the Habilitation thesis entitled **"Scientific achievements in the post-doctoral period"** I have illustrated the publications accumulated during the post-doctoral period in three main themes of study:

1. Researches regarding cardiovascular diseases in terms of risk factors. Clinical implications.

2. Researches regarding cardiovascular diseases in terms of Biochemical/genetics interrelations data. Clinical implications.

3. Researches regarding cardiovascular diseases in terms of comorbidities. Clinical implications.

In **the first subchapter of Section I**, some of the cardiovascular risk factors were discussed, their interactions and some of their implications in cardiovascular pathology.

The second subchapter of Section I brings into discussion the approach of cardiovascular diseases in relation to the biochemical and genetic factors involved in the occurrence of this pathology underlying on the practical implications of their research.

The third subchapter of Section I deals with the approach of cardiovascular pathology in terms of associated comorbidities, with special clinical implications in the complex approach and management of the patient with cardiovascular disease.

Most of Section I consists of the results published in the research topic in 12 ISI Thomson papers, 10 papers indexed in international databases, some of them presenting the results of the research grant obtained through the competition.

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

As future projects, I intend to continue my work using the same values that have guided me throughout my career and which, I consider, are essential for the teaching and research career - the desire for continuous improvement, honesty, communication, teamwork, the interest for research and information dissemination.

The research will focus on addressing more topics of interest. Thus, in order to continue my research activity in the future, I plan to make more efficient the professional interaction between the current and future collaborations, between my ability to coordinate original researches and the need to supervise young students and PhD students, respecting current standards in clinical and experimental research.

From the point of view of the approached themes, I will consider the continuation of the main topics debated so far, but also the development of new research themes.

• As my areas of interest are very extensive (as evidenced by the work done and the collaboration so far), one of my main objectives is to continue the researches started with the aim of identifying new aspects and implicitly results that could be based on the development of new scientific papers. In this respect, further collaboration through the organization of regular meetings with those involved in the above-mentioned research, namely the Department of Genetics, the Hospital Immunology Laboratory, the Discipline of Gastroenterology, are essential for assessing the current state of research as a starting point for future research.

• Furthermore, I aim to capitalize on the results of the research grants that I have participated either as a director or as a member for the purpose of conducting scientific papers and possibly extending the collaboration with related specialties. One area of interest for future research collaboration is Interventional Cardiology, extending the research started in the Grant I was directing, in order to detect resistance to clopidogrel on patients who have been enroled and to demonstrate that this resistance is one of the causes of early stent thrombosis. Expanding the research on the patients with liver cirrhosis could have a practical implication, helping to identify patients at risk of bleeding or of early thrombosis and implicitly unfavorable postangioplasty evolution.

I intend to expand the research theme of cardiovascular risk factors by trying to further analyze the evolution of some risk factors in associated systemic diseases, and, on the other hand, to

establish some relationships between these factors and genetic mechanisms and their implications in different comorbidities such as lower extremity artery disease.

Another direction of research is represented by the new biomarkers used in assessing the prognosis of patients with heart failure that associate other comorbidities, especially those with advanced liver disease.

Being confident that teamwork is essential for success in research and career development, I want to support the strengthening of research teams by attracting young enthusiasts. Since the beginning of my university career, I have always been involved in the teaching and mentoring of young doctors, both in clinical and research activities.

With the previously gained experience as a grant manager, investigator / sub-investigator in various clinical trials and the acquired experience over many years of training young doctors, I strongly believe that as a Ph.D. supervisor I will have the opportunity and the ability to transfer my abilities, my knowledge and my enthusiasm to the young people, helping them to achieve the ultimate goal of getting the title of Doctor of Medical Sciences.

In addition, the future career development plan will be based on the most effective harmonization between the three areas of activity presented, aiming to integrate into the efforts of the academic community to maintain and increase the prestige of our university, an institution in which I carried out my activity in the last 29 years.

Section III includes a list of bibliographic references used during the elaboration of the Habilitation thesis.

OVERVIEW OF PERSONAL PROFESSIONAL, ACADEMIC AND SCIENTIFIC ACHIEVEMENTS

The habilitation thesis entitled **"The complex approach of cardiovascular pathology in terms of risk factors and comorbidities"** presents the three main directions of clinical research which I assumed after the completion of my doctoral studies. In 20 april 2001 I had the public defence of my PhD thesis entitled „**The treatment of arterial hypertension with angiotensin converting enzyme inhibitors**." having as **Scientific coordinator**: Prof.dr. Georgeta Datcu and in November 2001 I was awarded my PhD title in medicine which was confirmed by the Ministry of Education (Diploma No. 4911/02.11.2001 The doctoral thesis has basically marked the beginning of my research activity opening up new possibilities for deepening in the field of cardiovascular diseases. Two principal points have validated my PhD thesis:

- a complex approach of arterial hypertension regarded as a cardiovascular risk factor and its involvement in the occurrence of other cardiovascular diseases based on noninvasive cardiovascular investigations; the clinical research was performed on a batch of patients in the cardiology clinic and was based mainly on the echocardiographic assessment of left ventricular function in order to establish some possible correlations between the evolution of left ventricular hypertrophy (as an independent risk factor) and the antihypertensive medication;

- secondly, I have acquired good practice skill in finding, harmonizing and coordinating a research team.

Having this background, I had the opportunity to continue the clinical research in three main directions that will be detailed in the habilitation thesis with their conceptualization and the related papers published in peer-reviewed journals. Clinical activity in the field of internal medicine and cardiology made me interested in studying some traditional risk factors and their implications in cardiovascular pathology and also allowed me the approach of cardiovascular pathology in the context of other comorbidities. In some published papers I emphasized the complex relationship which has developed between some cardiovascular risk factors and cardiovascular pathology and also I was interested in identifying some genetic and biochemical implications in cardiovascular pathology.

Throughout my career, I have been permanently interested to integrate the research activities with teaching, academic and professional development. Currently, I am associate professor at the Internal Medicine Department, Faculty of Medicine, Iasi "Grigore T. Popa" University of Medicine and Pharmacy. At the same time, I am working as senior physician in the field of cardiology and internal medicine at the Cardiology Clinic in St Spiridon Emergency Hospital.

Academic and Professional activity

I graduated from the Iasi "Grigore T. Popa" University of Medicine and Pharmacy in 1990 (Diploma Serie I nr 79/elib nr 75/24 01 1991) and the next year I was admitted as Substitute Professor's Assistant at the Discipline of Internal Medicine.

I got my professional experience working in the field of internal medicine and then in cardiology and I became a specialist in internal medicine in 1994 and Senior Consultant in Internal Medicine in 1998 (Certificate serie P1 No. 010205). In 1998 I became a specialist in cardiology as a second specialty and Senior Consultant in cardiology in 2003 (Certificate serie P1 No. 010204).

During my hospital activity I was interested in continuing medical education by enrolling and completing complementary studies: General Ultrasonography (Certificate of Competence serie C No. 001705/may 1999) General Echocardiography Course – "Diagnosis and Evaluation of Cardiovascular Diseases through eco M mode, 2D and Doppler and Doppler in color flow " at the Heart Institute Cluj, 01.02 - 05.03 1999 / graduated with general average 9.75. Certificate no. 1224/05 03 and other postgraduating courses in the field of cardiology.

Gradually, I developed skills in the majority of noninvasive cardiovascular diagnostic methods which I use in my daily practice: ABPM and Holter monitoring, effort test, echocardiography, general echography.

Between 2001 -2006 and from 2013 till present I am Coordinator of the USTACC Intensive Care Unit. This fact enabled me to improve in the field of emergency cardiology and to familiarize myself with the most complex clinical cases.

In the last years, a new area of interest was represented by the evaluation of patients with end stage liver cirrhosis. The majority of patients were on a waiting list for liver transplantation. Also, I contributed to the assessment and management of a large number of patients with different non – cardiovascular disorders. From clinical practice, I extended this collaboration to the organization of educational courses and scientific activity, fact which is reflected in the elaborated works and monographies.

I am proud to state that I belong to the cardiology school, initiated and developed by Professor Constantin Negoiță, a remarkable personality of Romanian medicine, to which generations of physicians and teachers owe him complete and complex professional training. He has managed, through his personal example, to outline the qualities of a complete medical personality that has served me as the first model I have always tried to achieve: excellent professional training and continual improvement, permanent attention to medical information but without neglecting the importance of the evidence gathered through personal experience, the knowledge of foreign languages that opens up new horizons, didactic talent and special human qualities reflected in the behavior and devotion to the patient.

Although the methods of academic evaluation are becoming more and more complex, the principle of transferring the performance of a good teacher to his/her student, permanently aware of his

chance and, above all, of his multiple responsibilities is always at the basis of successful teaching. In addition, medical education is characterized by special requirements, starting from the need for well-equipped laboratories and ending with meeting the ethical principles related to involving patients in clinical teaching.

In 1990, immediately after graduating from the Faculty of Medicine I had the opportunity to start a teaching career and I obtained by contest the position of Substitute Professor"s Assistant (1991-1994) at the Discipline of Internal medicine, Faculty of Medicine of "Grigore T. Popa" University of Medicine and Pharmacy. Since then I have occupied the following positions at the same discipline of the Iasi "Grigore T. Popa" University of Medicine: Assistant professor (1994 -2002), Lecturer (2002-2015) and Associate professor (2015 and present). I completed my didactic training by acquiring foreign language competence (English and French level B2). Since 2014 I teach the series from French section. I have enlarged my teaching activity by creating courses and internships for the residents in the specialties of cardiology, internal medicine but also from other specialties (taking into account the interdisciplinary path of cardiology). From 2005 until now I have participated as a lecturer at the postgraduate course of General ultrasonography offered by "Grigore T. Popa" University of Medicine and Pharmacy in order to obtain the Competence Certificate in General Ultrasonography.

My didactic activity has two main components through the thematic and approach specificities: **work with students and postgraduates (with resident doctors and postgraduate courses).**

I have been constantly involved in coordinating the teaching and scientific activities of residents and students by my tutor position for many series of students, coordinating and supervising some original papers presented at student congresses or dedicated local conference sections, organizing echocardiography workshops and presentations of clinical cases dedicated to medical students and residents.

Teaching with students starts from the premise that any career development plan for a university professional should maintain the student as a central element. My didactic activity in all these years included: Courses / practical internships / seminars, for 3rd and 4th year General Medicine students (Romanian and French section); I have been directing Students with papers / posters as part of Student Circle Activity and with participation in Student Conferences (National and International), including the French Teaching Series, some of these works being awarded.

Annually, from April 2015 to May 2018 I have participated as lecturer at the Workshops organized in CONGRESSIS on various topics related to emergency echocardiography one of them being "Echocardiography in normal conditions and in emergency".

Daily collaboration with French language students has led me to improve my communication skills, teamwork, gained during my teaching experience and at the same time adapting to different multicultural levels.

Having as main goal the education of the students in the spirit of developing a competent medical thinking, I have constantly tried to systematize the theoretical notions and to update the knowledge with medical news from the literature. Also, I have always sought to combine the theoretical and practical notions. Another thing I set out to do and realized was the adaptation of the subject matter

to the profile and needs of each series of students proof being the elaboration of textbooks and monographs adapted to each study formations.
Concerning my didactic activity with resident doctors, both as a specialist physician (1994 and 1998) and later as senior consultant in internal medicine (1999) and cardiology (2003) I was involved in coordinating the practical work of the residents. I directly contributed to the theoretical and practical training of resident physicians, both formal, supporting theoretical lectures and case reports, as well as informally, through the concrete daily discussions about patients especially in the Treatment Unit of Cardiac Critical Patients (USTACC) whose coordinator's I am currently.

Scientific activity

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

In scientific research, I approached interdisciplinary topics, aiming to offer my expertise in noninvasive clinical and subclinical assessment of some parameters of cardiovascular function or in the recognition of various aspects of cardiovascular risk especially in patients with different comorbidities.

As mentioned, scientific research has materialized in **3 main directions.**

The **first direction is reflected first, in my doctoral thesis** and the published results of the other two will be presented below, in the chapters of the habilitation thesis.

In my doctoral thesis I have approached the first direction of my research activity. The subject of the doctoral thesis refers to the investigation of the complex effects of angiotensin converting enzyme inhibitors in hypertensive patients, besides the decrease or the normalization of blood pressure values, in view of the fact that high blood pressure (HBP) is one of the major risk factors for subsequent cardiovascular damage. Effective control of blood pressure values is a first step in reducing cardiovascular risk by reducing morphological consequences of HBP, assessed by echocardiographic issues of left ventricular hypertrophy in hypertensive patients and the evolution of these issues after ACE inhibitors treatments. A number of 11 full-text articles, 21 abstracts were published and 5 were communicated at national and international congresses.

The assessment of some aspects of cardiovascular risk was a natural choice considering my activity in a cardiology department. Also, given my professional evolution in the field of cardiology, I had the opportunity to explore a significant number of cases with associated diseases especially hepatic disorders and to capitalize on some of these results in the field of scientific research and academic development. Some of the complex clinical cases assessed by me in collaboration with colleagues from other specialties were presented at scientific meetings or were published full-text in specialty journals.

Training in the field of scientific research is a long-term mission. So far, I have sought the way for improvement by finding collaborative relationships with researchers in different Departments related with this aim I have participated to:

- "Managing a Horizon 2020 Project" Seminar organized under the Project "Transnational Actions to Support Successful Participation in the EU Framework Program for Research and Innovation ORIZONT 2020 act HORIZ", Iasi, 12-13 October 2016.

- "How to Write a Successful Orizont 2010" Seminar organized under the project "Transnational Actions to Support Successful Participation in the EU Framework Program for Research and Innovation ORIZONT 2020 actHORIZ", Iasi, 10-11 oct 2016.

- in may 2017 I obtain the Certificate of Good Clinical Practice awarded by NIDA Clinical Coordinating Center (valability until 11 may 2020). This training has been funded in whole or in part with Federal funds from the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN27201201000024C.

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

The research activity has resulted in my participation in a number of clinical trials:

As the main investigator:

EPICOR - Long Term Follow-up of Antithrombotic Management Patterns in Acute Coronary Syndromes Patients. (2010 -2011) and the Pioneer study (Epidemiological study in heart failure patient with elevated heart rate) - 2012.

As sub-investigator (Principal Investigator Prof. Dr. Datcu MD).

EPHESUS trial (A double blind, randomized, placebo-controlled trial evaluating the safety and efficacy of Eplerenone in patients with heart failure following acute myocardial infarction). Dates: 1999-2002;

EPLA trial (open label extension study evaluating the safety of Eplerenone in patients with heart failure). Dates: 2003-2006;

PREAMI (Perindopril and remodeling after acute myocardial infarction). Period of implementation - 2002-2003;

MICHELANGELO OASIS 6 (Fondaparinux in acute myocardial infarction). Period of deployment - 2003-2004;

PALO 0809 - Multicenter Phase IV Open Label Uncontrolled Study to Assess the Efficacy and Safety of a Single Intravenous Dose of Palonosetron 0.25 mg Prevention of Chemotherapy Induced Nausea and Vomiting in Patients with Non-Hodgkin's Lymphomas Undergoing Repeated Cycles

of Moderately Emetogenic Chemotherapies. Protocol No. PALO 0809. Principal Investigator Dr. Dănăilă Cătălin, Hematology Clinic (2009).

PIONEER study (Epidemiological study in heart failure patients), sponsored by Servier Pharma SRL (2013).

The experience gained in these studies was extremely useful to me later and was reflected in the participation in 4 grants both as GRANT DIRECTOR and as a member as follows:

NOVEMBER 2011 - Internship GRANT won through UMF "Gr T Popa" Iasi with the title " ELABORATION OF AN INTEGRATED MODEL OF ANALYSIS OF RESISTANCE TO CLOPIDOGREL BY GENOTIPATION OF CYP2C19 CITOCROMES AND GENE ABCB1 "Grant Director - Irina Iuliana Costache. Contract number: 28214 / 16.12.2011, Internal Grants Competition 2011 of the University of Medicine and Pharmacy "Gr. T. Popa "Iasi. Period: 2011-2012, duration 12 months. Funding sources: "Grigore T. Popa" University of Medicine and Pharmacy Iasi. Funding amount: EUR 5000.

I was member of the following research grants and projects:

1) Research Grant Teams Ideas - Competition 2012 "Circulating cytocrome c, cardiac biomarkers and heart contractile function during and after cardiopulmonary resuscitation", Contract number: 19852 / 28.09 .2012, Research Grants Teams Ideas - Competition 2012. Period: 2013-2014, 16 months. Project Director: Dr. Antoniu Petriş, Head of Works. Project role: Sub-Investigator. Funding sources: "Grigore T. Popa" University of Medicine and Pharmacy Iasi. Funding amount: EUR 9000. Main results or published patents: ongoing.

2) Member of the Project Research Team "Complex characterization of active cytostatic extracts from Claviceps purpurea strains obtained by parasyxual hybrid biotechnology for use in veterinary therapeutics" under Program 4 - "Partnerships in Priority Areas" 2007-2013. - Between BIOLOGICAL RESEARCH INSTITUTE IAŞI and UMF "Gr. T. POPA "IAŞI. Grant Contract for Project Implementation No. 62065 / 2008. Partner Responsible: Prof dr Hâncianu Monica. Project role: Sub-Investigator.

3) Member of the ERASMUS + Project entitled "Massive open online course for palliative clinical and intercultural and multilingual medical communication" ongoing in 2014-2017; coordinator of UMF "Gr.T.Popa" Iasi, Project manager: Dr. Petriş Ovidiu Rusalim, Head of Works, Contract no: 2014-1-RO01-KA203-002940. Project role: Sub-Investigator.

4) Member of the national research team within the project titled ”Rationing Missed Nursing care : An International and multidimensional problem” – Project COST Action CA 15208 in progress during the period 2016-2020.

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

the opportunity to familiarize myself with the requirements for writing article and publication criteria as I was admitted in the Editorial board (for cardiology section) of the Annals of Vascular Medicine and Research (JSciMed Central Peer-reviewed Open Access Journals) and also I am peer reviewer for Journal of Advances in Medicine and Medical Research.

The scientific portfolio currently includes: a number of 29 articles rated by Thomson ISI Web of Science Core Collection (of which 19 as principal author), articles and ISI proceedings indexed by Thomson ISI Web of Science Core Collection (of which 11 as principal author) and 42 articles indexed by other international databases (of which 33 as principal author). As a result of national and international visibility, these articles were cited, with a total of 106 citations in the Thomson ISI Web of Science Core Collection and a Hirsch-index of 6.

Moreover, one of my articles were rewarded at the annual competition organized by UEFISCDI (Executive Agency for Higher Education, Research, Development and Innovation Funding) as follows:

UEFSCDI-2014 Prize (Prize for Research Results 2014 articles) : Ana Clara Aprotosoaie, Monica Hăncianu, **Irina-Iuliana Costache** , Anca Miron: Linalool: a review on a key odorant molecule with valuable biological properties, published in Flavour and flagrance Journal, 2014, 29 (4), 193–219. IF 1,82.

http://uefiscdi.gov.ro/userfiles/file/PREMIERE_ARTICOLE/ARTICOLE%202014/LISTA%206%20REZULTATE.pdf

In 2018 - First Prise Section ”Rapid fire” The 57th National Congres of Cardiologiy, Sinaia 2018 with the presentation: ”Heart fatty acid binding protein (HFABP) level in patients with chronic liver disease and rehabilitation treatment after coronary artery by pass grafting”. Authors: R. Al Namat, O. Mitu, MG Felea**, I. Costache**, V. Aursulesei, A. Petriș, AM Apostol, D. Al Namat, N. Al Namat, F.Mitu.

Scientific activity was completed by the publication of textbooks and books. Thus, I contributed to updating the specialty-focused information necessary for the training of students and residents of the Faculty of Medicine by publishing books and also monographies as follows: **34 books: 20 books main author, 13 books co –author, 1 book – coordinator, 37 chapters in 20 different publications, 2 chapters in international books.**

Connecting my research with academic activity, I was interested from the beginning of my career to join professional organizations, in my country and abroad, in the field of cardiology and internal medicine.

Actually, I am member of numerous scientific societies :

Member of the European Society of Cardiology .

Member of the Romanian Society of Cardiology.

Member of the Working Group of Emergency Cardiology from Romanian Society of Cardiology and Ischemic Cardiopathy Working group

Member of the Romanian Society of Bioethics .

Member of Society of Medicins and Naturalists from Iași, - Department of Internal Medicine and Cardiology.

Participation in congresses and conferences was a constant concern for me. Every year I have participated at the National Congress of Cardiology at Sinaia – Romania, with oral presentations/ original papers, clinical cases and posters. As a member of the scientific or organizational committees I have contributed to the organization of conferences organized under the aegis of the Iasi "Grigore T. Popa "University of Medicine and Pharmacy. For many years I have been a speaker at conferences, congresses or medical continuing education courses organized by the Romanian Society of Cardiology.

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

- investigator in clinical trials with international participation;

- participation in continuing education and educational courses;

- improvement of teaching and student evaluation methods;

- participation in original clinical collaborative researches, with the publication of research results in journals indexed by Thomson ISI Web of Science Core Collection and presentation of papers at European congresses;

- engaging in research programs obtained by competition (1 as project manager, 3 as a team member);

- co-author of 2 chapters published in international textbooks;

- publication of articles indexed and rated in international databases: 29 indexed in Thomson ISI Web of Science Core Collection, (19 as principal author), 42 indexed in other international databases.

- 1 award-winning ISI articles in competitions organized by UEFISCDI;

- citations in international databases: 106 citations in Thomson ISI Web of Science Core Collection and a Hirsch-index of 6.

SECTION I.

SCIENTIFIC ACHIEVEMENTS FROM THE POSTDOCTORAL PERIOD

I.1. Researches regarding cardiovascular diseases in terms of risk factors. Clinical implications.

I.1.1 The term ”risk factors” in the global context of cardiovascular diseases

Cardiovascular disease (CVD) is regarded as a major cause of disability and premature death throughout the world, substantially contributing to the growing costs of health care. **Atherosclerosis** is the underlying pathology**,** developing over many years usually being advanced by the time symptoms occur, generally in middle aged patients. A **”risk factor**” is defined as a measurable characteristic, causally associated with increased disease frequency, ~~a~~ significantly independent as a predictor of an increased risk of the disease (Dawber, T.R et al 1951).

The term “risk factor” (RF) appeared in the 1960's when a high correlation between some population characteristics and the prevalence of clinical manifestations of ischaemic heart disease was epidemiologically suggested. At the origin of the term ”risk factor” is **The Framingham Heart Study**, whose objectives were to identify the common factors or characteristics that contribute to CVD by following its development over a long period of time in a large group of participants who had not yet developed overt symptoms of CVD or suffered a heart attack or stroke.

In the 1950s, atherosclerosis was considered a normal phenomenon of aging and occurred universally as people became older. Also seen as normal consequences of aging were high blood pressure and elevated serum cholesterol. These and further risk factors, e.g., homocysteine, were gradually discovered over the years (Kannel et al., 1976, Lloyd-Jones et al., 2001, Sundstrom et al., 2005, O’Donnell et al., 2008). A microorganism involved in respiratory infections, **Chlamydophila pneumonia**, was first associated with atherosclerosis and coronary heart diseases in 1986, and this bacteria is highly likely to be a modifiable risk factor that may be a target of future therapies (Blasi et al., 2009). The Framingham Heart Study, along with other important large studies, e.g., the Seven Countries Study, Nurses’ Health Study, Women’s Health Initiative, emphasized the importance of healthy diet and regular exercise in maintaining good health and **underlined the differences in cardiovascular risk between men and women** (Millen et al., 2001). **Cardiovascular disease (CVD) has been seen as a men’s disease for decades, although being more common in women. The effects of the major risk factors (RF) on CVD outcomes are accepted as being the same in both genders. Cardiovascular disease (CVD) represents the leading cause of mortality in both men and women** (Mosca, Barret-Connor, & Wenger, 2011).

Despite the fact that the prevalence of coronary heart disease (CHD) is higher in men, **the prevalence of stroke and the absolute annual number of CVD deaths are higher in women** (Mosca, Barret-Connor, & Wenger, 2011). However, **physicians tend to underestimate the cardiovascular risk in the female population (**Jneid & Thacker, 2001; Mikhail, 2005). Moreover, **in women, a delayed diagnosis of CHD occurs due to the frequently atypical manifestation of CHD** and the underuse of diagnostic procedures **result in** (Jneid & Thacker, 2001; Hvelplund et al., 2010). New, potentially **independent, CVD risk factors exclusive to women** have been recognized following new evidence (Appelman et al., 2015). Accelerated atherosclerosis and development of CVD are associated with **common disorders of pregnancy, such as gestational hypertension and diabetes, as well as some endocrine disorders in women of reproductive age and early menopause**. Early detection of women with a high risk of CVD might enable the identification of **female-specific risk factors**. With respect to female-specific RF only associations could be found between preeclampsia, gestational diabetes and menopause onset with the occurrence of CVD. These reviews show that CVD is the main cause of death in men and women; the prevalence being, however, higher in women (Tziomalos et al., 2011). **Differences in the impact of major CV risk factors leading to a worse outcome in women** should be taken into account when determining the CV risk profile**.** Lifestyle interventions and primary prevention in women needs more consideration (Appelman, et al., 2015). While men and women have similar risk factors for cardiovascular diseases, many social behaviors in low and middle income countries differ by gender. For example, **women who smoke have three times the risk of heart attacks and also an earlier onset than men and non-smoking women** (Bernabe-Ortiz et al., 2012).

It was also confirmed that **cigarette smoking is a highly significant factor in the development of heart disease,** leading to angina pectoris, myocardial infarction (MI), and coronary death (Freund et al., 1993, Doll et al., 2004).

The debilitating and often fatal complications of cardiovascular disease (CVD) are usually seen in middle-aged or elderly men and women. Atherosclerosis – the main pathological process leading to coronary artery disease, cerebral artery disease and peripheral artery disease – begins early in life, gradually progressing throughout adolescence and early adulthood usually being asymptomatic for a long period. **Cardiovascular risk factors influence the rate of progression of atherosclerosis:** smoking, an unhealthy diet and physical inactivity (which together result in obesity), arterial hypertension, dyslipidaemia and diabetes. Continuous exposure to these risk factors leads to the clinical manifestations of these diseases including angina, myocardial infarction, transient cerebral ischaemic attacks and strokes. Risk factors have a high predictibility for atherosclerosis and ischemic heart disease, although the relationship is not strictly causal. They indicate more a **"predisposition"** for the disease (Elwood et al., 2010).

The major findings of the Framingham Heart Study, according to the researchers themselves were: **(1)** In 1960 it was established that **cigarette smoking, increased cholesterol and elevated blood pressure** increase the risk of heart disease; they **were considered major risk factors for ischaemic heart disease**. Exercise decreases risk of heart disease, while obesity increases it; **(2)**

In 1970 it was established that elevated blood pressure increases the risk of stroke. **In postmenopausal women, the risk of heart disease is elevated, compared with premenopausal women. Psychosocial factors** were also involved as risk factors for heart disease. In 1980 it was established that high levels of HDL cholesterol reduce the risk of heart disease; **(3)** In 1990 **left ventricular hypertrophy was incriminated to increase risk of stroke, while elevated blood pressure can progress to heart failure.** Framingham Risk Score is published, and correctly predicts 10-year risk of future coronary heart disease (CHD) events. At 40 years of age, the lifetime risk for CHD is 50% for men and 33% for women (Murabito, 1995); **(4)** In 2000 – a new term – the so called **"high normal blood pressure"** was discovered to increase risk of cardiovascular disease (high normal blood pressure is defined as a systolic pressure of 120–139 mm Hg and/or a diastolic pressure of 80–89 mm Hg). Lifetime risk of developing elevated blood pressure is 90%. **Hypertension** is the most important risk factor for cardiovascular diseases such as stroke (Lopez et al., 2001), coronary artery disease (Van der Hoogen et al., 2000; Flack et al., 1995), end-stage renal disease (Klag et al., 1996; Tozawa et al., 2003) and heart failure (Levy et al., 1996). It is estimated that about 25% of the world's adult population have hypertension, and it will be likely that by 2025 it will increase to 29% (Mittal & Singh, 2010). In Europe, an estimated 37%–55% of the adult population is affected by hypertension (Wolf-Maier et al., 2003; Firmann et al., 2008). **The prevalence of hypertension is even higher in some developing countries.** According to the analyzed results of 86% of the subjects enrolled into the SEPHAR III study, initiated by the Romanian Hypertension Society, 49.5% of the Romanians aged between 18 and 80 suffer from hypertension (Dorobantu et al., 2008, 2016). The same study showed that the hypertension prevalence in the Romanian adult population was on the rise, compared to the 40.4% incidence recorded following a 2012 SEPHAR study (Dorobantu et al., 2012, 2016). In Bucharest, preliminary data showed arterial hypertension prevalence beyond the national average of 50.6 %. Regionally, prevalence over the national coverage have been recorded in the Western (58.3%), South-Western (53.2%), Bucharest-Ilfov (51.9%) and North-Westerns (50.7%) areas, according to SEPHAR III preliminary results. On the other hand, areas with lower prevalence are the Central (47.4%), Southern (46.7%), North-Eastern (46.6%) and South-Eastern (44.3%). (Dorobantu et al., 2016). The importance of these data is connected to the fact that high blood pressure (HBP) is the main risk factor of cardiovascular diseases, the cause of most deaths, not only in Romania, but also world-wide. Regarding its impact on CVD, high blood pressure is linked to 62% of the deaths recorded in our country, which in the year 2014 reached 2.547.912. The onset of arterial hypertension is determined, mostly, by factors which depend on the patient's lifestyle (Dima-Cozma & Cozma, 2012), which could be changed.

Obesity is also considered to be a risk factor for heart failure. Predictors for the risk of elevated blood pressure are the serum aldosterone levels. Lifetime risk for obesity is approximately 50%. **Some genes increase risk of atrial fibrillation.** The Busselton Health Study has been carried out in a high proportion of the residents of Busselton since 1966, a town in Western Australia, over a period of many years. The Busselton Health Study investigated the influence of some factors that had not been investigated in the Framingham Heart Study, e.g., **sleep**

apnea (Knuiman et al., 1997, Marshall et al., 2009). The Caerphilly Heart Disease Study, also known as the Caerphilly Prospective Study (CaPS), is an epidemiological prospective cohort, set up in 1979 in a representative population sample from a small town in South Wales, UK. The study has led to over 400 publications in the medical press, through the collecting of wide ranging data, notably on vascular disease (The Caerphilly and Speedwell Collaborative Group, 1984, Elwood et al., 2013). Multidisciplinary research has identified **three major risk factors: hypertension, dyslipidemia and smoking**. Cardiovascular risk factors may be **modifiable** (ie those factors that can be modified by diet or pharmacological measures, such as smoking, dyslipidemia, hypertension, obesity, diabetes) or **nonmodifiable** (age, sex, genetic factors). A population-based study conducted more recently in 2004 in 52 countries, 5 continents (the INTERHEART study) led to the identification of **nine risk factors involved in the onset of acute myocardial infarction: smoking, dyslipidemia, hypertension, diabetes, obesity diet, physical inactivity, alcohol consumption, psychosocial factors.** A number of risk factors are involved in the occurrence of other cardiovascular disease, aside from coronary heart disease**: chronic alcoholism is associated with increased incidence of dilated cardiomyopathy and arrhythmias** (Annand et al., 2008).

Additional factors such as **stress, psychosocial factors, or inflammation** are becoming increasingly common as well. Framingham risk scoring system includes age, total cholesterol, HDL-cholesterol, smoking, systolic blood pressure, and gender. Based on this score, patients are divided into three categories: **low risk, less than 10%, intermediate risk, between 10-20%, and high risk, over 20%.** In practice, an improvement in risk assessment may be obtained by associating these scores with the measurement of **new biomarkers** (Upadhyay, 2015).

I.1.2.Why is important to identify risk factors?

Cardiovascular disease (CVD) is the primary cause of death in many developing countries, with the coronary heart disease (CHD) being the most preventable form of CVD. In the United States, CHD annually results in 502,000 deaths, of which 185,000 are due to myocardial infarction (MI); economic burden is $133 billion (Tamam, 2014). The American Health Association policy statement concluded that, by the year 2030, costs will rise to more than $1 trillion annually in the United States and, therefore, preventive measures are required (Weintraub et al., 2011). By the year 2020, CHD is estimated to become the leading cause of death and disability worldwide. Evidence show that preventive measures may hinder atherosclerotic disease and its consequences. Primordial prevention usually refers to healthy lifestyle choices in order to prevent the development of coronary risk factors (Strasser, 1978). A review which examined 55 trials that intended to reduce multiple risk factors suggested that intervention results in small reductions in risk factors (blood pressure, cholesterol, and smoking), but has little or no impact on the risk of CHD mortality or morbidity (Ebrahim et al., 2011). This suggests that especially in developing countries a different approach to behavior change is needed. **Global cardiovascular risk assessment represents an important goal to be achieved not only in individuals with overt clinical manifestations of atherosclerotic disease, but also in healthy individuals**. In order to

establish both primary and secondary preventive measures which may lead to decreased morbidity and mortality and reduced hospitalization costs, early detection of risk factors is important. In a much broader sense, risk factors include characteristics of lifestyle, certain biochemical and physiological characteristics and some individual modifiable factors (Mahmood et al., 2014).

Total CVD risk is defined as the probability of an individual's experiencing a CVD event (e.g. myocardial infarction or stroke) over a given period of time, for example 10 years. Total CVD risk depends on the individual's particular risk factor profile, sex and age; it will be higher for older men with several risk factors than for younger women with few risk factors. The total risk of developing cardiovascular disease is determined by the combined effect of cardiovascular risk factors, which commonly coexist and act cumulatively. Compared to someone with just one elevated risk factor, an individual with several mildly raised risk factors may be at a higher total risk of CVD (according to World Health Organisation - WHO 2007).

The knowlegde and the modification of risk factors has been shown to reduce mortality and morbidity in people with diagnosed or undiagnosed cardiovascular disease.

So, **the prevention of cardiovascular disease (primary, secondary and tertiary prevention) is necessary and must be an important tool in addressing any patient.**

I.1.3. Clinical implications of risk factors identification.

It is important to know and also **to recognize cardiovascular risk factors** and also CVD **in order to help primary care providers to prevent and optimize the care for patients with CVD.** Chronic diseases such as CVD are the result of complex interactions between genetic and environmental factors over extended periods of time.

Epidemiological methods allow us **to estimate individual risk** according to the level of exposure to different risk factors included in a mathematical function.

The study provides evidence-based recommendations on **how to assess and manage individuals with asymptomatic atherosclerosis,** on the basis of their estimated total, or absolute, CVD risk.

Timely and **sustained lifestyle interventions** and, when needed, **drug treatment** will reduce the risk of CVD events, such as heart attacks and strokes, in people with a high total risk of CVD, and hence will **reduce premature morbidity, mortality and disability**.

The predicted risk of an individual can be a useful guide for **making clinical decisions** on the intensity of preventive interventions: when dietary advice should be strict and specific, when suggestions for physical activity should be intensified and individualized, and when and which drugs should be prescribed to control risk factors (WHO 2007).

*

In the next paragraphs, I shall present the main researches I have participated in during the post-doctoral period on some risk factors, their interactions and impact on current cardiovascular diseases.

I.1.4. The concerns regarding the risk factors were materialized in the following publications:

ISI Articles

1. Irina Iuliana Costache, Egidia Miftode, Ovidiu Petris, Alina Delia Popa, Dan Iliescu, Eosefina Gina Botnariu. **Associations between area of residence and cardiovascular risk.** Revista de cercetare și intervenție socială, 2015;49: 68-79. **IF = 1,141.**

2. Irina Iuliana Costache, Egidia Miftode, Ovidiu Mitu, Viviana Aursulesei: **Sex differences in cardiovascular risk factors in a rural community from north romania region**, Revista de Cercetare și Intervenție Socială 2016; 55: 204-214. **IF= 0,424.**

3. Irina Iuliana Costache, Egidia Miftode, Ovidiu Mitu, Alexandru Dan Costache, Viviana Aursulesei: **Arterial hypertension prevalence in a Romanian rural community: correlations with social and economic status, age and gender,** Revista de Cercetare și Intervenție Socială 2017;59:62-74. **IF = 0,380**.

Starting from the theoretical premises previously stated, the present studies **aimed to:**

1) identify cardiovascular risk factors and their correlation with atherosclerotic cardiovascular disease in a rural community in Romania.

2) evaluate the gender differences with specific cardiovascular risk factors in a rural community of the North Romanian Region and also **to evaluate the pathological associations that would increase the cardiovascular and cardiometabolic risks in patients with cumulation of risk factors, differently in the two genders** and, finally**, to establish the influence of lifestyle on the incidence of risk factors in the studied population and their comorbidities.**

The secondary objectives of this second study were:

a. Creating a cross-sectional study in a rural population of Suceava county in order to determine the prevalence of cardiovascular risk factors and their different profile at the two genders;

b. Performing a study of the same rural population allowing complete characterization of the people with cardiovascular risk factors in terms of cardiovascular and cardiometabolic comorbidity profile and establish gender differences in the two inputs;

c. Evaluating the role of parameters related to lifestyle on the occurrence of some cardiovascular risk factors and comorbidities, analyzed separately in the two genders.

3) estimate the prevalence of hypertension among adults (n = 2659) in a rural community of the North Romanian Region and **to identify the risk factors involved in hypertension**.

Material and method

All three studies were conducted in a rural community of Suceava county, being chosen as representative in terms of number of inhabitants, population stability, uniformity in educational, religious and ethnic features. An important element was the ongoing collaboration with local healthcare professionals and their willingness to actively participate in data collection.

The study was conducted in collaboration with a General Practitioner (GP) from Suceava County, in Patrauti who provided all necessary data on the basis of existing medical records.

The exclusion criteria in this study were: previous personal history of cerebrovascular diseases (stroke, acute coronary syndrome), patients younger than 18 years old, patient refusal, the presence of psychiatric, cognitive or other disease that might alter the understanding of the study objectives or the ability to provide accurate information. Those who did not complete the interview were recorded as having refused to participate and were taken into account when calculating the refusal rate, but excluded from the final analysis.

The three studies included a number of patients ranging from 285- 2659 aged between 26 - 92 years. The rural community where the research was conducted has a population of 4,567 inhabitants, with relatively equal distribution on the two genders (2,262 males and 2,305 females). The distribution by age groups is as follows: between 18-65 years – 2,344 people (51.32%), of whom 1,203 males (26.34%) and 1,141 females (24.98%); over 65 years = 833 inhabitants (18.23%), 527 males (11.53%) , 306 females (6,7%). The number of inhabitants in the records of the family doctor who conducted collaboration is 2659 people, of whom 1244 males (46.78%) and 1415 females (53.21%), with the following distribution by age: between 18-65 years = 1457 (54.79%) , 826 females (31.06%), 631 males (23.73%), between 65-90 years = 304 (11.43%) , 153 females (5.75%), 151 males (5.67%) and over 90 years = 8 (0.30%), 6 females (0.22%), 2 males (0.08%). Population under 18 was not included in the study. For each participant an individual file

was completed (by GP) which included: age, gender, elements of lifestyle (smoking status, physical activity undertaken, drinking, type of diet), pathological personal history, anthropometric measures (weight, height , waist circumference, body mass index), systolic and diastolic blood pressure, and biochemical parameters (fasting plasma glucose, total cholesterol, HDL cholesterol, fasting serum triglycerides).

1) The first study group consisted in 364 patients, aged between 26 - 92 years from rural and urban area, followed by general practitioners.

Descriptive statistics was used to calculate the average and standard deviation of assessed parameters. The data didn't have Gaussian distribution so the nonparametric statistical tests were used. For all analyses a p value < 0.05 was considered significant; all were undertaken using the Statistical Package for Social Science (SPSS) program for Windows Version 13.0 (SPSS 13.0, Chicago, IL, USA).

The traditional risk factors for cardiovascular disease were assessed. A standardized questionnaire provided information on age, area of residence, smoking status and the number of cigarettes smoked on a daily basis. Weight, height and blood pressure were measured in duplicated and recorded according to the standard protocol. In order to determine cardiovascular risk scores plasma cholesterol levels, HDLc, LDLc, and glycaemia were determined. The presence of diabetes was considered for those patients with history of diabetes or in the presence of 2 values of glycaemia higher than 126 mg/dl.

Risk scores were calculated by using the University of Edinburgh Risk Calculator. Risk scores are for estimating the probability of cardiovascular disease for individuals who have not already developed any major atherosclerotic disease. This calculator was originally developed to provide online charts based on the design of the well known Joint British Societies (JBS) Cardiovascular Disease Risk Prediction Charts, developed by The University of Manchester, and published in the British National Formulary (BNF). The calculator can produce risk scores based on the following scores: Framingham, Joint British Societies (JBS) / British National Formulary (BNF), ASSIGN. Time period to calculate risk over can be varied between 4 and 12 years for any of the Framingham calculations, but it is fixed at 10 years for the BNF or ASSIGN scores. **The main parameters we used were: age, gender, smoking, number of cigarettes/day, family history of diabetes, Scottish Index of Multiple Deprivation (SIMD) which was considered as value of 20, systolic arterial blood pressure, total cholesterol and HDL (mmol/l). We used the Edinburgh Risk Calculator to determine the probability to develop a myocardial infarction, cerebral stroke and death in next 10 years** (Costache et al, 2015).

2) The second study group consisted of 285 patients aged between 26 - 92 years which were assessed for the main cardiovascular risk factors; 187 (65.6%) of them were females (Costache et al, 2016).

3) The third study was conducted on the inhabitants from the records of the family doctor who conducted collaboration: 2659 people, of whom 1244 males (46.78%) and 1415 females (53.21%). The patients fulfilled the defined diagnostic criteria for hypertension: systolic pressure 140 mmHg and/or diastolic pressure 90 mmHg, or have been diagnosed with hypertension or have taken antihypertensive drugs within two weeks. Patients with secondary hypertension were excluded. Blood pressure (BP) was recorded in a sitting and orthostatic position using the same device. Every measurement was taken after five minutes of resting quietly. If this reading did not indicate hypertension then it was accepted, however, if it did indicate hypertension then a second reading was taken and this reading was reported for the purpose of the study. Hypertension was defined as BP 140 mmHg systolic or 90 mmHg diastolic BP, in accordance with the Joint National Committee (JNC) V criteria (Frohlich, 1993). A higher cut-off of BP 160 mmHg systolic or 100 mmHg diastolic BP was used to identify those with severe hypertension. Finally the study group consisted of 285 patients aged between 26 - 92 years. It also was assessed the presence of traditional risk factors. The contribution of stress in cardiovascular disease is controversial, due to the improper definition of stress itself. The diagnosis of stress was established through case history investigation, out of which the existence of stressful situations was concluded, together with subjects' personality and lifestyle traits favorable for psychiatric stress. With the aid of the Family Doctor a self-evaluation questionnaire was composed, in order to evaluate stressful situations (existential situations which act as stressors), as well as subjects' personality traits (Costache et al, 2017).

Statistical analysis

Statistical analysis was performed using SPSS 20.0 software (Statistical Package for the Social Sciences, Chicago, Illinois). Data were expressed as mean ± standard deviation (SD) or number of cases with percentage, for continuous and ordinal variables. T-test was used for comparing the continuous variables and chisquare test for categorical comparisons. For all data, a two-sided p value < 0.05 was considered statistically significant.

Results

1) In the first analyzed group predominated women (n = 195; 61.1%) and people in rural areas (n = 228; 71.5%). It was assessed the presence of traditional risk factors. Thus 63 persons (19.7%) were smokers, 70 (21.9%) had one first degree relative with cerebrovascular disease and 55 (17.2%) were known to have diabetes. Smokers admitted to smoking an average of 11.05 ± 4.97 cigarettes/day. The mean value of systolic blood pressure was 130. 90 mmHg, total cholesterol of 3.39 mmol/ l, and HDLc 1.33 mmol/l. These parameters were assessed to estimate the risk of coronary heart disease, myocardial infarction, stroke and death due to cardiovascular disease over 10 years (Table 1.1). (Costache et al, 2015).

Table 1.1. Cardiovascular Risk Factors - descriptive characterization

	N	Minimum	Maximum	Mean	Std. Deviation
SBP	319	85	220	130.09	21.738
T-chol	318	2.1250	6.4300	3.394654	.7439428
HDLc	317	.5775	3.6500	1.333730	.4204228

SBP = Systolic Blood Pressure; T-chol = total cholesterol;HDLc = High Density Lipoprotein cholesterol

Using the University of Edinburgh Risk Calculator we noticed that in the study group, the probability to develop a myocardial infarction, in next 10 years, was 3.37%, and for cerebral stroke was 4.08%. The probability of death due to cardiovascular disease was 3.99% (Table 1. 2).

Table 1.2. The Risk of Cardiovascular Disease - descriptive data

	N	Minimum	Maximum	Mean	Std. Deviation
MI	317	.0099	24.7894	3.372604	4.2736717
STROKE	317	.2168	43.5244	4.080486	4.6289745
CVD	319	11.2022	40.8416	40.748682	1.6594873
CHD death	317	.0018	16.3834	1.757731	2.6533844
CVD death	317	.0236	37.2948	3.990897	5.5570532
BNF	317	.9221	61.8891	11.113396	9.6911638
ASSIGN	317	2.4295	90.6166	17.570963	14.5106022

MI = myocardial infarction; CVD = cardiovascular disease; CHD = coronary heart disease; BNF = British National Formulary; ASSIGN = ASSIGN definition- any cardiovascular death, CHD (ICD-9 410–414, ICD-10 I20-I25) including angioplasty and bypass grafting, cerebrovascular disease.

The risk of coronary heart disease (CHD) estimated by Framingham equation was significantly higher in urban than in rural (8.03% vs 6.6%; Std. Deviation 6.31; p = 0.034). **The risk of myocardial infarction (MI) was higher in urban than in rural**, too (4.5% vs 2.8%; Std. Deviation of 5.02; p = 0.002). There was no significant risk of stroke, cardiovascular disease, death due to coronary heart disease or death due to cardiovascular disease in urban or rural. The risk for cardiovascular disease estimated by ASSIGN equation was significantly higher in urban than in rural (19.4% vs. 12.9%; Std. Deviation 15.9; p = 0.002) (Table 1. 3). (Costache et al, 2015).

Table 1. 3. Assessment of Cardiovascular Risk Factors According to Aria of Residence

		N	Mean	Std. Deviation	95% Confidence Interval for Mean		p*
					Lower Bound	Upper Bound	
CHD	urban	91	8.033024	6.3188378	6.717062	9.348986	**.034**
	rural	226	6.630209	5.8517163	5.863167	7.397252	
MI	urban	91	4.590432	5.0230511	3.544331	5.636533	**.002**
	rural	226	2.882239	3.8363231	2.379374	3.385104	
STROKE	urban	91	3.592701	3.4451816	2.875208	4.310195	.331
	rural	226	4.276895	5.0210467	3.618737	4.935054	
CVD	urban	91	40.515888	3.1070524	39.868813	41.162963	.113
	rural	228	40.841596	.0000000	40.841596	40.841596	
CHD_DEATH	urban	91	1.721808	2.3134704	1.240004	2.203611	.705
	rural	226	1.772196	2.7832227	1.407371	2.137020	
CVD_DEATH	urban	91	2.800629	3.3392035	2.105206	3.496052	.057
	rural	226	4.470165	6.1730545	3.661001	5.279328	
BNF	urban	91	11.625725	9.1741489	9.715116	13.536334	.305
	rural	226	10.907104	9.9039703	9.608893	12.205316	
ASSIGN	urban	91	19.430564	15.9863413	17.335075	21.526052	.002
	rural	226	12.952614	8.3745706	11.208525	14.696703	

MI = myocardial infarction; CVD = cardiovascular disease; CHD = coronary heart disease; BNF = British National Formulary; ASSIGN = ASSIGN definition - any cardiovascular death, CHD (ICD-9 410–414, ICD-10 I20-I25) including angioplasty and bypass grafting, cerebrovascular disease.

2) In the second study the mean age of patients was 65.96 ± 11.93 years, with minimum age 26 and maximum age 92, 187 (65.6%) of them were females while the men group was represented by 98 (34.4%) subjects. More than half of individuals had normal weight, 14% were overweight and 28% were obese. 14.7% of population had type 2 diabetes mellitus and 13% were former and active smokers. The presence of chronic stress was reported by 27% of patients while 21% had a positive family history of CVD. 85% of patients were hypertensive, most of them being classified into classes 1 and 2 of high blood pressure. More than 50% of subjects presented, suspected or documented coronary artery disease while 12% had a documented stroke. Divided by gender, the cardiovascular risk factors presented important differences (Table 1.4). (Costache et al, 2016).

Tabel 1. 4. The presence of cardiovascular risk factors in the study population in differences by gender.

CV risk factor	**Total (n = 285)**	**Female (n = 187)**	**Male (n = 98)**	**p value**
Type 2 diabetes mellitus (%, no)	14.7 (42)	73.8 (31)	26.2 (11)	0.150
Obesity (%, no) • Normal weight • Overweight • Obesity grade I • Obesity grade II • Obesity grade III	57.2 (163) 14 (40) 13.7 (39) 11.9 (34) 3.2 (9)	35.8 9.1 10.2 8.1 2.5	21.4 4.9 3.5 3.9 0.7	0.618
Chronic stress (%, no)	27.7 (79)	70.9 (56)	29.1 (23)	0.154
Family history of CVD (%, no)	21.4 (61)	57.4 (35)	42.6 (26)	0.089
Arterial hypertension (%, no) • Normal blood pressure • Grade 1 • Grade 2 • Grade 3	14.4 (41) 38.2 (109) 37.5 (107) 9.8 (28)	7.7 24.6 24.9 8.4	6.7 13.7 12.6 1.4	0.052
Coronary artery disease (%, no)	59.6 (170)	63.5 (108)	36.5 (62)	0.220
Stroke (%, no)	11.9 (34)	70.6 (24)	29.4 (10)	0.328

Smoking status (%, no) • Non-smoker • Former smoker • Active smoker	86.3 (246) 7.0 (20) 6.7 (19)	

Almost all cardiovascular risk factors were more importantly represented in the female group: type 2 diabetes mellitus (73%), chronic stress (70%), family history of CVD (57%), suspected or documented coronary artery disease (63%) or stroke (70%). As well, the overweight and obesity predominance was higher in the female population but with no statistical difference. In women, 57.9% had arterial hypertension comparative to men where only 27.7% presented increased blood pressure, the statistical significance being almost insignificant (p = 0.052). Nonetheless, almost 98% of females were non-smokers, comparative to only 64% in men. 20% of men were former smokers and 15% were active smokers while only 2.1% of women smoked (p < 0.001) (Figure 1.1.). (Costache et al, 2016).

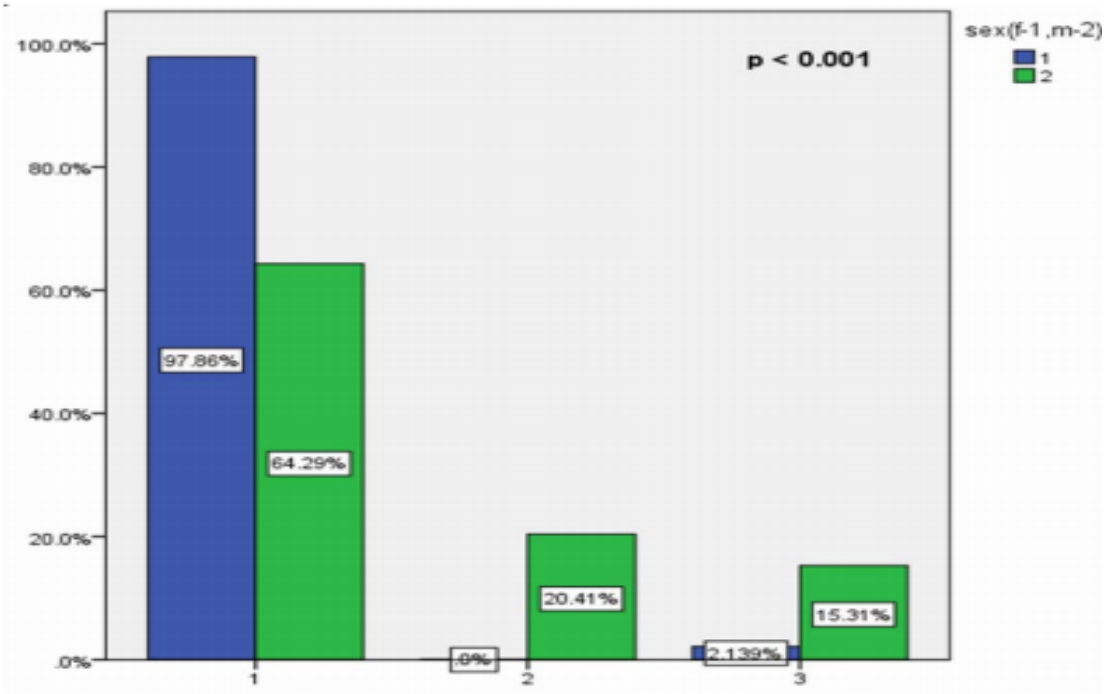


Figure 1.1. Smoking differences by gender (1 represents non-smokers; 2 – former smokers; 3 - active smokers)

Regarding biochemical markers, the patients involved in the study were dyslipidemic, both mean total cholesterol and triglyceride levels being over the superior values (208 mg/dl, respectively 156 mg/dl). However, HDL and LDL cholesterol were in normal ranges, while uric acid was high (5.83

mg/dl), maximum value being 9 mg/dl. In diabetic patients, the glycosylated hemoglobin, HbA1C had high values (average 8.04%) suggesting a bad control of diabetes in this specific region. Mean results can be found in Table 1.5. By analyzing biochemical markers by gender, women were significantly older than men in the study population (p = 0.013). **Total cholesterol, triglycerides and LDL cholesterol were rather similar between the two groups. However, HDL cholesterol was significantly decreased in the male group (49.52 ± 14.56 vs. 55.61 ± 16.86, p = 0.010). Even though the uric acid had higher values in men (6.83 ± 1.83 vs. 4.83 ± 1.94), the statistical significance was not reached (p = 0.09) probably due to the variation of values. The glycosylated hemoglobin, HbA1C presented close values after dividing by gender.** All biochemical results divided by gender can be also found in Table 1.5. (Costache et al, 2016).

Table 1.5. Biochemical values in the study population by gender

Parameter	Total (n = 285)	Minimum	Maximum	Female (n = 187)	Male (n = 98)	p value
Age (years)	65.96 ± 11.93	26	92	67.22 ± 11.61	63.55 ± 12.22	0.013
Total cholesterol (mg/dl)	208.62 ± 42.17	112	339	209.42 ± 39.85	207.43 ± 46.46	0.731
Triglycerides (mg/dl)	156.54 ± 99.27	39	895	152.21 ± 88.64	164.67 ± 116.71	0.316
HDL cholesterol (mg/dl)	53.50 ± 16.32	23	146	55.61 ± 16.86	49.52 ± 14.56	0.010
LDL cholesterol (mg/dl)	123.01 ± 36.48	29	232	124.38 ± 35.85	120.46 ± 37.76	0.469
Uric acid (mg/dl)	5.83 ± 2.08	3	9	4.83 ± 1.94	6.83 ± 1.83	0.097
Glycosylated hemoglobin (HbA1C) (%)	8.04 ± 2.56	4	15	8.29 ± 3.03	7.56 ± 1.33	0.399

The age-adjusted prevalence of obesity (BMI) was 13% and 35%, among men and women (p = 0.0003), respectively. The prevalence of abdominal obesity was 11% and 58% (p < 0.0001), and high WHR (men: >0.9, women: >0.85) was 51% and 73% (p = 0.002) for men and women respectively. **Women had 4.3 times greater odds of obesity** (95% CI: 1.9-10.1), 14.2 - fold **increased odds for abdominal adiposity** (95% CI: 5.8-34.6), and **2.8 times greater odds of high waist-hip ratio** (95% CI: 1.4-5.7), **compared to men**. **Women had more than three-fold greater odds of having metabolic syndrome (p = 0.001) compared to male counterparts, including abdominal obesity, low HDL-cholesterol, and high fasting blood glucose components.** In

contrast, **female participants had 50% lower odds of having hypertension, compared to men** (95% CI: 0.3-1.0). Among men, BMI and waist circumference were significantly correlated with blood pressure, triglycerides, total, LDL-, and HDL-cholesterol (BMI only), and fasting glucose; in contrast, **only blood pressure was positively associated with BMI and waist circumference in women.** (Costache et al, 2016).

3) The third study consists of 2659 patients from the same rural community of the North Romania, from which 285 persons have been included in the final analysis. The mean age of the study group was 65.96 ± 11.93 years, the age varying between 26 and 92 years. 98 persons (34.4%) were males. 42.8% of the persons were overweight or obese according to the WHO classification of obesity. 13.7% were smokers (6.7% active smokers and 7.0% former smokers) while 14.7% had type 2 diabetes mellitus. 21.4% presented family history of CVD and 27.7% reported increased levels of psychosomatic stress. 85.6% had increased blood pressure values, most of them having grade 1 or grade 2 of arterial hypertension (38.2% - grade 1, 37.5% - grade 2, respectively 9.8% - grade 3 arterial hypertension). Related to the total number of population, this represents 9.13%. Regarding biochemical values, the average values are above the superior reference values especially for total cholesterol (208.62 ± 42.17 mg %), LDL cholesterol (123.01 ± 36.48 mg %) and triglycerides (156.54 ± 99.27 mg %) (Costache et al, 2017).

By dividing the persons into 6 age categories, according to decades, **normal blood pressure values were more frequently seen in age extremities** (< 50 or > 80 years old) (Figure 1.2.). On the other hand, **most of the persons aged 50-69 had arterial hypertension grade 2 or 3 while those with age 70 – 79 presented rather similar percentages covering all classes of arterial hypertension and normal blood pressure (31.7%).** However, after dividing the blood pressure and decades by sex categories, the statistical significance has been lost (for women: $p = 0.191$; for men: $p = 0.293$). Nonetheless, **in the group 50 – 69 years, women had increased levels of arterial hypertension grade 2 and 3, while in the elderly (above 70 years) the blood pressure was rather normal in men's category ($p = 0.013$ between sex groups). A positive relationship was obtained between arterial hypertension and obesity** (Figure 1. 3). As the grade of arterial hypertension was increasing, the proportion of obese patients was higher ($p < 0.0001$). As well, more than 55% of patients with 3rd degree obesity had the most increased blood pressure values, **confirming the direct link between obesity and arterial hypertension**. (Costache et al, 2017).

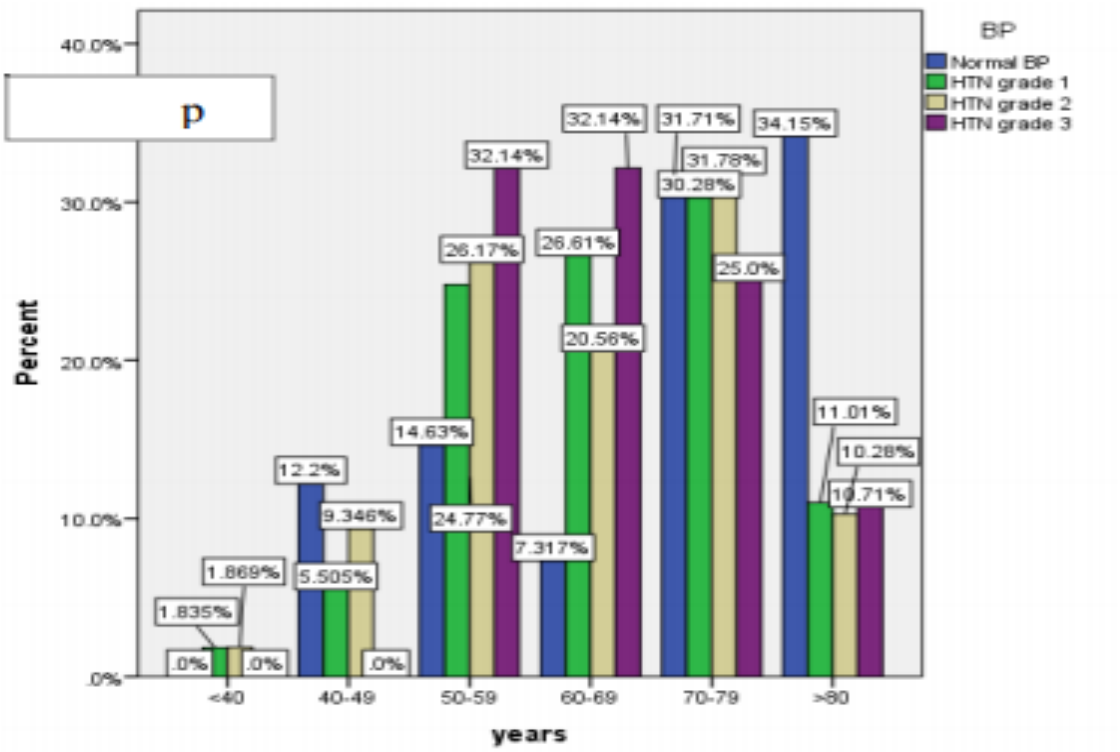


Figure 1.2. Arterial hypertension grades according to decades of age

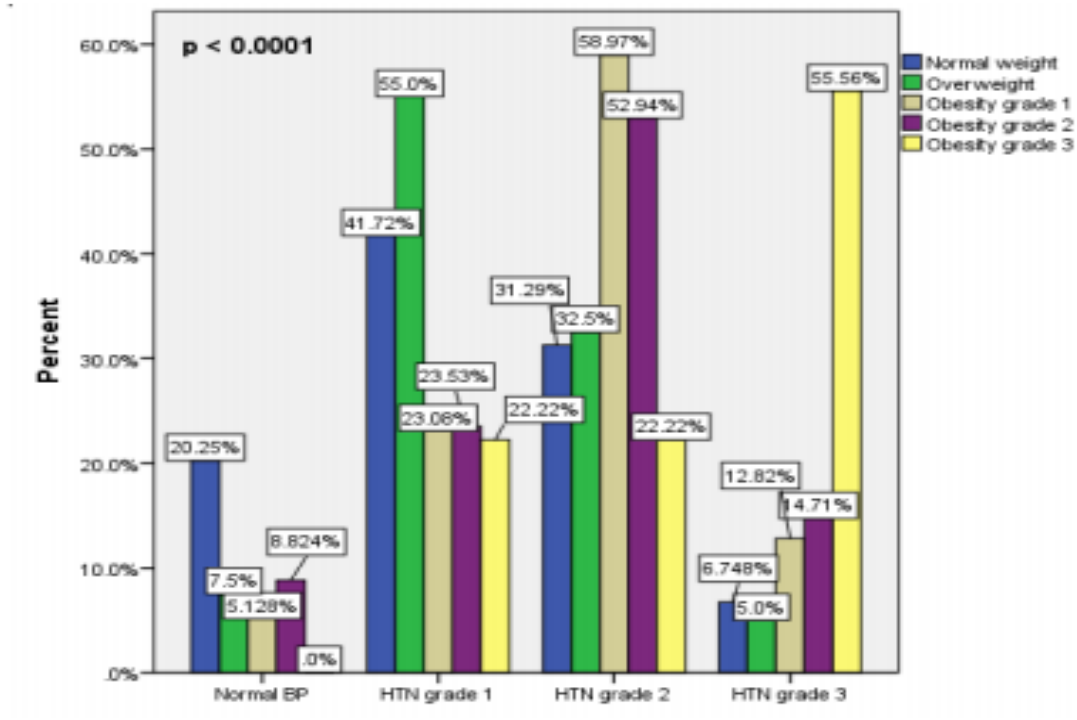


Figure 1.3. Relationship between arterial hypertension and obesity

There was **a direct correlation between arterial hypertension and type 2 diabetes mellitus** (Figure 1.4.). More than 71% of persons with diabetes had 2nd or 3rd degree arterial hypertension (p = 0.002) suggesting the powerful association between these two pathologies. As well, **most hypertensive patients had increased levels of stress,** especially in final stages of the arterial hypertension compared to normal blood pressure values where only 8.8% of persons presented stress (p = 0.025) (Figure 1. 5). In our study group, there was **no significant difference between arterial hypertension and smoking (p = 0.673) or family history of CVD (p = 0.094)** (Costache et al, 2017).

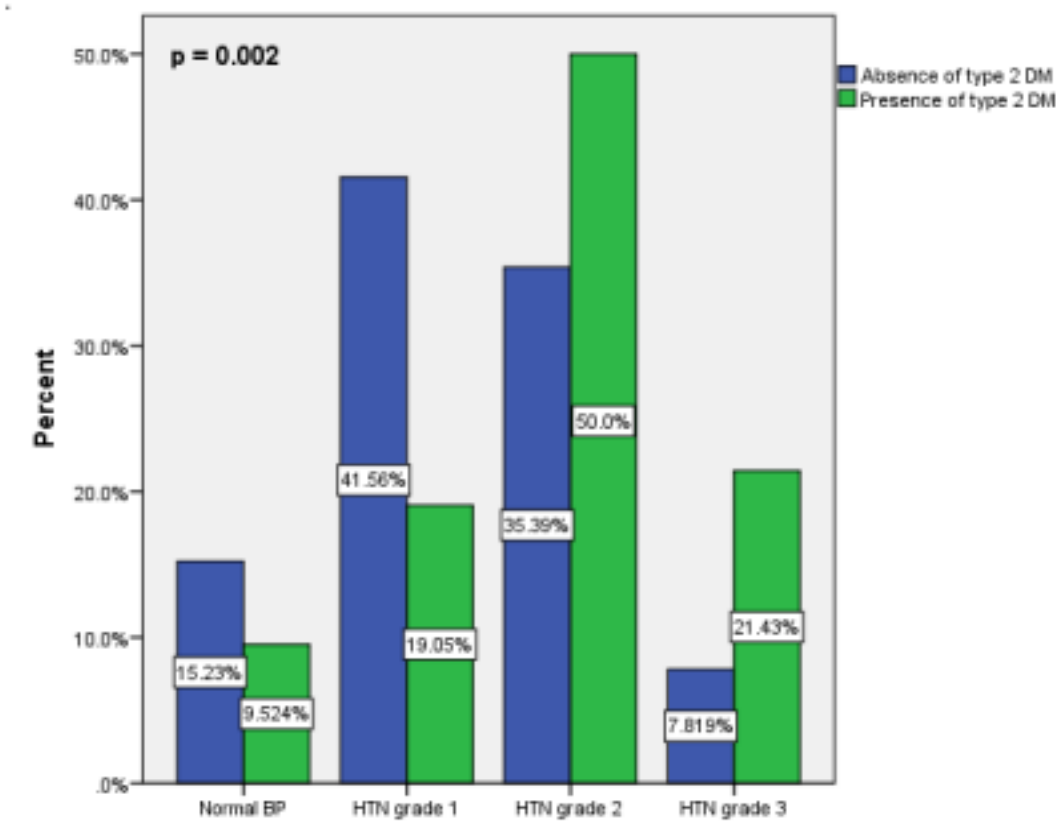


Figure 1.4. Relationship between arterial hypertension and type 2 of diabetes mellitus

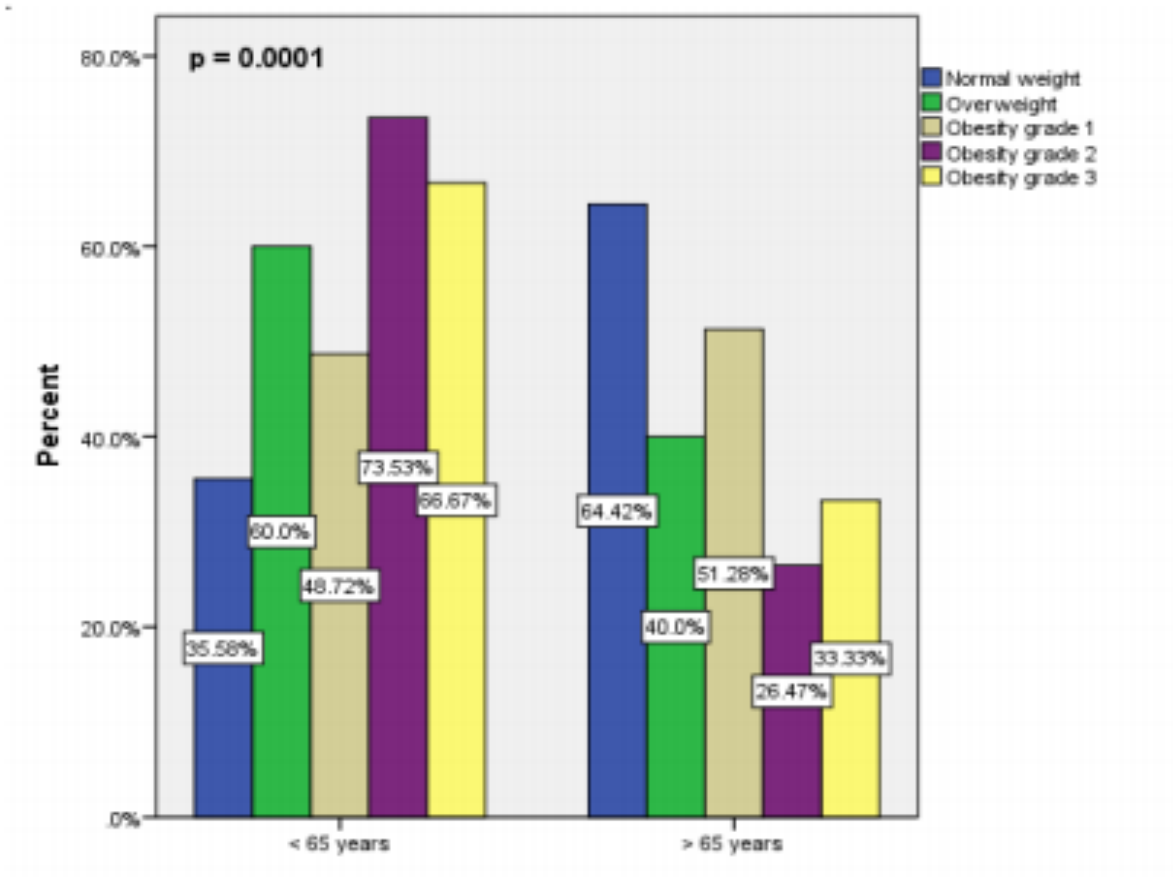


Figure 1.5. Relatioship between arterial hypertension and stress

Though **the values of lipid profile were higher as the grade of arterial hypertension was increasing, none of these reached the statistical significance,** HDL cholesterol and total cholesterol having the best associations (Table 1.6.). We continued to evaluate the study group referring to the working status and dividing the population into 2 subgroups: active – under the age of 65, respectively retired – over the age of 65. **Obesity was more frequently observed in persons younger than 65, especially 2nd and 3rd degree obesity, while more than 64% of normal weight subjects were retired (p = 0.0001)** (Figure 1. 6.). **Stress was more pronounced in the active population where 38.6% of persons declared that they were stressed compared to only 18.3% in the retired population (p = 0.0001).** There were no significant associations between age and type 2 diabetes mellitus (p = 0.154) or smoking (p = 0.135). Regarding biochemical values, total cholesterol and LDL cholesterol levels did not differ significantly between the two groups (Table 1.7.). However, the value of triglycerides was significantly increased in the active population (p = 0.0001) while HDL cholesterol was significantly decreased in the same group (p = 0.0001) showing marked dyslipidemia as the persons were active. (Costache et al, 2017).

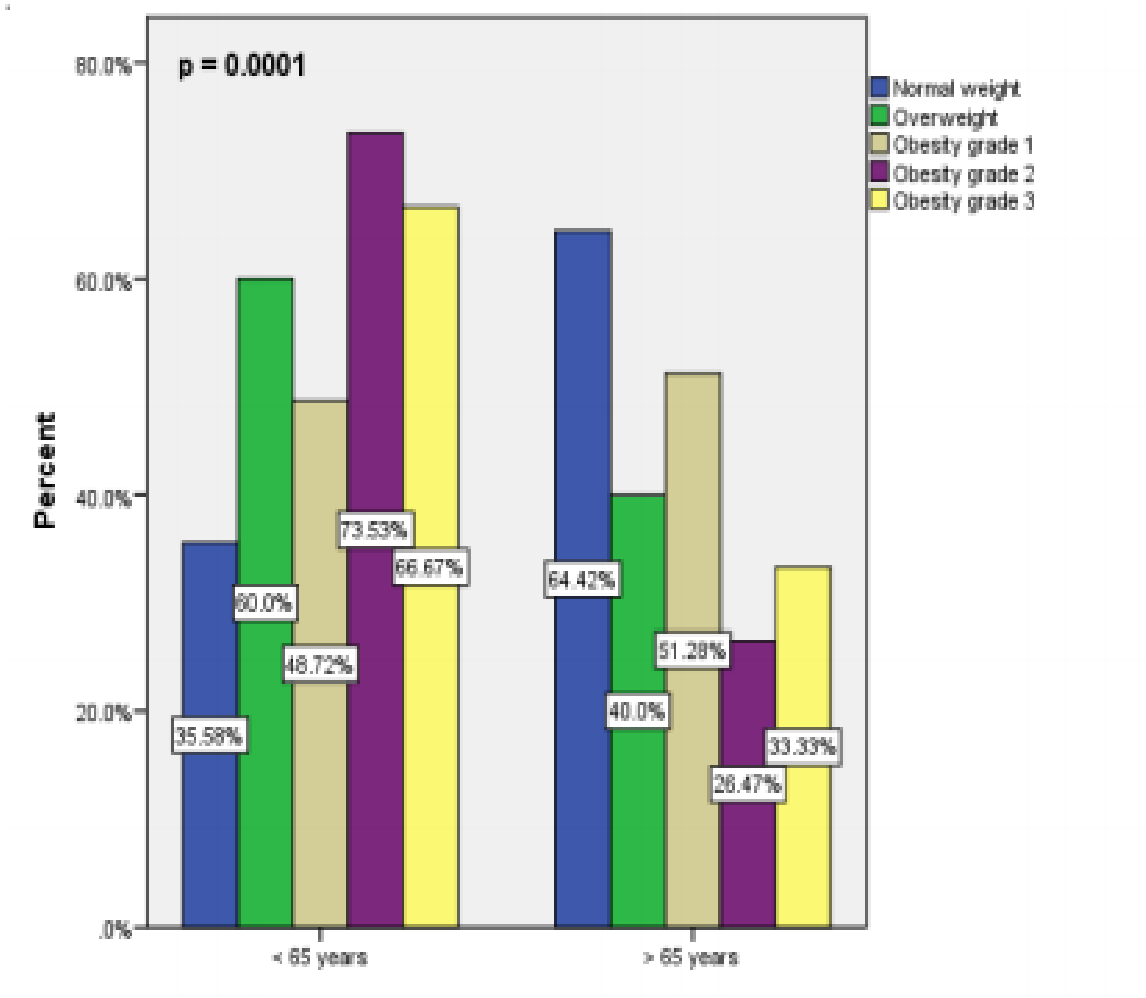


Figure 1.6. Relationship between obesity and age

Table 1.6. Association between lipid profile markers and arterial hypertension

Marker	**Normal BP**	**HTN grade 1**	**HTN grade 2**	**HTN grade 3**	**p value**
Total cholesterol (mg/dl)	194.59 ± 31.63	213.96 ± 41.27	207.94 ± 44.36	211.00 ± 47.82	0.093
Triglycerides (mg/dl)	138.02 ± 98.50	161.19 ± 105.48	157.57 ± 96.01	161.89 ± 88.41	0.627

HDL cholesterol (mg/dl)	60.07 ± 18.58	53.60 ± 15.01	52.56 ± 16.76	47.81 ± 13.70	0.056
LDL cholesterol (mg/dl)	110.71 ± 34.86	127.53 ± 34.97	121.47 ± 36.68	130.37 ± 41.30	0.156

BP = blood pressure; HTN = arterial hypertension

Tabel 1.7. Association between lipid profile markers and working status

Marker	**Active persons (< 65 years)**	**Retired persons (> 65 years)**	**p value**
Total cholesterol (mg/dl)	211.28 ± 44.36	206.33 ± 40.21	0.325
Triglycerides (mg/dl)	180.69 ± 120.86	135.89 ± 70.20	**0.0001**
HDL cholesterol (mg/dl)	48.71 ± 15.16	57.15 ± 16.30	**0.0001**
LDL cholesterol (mg/dl)	124.39 ± 35.82	122.03 ± 37.07	0.651

Urban rural differences may be, in part, due to diet and the stress of urban living. In our study in both males and females, age and high BMI were significant predictors of hypertension; which suggests that the main modifiable risk factor is obesity. Interestingly, stress was an independent predictor of hypertension in males. Although psychosocial stress has been implicated as a risk factor for hypertension in urban populations it has not been thought to be a major predictor of hypertension in those living rurally. **Females were significantly more likely to be overweight and have abdominal obesity than males, although this did not translate into a higher prevalence of hypertension. It seems likely that, in females, factors relating to obesity may be the main modifiable risk factor for hypertension, whilst in males' alcohol consumption and stress are also important risk factors** (Costache et al, 2017).

Discussions

1) In some studies, the authors concluded that case-fatality rates were highest in the middle- and low-income countries. In total, in high-income countries, 6.5% of individuals died following a MI, stroke, or heart-failure hospitalization in high-income countries vs. 15.86% in middle-income countries and 17.28% in low-income countries (O'Riordan, 2014). A population-based study of Ontario patients with chronic CAD found differences in use of laboratory testing and apparent access to physicians in rural vs. urban dwellers, but these did not affect the rates of hospitalization or 1-year survival or any differences in risk adjusted mortality or in cardiac hospitalizations (47.2% vs. 46.3%) (Busko, 2014). In a Tehran lipid and glucose study, performed in 15005 subjects, the prevalence and distribution of high blood pressure, cigarette smoking, dyslipoproteinemia, diabetes mellitus, and obesity was determined. In adults, 78% of men and 80% of women presented at least one CVD risk factor. The percentage of adult women with two or more risk factors was significantly greater than for men. The prevalence of diabetes was 9.8%, of hypertension 20.4%, of total cholesterol 19.3% and smoking was 22.3% (Azizi, 2002). Another study, including a cohort of 4535 Indian adults, investigated the prevalence of cardiovascular risk factors by socioeconomic position. The results of the study showed that lower fruit intake and higher tobacco and alcohol use were found in those with lower socio-economic conditions. They had less blood pressure, glucose or cholesterol screening and less knowledge of nine cardiovascular risk factors. Overweight, physical inactivity, diabetes, hypertension, family history of cardiovascular disease and previous CVD (men only) were greater in persons with higher socio-economic positions (Zaman et al., 2012). Unwin et al. (2010) showed in 206 subjects, in which were investigated the changes and their determinants in cardiovascular risk factors on rural to urban migration after 1 and 3 months of migration, that physical activity declined (79.4% to 26.5% in men, 37.8% to 15.6% in women, $p < 0.001$), and weight increased. There were positive correlations found between blood pressure, smoking habits, cholesterol (total and LDL), triglycerides, and body weight. A relationship between hypertension, smoking, diabetes, and obesity was observed. Cardiovascular mortality may be affected by marital status. Increased mortality risk was higher in subjects who were unmarried, probable based on physiologic pathways (Di Castelnuovo et al., 2009).

In AFINOS study, Martinez-Gomez et al (2010) investigated the association between sedentary behavior and cardiovascular risk factors in 210 adolescents, demonstrating that adolescents with a high level of sedentary behavior had less favorable systolic blood pressure, triglycerides levels and cardiac risk factors scores than adolescents with a high level of overall adiposity. In an observational study performed in 71018 women for the duration of 5 years, Chomistek et al (2013), starting free of cardiovascular disease, with sedentary behavior and normal weigh at baseline, demonstrated that prolonged sitting time was associated with increased cardiovascular disease risk, independent of leisure-time physical activity; combination of low physical activity and prolonged sitting augments cardiovascular disease risk. Changes in population diet are likely to reduce cardiovascular disease and cancer, but the effect of dietary advice is uncertain. In an update of a

previous review in Cochrane Database, Rees et al (2013) assessed the effects of providing dietary advice to achieve sustained dietary changes or improved cardiovascular risk profile among healthy adults. The authors concluded that dietary advice appears to be effective in bringing modest beneficial changes in cardiovascular risk factors over approximately 12 months. A systematically review of 58 epidemiological studies about the role of Mediterranean diet in the prevention of cardiovascular diseases showed favorable effects but a certain degree of controversy remains. Important methodological differences and limitations in the studies make difficult to compare results, thus further studies, particularly randomized clinical trials, are needed to finally substantiate the benefits of Mediterranean diet (Grossoa et al., 2014, Aragaki et al., 2013).

2) In existing literature it is stipulated that the classic risk factors for CVD are the same in women and men, but there are gender differences in the prevalence of risk factors. Mosca, Barret-Connor and Wenger (2011) mentions that although women and men overall have nearly equal percentages of hypertension (1 in 3 adults), data from the National Health and Nutrition Examination Survey (NHANES) showed that the prevalence of high blood pressure is greater in women > 65 years of age.The highest rate of hypertension is among black women, 44%, and is increasing. The death rate caused by hypertension in 2007 was 37.0 per 100 000 for black women compared with 14.3 per 100 000 for white women (Mosca, Barret-Connor, & Wenger, 2011). Another study suggested that the prevalence of hypertension in young men is higher than in young women but the situation was opposite before the age of 40, and after that the prevalence of hypertension did not differ between males and females in rural areas (Wu et al., 1995).

Diabetes mellitus is more prevalent among women than men (8.3% versus 7.2%). Type II diabetes mellitus imparts a greater risk of CHD in women than men and is not explained by differences in risk factors, but rather by the more favorable survival rate of women (than men) without diabetes mellitus. On the basis of the NHANES data, the age-adjusted prevalence of the metabolic syndrome is highest among Mexican-American women (40.6%), which is 22% higher than in Mexican-American men. The prevalence of high values of total cholesterol was 16.2% among women and 13.5% among men.

Bernabe-Ortiz and colab. (2012) in The Peru Migrant Study refers to a Brazilian study which demonstrated that high cholesterol and hypertension were more prevalent among women compared to men. In Peru, three studies have shown that metabolic syndrome, abdominal obesity, and low high density lipoprotein-cholesterol (HDL) are higher in women than men while there seems to be no differences in hypertension, hypertriglyceridemia or high fasting glucose. Appelman et al., (2015) is one of those who examined the available literature regarding the prevalence and effects of the traditional major RFs for CVD in men and women. This included large prospective cohort studies, cross-sectional studies and registries. Furthermore, a literature search was performed to examine the impact of female-specific RFs on the traditional RFs and the occurrence of CVD. He found that the effects of elevated blood pressure, overweight and obesity, and elevated cholesterol on CVD outcomes are largely similar between women and men; however prolonged smoking is significantly more hazardous for women than for men. This review shows

that CVD is the main cause of death in men and women; however the prevalence is higher in women. Determination of the CV risk profile should take into account that there are differences in impact of major CV risk factors leading to a worse outcome in women. As a consequence, lifestyle interventions and awareness in women needs more consideration.

3) According to the World Health Organisation, CVD are the leading cause of mortality in the world. In Romania, HBP prevalence varies between 8 - 16% at the average age, reaching 40% at people of 60 years of age (Dorobantu et al., 2008). It is higher in the rural environment. In women who took oral contraceptives for more than 5 years, the risk is 2-3 times higher. The cardiovascular risk is higher in men under the age of 55, while suffering a moderate growth in women as they reach the age of 55, so that it may rise significantly as the latter reach the age of 75. HBP control reduces the cardiovascular risk by 15%, considering as important smoking quitting, weight loss, reduction of sodium intake and physical activity performed regularly. Overweight and obesity are also important risk factors of hypertension, the pooled results showing that the prevalence increases with the growing rate of both. Hypertension is one of the leading causes of disease burden across the world. More information about hypertension prevalence could help to improve overall antihypertensive health care (Chen et al., 2014).

Among the factors associated with HBP onset, it is estimated that half of cases are connected with an unhealthy diet, while 30% are correlated with high salt intake, and almost 20% with a low potassium intake, generated a small amount of fruits and vegetables in alimentation, according to the World Hypertension League (Frohlich, et al., 1993). Compared to the European countries, in Asia the data regarding the HBP prevalence in rural communities is somewhat different.

Throughout the world, cardiovascular diseases (CVD) have become a major public health problem and have been recognized as a leading cause of death and disability in most developed and some developing countries (Lopez, et al., 2001; He, et al., 2005). So, the prevalence of hypertension in rural areas of China is 22.8% (18 years old) in the last decade, which is well-above the level in 2002 (18.0%) concluded by a national investigation and even 1.3% higher than the prevalence (21.5%) in the urban environment (Chen et al, 2014). In India, the prevalence of hypertension was 19.0% in a rural community (Kokiwar et al., 2012). Hypertension is a major risk factor for CVDs, including stroke and myocardial infarction, and its burden is increasing disproportionately in developing countries as they undergo demographic transition. In 2008, a cross-sectional study of rural community health status among 1078 adults (aged > or =18 years) shown that the crude prevalence of hypertension was 18.3% in Nigeria while a total of 30 peerreviewed publications were identified that reported the prevalence of hypertension in 33 143 patients was 32.6% in rural Ibero-America (Diaz & Tringler, 2014).

The prevalence of hypertension was substantially different, depending on the region. The prevalence was higher in north China than in south China (25.7% vs. 19.3%), and differences still exist between the urban and rural population (25.8% vs. 20.4%), which may be attributed to the discrepancy in eating habits (Kokiwar et al, 2012). The proportion of salt intake among the local

residents in Hainan and the longterm living from the north is 7.2% and 8.4%, while in the short-term residents from the north is more than16.9% (Lin et al, 2014). High blood pressure is also an important public health concern in both urban and rural settings in African population, but the risk factors associated with high BP are different in women and men, therefore requiring different prophylactic methods (Agyemang, 2006).

Conclusions

1) In our study we noted a predominance of women (n = 195; 61.1%) and persons with environment of origin from rural (n = 228; 71.5%). **The risk of coronary heart disease (CHD) estimated by Framingham equation was significantly higher in urban than in rural (8.03% vs. 6.6%). The risk of myocardial infarction (MI) was higher in urban than in rural, too (4.5% vs. 2.8%).** There was no significant risk of stroke, coronary heart disease or death due to cardiovascular disease in urban or rural. The risk for cardiovascular disease estimated by ASSIGN equation was significantly higher in urban than in rural (19.4% vs. 12.9%). Further studies will establish the main mechanism of linkage between environment of origin and tightly correlation (Costache et al, 2015).

2) Our study revealed that divided by gender**, the cardiovascular risk factors presented important differences in our group meaning that almost all cardiovascular risk factors were more importantly represented in the female group apart from smoking.** Women had more than three-fold greater odds of having metabolic syndrome (p = 0.001) compared to male counterparts, including abdominal obesity, low HDL-cholesterol, and high fasting blood glucose components. In contrast, female participants had 50% lower odds of having hypertension, compared to men (95%CI: 0.3-1.0). The present study has clear practical implications in terms of the initiation of the targeted measures for primary prevention into the studied population. **Data obtained from the survey on the prevalence of risk factors could be extrapolated to other population and can be used in the future for cardiovascular and cardiometabolic risk assessment and early initiation of preventive measures** (Costache et al, 2016).

3) **HBP values in an analyzed rural community were 9.13%, lower than the national data**. Most of the persons aged 50 – 69 had 2nd or 3rd degree arterial hypertension while those between the ages 70 – 79 presented rather similar percentages covering all classes of arterial hypertension and normal blood pressure. In the group 50 – 69 years, women had increased levels of 2nd or 3rd degree arterial hypertension, while in the elderly (above 70 years of age) the blood pressure was rather normal in men's category. A positive relationship was obtained between arterial hypertension and obesity. **There was a direct correlation between arterial hypertension and type 2 diabetes mellitus. Most hypertensive patients had increased levels of stress, especially in final stages of arterial hypertension compared to normal blood pressure values where only 8.8% of persons presented stress (p = 0.025). In our study group, there was no significant difference between arterial hypertension and smoking (p = 0.673) or family history of CVD**

(p = 0.094). Marked dyslipidemia was associated with the working status (Costache et al, 2017).

Clinical implications

The results of these studies may be used to design prevention programs that have already proven to be absolutely necessary to promote a healthy lifestyle starting with teenagers, the influence (when possible) of risk factors in order to increase the life expectancy of the population and quality of life. The findings can contribute to strategies for state and municipal health services to monitor and prevent cardiovascular events.

*

I.2. Researches regarding cardiovascular diseases in terms of genetics/biochemical relationships. Clinical implications.

I.2.1. The approach of cardiovascular pathology, especially the ischemic one, in terms of genetics relationships. Resistance (genetically determined) to the platelet antiaggregation treatment.

Acute coronary syndromes (ACS) define a spectrum of cardiac conditions: unstable angina, non-Q myocardial infarction (NSTEMI) and myocardial infarction with ST-segment elevation (STEMI), associated with a major risk of complications and death. According to the current American College of Cardiology/American Heart Association and European Society of Cardiology guidelines, oral antiplatelet agents are the cornerstone of modern pharmacotherapy in cardiovascular atherothrombotic diseases (Neki, et al 2016), its efficiency having strong evidence according to many randomized trials (Antman et al 2008). **Clopidogrel is one of the most recommended oral antiplatelet drugs worldwide for the treatment of atherosclerotic events in patients with acute coronary syndrome, stroke, myocardial infarction or peripheral arterial disease or to prevent stent thrombosis** (Liu et al 2016, Alexandrescu et al 2016). It is a second-generation oral thienopyridine prodrug that requires enzymatic biotransformation into the active compound (Angiolillo et al 2009).

Recent studies have shown that **approximately 31% of the patients with ACS present Clopidogrel resistance and also a bad evolution.** The cumulative risk for an ischemic event during the acute phase and the next three months is about 50% due to the long time needed to stabilize atherosclerotic plaque (Bertrand et al. 2002).

In the last 10 years there have been made remarkable progressis in the treatment strategy of ACS, including antiplatelet therapy, doses, duration of treatment and the use of dual or triple therapy with these agents (Husted et al 2009). It is known that ACS have a common anatomical substrate. Pathological, biological and angioscopic observations showed that unstable angina and myocardial infarction are different clinical presentations resulting from a common pathophysiological mechanism, such as atherosclerotic plaque rupture or erosion, with different degrees of thrombosis and distal embolization (Davies et al 1993, Nguyen et al 2005). **Antiplatelet therapy is routinely used in patients at high risk of atherothrombosis and became the "cornerstone" in the treatment of coronary artery disease** (Antithrombotic Trialists' Collaboration 2002, Simon et al 2009). Despite discoveries and developments in the field of antiplatelet therapy, even the treated patients even are at increased risk for developing a new thrombotic event (Eisenstein et al 2007). Thienopyridines (ticlopidine and clopidogrel) are inhibitors of adenosine diphosphate (ADP), resulting in inhibition of platelet aggregation. Because of side effects, ticlopidine was replaced by clopidogrel. **Clopidogrel is one of the most recommended platelet inhibitors, although**

pharmacodynamic vary substantially in each individual (Balsano et al 1990, Gurbel et al 2003), **so that patients with suboptimal response are at a major risk of future cardiovascular events** (Hochholzer et al 2006).

In patients with acute coronary syndromes, clopidogrel is recommended for acute treatment and long-term treatment of at least 9-12 months. Clopidogrel may be recommended for immediate or long-term therapy in patients who are intolerant to aspirin (CAPRIE Steering Committee 1996) and patients receiving a stent (Bertrand et al, 2000). Over time there have been developed a series of tests to assess the inhibition of platelet in patients treated with clopidogrel: LTA - light transmision aggregometry (gold standard for predicting cardiovascular events), VASP - vasodilator stimulated phosphoprotein and PRI - platelet reactivity index (Angiolillo et al 2007). VASP is considered the most specific test to identify the degree of inhibition of platelet P2Y12 receptor (Costache et al, 2012, Bonello et al 2010).

Some studies have reported an inhibitory effect of clopidogrel in combination with aspirin (Gurbel et al 2006). **Although clinical benefit has been demonstrated in the case of clopidogrel, individual genetic variations exist, trying to explain the inadequate platelet inhibition, which is the major risk factor for new thrombotic events** (Nguyen et al 2005).

The concept of "resistance to clopidogrel" dates back several years and has two implications: a clinical one - related to the evolution and hence the poor prognosis of some patients with ACS and the second - the practical understanding that a new antiplatelet agent that do not develop resistance phenomenon is neccessary (Nguyen et al 2005, Giusti et al 2009). Recent studies have shown that approximately 31% of patients with ACS present **Clopidogrel resistance, which is defined as poor response regarding antiplatelet platelet, these patients having a clinical course and implicitly unfavorable prognosis** (Gurbel et al 2003, Collet et al 2009). In this category are included the patients with early intrastent thrombosis (Sibbing et al 2009). Regarding the mechanisms of this resistance several theories were issued. It is known that clopidogrel is a prodrug that requires a series of processing to generate the active metabolite, the latter being the real inhibitor of the ADP receptor, P2Y12 (Frere et al 2008). It is a second-generation oral thienopyridine prodrug that requires enzymatic biotransformation into the active compound (Angiolillo et al 2009).The active clopidogrel metabolite inhibits adenosine diphosphate (ADP)-induced platelet aggregation by binding and blocking the platelet P2Y12 receptors (Feher et al 2010, Norgard et al 2017). After its digestive absorbtion, the major part of clopidogrel is hydrolyzed by esterases to an inactive carboxylic acid derivative. A small proportion of clopidogrel (15%) is activated via two sequential oxidation reactions catalyzed by the hepatic cytochrome P450 (CYP450) enzymes, mainly CYP2C19, CYP23A4, CYP3A5 and CYP2C9. The first bioactivation stage is characterized by the oxidation of the thiophene ring of clopidogrel to 2-oxoclopidogrel which is further oxidized in the second stage, with the opening of the thiophene ring and the formation of both a carboxyl and a thiol group (fig. 1.2.1) (Liu et al 2016, Feher et al 2010, Dean et al 2015, Giusti et al 2010).

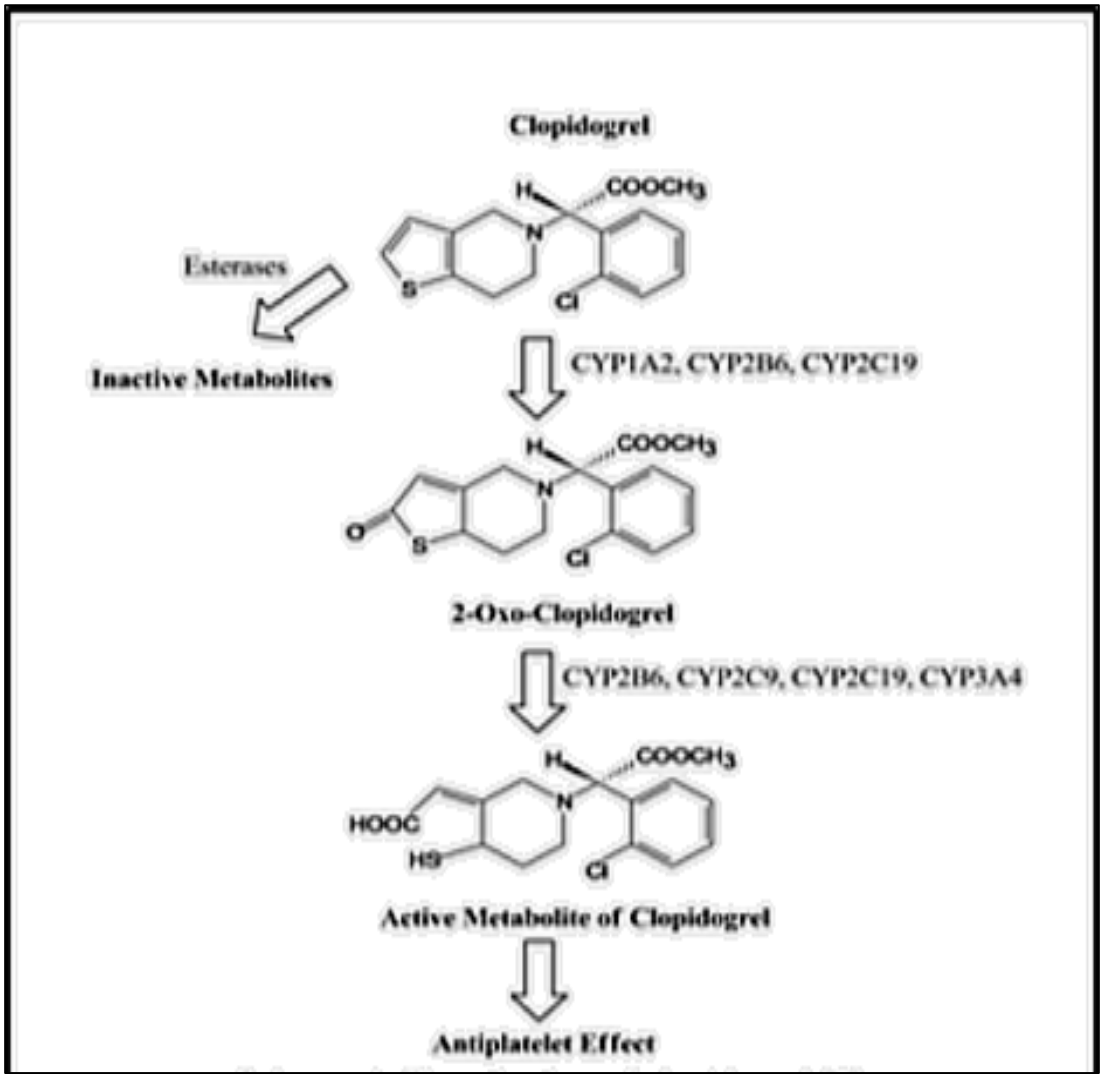


Figure 1.2.1. Bioactivation of Clopidogrel

Transformation "in vivo" of the prodrug – clopidogrel into active metabolite, is a critical step in achieving antiplatelet effect. Converted into the active metabolite – clopidogrel will inhibit ADP receptor - P2Y12, and secondary inhibits ADP-mediated platelet activation. This metabolism is dependent on the activity of liver - cytochrome P450 isoenzymes belonging to the family (CYP2C19, CYP1A2, CYP2B6, CYP2C9 and CYP3A4). The response to clopidogrel is therefore influenced by a cascade of events, responsible for the formation of the active metabolite (Savi et al 2000). Clopidogrel metabolism in the liver is the first step in its transformation 2-oxo-clopidogrel and subsequent conversion to the active metabolite. CYP1A2, CYP2B6, CYP2C9 and CYP3A4 / 5 are known to be involved in the metabolism of clopidogrel. The importance of each Cytochrome before mentioned and exactly where they are involved is very controversial in the literature (Savi et al 2000). Some refferences indicate CYP3A4 (Farid et al 2007) as a major factor involved in clopidogrel metabolism, while others confer cytochrome CYP1A2, CYP2B6 and

CYP2C19 this role (Kurihara et al 2005). Kazue et al (2010) showed that CYP2C19, substantially contributes to the metabolism of clopidogrel, while CYP3A4 plays a role only in the conversion of the 2-oxo-clopidogrel. According to other studies, it appears that there is a direct link between CYP3A5 and an antiplatelet effect of clopidogrel. Currently, at least 25 polymorphisms (single nucleotide polymorphisms - SNPs) have been described in the gene encoding CYP2C19 allele, the CYP2C19 * 2 (exon 5), being the best known and studied. It appears that it induces a significant decrease in serum concentration of the active metabolite and thus reduces the inhibition of platelet aggregation (Simon et al 2009). The allele that causes loss of function enzyme (CYP2C19 * 2) is the most studied polymorphism without having until now reached a consensus. JM Lee showed that there are ethnic differences in the frequency of polymorphisms. Allele CYP2C19 * 3A appears to be more common in Asian populations than in the US (Lee et al 2009).

It is reported that 20-30% of the US population is heterozygous for polymorphisms CYP2C19 * 2, while 3.2% were homozygous with enzyme activity canceled, similar to the prevalence rate in the European population (Mega et al 2009). Although other SNP are rare, more studies are needed to elucidate the role of less known CYP2C19 polymorphisms. Recently it was reported that the presence of allele CYP2C19 * 3A allele CYP2C19 * in connection with 2A, leads to an increased risk of acute stent thrombosis (Harmsze et al 2010). Patients treated with clopidogrel, who have CYP2C19 * 2A allele, appear to have an increased risk for recurrent ischemic events (Bonello et al, 2010).

On the contrary, CYP2C19 * 17 allele was associated with an exacerbation of enzyme activity, the allele called "gain function" that can increase the risk of bleeding during treatment with clopidogrel (Sibbing et al 2010). Two recent studies show the influence of allelic variant C3435T (rs1045642) ABCB1 gene in intestinal absorption of clopidogrel in patients with cardiovascular disease (Taubert et al 2006).

These records argue on the one hand of the necessity of tests or assays to identify patients who could show this phenomenon of resistance before treatment, on the other hand is necessary to find and introduce new drugs without this phenomenon of resistance and possibly with no hepatic metabolism.

The best known (specific and sensitive) and used methods for determining SNPs (which may vary depending on the targeted polymorphisms), are:

- TaqMan assays (Applied Biosystems, Life Technologies, Pleasanton, CA, USA), is based on allelic discrimination by Real-Time PCR TaqMan system. For each allele is used the same set of primers and one sonde with specific fluorescence. By degradation of this sonde by polimerase (with properties 5'-exonuclease 3') during amplification will be issued (by probe hybridized only), a specific fluorescence. This will allow allelic discrimination as a color code.

- Reverse Hybridization is based on PCR amplification, followed by hybridization of the amplicon formed on a "strip", marked in advance with probes specific to each allele, and then by a reaction with a biotinylated antibody and an enzyme, to obtain a characteristic color reaction.

- Sequencing of the amplicon of interest is the only method able to identify all mutations and polymorphisms in the region of interest. Also, sequencing is a method of validation of the results

obtained by alternative methods. However sequencing remains a very complex, time-consuming and funds-consuming method.

Clinical implications:
Identifying patients with clopidogrel resistance in early stages, finding rapid therapeutic alternatives to reduce the risk of complications related to early intrastent thrombosis and to avoid the occurrence of other severe complications in acute coronary syndromes related with re-thrombosis phenomen.

My concerns in this direction have been materialized through the following research project and a series of publications:

GRANT DIRECTOR:
November 2011 – GRANT won by internal competition "Grigore T Popa" University, entitled " THE EVALUATION OF CLOPIDOGREL RESISTANCE IN PATIENTS WITH ACUTE CORONARY SYNDROMES USING AN INTEGRATED EXPERIMENTAL MODEL" Grant director - Iuliana Irina Costache . Contract number : 28214 / 16.12.2011 , Internal Grants Competition 2011 for the University of Medicine and Pharmacy " Gr . T. Popa " . Period: 2011-2012 , duration 12 months. Funding sources : University of Medicine and Pharmacy " Grigore T. Popa" .

The project was interdisciplinary as it brings together the expertise of large areas: cardiology, molecular genetics, epidemiology and pharmacology. The grant was initially realized on a sample of 80 patients, which was analysed based on the response to clopidogrel, by genotyping of cytochrome CYP2C19 and ABCB1 gene. The experience in the field is also supported by publications on the topic addressed in journals (BDI / ISI).

I.2.1.1. Published papers in relation with the GRANT:

ISI Articles:

1. Irina Iuliana Costache, Ana Clara Aprotosoaie, Iuliu Cristian Ivanov, Dan Iliescu, Irina Gârleanu, Antoniu Octavian Petriş: **Screening methods using clopidogrel by genotyping CYP2C19 cytochrome and ABCB1 gene in patients with acute coronary syndromes**, Biomedical Research 2015; 26 (2): 266-272.**IF = 0,196.**

2. Irina Iuliana Costache, Florin Mitu, Razan AL Namat*, Iuliu Ivanov, Roxana Popescu, Ovidiu Mitu, Alexandru Dan Costache, Ana Clara Aprotosoaie: **Is There a Link Between Clopidogrel Resistance and Common Risk Factors for Atherosclerosis in Patients with Acute Coronary Syndrome?** REV.CHIM. ♦2017; 68 (11):2726-2730, **IF= 1.232**

BDI Articles:

3. Irina Iuliana Costache, C. Rusu, I. Ivanov, Roxana Popescu, AO Petriş **"Resistance to clopidogrel - a risk factor for patients with acute coronary syndromes.** Rev Med Chir 2012; 116 (2): 383- 388.

4. Irina Iuliana Costache, Cristina Rusu, I.Ivanov, Roxana Popescu, A. Petriş. **"Impact of clopidogrel response on clinical evolution in patients with acute coronary syndromes"** Rev Med Chir 2012; 116 (4): 960 – 967.

5. Irina Gîrleanu, DM Alexandrescu, AO Petriş, **Irina Iuliana Costache: "Barriers of antiagregant treatement".** Rev Med Chir 2014; 118 (2): 333-338.

Poster:

Irina Iuliana Costache, Cristina Rusu, Iuliu Ivanov, Roxana Popescu, AO Petriş: **Evaluation of Clopidogrel resistance by genotyping CYP2C19 cytochrome and ABCB1 gene in patients with acute coronary syndromes** – the 6rd Conference of Genetics, Iaşi, oct 2012, published in Abstracts Volume, pages 32-33.

The antiplatelet effect of clopidogrel prodrug is characterized by a wide inter-individual variability that has a significant clinical relevance. Among varios factors that are involved in the occurrence of clopidogrel resistance, the genetic polymorphisms play a key role.
Thus the **aims of the studies** were:
- to analyze all aspects of the phenomenon of resistance to clopidogrel in ACS patients, by correlating clinical data with laboratory reference mainly to genetic methods or genotyping gene ABCB1 and CYP2C19 - analysis of polymorphisms with hypo and hyperactivity (CYP2C19 * 2, * 3, * 17) using TaqMan Assay and High Resolution Melting (HRM) technique;
- to identify alternative genotyping techniques quickly and efficiently targeted polymorphisms (Allele-Specific PCR, PCR-RFLP (restriction fragment length polymorphism) or multiplex PCR);
- to investigate the frequency of resistance to treatment with clopidogrel, alone or in combination with other platelet inhibitors, in patients with ACS, corroborating the results obtained from both techniques (available) to assess platelet function and CYP2C19 and ABCB1 genotyping techniques.
- to determine the relationship between clopidogrel genetic response (based on laboratory specific tests) and the worse evolution of the patients with acute coronary syndromes.
- to investigate the impact of some risk factors for atherosclerosis on the antiplatelet effect of clopidogrel in patients with acute coronary syndrome and the possible correlation with metabolizer phenotype of patients based on CYP2C19 polymorfisms.
- to evaluate the side effects of antiplatelet therapy in order to establish correlations with medication type, doses and association with other therapies.

The conclusions represented the starting point in achieving an integrated experimental model, viable and reproducible, which allows testing effectiveness of clopidogrel therapy and identifying patient's resistant to treatment, who will need alternative antiplatelet therapies.

Materials and methods

The study included an initial group of 80 patients (86.25% of the patients were male and 13.75% were female, aged between 45-85 years) admitted to the Cardiology Clinic of the St. Spiridon Emergency Districtual Clinical Hospital Iasi during the period January - December 2012. The group was then completed up to 96 patients (15 women and 81 men).

Informations on the general condition, medical history (hypertension, diabetes or coronary heart disease), personal history (smoking and drinking) were obtained from all patients.The study was approved by the Ethics Committee of University of Medicine and Pharmacy Grigore T.Popa Iasi, and all patients enrolled were previously informed about the subject of the study and they signed an informed consent. DNA extraction and genotyping The Wizard Genomic DNA Purification - Promega Kit (Promega Inc., Madison, WI, USA) was used to isolate DNA from blood samples.

CYP2C19 polymorphisms were investigated using TaqMan assay (Applied Biosystems, Life Technologies, Pleasanton, CA, USA) that is based on the allelic discrimination by Real-Time PCR.

The patients eligible for the study met the following criteria: a) clinical – previous anginal chest pain present during hospitalization or a history of severe myocardial infarction or previously diagnosed coronary graphic changes with or without subsequent angioplasty; b) paraclinical – suggestive electrocardiographical changes - subendocardic or subepicardic ischemic lession or recent branch block associated with changes in myocardic cytolysis enzymes and positive troponin. Patients with acute coronary syndrome, those without clopidogrel therapy, contra-indications for platelet anti-platelet therapy (ex antecedents of haemorrhagic accidents or known haemorrhagic diathesis, known liver disease), and patients with psychiatric disorders and non-compliant ones were excluded from the study.

The paraclinical exploration consisted of:

- investigation of associated risk factors: glycemia, uric acid, complete lipid profile (analysis of total cholesterol, triglycerides), hepatic enzymes (alanine transaminase, ALT; aspartate transaminase, AST), myocardial enzymes (creatine kinase MB isoenzyme, CK MB), serum creatinine, serum urea and thrombocytes count.

- complete functional balance for target organ damage in hypertensive and / or diabetic patients (renal function, electrolytes)

- electrocardiogram 12 derivates - both for the positive diagnosis of acute coronary syndrome and for the diagnosis of various complications (eg rhythm and / or conduction disorders) and the evolution over time.

- echocardiogram - 2D, M mod and Doppler – to appreciate: left ventricule (LV) and left atrium (LA) dimensions, wall thickness (for LV hypertrophy assessment), systolic function of LV (expressed by LV ejection fraction and shortening LV fraction), LV diastolic function, abnormalities in LV regional motion, presence of valvular regurgitation and other complications (intracavitary thrombi).

Patients were initially evaluated at the time of inclusion in the study, taking the complete check-up described above. After establishing with certainty the diagnosis and therapeutic regimen, it was decided to include it in the study.

All patients in the study received antiaggregant treatment with clopidogrel in some cases associated with aspirin. In some cases, patients were already on clopidogrel therapy at the time of inclusion, others were initiated on inclusion.

All patients were previously informed about the purpose of the study, and those who agreed to be included in the study signed an informed consent, after which the blood sample for DNA extraction was collected. (Costache et al, 2015, Costache et al, 2017).

Statistical analysis

Statistical analysis was performed using the IBM SPSS 20.0 software (Statistical Package for the Social Sciences, Chicago, Illinois). Data were expressed as mean ± standard deviation or number of cases with percentage, for continuous and ordinal variables. Cross-tabulation and Pearson Chi-Square test were used for describing the relationship between two categorical variables. The oneway analysis of variance (ANOVA) was used to determine the significant differences between the means of continuous variables and an independent categorical variable. For all data, a two-sided p value < 0.05 was considered statistically significant.

Experimental protocol

Two 2-ml tubes of peripheral blood were harvested per patient.

It was used EDTA as anticoagulant. Two extractions were performed simultaneously, one in each of the harvested tubes. The extractions were performed using the Wizard Genomic DNA Purification - Promega kit.

The extraction process followed 5 steps: 1. red cells lysis; 2. lysis of white blood cells and of nuclei; 3. cellular proteins precipitation; 4. genomic DNA precipitation: 5. DNA Rehydration .

The TaqMan assay genotyping method for the targeted SNPs is based on allelic discrimination through Real-Time PCR in the TaqMan system.

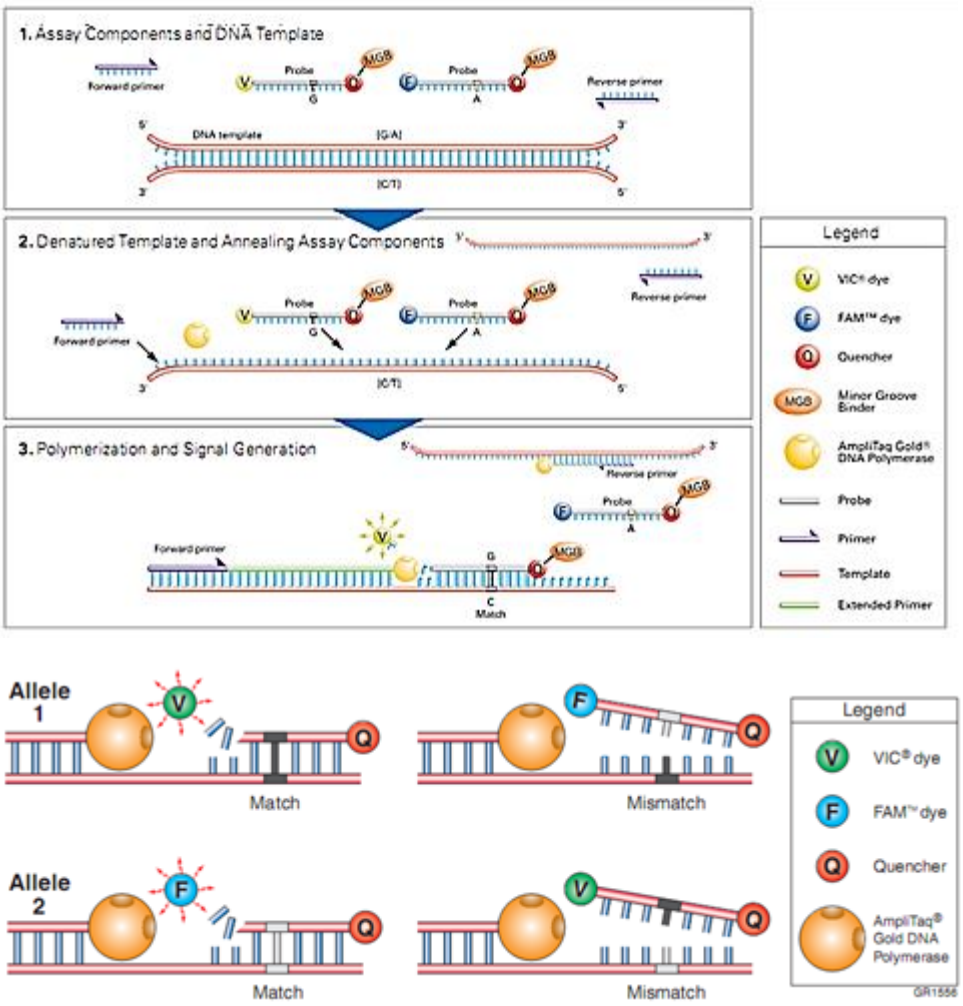


Fig 2. 1. TaqMan Assay method (Product Bulletin TaqMan®

Genotyping Assays - Applied Biosystems)

In our study, specific probes were used for the CYP2C19 mutant * 2A, * 3, * 17 alleles versus wild-type alleles and also for the 3435C> T mutant allele versus the wild-type allele in the ABCB1 gene.

Working protocol: Amplification was performed using the Real Time PCR MX3005P Stratagene / Agilent thermocouple.

Together with the optimization and performance of the TaqMan Sssay determinations, we attempted to identify an alternative method with lower costs that could allelically discriminate with a confidentiality of over 90% and decided to approach the High Resolution Melting (HRM) technique.

HRM is a technique that consists in analyzing post-amplification PCR of the melting curves of interest fragments (amplicons).

In our study, HRM genotyping was performed for all ABCB1c polymorphisms C3435T (rs1045642), CYP2C19 * 2 (c.G681A; rs4244285), CYP2C19 * 3 (c.G636A; rs4986893), and CYP2C19 * 17 (c.C806T; rs12248560). (Costache et al, 2015, Costache et al, 2017).

DNA isolation.

The extractions were carried out by using Wizard Genomic DNA Purification - Promega Kit (Promega Inc., Madison, WI, USA). The DNA was quantified and then aliquoted and labeled in order to achieve DNA collection in Molecular Biology Laboratory (Regional Institute of Oncology, Iasi). Some of the DNA was stored at -20°C for the PCR experiments. The other samples were frozen at -80°C.

Genotype analysis

TaqMan assay (Applied Biosystems, Life Technologies, Pleasanton, CA, USA), used for the aimed single nucleotide polymorphism (SNP), is based on the allelic discrimination by Real-Time PCR in a TaqMan system. The same set of primers and one specific fluorescence probe are used for each allele. Through the degradation of the probes by the polymerase (with 5'-3' exonuclease properties) during amplification, a specific fluorescence will be released (only by the hybridized probe). This allows allelic discrimination as a color code. The probes used for the wild allele had a Reporter-VIC fluorescence, and the probes for the mutant allele had a Reporter-FAM fluorescence. All the probes that were used had a MGB unit (Minor Grove Binder) to the 3' end. TaqMan probes that have MGB incorporated at the 3' end have a very high specificity for the specific allele, therefore they are ideal for allelic discrimination experiments. MGB molecules are caught on the minor notch of the double helix, improving the hybridization process and stabilizing the probe matrix complex. The current study used specific probes for mutant alleles *2A, *3, *17 of the CYP2C19 gene versus specific probes for the wild allele, as well as probes for mutant allele 3435C>T versus wild allele of the ABCB1 gene (Table 2.1). (Costache et al, 2015, Costache et al, 2017).

Table 2. 1. Rs codes, location and sequences of the studied polymorphisms

Gene	**Code**	**Location on the chromosome**	The sequence and the fluorescence of the used TaqMan [VIC/FAM]
Cyp2c19 *2A c.681G> A	rs4244285	Chr.10: 96541616 - 96541616	TTCCCACTATCATTGATTATTTCCC[A/G]GGAACCCATAACAAATTACTTAAAA
Cyp2c19 *3 c.636G> A	rs4986893	Chr.10: 96540410 - 96540410	ACATCAGGATTGTAAGCACCCCCTG[A/G]ATCCAGGTAAGGCCAAGTTTTTTGC
Cyp2c19 *17 g.-806C> T	rs12248560	Chr.10: 96521657 - 96521657	AAATTTGTGTCTTCTGTTCTCAAAG[C/T]ATCTCTGATGTAAGAGATAATGCGC
ABCB1 3435C> T	rs1045642	Chr.7: 87138645 - 87138645	TGTTGGCCTCCTTTGCTGCCCTCAC[A/G]ATCTCTTCCTGTGACACCACCCGGC

The amplification was performed using the Real Time PCR MX3005P Stratagene/Agilent thermal cycler. The amplification program (40 cycles): 95°C-10 min; 92°C-15 sec; 60º C – 1 min - acquisition on the FAM and VIC channels throughout the elongation. Simultaneously with the optimization and the determinations made through the TaqMan Assay, it was attempted to identify an alternative method with lower costs, that could make allelic discrimination with a confidence of over 90%, and it was decided to approach the High Resolution Melting technique (HRM). HRM is a technique which analyzes the melting curves of the fragments of interest post-PCR amplification (amplicons) (Figure 2. 2). (Costache et al, 2015).

Figure 2.2. Sequences of amplicons used in HRM technique

The method is based on using the dissociation melting curves of the products of interest, being available due to the improvement of the dyes that emit fluorescence while binding on doublestranded DNA and the evolution of RealTime PCR equipment and the analysis programs associated to each machine. Discrimination between the types of polymorphisms by the dissociation curve is based on the composition of the amplicon, its length, the content of CG and the complementarity of the two strands. HRM begins with a PCR amplification of the region of interest, in the presence of dyes that bind to the double-helix and emit fluorescence. These fluorochromes emit a very high fluorescence when bound to double-stranded DNA (dsDNA) and they emit a very weak fluorescence when they are not bound to dsDNA. The amplification is followed then by a melting curve at a very high resolution, made of very narrow steps of temperature (0.1ºC) and of the reading of a very large number of fluorescent events for each step with a very high precision. When dsDNA is dissociated into component strands, the fluorochrome is released leading to a decrease in the overall fluorescence from the tube. The result is obtaining a melting curve characteristic for each amplicon.

The HRM reaction can take place in a single step (PCR + MELT) or the PCR reaction can be performed in a standard thermal-cycler, and only HRM in a Real-Time PCR. Genotyping through HRM was performed for all ABCB1c polymorphisms: C3435T (rs1045642), CYP2C19*2 (c.G681A; rs4244285), CYP2C19*3 (c.G636A, rs4986893), and CYP2C19*17 (c.C806T, rs12248560), using RealTimePCR RotorGene 6000® (Qiagen, Courtaboeuf, France) and primers from Table 2. 2. (Costache et al, 2015).

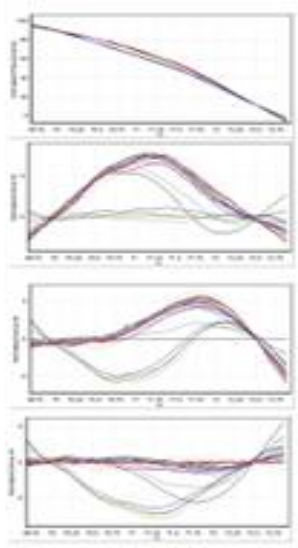
Table 2.2**.** Sequences of primers used in HRM technique

Mutation	Primer sequences
ABCB1c.C3435TF	5’ GGGTGGTGTCACAGGAAGAG 3’
ABCB1c.C3435TR	5’ AGGCAGTGACTCGATGAAGG 3’
CYP2C19*2 F	5’ TGCAATAATTTTCCCACTATCA 3’
CYP2C19*2 R	5’ TCACTTTCCATAAAAGCAAGG 3’
CYP2C19*3 F	5’ TGAAAACATCAGGATTGTAAGCA 3’
CYP2C19*3 R	5’ TGGTTTCTCAGGAAGCAAAAA 3’
CYP2C19*17 F	5’ AAATTTGTGTCTTCTGTTCTCAAA 3’
CYP2C19*17 R	5’ TAGCTGGCAGAACTGGGATT 3’

The design of primer sequences was performed using, ”Primer 3 imput” software. PCR reaction was done with addition of Bryte Green (Promega) fluorochrome. The conditions of PCR amplification were enzyme activation at 95ºC – 10 min followed by 40 cycles of: denaturation 95ºC - 15 sec, primers hybridization (different temperature depending on the melting temperature of each primer set)-15 sec, elongation 72ºC - 40 sec. The reading of fluorescence was made after every cycle of amplification on the Green channel. The curve of dissociation was achieved at a ramp from 70 to 94°C. The confidence threshold was established at 90%. HRM curves were normalized using pre- and post melting regions.

Results

The genotypes obtained by TaqMan assay are shown in Table 2.3. Also, the amplification by BrytGreen master-mix was performed for the HRM technique for the 96 patients. Very good results were obtained from the first stage of the study for CYP2C19*2 and CYP2C19*17 polymorphisms for a group of 36 patients (Fig. 2.3).



No.	Colour	Name	Genotype	Confidence %
1		1	wt	100.00
2		2	hz	100.00
3		3	wt	98.93
5		5	wt	98.09
6		6	wt	99.34
7		7	wt	98.59
8		8	wt	98.77
9		9	hz	99.59
10		10	wt	96.97
11		11	mt	100.00
12		12	wt	96.47
13		13	wt	97.67
14		14	wt	98.99
15		15	wt	99.55
16		16	wt	99.67
17		17	wt	98.74
18		18	wt	98.53
19		19	wt	99.77
20		20	wt	99.80
21		21	wt	98.82
22		22	wt	99.15
24		24	wt	98.22
25		25	wt	98.79
26		26	wt	99.60
27		27	wt	99.66
28		28	wt	99.57
29		29	wt	99.06
30		30	hz	99.40
31		31	mt	96.21
32		32	hz	99.34
33		33	wt	99.39
34		34	wt	99.54
35		35	hz	98.59
36		36	wt	98.00

Figure 2.3. HRM interpretation : wt – wild type; hz- heterozygous; mt - mutant.

Table 2. 3. Genotypes frequencies for the studied genes and mutant alleles

Polimorfism	Genotype	Probe number	Frequency
ABCB1 c.C3435T	CC	12	15%
	CT	48	60%
	TT	20	25%
	Alela T	55%	
CYP2C19*2,c.G681A	GG	62	77.5%
	GA	15	18.75%
	AA	3	3.75%
	Alela A	15.3%	
CYP2C19*3,g.-806C>T	CC	80	100%
	CT	0	0%
	TT	0	0%
	Alela T	0%	
CYP2C19*17, c.C806T	CC	4	5%
	CT	27	33.7%
	TT	49	61.25%
	Alela T	78.1%	

Primers functioned for these polymorphisms, giving specific amplicons (without non-specific binding and dimerization), and the results were 100% correlated with the genotyping through TaqMan. The obtained allelic discrimination confidence was of over 95%. 100% correlation between TaqMan genotyping and the one achieved through HRM was also confirmed by continuing RealTime PCR amplifications for the rest of the lot as well (44 patients). For the CYP2C19*3 and ABCB1 allele, the primers set initially were not suitable, forming nonspecific amplifications, that in a nonspecific RealTimePCR (with fluorochromes emitting fluorescence by conjugation with dsDNA) can alter the results.The design was made for other two sets of primers (through the primer 3 imput program). With these primer sets, optimal amplification we achieved. For the ABCB1 gene, where samples of wild-type (wt), heterozygous (hz) and mutant (mt) were previously determined (by TaqMan), the parameters of analysis were correctly set. Thus, the confirmation of TaqMan genotyping (100% correlation) was successful for all 96 patients. For the CYP2C19*3 allele, the RealTime amplification has been optimized so that it does not result in nonspecific binding (the appearance of dimers), but because there was no evidence of hz and mt control, parameter settings for the HRM analysis could not be performed. (Costache et al, 2015, Costache et al, 2017).

Based on the literature data, depending on the genotypic constellation of the alleles studied for the CYP2C19 gene, patients were divided into several categories of prediction:

1. Normal metaboliser (N): wild-type CYP2C19 * 2 * 3 * 17;
2. Intermediate metaboliser (MI): heterozygot for * 2 and / or * 3 and wild-type * 17;
3. Slow metaboliser (MS): * 2 / * 2, * 2 / * 3, * 3 / * 3, indifferent whether they are herezygotes or homozygotes on * 17;

4. Very quick metaboliser (MFR): homozygous or heterozygous for * 17 and wild-type for * 2 and * 3;

5. Unpredictable metaboliser (MN): hererozygote or homozygote on * 17 and heterozygote on * 2 and / or * 3.

Thus, in our study group we identified: 4 patients with normal metabolic phenotype (5%), 3 poorly metabolic patients (3.75%), 58 patients with very rapid metabolic phenotype (72.5%) and 15 patients with unpredictable phenotype (18.75 %). No patients with intermediate phenotype were identified. (Costache et al, 2012, Costache et al, 2015).

From the point of view of clinical evolution, the 3 patients with poor metabolic phenotype exhibited repeated episodes of aggravation of angina pains that required frequent admissions and review of the treatment regimen in the direction of increasing doses of nitrate and beta-blocker. One patient evolved into ischemic dilated cardiomyopathy with LV dilation and significant decrease in ejection fraction. Two of the patients were referred to the interventional cardiology department for coronarography and transluminal stent angioplasty.

Of the 15 patients with unpredictable phenotype, 10 had a favorable evolution during hospitalization without repetition of angina pains and no other complications. The control at 3 months was in optimal clinical and functional parameters. 5 of the patients, with unpredictable phenotype presented readmissions during the follow-up period, due to the recurrence of angina pain with characteristics of unstable angor, resulting in changes in the treatment regimen. 4 of these patients experienced left ventricular failure (cardiac asthma - 3 cases and acute pulmonary edema - 1 case). One patient (83 years old) had a peripheral arterial embolism under sinus rhythm conditions), which required hospitalization in the department of vascular surgery and embolectomy in an emergency. Further development was favorable.

Among the patients with rapidly metabolizing phenotype were the following events: intense macroscopic hematuria (2 males, age 75 and 82 years, respectively) - both needed removal of clopidogrel from the therapeutic regimen; - cutaneous ecchymosis - 7 cases (4 women, 3 males); - purpura - 1 female case, associated with thrombocytopenia - 75,000 / mmc; - gingival bleeding, independent of other causes - 2 female cases. Bleeding stopped after clopidogrel was removed from the treatment schedule; - upper gastrointestinal haemorrhage - melena - 1 male case where clopidogrel was associated with aspirin. Evolution was without hemodynamic degradation; - Haemoptisis - 1 male case (75 years).

In the rest of the patients in this group no bleeding events were recorded during the follow-up period, but 15 of the patients needed readmission for aggravation of angina pectoris (6 patients) and for left ventricular failure (12 patients). The 4 patients with normal metabolic phenotype had normal evolution uncomplicated during the follow-up period. (Costache et al, 2012, Costache et al, 2015, Gîrleanu et al 2014).

The majority of the studied patients were males and most subjects were hypertensive (98.8%), diabetic (63.8%), smokers (80%), dyslipidaemic (93.8%) or obese (83.8%). Mean cholesterol was above the normal limit with the highest value being 375 mg/dL. As well, most patients had values

of triglycerides over the superior limit, the highest value being 450 mg/dL. The average value of CK MB enzyme was 38.46 U/L, and 36.25% of the patients showed CK MB values about the normal limit (24 U/L). Their clinical data and electrocardiogram supported the diagnosis of unstable angina. The hepatic transaminases reached normal levels, the higher values being for AST since some patients were admitted with myocardial infarction in different stages. The renal markers were close to the superior limit, while the thrombocytes were normal in most patients. All data can be found in table 2.4. (Costache et al, 2017).

Table 2.4. Cardiovascular risk profile in the studied population

Cardiovascular risk factor	Result
Age (years)	69.30 ± 9.39
Male sex (%)	86.3
Arterial hypertension (%)	98.8
Type 2 diabetes mellitus (%)	63.8
Current smokers (%)	80
Dyslipidaemia (%)	93.8
Obesity (%)	83.8
Total cholesterol (mg/dl)	229.56 ± 46.28
Triglycerides (mg/dl)	163.81 ± 57.79
AST (U/L)	46.40 ± 26.44
ALT (U/L)	41.30 ± 12.37
Serum creatinine (mg/dl)	1.36 ± 0.63
Serum urea (mg/dl)	56.79 ± 21.39
Thrombocytes (/mm^3)	263,000 ± 124,046

By comparing the phenotypes based on gender, no significant differences were noticed, most males and females having an ultrarapid metabolizer phenotype (73.9% and 63.6%, respectively; p = 0.0524). As well, no significant differences were remarked between the type of phenotype and major cardiovascular risk factors, such as arterial hypertension (p = 0.222), type 2 diabetes mellitus (p = 0.225) or obesity (p = 0.462). However, we noticed a trend increase of poor metabolizer phenotype in the case of hypertensive and obese patients compared with normotensive and non-obese patients (table 2.5). (Costache et al, 2017).

Table 2.5**.** The phenotype metabolizer prevalence according to major cardiovascular risk factors

Variable	Category	Phenotype (%)				p value
		Extensive metabolizer	Unpredictable metabolizer	Poor metabolizer	Ultrarapid metabolizer	
Hypertension	No	0	100	0	0	0.222
	Yes	5.1	17.7	3.8	73.4	
Diabetes	No	0	13.8	6.9	79.3	0.225
	Yes	7.8	21.6	2	68.6	

Obesity	No	0	30.8	0	62.9	0.462
	Yes	6	16.4	4.5	73.1	
Dyslipidaemia	No	0	60	20	20	0.012
	Yes	5.3	16	2.7	76	
Smoking	No	0	31.3	12.5	56.3	0.050
	Yes	6.3	15.6	1.6	76.6	

Also, the diabetic patients showed a decrease by about 13.5% of ultrarapid metabolizer phenotype proportion and an increase of more than 50% in the proportion of unpredictable metabolizer phenotype. In our study, we found that more than 76% of dyslipidaemic patients presented an ultrarapid phenotype while 20% of non-dyslipidaemic patients had a poor response based on genetic testing (p = 0.012) (fig. 2.4.). (Costache et al, 2017).

As well, the same trend was seen when comparing the smoking status where most smokers had an ultrarapid phenotype while more non-smokers were categorized in unpredictable and poor group response (p = 0.050) (fig. 2.5.).

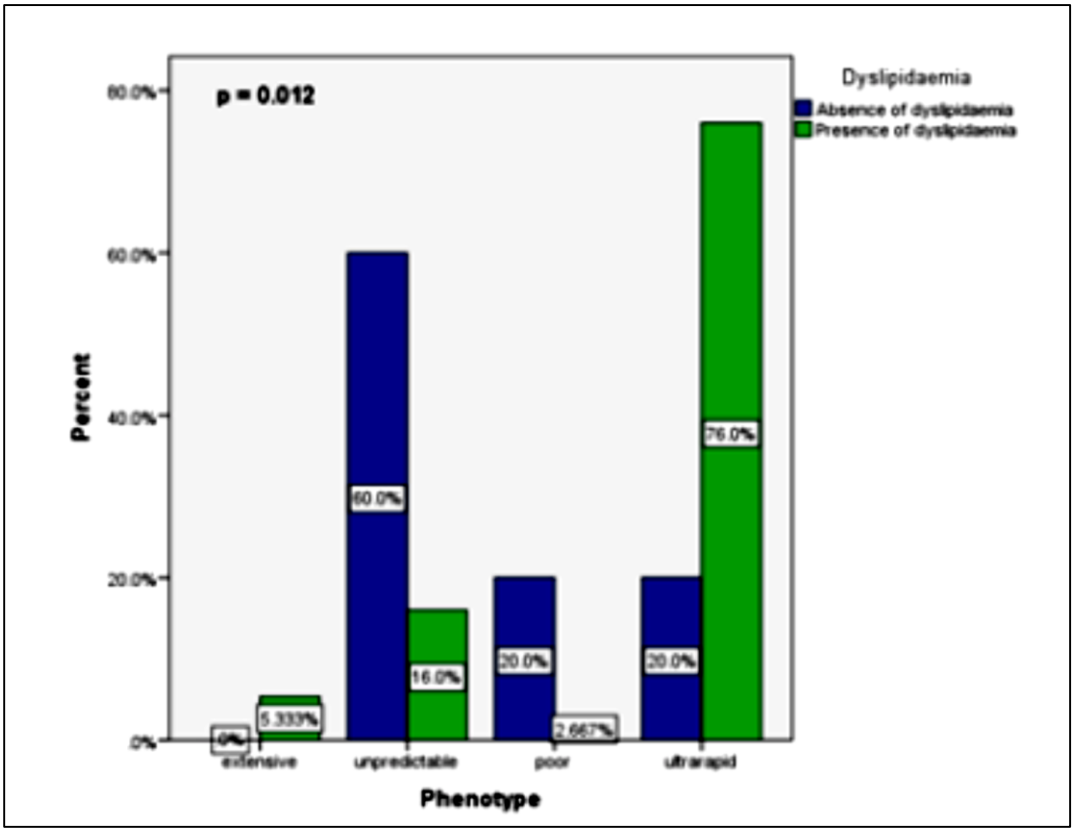


Fig. 2.4. The phenotype prevalence according to dyslipidaemia

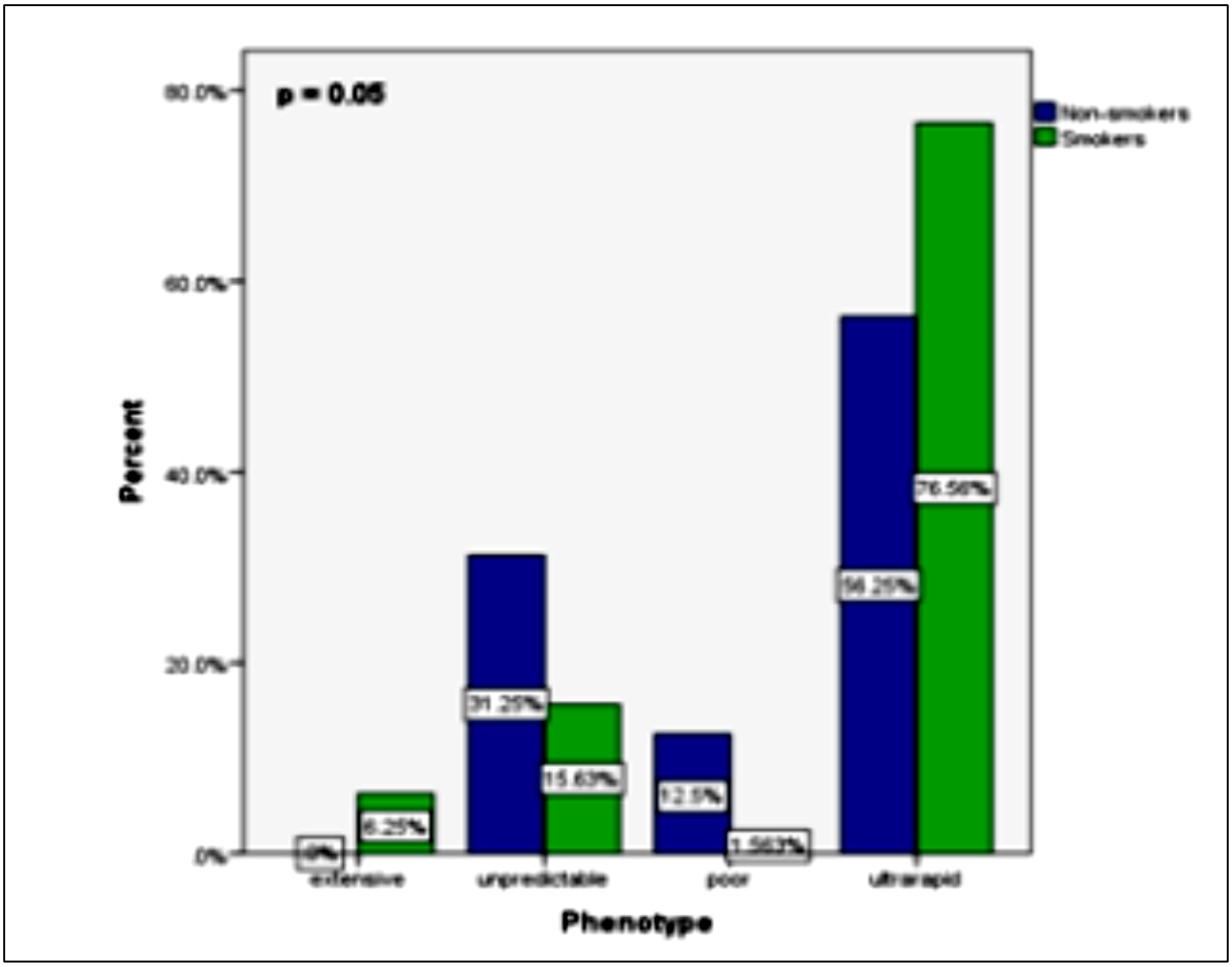


Fig. 2.5. The phenotype prevalence according to smoking status

Older patients had an ultrarapid metabolizer phenotype, but without statistical significance (p = 0.661). No significant differences were noticed for the values of total cholesterol and those of triglycerides. AST and ALT did not differ between the four groups, as well. The renal function was impaired in the extensive and ultrarapid metabolizer categories as compared to the poor and unpredictable ones (p = 0.762). The thrombocytes values were basically the same among all phenotypes (table 2.6). (Costache et al, 2017).

Table 2.6. Biochemical values according to the genetic testing

CV risk factor	Extensive metabolizer	Unpredictable metabolizer	Poor metabolizer	Ultrarapid metabolizer	p value
Age (years)	66.50 ± 9.74	67.73 ± 10.77	65.67 ± 11.37	70.09 ± 9.04	0.661
Total cholesterol (mg/dl)	236.50 ± 21.81	237.07 ± 46.43	221.00 ± 45.17	227.59 ± 48.13	0.879
Triglycerides (mg/dl)	175.75 ± 16.50	173.07 ± 48.76	143.67 ± 24.09	161.64 ± 62.86	0.807

AST (U/L)	46.25 ± 9.32	51.53 ± 31.52	45.00 ± 5.00	45.16 ± 26.66	0.877
ALT (U/L)	42.50 ± 6.45	42.80 ± 16.32	43.33 ± 7.63	40.72 ± 11.89	0.930
Creatinine (mg/dl)	1.30 ± 0.11	1.52 ± 1.24	1.40 ± 0.34	1.32 ± 0.39	0.762
Thrombocytes (/mm³)	282500 ± 72743	269400 ± 101445	280000 ± 140000	259120 ± 133284	0.970

Clopidogrel is considered superior to aspirin for secondary prevention after cardiovascular events. (Deepak et al 2006). Combined aspirin and clopidogrel therapy is even more effective in preventing cardiovascular events and associated moratlity but that regimen may significantly increase the risk of bleeding (Markus et al, CARESS trial 2005)

In order to evaluate the side effects of antiplatelet therapy in order to establish correlations with medication type, doses and association with other therapies the study was completed up to 125 patients. The baseline characteristics of the studied group are illustrated in table 2.7. Treatment received by the patients included in the study upon discharge is mentioned in figure 2.6. (Gîrleanu et al, 2014).

Table 2.7. The baseline characteristics of the study group

Characteristics	Patients (n = 125)
Age, yrs (mean ±SD)	69,3±9,39
Male, n %	66 (52,8%)
Diabetes n,%	51 (40,8%)
Smoking n, %	64 (51,2%)
Blood hypertension n,%	79 (63,2%)
Cholesterol, mg/dl (mean ±SD)	229,5 ±45,99
Creatinine, mg/dl (mean ±SD)	0,98 ± 0,39

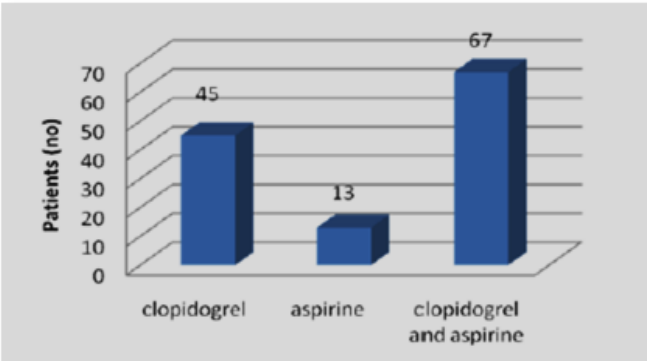


Fig. 2.6. Treatment received by the patients included in the study upon discharge

Side effects reported (possibly correlated with antiplatelet therapy) were: macroscopic hematuria (7 cases), cutaneous ecchymosis (7 cases), purpuric lesions (9 cases), gingival bleeding (12 cases), upper gastrointestinal bleeding (6 cases), hemoptysis (2 cases) (Table 2.8) (Gîrleanu et al, 2014).

Table 2.8. Side effects in patients with antiaggregant treatment

Side effect	Clopidogrel (n = 45)	Aspirin (n = 13)	Clopidogrel and aspirin (n = 67)
Macroscopic hematuria n, %	0	0	7 (5,6%)
Cutaneous ecchymosis n, %	2 (1,6%)	1 (0.8%)	4 (3,2%)
Cutaneous purpura n,%	5 (4%)	0	1 (0.8%)
Gingival bleeding n,%	4 (3,2%)	0	8 (6.4%)
Upper digestive hemorrhage n,%	0	0	7 (5,6%)
Hemoptysis n,%	2 (1,6%)	0	0

Discussion

CYP2C19 cytochrome contributes significantly to the metabolizing of clopidogrel and it influences the therapeutic response (Kazui et al 2010). At least 25 polymorphisms have been described for the gene encoding CYP2C19, of which the **CYP2C19*2 allele (exon 5) is the best known and studied**. It appears that it induces a significant drop in the concentration of the serum active metabolite and thus it reduces the inhibition of platelet aggregation (Simon et al 2009). **Patients who have the CYP2C19*2A allele and who are treated with clopidogrel seem to have an increased risk for recurent ischemic events** (Bonello et al 2010). In addition, the presence of **the CYP2C19*3A allele, in conjunction with the CYP2C19*2A allele, results in an increased risk of intrastent acute thrombosis** (Harmsze et al 2010). On the contrary**, the CYP2C19*17 allele was associated with an exacerbation of the enzymatic activity, being the allele with a "gain of function", that can increase the risk of bleeding during treatment with clopidogrel** (Sibbing et al 2010). Polymorphisms that induce loss of function (CYP2C19*2, *3) were investigated in the present study (the most frequent in European statistics), as well as those that potentiate it (CYP2C19*17). Since the C3435T (rs1045642) allelic version of the ABCB1 gene influences the intestinal absorption of clopidogrel in patients with cardiovascular diseases (Taubert et al 2006), this polymorphism was also investigated. **The genotyping results obtained by both TaqMan and HRM assays were similar.** The proportion of normal metabolizer patients (wild-type CYP2C19*2*3*17) in our study is much lower compared to the specialized literature, which stresses the need for such studies on much larger groups of patients. The absence of the *3 allele in our study does not exclude the existence of individuals with slow metabolizing or intermediate phenotype (heterozygous for *2 and/or *3 and wild-type *17) resulting from the presence of other alleles with loss of function.

Arterial hypertension, diabetes and obesity have been described as independent risk factors for resistance to clopidogrel. Hypertension is known for its enhanced platelet aggregability and adhesiveness but the relationship between this condition and the incidence of clopidogrel resistance remains unclear (Liu et al 2016, Kim et al 2009). Generally, the metabolic features of obesity with major resistance to insulin and chronic inflammatory status, high platelet reactivity, increased platelet turnover, endotoxemia, and suppression of CYP450 enzyme activity predispose to the alteration of the pharmacological response to clopidogrel and may cause an increased risk of thrombotic events (Norgard et al 2017). Also, **diabetic patients tend to be low responders to clopidogrel due to a prothrombotic condition with high platelet reactivity that might trigger atherothrombotic burden** (Kubica et al 2014). **The prothrombotic state in type 2 diabetes mellitus is related to the endothelial dysfunction, impaired fibrinolysis and coagulation, and also to the impaired platelet function** (adhesion, aggregation and activation) (Angiolillo et al 2009). However, various studies reported conflictual data. Liu et al. (2016) found a significant association of both hypertension and CYP2C19*2 allele with clopidogrel resistance in Chinese patients with ischemic stroke but they did not observe any correlation between poor response to clopidogrel and diabetes in the same subjects. Another study did not identify arterial hypertension, diabetes, obesity, age, platelet count, cholesterol level or concurrent drug intake as clinical predictors of clopidogrel resistance in Indian patients with acute coronary syndrome (Kumar et al 2007). Pankert et al. (2011) reported an impaired responsiveness to clopidogrel only in obese patients with metabolic syndrome, while obese patients without metabolic syndrome showed no significant differences in platelet reactivity compared to nonobese subjects. Also, Gaglia et al. (2011) did not found an association between body mass index and on treatment platelet reactivity (clopidogrel plus aspirin).The same discrepancies were noticed as regards to the influence of smoking. Commonly, the **current smoking was proposed as an independent predictor of low antiplatelet effect of clopidogrel** (Bliden et al 2008). On the contrary, Liu et al. (2016) did not observe any correlation between smoking habit and clopidogrel resistance in ischemic stroke patients.**The identification of a statistically significant association of smoking with the ultrarapid metabolizer phenotype of clopidogrel as in our study is in agreement with data reported by Matetzky et al. (2004) and Desai et al. (2009)**. They showed an enhanced antiplatelet effect of clopidogrel in smokers. A possible explanation is the activation of CYP1A2 enzyme by the polycyclic aromatic hydrocarbons from tobacco smoke. CYP1A2 is the enzyme responsible for the first oxidative step in the metabolic activation of clopidogrel (Feher et al 2010, Matetzky et al. 2004). Certainly, **the response to clopidogrel is not uniform and it is related to multifactorial causes.** Although it has been suggested that CYP2C19 genotype largely contribute to the clopidogrel resistance this should not be interpreted unidirectionally. A systematic meta-analysis performed by Holmes et al (2011) did not demonstrate a clinically significant association of CYP2C19 genotype with cardiovascular outcomes. Besides, Bauer et al. (2011) showed that the data from genetic association studies does not indicate a consistent influence of CPY2C19 polymorphisms on the clinical efficacy of clopidogrel. In the line with these findings, a recent

metabolomic analysis suggested that the CYP3A isoenzymes are involved in the primary metabolism of clopidogrel but not CYP2C19 (Bouman et al 2011). Overall, **the relationship between traditional cardiovascular risk factors with genetic factors involved in clopidogrel resistance is not a simple linear association. Besides genetic makeup, the effect of major risk cardiovascular factors as hypertension, diabetes, obesity, smoking, dyslipidaemia on pharmacological response to clopidogrel is modulated by race- and population-specific differences, clinical conditions, platelet phenotype, clopidogrel treatment schedule, and their particular interactions.**

Although some studies demonstrated that clopidogrel increases the risk of bleeding (Costache et al, 2012, Diener et al, 2004) our findings suggested that hemorrhagic acute events are rare in patients on antiaggregant therapy. These resutlts may have important implications in antiplatelet therapies for patients with or at risk for cardiovascular disease.

The reported risk of bleeding during hospitalization for ACS varies between 1-10% and is influenced by patients comorbidities, age, sex, rates of treatment by revascularization or invasive procedures such as angiography, intraaortic ballon pumps, or coronary artery by pass grafting (CABG) and the antiplatelet /antithrombotic therapy regimen used for treatment (Eisentein et al 2007).

Prior studies have consistently identified female gender, older age, lower body mass index, low creatinine clearence and the use of percutaneous interventions are risk factors for bleeding complications (Eisentein 2007, Ng et al 2008).

Recently, Subhewal et al (2009), published the CRUSADE Bleeding Score, using clinical criteria available at presentation such as: baseline hematocrit, creatinine clearence, heart rate, sex, systolic blood pressure, diabetes, prior vascular disease and the presence of cardiac heart failure upon presentation.

Current ACC/AHA Guidelines for the treatment of patients with ACS recommended dual antiplatelet therapy due to numerous studies demonstrating a reduction in ischemic complications (Patel et al, 2010). The addition of an additional antiplatelet agent does increase the incidence of bleeding, results which were also confirmed in our study.

In the CURE trial of patients with ACS randomized to clopidogrel plus aspirin versus aspirin alone, the rate of major bleeding in patients assigned to clopidogrel plus aspirin was 3.7%, while rate in patients assigned to aspirin alone was 2.7% (RR = 1.33; p=0,001) (Mehta et al, 2000).

Bleeding risk was also significantly higher among patients treated with aspirin and clopidogrel who requiered CABG.

Posthoc analysis of CURE trial demonstrated tahat the bleeding risk was especially high whem CABG was performed within last 5 days of the last dose of Clopidogrel (Mehta et al, 2000).

Patients who suffer from bleeding complications or require transfusions are often treated less aggressively with the evidence based medications placing them at higher risk of future ischemic events.

The Prospective Registry Evaluating Myocardial Infarction Events and Recovery (PREMIER), a registry of 2498 patients with Acute Myocardial Infarction found that patients with bleeding during

the index MI hospitalization were less likely to be treated with antiplatelet therapy and other agents during the first 6 months after discharge. (Al Badarin et al, 2013).
These findings offer insight into the current treatment in patients with bleeding complications and may help explain why patients with bleeding complications are at a significantly higher risk for ischemic events. Bleeding events which occurred during antiplatelet therapy are relatively rare when compared to the large number of patients treated with an antiaggreggant drug in mono or in dual therapy. Bleeding side effect can manifest in different territories and dissapear with cessation of treatment.
Association between double antiaggregation and heparin therapy increased the risk of upper digestive bleeding.

Conclusions:

1) Being relatively less expensive than TaqMan Assay, HRM technique would allow an easier access for a wider number of patients with acute coronary syndromes, that are treated with Clopidogrel. (Costache et al, 2012, Costache et al, 2015).
2) Our results did not demonstrate a statistically significant association of cardiovascular risk factors as hypertension, obesity and type-2 diabetes with CYP2C19 genotypes in our research population. (Costache et al, 2017).
3) On the contrary, a statistically significant correlation was found between smoking or dyslipidaemia and the presence of the ultrarapid metabolizer phenotype for clopidogrel. Further investigations should be conducted on a high number of patients. The analysis of all genetic polymorphisms and the measurement of platelet reactivity may contribute to a strength of research. (Costache et al, 2017).
4) Although performed on a relatively small group of patients, the study allowed a clear positive correlation between the rapid metabolite phenotype for clopidogrel and the frequency of non-fatal haemorrhagic events. Haemorrhagic events occurring with clopidogrel are small in size, but may manifest differently in various areas (cutaneous, oral, mucous, digestive tract, urinary) and disappear when stopping treatment. The rapid metabolic phenotype does not provide complete protection against the repetition of ischemic episodes. Also, all patients with poor metabolic phenotype had a recurrence of angina pains and the need for rapid guidance to interventional cardiology. Patients with unpredictable phenotypes may have unpredictable evolution, some with no angina events, others with recurrence and aggravation of angina, evolution that certainly involves other factors. (Costache et al, 2012, Costache et al, 2015).
5) Haemorragic events under the treatment with antiplatelet agents are rare in comparison of the large number of patients treated. Clinical manifestations are very different depending on the drug and also on the drug associations used. Hemorrhagic accidents may be sometimes be very serious determining the specific therapeutic measures. Our study did not demonstrate an increased risk of bleeding in patients treated with clopidogrel versus aspirin but the double antiaggregation was associated with more hemorrhagic adverse events. (Gîrleanu et al, 2014).

Elements of originality and innovation

- Optimization Protocol Assessment for patients with ACS in terms of response to antiplatelet therapies;
- The introduction of modern tests that provide quality precise, results (which are able to indicate a specific therapy), but that can be achieved with existing lab an affordable cost;
- The prediction since the diagnosis, of the patient's evolution under the treatment with clopidogrel, according to the presence of genetic abnormalities that can lead to defects in the prodrug metabolism;
- The initiation of a collection of DNA enabling application and other molecular methods of investigation when the first test provides unsatisfactory results; thus avoiding excess stress on patients by repeated medical visits and the possibility to optimize laboratory testing.

The estimated impact of the project

- Services are addressed to patients (establishing a scheme for precise treatment);
- Creation of a starting point for future projects (more research-related and other polymorphisms in the cytochrome study or superfamily of CYP450 that can cause resistance to anticoagulant therapy, use of data / methods obtained for evaluation / explanation other drug resistance using the same metabolic line - antipsychotics, antiepileptics, etc, and the interactions of these drugs), and premises in order to become part of similar European projects.

The interdisciplinary character

- The project was interdisciplinary as it brings together the expertise of large areas: Cardiology, Molecular Genetics, Epidemiology, Pharmacology.

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I.2.1.2. Other scientific papers published in the proposed theme:

Books:

1. "Ischemic preconditioning and reperfusion" - Author: **Iuliana Irina Costache**, Edit. Junimea, Iasi, 2005.

2. "Left ventricular remodeling in acute myocardial infarction" Author Irina Costache, edit Junimea, 2006.

3. Coordinator for the book "Integrative METHOD FOR STUDY OF HEART - myocardial ischemia" Publisher UMF "Grigore T.Popa" Iasi, 2014 The project "e-Mediqual" POSDRU 86 / 1.2 / S / 63 815.

Coordinators (in alphabetical order): Doina Azoicăi, Catalina Arsenescu Georgescu, Irina Draga Căruntu, Diana Cimpoeşu, Manuela Ciocoiu, **Irina Iuliana Costache**, Daniela Cristina Dimitriu, Beatrice Ioan Radu Iliescu Florin Mitu Florin Dumitru Pietrariu, Antony Octavian Petris, Ovidiu Petris, Dragomir Nicolae Serban Lăcrămioara Ionela Serban, Gregory Tinică Traian Taranu.

ISI Articles:

1. Antoniu Petris, Diana Cimpoesu, **Irina Costache**, Irinel Rotariu **"Do not resuscitate decision (I). Ethical during cardiopulmonary resuscitation ".** Rev Rom Bioethics 2011; 9 (2): 40 - 49. **IF = 0, 683**

2. Diana Cimpoesu, Irinel Rotariu, **Irina Costache,** A.Petriş. **"Do not resuscitate" decision (ii). Ethics and law in cardiopulmonary resuscitation** "Rev Rom Bioethics 2012; 10 (2): 29 - 41. **IF =1.**

Other publications

1. Irina Costache, Iliescu Dan Anca Rogojanu. **"Ventricular septal defect secondary acute myocardial infarction"** - clinical case. Rev Chir Soc Med Suppl Clinic Magazine, 2003; vol. VIII.

2. Irina Costache, A.Petriş, M.D. Datcu. **"Peculiarities of clinical course of acute myocardial infarction in women - study 80 cases."** Rev Chir Soc Med Suppl Clinic, 2002; vol.VII, (6) 2002. Presented at the oral form XLI Congress of Cardiology Sinaia 2002, published in abstracts, p 81

3. Irina Costache. "Acute myocardial infarction in women - ethiopatogenic features and clinical course." General review. Rev Chir Soc Med Suppl Clinic Magazine, 2002 Vol. VII (2).

4. Datcu MD, A. Petris, **Irina Costache** et al: **"Acute myocardial infarction in Elderly Patients: walking in the twilight zone of guidelines "**Eur Heart J 2006, 27 Suppl

5. Datcu MD, A.Petriş, **Irina Costache** et al **"The current treatment very elderly patients (over 75 years) with acute myocardial infarction: limits and progress".** Rom Cardiol Rev. 2006, Vol XXI, Suppl A: 42

6. Datcu MD, Viviana Aursulesei**, Irina Costache,** et al. "Stimulating catheter thrombosis - clinical case" Rom Cardiol Rev 2008: 18 work awarded at the 47 th National Congress of Cardiology with the prize on atherothrombosis

I. 2. 2. The approach of cardiovascular pathology, especially the ischemic one, namely in terms of biochemical data.

The objectives of the researches were:
1) To assess **the new biochemical markers** involved in the pathogenesis, the evolution and prognosis of CVD (especially ischemic one);
2) To assess **biochemical markers usefull in emergencies.**
3) To perform an **experimental model with direct implications on cardiovascular risk factors (CVRF)**. New therapeutic perspectives.

I.2.2.1 New biochemical markers involved in the pathogenesis, the evolution and prognosis of CVD (especially ischemic one).
Background:
a) Coronary artery disease (CAD) is one of the major predictors of future cardiovascular events (CVE). Besides the traditional risk factors**, a high correlation with a future CVE is being exhibited by new biomarkers such as high-sensitivity C-Reactive Protein (hsCRP), fibrinogen, homocysteine, and free fatty acid (FFA).** The Framingham study design remains a milestone in identifying the CVD risk factors. By using the Framingham Risk Score many clinical trials showed that treating **modifiable risk factors** with evolution towards normal range of the values, the likelihood of developing CVD can be reduced. The Framingham investigators elaborated these functions through multivariate algorithms, where the dependent variable was the CVD, and, as for the explanatory variables, they considered the following independent variables: **age, gender, systolic blood pressure, total cholesterol, highdensity lipoprotein cholesterol, smoking behavior, and diabetes status.** The above mentioned association allows the estimation of the risk over a fixed time, as in ten years from now, of developing a cardiac or vascular condition (coronary heart disease, stroke, peripheral vascular disease, or heart failure). The Framingham study highlighted the multifactorial aspect of the cardiovascular risk and the time-correlation between the risk factors to induce the CVD onset, and the imperious need for drug treatment recommendations for dyslipidemia and arterial hypertension (D'Agostino et al 2013).
Half of cardiovascular mortality is accounted for by sudden cardiac death (SCD), of which 50% of patients were not previously diagnosed with heart disease (Huikuri et al, 2001, Wellens et al, 2014). **Additional risk factors for the onset of CVD are homocysteine, fibrinogen, lipoprotein(a), low density lipoprotein particle size and C-reactive protein** (Bandara et al 2016). **Hypercholesterolemia is an independent risk factor for ischemic heart disease** (Lloyd-Jones et al 2010). It is well-known that serum **low-density lipoprotein (LDL) concentration** has a direct positive correlation with the incidence of cardiovascular complications, mainly ischemic events, being set as the target for the lipid control. Different lipoproteins, as high-density

lipoproteins (HDL) and triglycerides are responsible for the onset, development, and destabilization of the entire atherosclerotic process (Assmann et al 2007, Bayturan et al, 2010, Faergeman et al 2009). Recent data suggest that **chemerin** could be important for the pathophysiology of obesity. However, its relation with clinical indices of obesity and metabolic parameters is controversial and less studied in metabolic healthy patients with morbid obesity (Benjamin et al 2017).

The Framingham Risk Score is a gender-specific algorithm used to estimate the 10-year cardiovascular risk of an individual. Cardiac rehabilitation is an instrument of medical management in cardiovascular diseases; beyond prevention, it can improve heart and muscle functioning in patients that were undergoing CABG and cardiac and vascular adaptation.

b) Heart-type Fatty Acid-Binding Protein (H-FABP is a non-invasive bio-marker, with high sensitivity and specificity, capable of pointing out the myocardial injury and predicting major adverse cardiovascular events (MACE) (Connolly et al 2018). Heart-type Fatty Acid-Binding Protein (H-FABP; FABP3) is a small cytoplasmic unbound protein of 15 kDa (smaller than Myoglobin =18 kDa, Troponin I =22 kDa, Troponin T=37 kDa and CK-MB =86 kDa) released from the cytoplasm of the cardiac myocytes following an ischemic cardiac episode. H-FABP takes part in the intracellular uptake of long chain fatty acids in the myocardium [Glatz et al 1988]. H-FABP binds long chain fatty acids in the cytosol and thus protects myocardial cells, whilst it is less protective for skeletal muscle, brain and kidney cells. **Myocardial ischemia leads to mitochondrial edema and membranous disintegration, releasing intracellular constituents into the blood and increasing plasma levels of cardiac enzymes in acute myocardial infarction and after coronary artery bypass grafting (CABG).** H-FABP is released earlier than Troponin T (TnT) due to its smaller size (15 kDa). A high level of H-FABP has been shown to be a marker of cell necrosis correlated with the infarct size, and it can predict subsequent cardiovascular events (postoperative atrial fibrillation and acute renal injury) in patients with negative TnT and acute coronary syndrome (Liu et al 2010). **There is a relationship between high H-FABP preoperative levels in patients undergoing coronary bypass surgery (CABG) and the higher probability of acute renal failure in postoperative time** (Otaki et al 2014). H-FABP concentration increases in the first 1.5 h, reaching a peak within 5-6 hand tends to decrease after 6 h; it returns to normal levels 24- 30 h later.

Cardiac rehabilitation (CR) program plays an important role in reducing the plasma levels of H-FABP protein, confirming that an early and sustained post-interventional rehabilitation treatment plays an important role in reversing the atherosclerotic process and preventing postoperative complications such as: arrhythmias and cardiac insufficiency, myocardial ischemia, biological changes, and renal failure (Al Namat et al 2017).

The specific feature of this protein is the association with myocardial damage area size in patients undergoing cardiac surgery. Elevated H-FABP serum concentrations could also be found in other conditions, like heart failure, chronic kidney disease, diabetes mellitus, and metabolic syndrome. Up to now, 10 variants were found in this family, expressed in different

tissues. H-FABP is released less than 30 min after myocardial injury in both humans and animals, and its renal excretion is within 24 hours (Oezkur et al 2014). The state of the art in research offers new directions in detecting cardiac lesions (myocardial infarction, postoperative myocardial injury and ongoing ischemic damage in heart failure) by discovering the H-FABP (Huang et al 2014, Otaki et al 2014). In the conditions mentioned above, high H-FABP levels are strongly associated in case of death (Zhang et al 2012, Parikh et al 2013, Schaub et al 2015). Otaki and colleagues published in 2014 a study upon the relation between the elevated pre-operative H-FABP levels in patients undergoing elective coronary artery bypass graft (CABG) surgery and the higher likelihood to experience post-operatively acute kidney injury (AKI). In perioperative and postoperative period of cardiac surgery, a severe and frequent complication that can occur is myocardial infarction (Pretto et al 2015). There are recent studies which state that it can be used especially in the early diagnosis of acute coronary syndrome, cardiac failure, kidney and liver injury, pulmonary embolism and some poisonings (Akpinar et al 2017, Liu et al 2010). Among the instruments of medical management in CVD, the guidelines have highlighted the role of CV prevention and its usefulness in primary, secondary, and tertiary prevention. **The cardiac rehabilitation (CR) program is comprised of**: medical evaluation and treatment, supervised exercises, education and counseling of patients, CR proving to be an essential and safe part of the care in postoperative program after CABG (Niebauer, 2016). Regular physical activity is fundamental in CR program, improving heart and muscle functioning in patients that were undergoing CABG. The benefits of exercise training are seen in cardiac and vascular adaptation with an enhanced blood flow towards the muscles, a reduction in the oxidative stress, and an amelioration of the endothelial dysfunction and arterial stiffness (Wilson et al 2016). Many studies that measured the modifying effects on the coronary risk factors, as well as hypertension, depression, and obesity, have underlined the positive effects of CR and exercise training programs (Ghashghaei et al 2012, Khalife-Zadeh et al 2015). By following the CR programs it was pointed out the very important risk reduction of 40% of cardiac morbidity and mortality (Raja et al 2012). The diagnosis of elevated H-FABP levels in postcardiac surgery regional acute myocardial infarction is occasionally challenging (Carmona et al 2015).

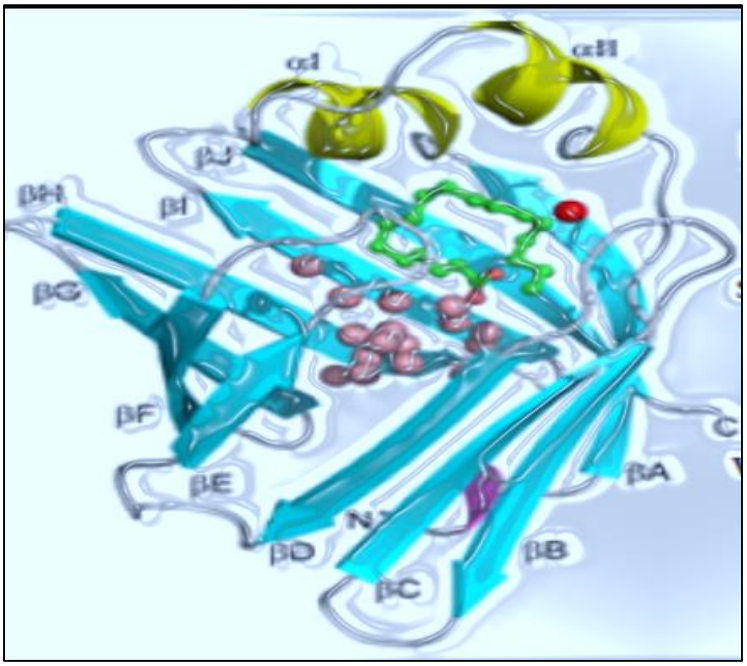

Fig. 2.2.1. Structure of human heart-type fatty acid-binding protein. FABP3 has a clum-like shell structure which consists of a pair of five- stranded antiparallel β –sheets that surround a large water-filled cavity (13 water molecules and a fatty acid covered on the top by two short parallel α –helices). (modificat după http://resou.osaka-u.ac.jp/en/research/2015/20150107_1)

c) Obesity is one of the most important modifiable CVR factors. The dramatical increase of obesity in recent years has lead to a high rate of cardiovascular and metabolic diseases and also of mortality (Bastien et al 2014). Abdominal perivascular fat tissue (PVAT) is recognized as an important player in obesity-mediated disorders, being a wide source of biologically active molecules termed adipokines, that can act in both autocrine and paracrine fashion (Chang et al 2013). Obesity could cause PVAT dysfunction which is induced by complex but not fully elucidated mechanisms involving adipocyte and hypoxia, insulin resistance, oxidative stress, vascular inflammation, and macrophage activation as early stages of atherosclerosis (Greenstein et al 2009, Lim et al 2014, Brown et al 2014). **Chemerin is a novel adipokine with controversial role in obesity** (Mattern et al 2014) which may link obesity to vascular inflammation, metabolic changes and atherosclerosis (Ouchi et al 2011). The relation of chemerin with clinical indices of obesity is conflictual (Sell et al 2010, Bozaoglu et al 2007, Kim et al 2014, Chakaroun et al 2012). Bozaouglu et al first demonstrated that **circulating chemerin levels correlate with the components of metabolic syndrome such as body mass index (BMI), triglycerides and blood pressure**. A very recent meta-analysis also confirms that **chemerin is related to BMI and insulin resistance, so it could be important for the pathophysiology of obesity** (Li et al 2014).

d) It is a well known fact that **the association of CV risk factors leads to a high increase of mortality and morbidity among cardiovascular patients**. The old patients and ”old-old” patients are considered at high risk. The number of individuals ≥85 years of age represents a

growing group in many countries. During the efforts to improve the life expectancy in older adults have been identified a number of relevant differences between the "young old" (65 to 74 years of age), the "older old" (75 to 84 years of age), and the "oldest old" (≥85 years of age) (Aronow et al 2011). The patients older than 70 years are becoming more a more common in the admissions for acute pulmonary edema (Chioncel et al 2014) or which are directed to ICU (Chioncel et al 2015). Two recent Italian surveys (TEMISTOCLE and CONFINE studies) found that the mean age of patients admitted for CHF increased from 77 years in 2002 to 79 years in 2008 and that the number of very old patients (>85 years) was substantial and increasing year-by-year (Biagi et al 2014). Although the incidence and prevalence of over 85-year-old HF patients in 'real-life' is increasing they have many issues still doubtful, primarily due to the exclusion of elderly people from most randomized controlled trials on HF (Heiat et al 2002). Octogenarians HF patients are managed despite the fact that clinical benefits at this age of some drugs are not clear proven (Vorilhon et al 2015). For the old old patients (pts) with congestive HF the use of combinations of drugs can easily lead to a sort of "evidence-based" polypragmasie. **Hyponatremia, anemia, increased BNP, blood urea nitrogen and serum uric acid are associated with increased mortality of old old patients.**

e) Heart failure is associated and related with an enormous burden on both patients and health care systems. Several national policy initiatives have concentrated on improving the quality of heart failure care, including reducing readmissions following a first hospitalization, which are common, costly, and, at least in part, preventable or avoidable. Several studies suggest that attention to details regarding patient comorbidities, barriers to care, optimization of both diuretic and neurohormonal therapies, and evaluation of prognosis would improve patient outcome. In the Cardiology Department, the most common indication for hospitalization and readmission within 3 to 6 months from initial discharge is the congestive heart failure. Moreover, both the reccurence and prevalence of congestive heart failure are increasing and the rate of risk is higher in patients over 75 years of age than in those 65 years old or younger (Krumholz et al 2009, Joynt et al 2011, Chun et al 2012, Lindenauer et al 2007). It can be appreciate that early rehospitalization in patients with congestive heart failure may be avoidable in up to 50% of cases. Identification of high risk patients is possible and also necessary shortly after admission in order to identify nonpharmacological interventions designed to decrease readmission frequency.

I.2.2.1.1.The concerns regarding the risk factors were materialized in the following publications:

1. Al Namat Razan, **Irina Iuliana Costache*, (corresponding author)** Maura Gabriela Felea, A. Petris, Viviana Aursulesei, O. Mitu, Nadia Al Namat, Dina Al Namat, M. Constantin, F. Mitu: **Lipid Profiles and Framingham Risk Score in Patients with Coronary Artery Bypass Graft Surgery undergoing Cardiac Rehabilitation Program;** Rev.Chim. 2017; 68 (10): 2219 – 2223; **IF= 1.412.**

2. Al Namat Razan, Viviana Aursulesei*, Maura Gabriela Felea,, **Irina Iuliana Costache**, A. Petris, O. Mitu, Nadia Al Namat, M. Constantin, Dina Al Namat, Cristina Ghiciuc, Catalina Elena Lupusoru, G. Tinica, F. Mitu: **Heart-type fatty acid-binding protein (H-FABP) in patients with coronary artery bypass graft surgery undergoing cardiac rehabilitation program**; Rev.Chim. 2017; 68 (7): 1485 -1489, **IF = 1.412**

3. Al Namat Razan, Maura Gabriela Felea, **Irina Iuliana Costache* (corresponding author),** Viviana Aursulesei*, A. Petris, O. Mitu, M. Constantin, Victorita Sorodoc, L. Sorodoc, Ionela Larisa Miftode, R. Miftode, Nadia Al Namat, Dina Al Namat, Andreea Luta, Adriana Ion, Mirela Mihaela Mihalcia, Manuela Ciocoiu, G. Tinica, F. Mitu: **"Heart-Type Fatty Acid-Binding Protein (H-FABP) in Patients with Type 2 Diabetes Beneficiaries of Rehabilitation Program Post Coronary Artery Bypass Grafting"** Rev.Chim. 2018; 69 (10):, pag 2712-2717, **IF = 1,412.**

4. Aursulesei Viviana*, Daniel Timofte, Liliana Mititelu Tarțau, Veronica Mocanu, Razan Al Namat, Victor Cristian Aursulesei, **Irina Iuliana Costache: Circulating chemerin levels, anthropometric indices and metabolic profile in morbid obesity,** Rev.Chim. 2018; 69 (6): 1412 – 1423, **IF = 1.412.**

5. Al Namat Razan, Mihai Constantin*, Ionela Larisa Miftode*, Andrei Manta, A. Petris, R. Miftode, A. D. Costache, D. Iliescu, **Irina Iuliana Costache**: **Biochemical Markers in Patients with Readmission for Congestive Heart Failure,** Rev.Chim. 2017; 69 (7): 1687-1691. **IF = 1.412.**

6. Petris A., G. Tatu-Chitoiu**, Irina Costache**, Diana Tînț: **"Survival variables in old old patients (> 85 years) with chronic heart failure".** Mol Cryst Liq Cryst, 2016;628: 7-14; ISSN: 1542-1406 (print), 1563-5287 (online). http://dx.doi.org/10.1080/15421406.2015.1137411. **IF = 0.571**

Other Publications in relation with the section I.2.2.

1) **Costache II**, Cristiana Vlad, Alexandru Dan Costache, Victor Cristian Aursulesei, and Viviana Aursulesei: **Role of Genetics in the Ethiopathogeny of Lower Extremity Artery Disease.** Ann Vasc Med Res 2017; 4(7): 1078.
2) Miftode RS, Viviana Aursulesei*, Larisa Miftode, Amalia Stefana Darie, Ana Maria Buburuz, Adriana Ion, Alexandru Dan Costache, and **Irina Iuliana Costache. Syndecan-1: New Perspectives of Risk and Prognostic Assessment in Heart Failure.** Ann Vasc Med Res 2018; 5(1): 1083.
3) Aprotosoaie Ana Clara, Anca Miron, Adriana Trifan, Vlad Simon Luca, **Irina Iuliana Costache: The Cardiovascular Effects of Cocoa Polyphenols – An Overview;** Diseases 2016, dec 4(4): 39, published online dec 17; Doi: 10.3390/diseases4040039;

Ethics

All the studies were conducted in accordance with the Declaration of Helsinki. Patient data were extracted from the observation charts with respect of their confidentiality and anonymity. On admission, each patient signed an informed consent form according to the regulations of the Rules of Procedure of the Emergency County Hospital 'St. Spiridon' Iasi, Romania.

1) New biochemical markers involved in the pathogenesis, the evolution and prognosis of CVD (especially ischemic one).

1. Razan Al Namat, **Irina Iuliana Costache*, (corresponding author)** Maura Gabriela Felea, A. Petris, Viviana Aursulesei, O. Mitu, Nadia Al Namat, Dina Al Namat, M. Constantin, F. Mitu: **Lipid Profiles and Framingham Risk Score in Patients with Coronary Artery Bypass Graft Surgery undergoing Cardiac Rehabilitation Program;** Rev.Chim. 2017; 68 (10): 2219 – 2223; **IF= 1.412.**

2. Razan Al Namat, Viviana Aursulesei*, Maura Gabriela Felea,, **Irina Iuliana Costache**, A. Petris, O. Mitu, Nadia Al Namat, M. Constantin, Dina Al Namat, Cristina Ghiciuc, Catalina Elena Lupusoru, G. Tinica, F. Mitu: **Heart-type fatty acid-binding protein (H-FABP) in patients with coronary artery bypass graft surgery undergoing cardiac rehabilitation program**; Rev.Chim. 2017; 68 (7): 1485 -1489; **IF = 1.412**

3. Razan Al Namat, Maura Gabriela Felea, **Irina Iuliana Costache [1]*(corresponding author),** Viviana Aursulesei*, A. Petris, O. Mitu, M. Constantin, Victorita Sorodoc, L. Sorodoc, Ionela Larisa Miftode, R. Miftode, Nadia Al Namat, Dina Al Namat, Andreea Luta, Adriana Ion, Mirela Mihaela Mihalcia, Manuela Ciocoiu, G. Tinica, F. Mitu: **"Heart-Type Fatty Acid-Binding Protein (H-FABP) in Patients with Type 2 Diabetes Beneficiaries of Rehabilitation Program Post Coronary Artery Bypass Grafting"** Rev.Chim. 2018; 69 (10): 2712-2717, **IF = 1,412.**

Aim of the sudies:

1) to determine whether lipid profiles and Framingham Risk Score would change in patients post CABG surgery, undergoing a cardiovascular recovery program;

2) to compare the plasmatic level of H-FABP in patients post CABG surgery, undergoing a cardiovascular recovery program. The levels were compared between the first phase developed during the first postoperative week, and the third phase carried out after 6 months;

3) to compare the plasmatic level of H-FABP in patients post CABG surgery, undergoing a cardiovascular recovery program, comparing results between patients with Type 2 Diabetes Mellitus and patients free of diabetes.

Material and methods

This was a prospective study including 120 patients admitted in the Clinic of Cardiovascular Surgery of the Institute of Cardiovascular Disease, following the cardiovascular recovery program immediately, at 3 and at 6 months after the cardiac surgery in the Cardiovascular Rehabilitation Clinic of the Rehabilitation Hospital of Iasi.

The **inclusion criteria** were: CABG patients (less than 1 week), aged 40-80 years old, BMI > 25 kg/m², and mixed dyslipidemia and intellectually capable to follow the rehabilitation cardiovascular program. The age range considered was optimal because the cardiovascular risk in this age group should be determined steadily, and any additional investigations are needed in order to evaluate the true vascular age.

The study was approved by the University Ethics Committee and all participants signed an informed consent. In both phases, for every patient, it was performed a clinical examination, a set of hematological, biochemical, (lipid, coagulation and inflammatory profile), and ECG and echocardiography (LVDd, LVSd, IVSd, PWd, LVM, LVMI, EF, and SF) were performed in both phases. Blood pressure, heart rate and effort capacity (METs) were also monitored during the rehabilitation program.

The following parameters were determined: glucose, serum urea, creatinine and uric acid level; Alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transferase (GGT) were assessing the hepatic function. High–density lipoprotein (HDL) cholesterol, low–density lipoprotein (LDL) cholesterol, total cholesterol and triglycerides were assessing the lipid profile.

Glomerular filtration rate (GFR) was calculated according to the most accurate formula of CKDEPI (Chronic Kidney Disease Epidemiology Collaboration):

eGFR = 141 x min(SCr/k,1)α x max(αCr/k,1)–1.209 x 0.993Age x [1.018 if Female] (where SCr is serum creatinine (mg/dL), k is 0.7 for females and 0.9 for males, α is –0.329 for females and –0.411 for males, min indicates the minimum of SCr/k or 1, and max indicates the maximum of SCr/k or 1) (Levey et al 2009).

Uric acid increase CV risk in case of high values. Serum fibrinogen level was used to determine the state of inflammation. Fibrinogen acts in the normal blood coagulation cascade and as a key regulator of inflammation in disease.

Ejection fraction (EF) and other cardiac parameters (LVDd, LVSd, IVSd, PWd, LVM, LVMI, and SF) were performed by echocardiography.

Statistical Analysis

The database was compiled in Microsoft Office Excel 2010 version, and statistical analysis was performed in the IBM SPSS Statistics v.20, calculating the averages, frequencies, standard deviations, differences between the maximum and minimum values of the numerical parameters. The statistical significance of the difference between two frequencies was determined by the Chisquare test of independence. The t Student test was used to reveal the significance of the difference between two average values. The threshold values for p were considered < 0.05, providing a statistical significance level of the test. The regression equations and correlation coefficients were also calculated.

Results

The mean age of the patients under study was 65.70 ± 9.91 years old, of which male age was 65.26 ± 10.26, respectively 66.96 ± 8.89 for female patients. Approximately one third of these, 29 patients, were female and 81 were male. The median age was 65 years. Most people were from urban areas (89%) and only 11% from rural areas. The smoking status was present in 58% of the cases and absent in 42% of the cases. Electrocardiogram (ECG) changes showed that atrial fibrillation was relatively frequent in Phase I (66.33%), whereas 91% regained sinus rhythm in Phase III. In the first stage, white blood cells (WBC) were high, probably indicating an inflammatory syndrome. Inflammation and postoperative hemorrhage could explain the low hemoglobin (Hb) and hematocrit (Ht) values, ameliorated six months later. An increase in the blood platelet counts was noticed. Plasma glucose values decreased in the IIIrd Phase (table 2.2.1) (Al Namat et al 2017).

Table 2.2.1 Biochemistry - comparative data between the rehabilitation phases

Biochemical values	Mean	Std deviation	Std Error mean	Sig. (2-tailed)
TGP 1	41.02	39.928	3.993	.000
TGP 3	26.50	14.749	1.475	
TGO 1	34.99	27.235	2.724	.000
TGO 3	25.03	18.449	1.845	

GGT 1 GGT 3	41.82 41.55	25.786 48.110	2.579 4.811	.955
Glycaemia 1 Glycaemia 3	139.55 121.28	58.081 46.451	5.808 4.645	.000
Urea 1 Urea 3	46.17 42.13	14.424 12.139	1.442 1.214	.000
Creatinine 1 Creatinine 3	1.2972 1.0924	.46679 .21551	.04668 .02155	.000

For biochemical samples (table 2. 2.1), **we obtained high statistical significance for all pairs of data that were compared, except for the serum gamma-glutamyl transpeptidase (GGT).** According to the calculated means, a decrease of the values in Phase III was observed for all the studied variables. GGT did not undergo Phase III changes compared to Phase I. **The renal filtration function registered a statistical significant improvement, and both creatinine and blood urea were diminished. Lipid profile values showed a statistically significant decrease, but with no change regarding the cardiovascular risk range** (table 2.2.2.). (Al Namat et al 2017).

Table 2.2.2. Lipid markers - comparative data after 6 months of rehabilitation

Biochemical tests	Mean	Std deviation	Std Error Mean	Sig. (2-tailed)
Chol 1	182.69	47.945	4.795	.003
Chol 3	170.31	50.619	5.062	
HDL 1	40.23	22.762	2.276	.000
HDL 3	50.04	26.586	2.659	

Fibrinogen, supported by C-reactive protein (CRP) level changes, showed significant phase-to-phase reductions. For bleeding times, there was statistical significance in all variables: $p < 0.001$. APTT decreased in Phase III, INR increased in Phase III, and TQ decreased in Phase III (table 2.2.3.) (Al Namat et al 2017).

Table 2.2.3 Coagulation and inflammation – comparative data between phase I and III.

Bleeding time	Mean	Std deviation	Std Error mean	Sig. (2-tailed)
APTT 1	34.02	7.374	.741	.000
APTT 3	31.69	5.912	.594	
INR 1	1.2845	.65564	.06589	.000
INR 3	2.087	.6331	.0636	
TQ 1	17.29	6.873	.691	.000
TQ 3	13.82	4.012	.403	
CRP 1	3.88	3.179	.318	.000
CRP 3	1.725	2.6791	.2679	
Fibrinogen 1	640.79	175.120	17.512	.000
Fibrinogen 3	442.75	115.201	11.520	

Regarding the echocardiographic parameters, statistical significance was obtained for the first 8 variables. These variables LVDd, LVSd, IVSd, PWd, LVM, and LVMI showed a reduced value in phase III (confirmed by the mean values) compared to first phase. EF and SF variables registered an increased value in Phase III, in concordance with symptomatology and effort capacity. The statistical significance confirmed that these changes were based on a factor that had a major influence upon the values in Phase III compared to Phase I. Patients were grouped accordingly by the degree of physical exercise that they were able to achieve immediately after CABG. In the first recovery phase (one week after myocardial infarction), most patients were able to undergo only a minimal effort of one MET, less than 38% an exercise of two METs, and less than 10% an effort of 3 METs. No one did more than 3 METs. In the third phase of cardiovascular recovery, all patients have improved and even exceeded their poor physical condition by performing average efforts of 4-5 METs, with nearly 2/3 of patients reaching 5 METs. The effects of the cardiac rehabilitation program in patients after cardiac surgery was seen in decreasing complications and improving QoL. (Al Namat et al 2017).

In our prospective study on hospitalized patients undergoing CABG, by comparing the Phase I and Phase III results, we observed that **the median 10-year Framingham cardiovascular risk score was approximately 6% lower ($p < 0.05$), reflecting the survival benefit gained by patients under the intensive cardiovascular recovery program** (fig. 2.2.2). (Al Namat et al 2017).

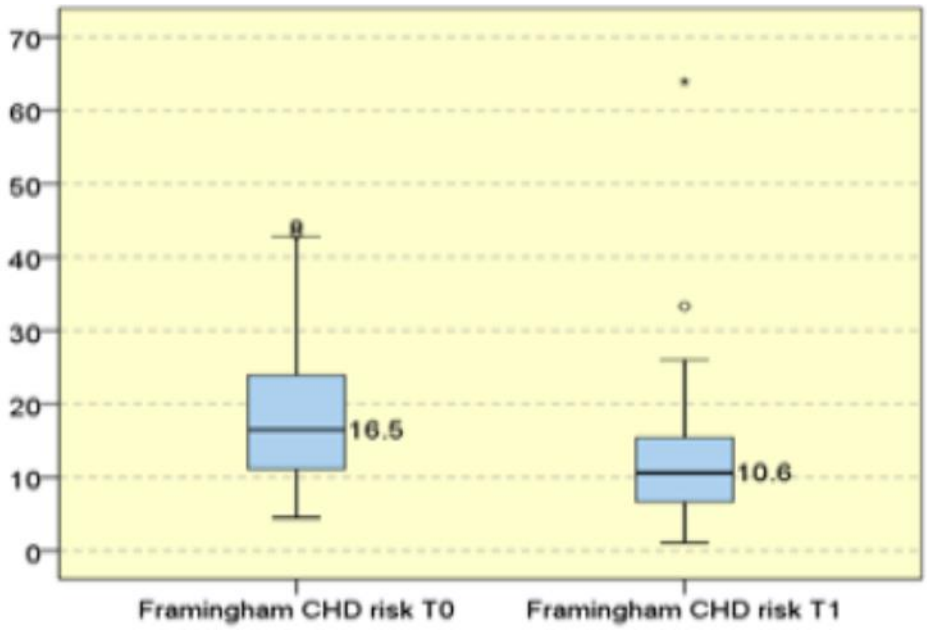

Fig. 2.2.2 Box-plot Diagram – Patients distribution on the Framingham CHD score in Phase I and III of cardiac rehabilitation after CABG

The weight (W) in phase I (W1) was compared with the weight in phase III (W3), respectively the BMI at the two moments, resulting in a difference of high statistical significance, resulting by following both diet and a balanced lifestyle, and cardiovascular gymnastics recommended in the rehabilitation program (table 2.2.4). (Al Namat et al 2017).

Table 2.2.4 Anthropometric parameters in phase I and III post cardiac surgery

Weight and BMI	Mean	Std deviation	Std error mean	Sig. (2-tailed)
W 1	81.80	12.595	1.260	.000
W 3	78.41	13.020	1.302	
BMI 1 (kg/m2)	29.55	3.147	.315	.000
BMI 3	28.36	3.492	.349	

For the H-FABP, the mean value in the Phase I was 67.40 ± 9.81, while the mean value in Phase III was 4.80 ± 2.30. The difference registered between the H-FABP plasmatic value in the first 24 h after cardiac surgery and the value in 6 months after the onset of cardiac rehabilitation program was important and statistically significant as $p < 0.05$. Systolic blood pressure (SBP) and diastolic blood pressure (DBP), as well as heart rate, were controlled under rehabilitation treatment,

reaching diurnal values within the normal range. For the echocardiographic parameters, we obtained statistical significance for the first 8 variables. These variables LVDd, LVSd, IVSd, PWd, LVM, and LVMI showed a reduced value in phase III (confirmed by the mean values) compared to first phase. EF and SF variables registered an increased value in Phase III. The statistical significance was a confirmation that these changes were not random, but based on a factor that actually influenced the values in Phase III compared to Phase I (table 2.2.5). (Al Namat et al 2017).

Table 2.2.5 Comparative analysis of anthropometric and echocardiographic parameters between the stages of cardiovascular recovery: phase I – phase III.

Echocardiographic parameters	Mean	Std. deviation	Std error mean	Sig. (2-tailed)
LVDd 1 (mm)	52.14	8.914	.891	.000
LVDd 3	48.89	9.195	.919	
LVSd 1 (mm)	36.65	8.776	.878	.000
LVSd 3	33.02	9.497	.950	
IVSd 1 (mm)	13.10	2.389	.239	.000
IVSd 3	12.26	2.623	.262	
PWd 1 (mm)	12.25	1.445	.145	.000
PWd 3	11.49	1.648	.165	
EF 1 (%)	43.55	7.615	.761	.000
EF 3	52.35	10.593	1.059	
SF 1 (%)	25.55	6.420	.642	.000
SF 3	27.78	6.907	.691	
LVM 1 (g)	276.43	94.761	9.476	.000
LVM 3	228.86	89.106	8.911	
LVMI 1 (g/m^2)	143.11	47.403	4.740	.000
LVMI 3	120.45	46.232	4.623	
RWT 1 (mm)	.4805	.08739	.00874	.680
RWT 3	.4834	.10875	.01088	

Plasma glucose values have been decreasing in both absolute and percentage terms, proving to improve the profile of carbohydrate metabolism, with an important role in the cardiovascular risk. For biochemical samples, we obtained high statistical significance for all pairs of data that were compared, except for the serum gammaglutamyl transpeptidase (GGT). According to the calculated means, a decrease of the values in Phase III was observed for all the studied variables. GGT did not undergo Phase III changes compared to Phase I. Inflammatory samples, represented by C-reactive protein (CRP), and fibrinogen showed significant phaseto-phase reductions. In the first stage, white blood cells (WBC) were elevated, most likely indicating an inflammatory syndrome, also confirmed by low hemoglobin (Hb) and hematocrit (Ht). These latter two values may also have been low in the context of postoperative hemorrhage. At six

months later, in the third phase, the anemic syndrome was absent and it was confirmed by the improvement of hematological profile. In the same context, an increase in the blood platelet counts also occurred (table 2.2.6.). (Al Namat et al 2018).

Table 2.2.6. Biochemical/haematological parameters comparative data.

Biochemical tests	Mean	Std. Deviation	Std. Error Mean	Sig. (2-tailed)
CRP 1	3.88	3.179	.318	.000
CRP 3	1.725	2.6791	.2679	
Fibrinogen 1	640.79	175.120	17.512	.000
Fibrinogen 3	442.75	115.201	11.520	
Chol 1	182.69	47.945	4.795	.003
Chol 3	170.31	50.619	5.062	
HDL 1	40.23	22.762	2.276	.000
HDL 3	50.04	26.586	2.659	
LDL 1	143.95	31.067	3.107	.000
LDL 3	122.06	29.619	2.962	
TG 1	146.63	55.629	5.563	.001
TG 3	131.62	56.238	5.624	
Na 1	141.13	5.200	.520	.000
Na 3	138.62	5.908	.591	
K 1	4.6370	.36700	.03670	.000
K 3	4.38	.392	.039	
Urea 1	46.17	14.424	1.442	.000
Urea 3	42.13	12.139	1.214	
Creatinine 1	1.2972	.46679	.04668	.000
Creatinine 3	1.0924	.21551	.02155	
Hematological tests	Mean	Std. Deviation	Std. Error Mean	Sig. (2-tailed)
WBC 1	10497.40	10.6417	10.6417	.007
WBC 3	8500.40	82.3686	823.686	
RBC 1	3.7707	.55200	.05520	.000
RBC 3	4.2347	.56213	.05621	
Hb 1	10.95	1.158	.116	.000
Hb 3	12.57	1.819	.182	
Ht 1	34.07	4.279	.428	.000
Ht 3	37.96	5.386	.539	
MCV 1	91.1373	9.27110	.92711	.113
MCV 3	89.7194	5.52901	.55290	
MCH 1	29.3926	3.41060	.34106	.388
MCH 3	29.7209	2.30598	.23060	
MCHC 1	32.2865	2.24967	.22497	.005
MCHC 3	33.1510	2.04881	.20488	
PLT 1	362260.00	90041.586	9004.159	.000
PLT 3	316880.00	130334.866	13033.487	

The return to sinus rhythm was spontaneous or under medication in most of the cases, within one hour to one week after CABG. (Al Namat et al 2017).

In our prospective study on hospitalized patients undergoing coronary artery bypass grafting, we noticed, like other researchers, the correlation between the occurrence of postoperative atrial fibrillation (POAF) and the plasmatic level of H-FABP, recognized as a sensitive marker for myocardial ischemic lesion. (Al Namat et al 2017).

In addition, **patients at extreme age enrolled in the study demonstrate the benefit of interventional cardiology and cardiovascular surgery in very old male patients.**

Between phase I and phase III of the cardiovascular recovery program, for six months duration, patients with atrial fibrillation converted to sinus rhythm. (Al Namat et al 2017).

Lipid profile values showed a statistically significant decrease, although in those in whom the initial values exceeded the upper limits of normality, the reduction did not bring the patient to a lower cardiovascular risk range. **The plasma ionogram showed an improvement of electrolytes values between the two phases, but with no importance for the post-infarction clinical evolution** (table 2.20). (Al Namat et al 2017).

The renal filtration function evaluated by plasma creatinine showed statistically significant improvement and, in terms of absolute values, creatinine level was reduced in a range between 0.2-0.4 mg/dL. Also, it was recorded **a significantly lower level of blood urea.**

The aspect of electrocardiogram (ECG) changes revealed the presence of atrial fibrillation in Phase I in 76 patients, whilst in Phase III only 9% of patients experienced this rhythm disorder. In the cardiovascular rehabilitation program, the cycloergometer testing has an important place, both immediately after myocardial infarction and in the first stage of rehabilitation, followed by physical endurance training, which is recognized for increasing physical exercise capacity, walking distance and improving the quality of life. Patients were grouped accordingly by the degree of physical exercise that they were able to achieve during the first phase and, later, in the third phase of cardiovascular recovery. (Al Namat et al 2017).

In our study, 55 patients were free of diabetes, and 65 had been diagnosed with Type 2 DM. **The H-FABP values decreased in both groups between the two phases of CR, 6 months away from CABG**. The decrease in H-FABP in diabetics **was also statistically significant** ($p = 0.03$) (fig. 2.2.3). As shown in the box-plot, more than half of the patients had important reduction of H-FABP, at 6 months after the onset of CR program. Half of the group registered a smaller reduction of H-FABP, but more noticeable in diabetics. The median value is a little bit lower and better for non-diabetics (fig. 2.2.3.). (Al Namat et al 2017, Al Namat et al 2018).

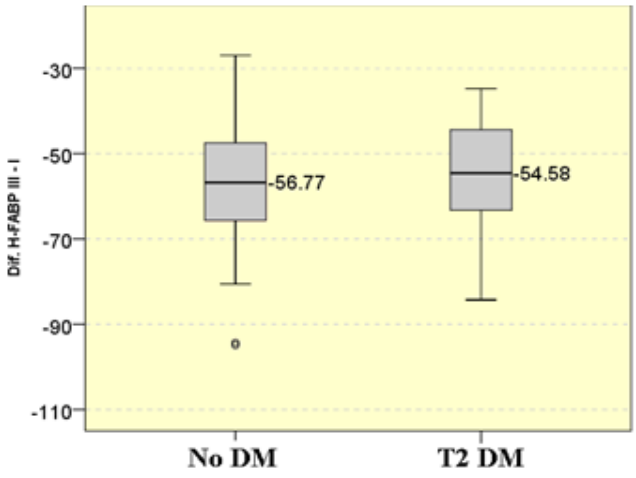

Fig. 2.2.3. H-FABP difference between phases in diabetics vs. nondiabetics

Compared to single bypass patients, in those who benefited from bi- or tri-coronary revascularization, serum H-FABP values reduced more significantly (p = 0.000) (fig. 2.2.4.). In patients with multiple coronary artery by-pass, the ~20 ng/mL reduction in H-FABP levels showed the benefit of multiple interventions on atherosclerotic lesions which caused coronary flow obstruction. (Al Namat et al 2017).

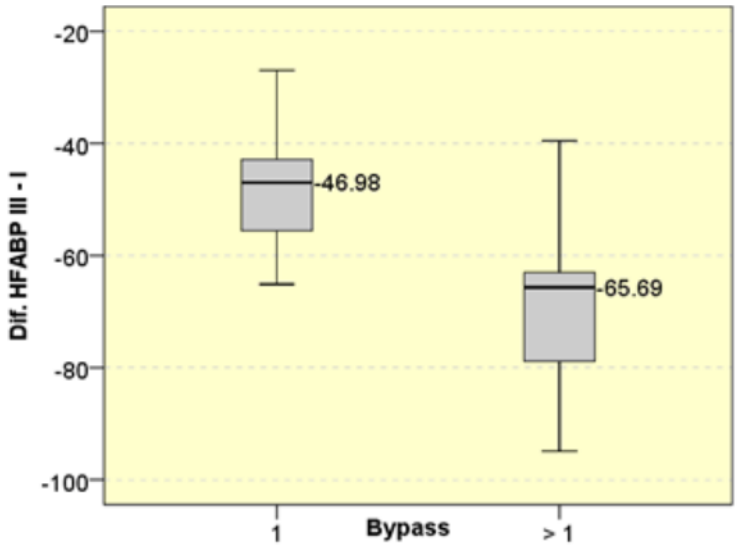

Fig. 2.2.4. Benefits of multiple bypass on H-FABP difference between

CR phases

Discussions

The enrollment in the cardiac rehabilitation programs is very low, despite the specific recommendations after CABG surgery. In some countries, healthcare providers turned to homebased cardiac rehabilitation program instead of centrebased care (Robinson et al 2009). **The Framingham Risk Score (FRS) is a useful clinical tool enabling to assess the risk level of coronary artery disease and to identify the potential modifiable risk factors in vulnerable individuals. FRS is the most appropriate method of measuring a person's likelihood of developing long-term cardiovascular disease**. **The FRS helps identify population (men and women) at high risk in order to prevent future cardiovascular events.** However, despite the applicability of this tool, **it has no power in assessing key factors influenced by diet and metabolic change.** So, it is not known whether FRS is a good predictor of the metabolic disorders that underlie ischemic heart disease. It has been demonstrated that FRS overestimates the risk of coronary artery disease in Europeans and therefore recalibration is recommended for special populations (Davalos et al 2012). Framingham's traditional risk factors such as age, hypertension, smoking, diabetes and cholesterol form the basis of the guidelines provided by Adult Treatment Panel III (ATP III). **Abnormal values for lipid profile (non-HDL, LDL, total cholesterol, triglycerides), uric acid and renal function (creatinine, urea and GFR) present the best**

correlations with increased markers of subclinical atherosclerosis. That confirms the necessity to assess biochemical profile regularly in order to prevent CVD (Yousefzadeh et al 2015). Cardiovascular risk is also related to family history, inflammation markers such as high sensitivity reactive C protein, and glycated hemoglobin in diabetics. These additional biomarkers are included in the Reynolds Risk Score, an alternative risk algorithm developed in 2007 for men and women. Both ATP-III and Reynolds scores received Class I recommendations from the American College of Cardiology and the American Heart Association, both scores being approved as part of the National Direction for the prevention of cardiovascular disease in Canada. A Framingham prediction model for all cardiovascular disease has recently been developed, but this model has not yet been validated on an external population (Mitu et al 2016).

The results obtained by calculating cardiovascular risk scores revealed important and useful correlations between several risk factors (myocardial infarction, atrial fibrillation, hypertension, sex, obesity, diabetes). This is one of the few studies that have attempted to investigate the possible association between lipid profiles and Framingham Risk Score.

We compared our results with those obtained from the Framingham Heart Study in April 2002, which identified major cardiovascular risk factors: hypertension (HTA), hypercholesterolemia, smoking, obesity, diabetes, sedentary, and other lipid fractions (HDL cholesterol, LDLcholesterol, triglycerides), peculiarities related to gender and age group. Numerous prospective studies following the Framingham trial confirmed the major impact of these risk factors on the development of cardiovascular disease and acute myocardial infarction (Nancy et al 2007).

In order to predict mortality in the short and long term after a coronary bypass surgery, various statistical models have been developed. A simplified risk score has been created, in order to reduce the complexity of the prediction of death risk calculator, by using these statistical models in health care units. This information can be used by clinicians and patients in choosing the optimal treatment for severe coronary artery disease (Cook et al 2012). It is of great probability that the reduction of body weight during the 6 months period had influenced the lowering of plasma H-FABP level and the metabolic control in diabetics, revealed by the greater decreased of H-FABP in diabetics.

The Framingham Risk Score (FRS) is a common and simplified clinical tool used to assess the risk level of coronary artery disease (CAD), and to individualize for each patient the maximum benefit of changing certain risk factors. This instrument is made up of components of coronary risk including gender, age, smoking status, systolic blood pressure and lipid profile. FRS is the most appropriate method of measuring a person's likelihood of developing long-term cardiovascular disease. Due to the fact that this risk score provides a view over the possible benefits of prevention, its utility can be extended, patients and clinicians being able to choose between lifestyle modification and/or preventive medical treatment (D´Agostino et al 2008).

Comparing the Phase I and Phase III results of the Framingham score, it was noted that the median value of the cardiovascular risk at 10 years could have a reduction of nearly 6% ($p < 0.05$).

Rehabilitation service appears to be greater for male patients (as has been shown in other studies), with cardiovascular pathology being at higher risk for them compared to women.

The echocardiographic parameters, as left ventricular (LV) telediastolic and telesistolic diameter, left ventricular wall thickness (interventricular septum, posterior wall), and the LV mass showed improvement in the third phase of the cardiovascular rehabilitation program with statistically significant reduction of volumes, diameters and LV mass. Significant echocardiographic changes were not recorded for the right ventricle (RV), as patients only presented an impairment of the left anterior descending artery (LAD) during acute myocardial infarction. Regarding glucose metabolism, glycemic values improvement may be the result of patient participation in the kinetotherapy program, their compliance with the hygiene-dietary regimen, and the significant reduction of anthropometric indices.

The effects of the cardiac rehabilitation program in patients after cardiac surgery can be showed by the decreasing complications, improving quality of life and decreasing ten year mortality. Despite of the existing recommendations after CABG surgery, there is a low participation rate in centre-based cardiac rehabilitation programs. For that reason, the healthcare providers in Denmark have focused on homebased cardiac rehabilitation program (Liebetrau et al 2014). By comparing the settings and the medical care programs in elderly patients with coronary artery disease, it was concluded that home-based cardiac rehabilitation can improve exercise capacity in elders versus the standard care, result that was confirmed by many other researchers (Salavati et al 2016, Fefer et al 2012, Sakakura et al, 2014). In our prospective study on hospitalized patients undergoing CABG, we observed as other researchers did, **the correlation between the occurrence of postoperative atrial fibrillation (POAF) and the plasmatic level of H-FABP, as a sensitive marker of ischemic myocardial injury** (Rader et al 2013). Ischemic injury during open-heart surgery leads to mitochondrial edema and disruption of membranes, releasing intracellular constituents into the blood and increasing the plasmatic level of cardiac enzymes in acute myocardial infarction and after CABG. **The return to sinus rhythm was spontaneous or under medication in most of the cases, within one hour to one week after CABG.**

In our prospective study on hospitalized patients undergoing coronary artery bypass grafting, we noticed, like other researchers, the correlation between the occurrence of postoperative atrial fibrillation (POAF) and the plasmatic level of H-FABP, recognized as a sensitive marker for myocardial ischemic lesion. In most cases, restoration of sinus rhythm was spontaneous or under medical treatment, within one hour to a week after CABG. Thus, ischemic lesion during open heart surgery should represent a therapeutic objective in order to reduce the occurrence of POAF (Akpinar et al 2014). The effects of cardiac recovery program in patients who have had a heart surgery can be seen in reducing complications, improving QoL, lowering mortality rate at 10 years.

Lipid profile values showed a statistically signifyant decrease, although in those in whom the baseline exceeded the normality limit, the reduction experienced did not return the patient to the limit of no cardiovascular risk. Even when the lipidic values are corrected, a residual cardiovascular risk remains. Thus, we have to test further the ratio between TG and HDL-cholesterol, the Reaven index; where the ratio exceeds the normal limit of 3.5, it will notify the presence of insulin resistance – an important underlying mechanism for hypertension, as well as for diabetes and metabolic syndrome. In this regard, statistical analysis can bring to front the implication of every parameter in the positive or negative clinical evolution and in lowering the H-FABP after CABG.

Conclusions

1) Due to the fact that the Framingham Risk Score provides an indication of the possible benefits of prevention, it may also be useful for the patient and clinicians to choose earlier the lifestyle changes and preventive medical treatment. **The decrease of plasmatic glucose in the IIIrd Phase proved that the better the carbohydrate metabolism profile is, the lower the cardiovascular risk will be.** In the cardiovascular rehabilitation program, the cycloergometer testing has an important place, both immediately after myocardial infarction and in the first stage of rehabilitation, followed by physical endurance training, which is recognized for increasing physical exercise capacity, walking distance and improving the quality of life. After a thorough research into major international databases, this study is probably the first to attempt to evaluate the relationship between cardiovascular risk scores and aortic-coronary post-bypass cardiovascular recovery outcomes. **In our study, by computing Framingham Risk Score we have been able to translate clinical practice data into statistically significant results, comparing the results from the first phase of the recovery to the final phase.** (Al Namat et al 2017, Al Namat et al 2018).

2) **The reduction of plasma H-FABP values were registered between the first phase (the first 24 h) after cardiac surgery and the third phase of the cardiac rehabilitation program. H-FABP protein had a higher sensitivity and specificity when compared to other enzymes of myocardial cytolysis.** The improvement in H-FABP values were linked to an increase in quality of life, blood pressure and heart rate, cardiac and renal function, inflammation and coagulation, as well as the improvement in all metabolisms. In this prospective study conducted on 110 patients receiving coronary-artery bypass, we have demonstrated that an early and sustained post-interventional rehabilitation treatment plays an important role in reversing the atherosclerotic process and preventing postoperative complications such as: arrhythmias and cardiac insufficiency, myocardial ischemia, biological changes, and renal failure. (Al Namat et al 2017).

3) Type 2 Diabetes Mellitus complicated with hyperglycemia, dyslipidemic syndrome, and obesity, can accelerate the atherosclerosis process with very high cardiovascular risk for major adverse cardiovascular events and for mortality. The H-FABP values decreased both in

diabetics and in non-diabetics between the two phases of CR, 6 months away from CABG. More than half of the patients had important reduction of H-FABP, at 6 months after the onset of CR program. Half of the group registered a smaller reduction of H-FABP, but more noticeable in diabetics. Ischemic lesion during open heart surgery is linked to high levels of H-FABP and with an occurence risk of postoperative atrial fibrillation, that can be also triggered and sustained by multiple endocrine conditions related to aging. Thus, metabolic control should always remain a target of the complex management in cardiac rehabilitation. (Al Namat et al 2018).

Aursulesei Viviana*, Daniel Timofte, Liliana Mititelu Tarțau, Veronica Mocanu, Razan Al Namat, Victor Cristian Aursulesei, **Irina Iuliana Costache: Circulating chemerin levels, anthropometric indices and metabolic profile in morbid obesity,** Rev.Chim. 2018; 69 (6): 1412 – 1423, IF = 1.412.

This study aimed to investigate the relationship of plasma chemerin levels with anthropometric indices and metabolic parameters in morbidly obese individuals.

Material and methods

It was a cross-sectional study which included 50 subjects, i.e. 25 morbidly obese patients (BMI ≥40 kg/m2) who were admitted for bariatric surgery and 25 age- and gender matched non-obese control patients (BMI < 30 kg/m2). The study protocol was approved by the University and the Hospital Local Ethics Committees and all the patients signed the informed consent. None of the enrolled patients had more than two criteria for defining metabolic syndrome (Alberti et al 2009). The exclusion criteria were strictly respected, i.e. subjects with diagnosed or treated cardiovascular disease or diabetes, enrolled in concurrent studies.

We used the classical anthropometric indices such as BMI (kg/m2), waist circumference and waist to hip circumference ratio (WHR), and also the index of central obesity defined as waist circumference to height ratio. Waist circumference was measured midway between the lowest ribs and the iliac crest. Systolic and diastolic blood pressure, as components of metabolic syndrome, were also determined according to the classical rules of office blood pressure measurement. Venous blood samples were collected after 12 hours fasting for the assessment of biochemical parameters linked to metabolic syndrome (plasma cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, fasting plasma glucose, uric acid, insulinemia). Fasting plasma glucose, total cholesterol and triglycerides were determined using enzymatic colorimetry, while imunoturbidimetry was used for HDLcholesterol measuring. LDL-cholesterol was calculated by Friedewald equation, as described elsewhere (Friedewald et al 1972). Fasting insulinemia was assessed using chemiluminiscence immunoassay kits (Siemens Healthcare GmbH., Germany)

automated by Immulite 1000 analyzer. **Insulin sensitivity (IS) and insulin resistance (IR) were also performed as parameters of metabolic health.** IS was calculated using quantitative check index (QUICKI) and IR by Homeostasis Model Assessment (HOMA-IR = fasting insulin (μU/mL) × fasting glucose (mg/dl)/225 × 18; reference normal values < 2.5) (Gutch.,et al 2015). The venous blood samples collected for assessment of chemerin and adiponectin levels were stored at -20°C for processing. (Aursulesei et al, 2018).

Serum chemerin and adiponectin were measured quantitatively by specific Human ELISA (enzyme-linked immunosorbent assay) kits (ab155430, ab99968, respectively) supplied by Abcam Cambridge, U.K., for research use only. Chemerin (known as retinoic acid receptor responder protein 2 - RARRES2) is a 14 kDa protein that becomes functional after activating by different proteases. Chemerin has an established role in adipocyte differentiation and glucose uptake (Mattern et al 2014). Adiponectin is an antiatherogenic adipokine, involved in carbohydrate and lipid metabolism. Although is mainly secreted by adipose tissue, a negative relation between circulating adiponectin and the amount of visceral adiposity is described **Adiponectin is also considered a marker of IS with controversial metabolic protection** (Kishida et al 2014).

Statistical analysis

Data analysis was performed using IBM SPSS Statistics Version 22.0. The variables were described using mean values ± standard deviation (SD). Independent two-sample test was used to study the differences between the obese and non-obese samples. A natural logarithmic transformation was performed for the variables without a normal distribution. Thus, Pearson coefficient was used to analyze the linear correlation between circulating chemerin, adiponectin, and the studied variables. Multiple linear regression models were created to analyze the factors that determine anthropometric indices, levels of HDL-cholesterol, triglycerides, fasting plasma glucose, uric acid, insulinemia, IS and IR in the obese group. The most important condition when creating these models was for circulating chemerin to be an independent variable.

Results

The baseline clinical and biochemical characteristics of the study population are presented in Table 2.2.7. (Aursulesei et al, 2018).

Table 2.2.7. Baseline clinical and biochemical characteristics of the study population

	Non-obese (control group) n=25	Obese n=25	P value
Age (years)	43.36 ±13.9 (37.62-49.10)	39.24±8.74 (35.63-42.85)	0.021
Female sex (%)	68	84	
Systolic blood pressure	118.04±11.72	129.36±13.03	0.0022
Diastolicblood pressure	67.08±7.89	75.28±11.12	0.0044

BMI (kg/m^2)	24.24±3.15 (22.36-24.96)	43.9±6.07 (40.49-45.50)	0.0001
Waist circumference (cm)	83.04±8.75 (79.43-86.65)	125.5±18.68 (117.09-133.21)	0.0001
WHR	0.83±0.08 (0.80-0.86)	0.96±0.10 (0.92-1)	0.0001
Index of central obesity	0.50±0.06 (0.48-0.52)	0.75±0.08 (0.71-0.78)	0.0001
Cholesterol (mg/dl)	197.80±41.39(180.71-214.89)	201.4±27.17 (190.18-212.62)	0.718
HDL Cholesterol (mg/dl)	50.36±14.94(44.19–56.53)	50±9.98 (45.88-54.12)	0.92
LDL Cholesterol (mg/dl)	125.04±39.97(109.37-140.71)	127.68±23.48 (117.98-137.37)	0.76
Triglycerides (mg/dl)	121.24±25.74(100.29-142.19)	124.32±17.96 (94.61-154.03)	0.86
Plasma glucose (mg/dl)	88.32±8.80(84.69-91.95)	99.28±14.62 (93.24-105.32)	0.0026
Insulinemia (µU/ml)	5.98 (2.81-12)	18.80 (13.50-30.10)	0.0004
Insulin sensitivity	0.16±0.02 (0.15-0.17)	0.13±0.02 (0.13-0.14)	0.0001
HOMA - IR	1.28 (0.63-2.87)	4.91 (3.38-6.62)	0.0012
Uric acid (mg/dl)	5.29±1.48 (4.68-5.90)	6.79±2.19(5.88-7.69)	0.0067
Adiponectin (ng/ml)	16.36±1.49	18.05±1.55	0.0003
Chemerin (ng/ml)	9.10 (8.13-10.60)	11.56(10.39-13.10)	0.0001
Chemerin/adiponectin x 10^{-3}	0.55±0.12	0.67±0.18	0.0052
Data are presented as means± standard deviations and confidence interval and median are interquartile range 25%-75% according to the normality of distribution.			

The obese and non-obese patients didn't fulfill the criteria for defining metabolic syndrome, the mean age was comparable between the groups, and females accounted for over 2/3 of all patients in both samples. Similar to other studies (Sell et al 2010, Bozaoglu et al 2007, Fulop et al 2014, Chang et al 2016, Bozaoglu et al 2009), **chemerin levels were significantly higher in obese patients compared to the non-obese group** (11.56 (10.39– 13.10) vs 9.10 (8.13–10.60) ng/mL, p = 0.0001). However, in our studied obese group chemerin was significantly lower compared to

other reported series (Sell et al 2010, Catoi et al 2018, 2014), possibly related to the status of metabolic health. **Adiponectin levels were also significantly different between the two samples** (18.05 ± 1.55 vs 16.36 ± 1.49 ng/mL, p = 0.0003). It is worth mentioning that unlike other studies (Geloneze et al 2009), we report higher values in the obese subjects. This finding could sustain that not circulating adiponectin, but the local expression in perivisceral fat and also the distribution of adipose tissue, are related with its metabolic functions (Kishida et al 2011, Sirbu et al 2018). An alternative explanation could be the adiponectin resistance phenomena, recently described by Engin (2017), as a compensatory response caused by adiponectin unresponsiveness to IR at different stages of obesity. We also studied the **chemerin/adiponectin ratio for the assessment of metabolic health** (Chu et al 2012). In our study the mean value was significantly higher in obese group (0.67 ± 0.18 vs 0.55 ± 0.12, p = 0.0052). Regarding the status of metabolic health, all biochemical parameters were within the normal range in the two groups (plasma fasting glucose 88.32 ± 8.80 vs 99.28 ± 14.62 mg/dL, uric acid 5.29 ± 1.48 vs 7.9 ± 2.19 mg/dL, cholesterol 197.80 ± 41.39 vs 201.4 ± 27.17 mg/dL, HDL-cholesterol 50.36 ± 14.94 vs 50 ± 9.98 mg/dL, LDL-cholesterol 125.04 ± 39.97 vs 127.68 ± 23.48 mg/dL, triglycerides 121.24 ± 25.74 vs 124.32 ± 17.96 mg/dL). Only for plasma fasting glucose and uric acid there were significant differences between groups ($p < 0.05$). All anthropometric indices were within the normal range in the non-obese group, including the mean value of 0.50 admitted for index of central obesity (Wise 2017). Also, the same indices were significantly higher in obese group (BMI 24.24 ± 3.15 vs 43.9 ± 6.07 kg/m2; waist circumference 83.04 ± 8.75 vs 125.5 ± 18.68 cm; WHR 0.83 ± 0.08 vs 0.96 ± 0.10; index of central obesity 0.50 ± 0.06 vs 0.75 ± 0.08; p = 0.0001). Similar findings were documented for insulinemia (0.16 ± 0.02 vs 0.13 ± 0.02, p = 0.0004) and the derived parameters of metabolic health (IS 0.16 ± 0.02 vs 0.13 ± 0.02, p = 0.0001; HOMA-IR 1.28 vs 4.91, p = 0.0012). Systolic (SBP) and diastolic (DBP) blood pressures, as components of metabolic syndrome, had also normal mean values in both groups (SBP 118.04 ± 11.72 vs 129.36 ± 13.03 mmHg, p = 0.0022; DBP 67.08 ± 7.89 vs 75.28 ± 11.12 mmHg, p = 0.0044). (Aursulesei et al, 2018).

In our study **circulating chemerin was not correlated with BMI** (r =0.25, p =0.08) as other reports demonstrate in obesity (Sell et al 2010, Bozaouglu et al 2007, Kim et al 2014, Li et al 2014, Bozaoglu et al 2009, Chu et al 2012, Alfadda et al 2012]. On the other hand, the reported data in morbid obesity are controversial, mainly based on results after bariatric surgery (Sell et al 2010, Catoi et al 2014, 2018). According to other clinical researches (Bozaoglu et al , 2007, Kim et al 2014, Chakaroun et al 2012, Li et al 2014, Bozaoglu et al 2009) the results of our study demonstrate **the correlation between circulating chemerin and waist circumference** (r =0.37, p = 0.012) and WHR (r = 0.36, p = 0.012). Also, our study is the first to report the correlation of serum chemerin with index of central obesity (r = 0.47, p = 0.003). It is worth mentioning that chemerin/ adioponectin ratio offers similar findings when relation with anthropometric indices is studied, in contrast to serum adiponectin which was not related to any clinical or biochemical parameter. **Chemerin/adiponectin ratio was not related to BMI** (r = 0.23, p = 0.11), but **a positive correlation was found with waist circumference** (r = 0.39, p = 0.008), WHR (r = 0.41, p = 0.005**) and index of central obesity** (r = 0.45, p = 0.002). **Our results suggest that**

chemerin/adiponectin ratio is superior to circulating adiponectin levels when relation with anthropometric parameters is assessed. Another finding of our study is that chemerin and chemerin/adiponectin ratio are not related with parameters of glucose metabolism homeostasis, including fasting plasma glucose, insulinemia, IS or IR, and also with HDL-cholesterol, triglycerides (p > 0.05). Taken together and considering that our obese patients are metabolic healthy, these results cannot be extrapolated to the whole spectrum of obesity. Li et al. (2014) underline this point due to the heterogeneity of design between studies, while Chu et al. highlight the importance of bias factors (CHU et al 2012). All the mentioned correlations are detalied in table 2.2.8. **Regarding the hemodynamic parameters related to metabolic health, our study demonstrates that serum chemerin and chemerin/adiponectin ratio are related to systolic blood pressure (r = 0.33, p = 0.022; r = 0.29, p = 0.044, respectively). Also, serum chemerin is related to mean arterial pressure (r = 0.3, p = 0.041), a parameter representative to peripheral resistance and estimated using the formula: [(2 x DBP) + SBP]/3**. This finding suggests the role of chemerin in modulating vascular tone. In fact, in a previous report we demonstrated that chemerin is an independent predictor for several parameters of arterial stiffness in metabolic healthy subjects with morbid obesity (Aursulesei et al 2017).

Table 2.2.8. Correlations of chemerin and chemerin/adiponectin ratio with parameters of metabolic health.

Parameter	Chemerin		Chemerin/Adiponectin ratio	
	r	p	r	p
BMI (Kg/m2)	0.25	0.08	0.23	0.11
Waist circumference	0.37	0.012	0.39	0.008
Waist to hip ciorcumference ratio (WHR)	0.36	0.012	0.41	0.005
Index of central obesity	0.47	0.003	0.45	0.002
Fasting plasma glucose (mg/dl)	0.24	0.095	0.23	0.11
Insulinemia (µU/ml)	0.21	0.143	0.21	0.150
IS	-0.25	0.082	-0.25	0.087
HOMA IR	0.27	0.08	0.29	0.08
HDL Cholesterol (mg/dl)	0.03	0.8	0.05	0.7
Triglycerides (mg/dl)	0.03	0.8	0.004	0.98
Systolic blood pressure (mmHg)	0.33	0.022	0.29	0.044
Diastolic blood pressure (mmHg)	0.27	0.06	0.21	0.13
Mean blood pressure (mmHg)	0.3	0.041	0.26	0.07

Table 2.2.9. Linear regression models g chemerin as an independent variable

Dependent variables	Independent variable - CHEMERIN			
	Coeficient	p-value coefficient	p-value Model	R^2 adjusted
BMI (Kg/m^2)	-0.23	0.21	0.946	0.0001
Waist circumference	0.992	0.24	0.858	0.0001
WHR	0.016	0.013	0.642	0.0001
Index of central obesity	0.005	0.21	0.862	0.0001
Insulinemia (µU/ml)	1.019	0.066	0.874	0.0001
IS	-0.00269	0.003	0.0001	0.845
HOMA-IR	0.231	0.13	0.0001	0.894

When multiple linear regression models were created to analyze chemerin as an independent variable that determine anthropometric indices and glucose homeostasis in obese group, we found that circulating levels still correlated with WHR ($p = 0.013$), but also with IS ($p = 0.003$) (table 2.2.9.). (Aursulesei et al, 2018). As we previously reported in a separate linear regression model where chemerin was a dependent variable, fasting plasma glucose determined the circulating chemerin level. Despite the presence of IR in our obese group, chemerin was not related with this parameter. (Aursulesei et al 2017).

Discussions

Our results are concordant with the previous report of Bozaoglu et al (2009). The authors concluded that chemerin is not a predictor for IR in patients with normal glucose tolerance. Corona-Meraz et al. (2016) also reported that serum chemerin levels are higher in obesity without IR versus obesity with IR. This could explain the levels of chemerin much lower in our study in comparison with similar clinical studies. The properties of perivisceral fat in our obese sample could be also determinant of chemerin and adiponectin profiles (Mattern et al 2014, Piya et al 2013, Bryan et al 2013).

Although we demonstrated that serum chemerin levels were related to anthropometric indices, our study has several limitations. First of all, we didn't study the expression of CMKLR1 receptor, mentioned to better characterize the role of chemerin in obesity-related metabolic and clinical changes (Mattern et al 2014, Piya et al 2013, Bryan et al 2013). Secondly, we didn't use other

relevant indices for morbid obesity such as body fat percentage, the subcutaneous fat mass amount and distribution (Piya et al 2013). Finally, due to the small size of samples and the method of chemerin measurement, not specifically addressed to the different isoforms, our results should be confirmed by further research.

Conclusions.

The relation between circulating chemerin and anthropometric indices in metabolic healthy morbidly obese patients is suggestive for the potential role of this adipokine in the pathophysiology of obesity. In morbidly obese patients without criteria for defining metabolic syndrome, chemerin is an independent predictor for WHR and decreased insulin sensitivity, but not for other metabolic changes. Chemerin/adiponectin ratio is also a useful parameter, but not superior to chemerin levels.

Our study demonstrated that circulating chemerin is related to anthropometric indices and it is an independent predictor for waist to hip circumference ratio as well as for decreased insulin sensitivity, but not for other metabolic changes. Chemerin/adiponectin ratio is also a useful parameter, but not superior to chemerin levels (Aursulesei et al 2018).

Petris A, G. Tatu-Chitoiu**, Irina Costache**, Diana Tînț: **"Survival variables in old old patients (> 85 years) with chronic heart failure".** Mol Cryst Liq Cryst, 2016, 628: 7-14. ISSN: 1542-1406 (print), 1563-5287 (online). http://dx.doi.org/10.1080/15421406.2015.1137411. **IF = 0.571**

Aim of the study was to analyze the variables involved in the evolution and survival in old patients hospitalized with symptomatic congestive heart failure.

Methods

In this study we used data of a single center database on 125 pts with symptomatic congestive heart failure older than 85 years screened consecutively between January 2011 – December 2012 in an academic hospital from Iasi, Romania. The recruited patients are admitted with dyspnoea, the verification of HF based on the presence of symptoms and signs of HF being done by the investigators according to the guidelines on acute and chronic HF published by the European Society of Cardiology (McMurray et al 2012). Clinical history, symptoms, signs, cardiovascular and associated comorbidities, standard biology, chest X-ray and echocardiographic data, and medications were recorded (Tables 2.2.10, 2.2.11). The creatinine clearance was calculated according to the Cockcroft and Gault formula. Hypertension was defined as systolic blood pressure>140mmHg or diastolic blood pressure>90mmHg. An assessment of preserved left

ventricular systolic function was given by echocardiography left ventricular ejection fraction (EF) ≥45%. (Petriș et al 2016).

Table 2.2.10. Cardiovascular and associated comorbidities (S = survival, D = Death)

Characteristics	S (n = 112)	D (n= 13)	p
Hypertension (%)	76,8	76,9	0,991
Ischaemic heart disease (%)	59,8	38,5	0,142
Previous stroke (%)	16,1	15,4	0,950
Atrial fibrillation (%)	58	38,5	0,021
Peripheral arterial disease (%)	11,6	0	< 0.001
Venous insufficiency (%)	19,6	15,4	0,715
Smoker (%)	10,7	30,8	0,165
Chronic alcoholism (%)	28,6	15,4	0,258
Diabetes (%)	15,2	23,1	0,466
COPD (%)	20,5	23,1	0,832
Renal failure (%)	40,2	53,8	0,348
Liver failure (%)	9,8	23,1	0,307
Cancer history (%)	14,3	15,4	0,916

- Defined as creatinine clearence < 30 ml/min calculated from serum creatinine values using the Cockroft and Gault formula; COPD = chronic obstructive pulmonary disease.

Table. 2. 2.11. Treatment on admission/discharge (S = survival, D = Death)

Characteristics	S (n = 112)	D (n= 13)	p
Furosemide (%)	56,3	76,9	0.133
Spironolactone (%)	27,7	46,2	0.170
Indapamide (%)	8,9	77	0.883
Statin (%)	12,5	0	< 0.001
Dopamine (%)	4,5	46,2	< 0.014
Digoxin (%)	21,4	30,8	0.449
Anticalcics (%)	25	23,1	0.880
ACE inhibitors (%)	53,6	38,5	0.306
Angiotensin receptor blockers (%)	4,5	0	0.441
Betablockers (%)	57,1	69,2	0.406
Amiodarone (%)	3,6	15,4	0.284
Aspirine (%)	64,3	15,4	< 0.001
Clopidogrel (%)	25,0	23,1	0.880
Antivitamin K (%)	17,9	38,5	0.178

Nitrates (%)	65,2	53,8	0.424
Trimetazidine (%)	32,1	23,1	0.508
Aminophylline (%)	51,8	76,9	0.072
Neurologic drugs (%)	14,3	23,1	0.407
Antidiabetic drugs (%)	11,6	23.1	0.304

Statistical analysis

For continuous variables, mean, standard deviation (SD), median, minimum and maximum ere assessed. For non-continuous variables, the frequency distribution was considered. In this analysis was used the independent-samples t-test to compares the means between two unrelated groups on the same continuous, dependent variable. All statistical analyses were two-tailed ith a threshold for significance set at a P value <0.05. The Pearson product moment correlation coefficient (r) was used as measure of the strength and direction of association that exists between two variables measured on at least an interval scale. All final analyses were conducted using SPSS software, version 20 (SPSS Inc, Chicago, Illinois, USA).

Results

We have analyzed a lot of 125 patients with congestive heart failure, older than 85 years (mean 87.43 +/− 2.54 years, median 87 year, range 85–99) (Figure 2.2.5.), 53.6% male, enrolled consecutively between January 2011 - December 2012. The old old patients group included patients with length of hospitalization of 8.10+/−3.98 days and the rate of rehospitalization 6.4% and the death rate 10.4%. We have compared some features of two groups: survivors' (S = 112 pts) vs deceased (D = 13 pts). Heart failure etiology in this groups (S vs D) was: ischemic dilated cardiomyopathy 52 pts (46.4%) vs 5 pts (38.5%), alcoholic cardiomyopathy 4 pts (3.6%) vs 0, hypertensive cardiopathy 33 pts (29.5%) vs 5 pts (38.5%) and mixed in 23 pts (20.5%) vs 3 pts (23.1%). The New York Heart Association (NYHA) class distribution (S-group vs D-group) was the following: in NYHA class II 59 pts (52.7%) vs 2 pts (15.4%), in NYHA class III 46 pts (41.1%) vs 9 pts (69.2%) and in NYHA class IV 59 pts (6.3%) vs 2 pts (15.4%). (Petriș et al 2016).

Chest X-ray was performed in 89.3% cases (S-group) vs 76.9% in D-group (p 0.572) and echocardiogram in 100% cases (S-group) vs 84.6% in D-group (p 0.165). Nor difference between cardio-thoracic index (on chest X-ray) in S-group (0.54+/−0.06) and D-group (0.51+/−0.06) (p 0.363), left and right ventricular diameters (echocardiogram) (for left ventricle diameter 52.56+/−9.72mmin S-group and 53.00+/−8.08mmin D-group, p 0.886; for right ventricle diameter 34.77+/−8.92 mm in S-group and 38.40+/−7.97 mm in D-group, p 0.223). There was a significant difference in the left ventricle ejection fraction between the two groups (44.36+/−13.12% in S-group and 38.33+/−5.59% in D-group, p 0.015). BNP values are 524.49+/−116.87 vs 2480.75+/−951.99 pg/ml, p = 0.131. (Petriș et al 2016).

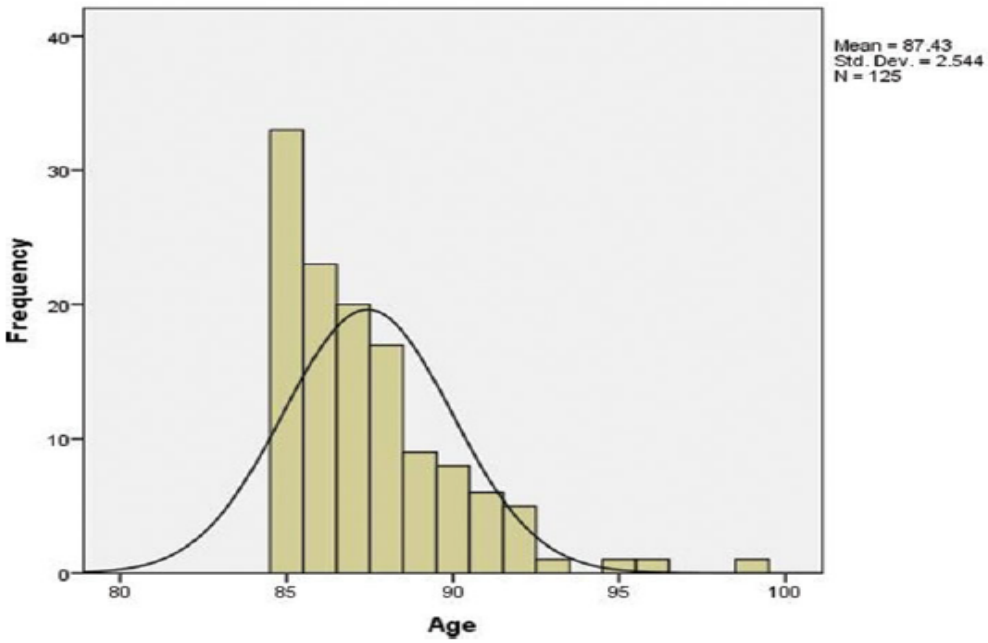

Fig. 2.2.5. Distribution of the analyzed cohort according to age.

Number of drugs taken was 5.53+/−1.86 in S-group vs 5.85+/−1.86 in D-group (p = 0.560) and the length-of-stay was 8.18+/−3.79 in S group vs 7.38+/−5.53 in D group (p = 0.494) (figure 2.9.). There were significant differences between group S vs D in: NYHA class (p = 0.011), atrial fibrillation (p = 0.021), left ventricle ejection fraction (p = 0.015), hemoglobin level (12.57+/−1.92 vs 11.20+/−1.87 g/dl, p = 0.017), blood urea nitrogen (58.38+/−28.92 vs 77.30+/−47.99 mg/dl, p = 0.04) and serum sodium (139.97+/−4.55 vs 136.69+/−7.12 mg/dl, p = 0.024). (Petriș et al 2016).

Evolution (survival vs death) was correlated with presence of atrial fibrillation (r = 0.207∗), NYHA class (r = 0.228∗), smoker status (r = 0.183∗), BUN level (r = 0.183∗), uric acid level (r = 0.322∗∗), serum sodium level (r = −0.206∗), brain natriuretic peptide (BNP) level (r = 0.658∗∗), and with administration of dopamine (r = 0.449∗∗) and aspirin (r=−0.304∗∗, a negative relation:more aspirine administrated in the survival group) but not with the number of drugs administrated per patient (r=0.053) or length-of-stay (r=−0.062) (Figure 2.2.6). Length-of stay was correlated with the number of drugs administrated per patient (r = 0.182∗), age (r = −0.209∗), NYHA class (r = 0.192∗), nr. of admission in the last 12 months (r = 0.234∗∗), smoker status (r = −0.185∗∗), GPT value (r = −0.207∗) and the left ventricle ejection fraction (r = −0.331∗∗). The number of drugs administrated is correlated with the length-of stay (r = 0.182∗), systolic blood pressure (r = 0.218∗), history of HBP (r = 0.176∗), left ventricle ejection fraction (r=−0.321∗∗), and some drug administration: furosemid (r = 0.430∗∗), spironolactone (r = 0.249∗∗), indapamide (r = 0.226∗), statin (0.317∗), digoxin (r=0.231∗∗), anticalcics (r=0.246∗∗), angiotensin receptor blockers – ARB (r = 0.247∗), beta-blockers (r = 0.220∗), clopidogrel (r = 0.286∗∗), nitrates (r = 0.389∗∗), trimetazidine (r = 0.235∗∗), aminophylline (r = 0.329∗∗), neurologic drugs (r = 0.341∗∗), antidiabetic drugs (r = 0.245∗∗). (Petriș et al 2016).

Legend: ∗correlation is significant at the 0.05 level (2-tailed); ∗∗correlation is significant at the 0.01 level (2-tailed).

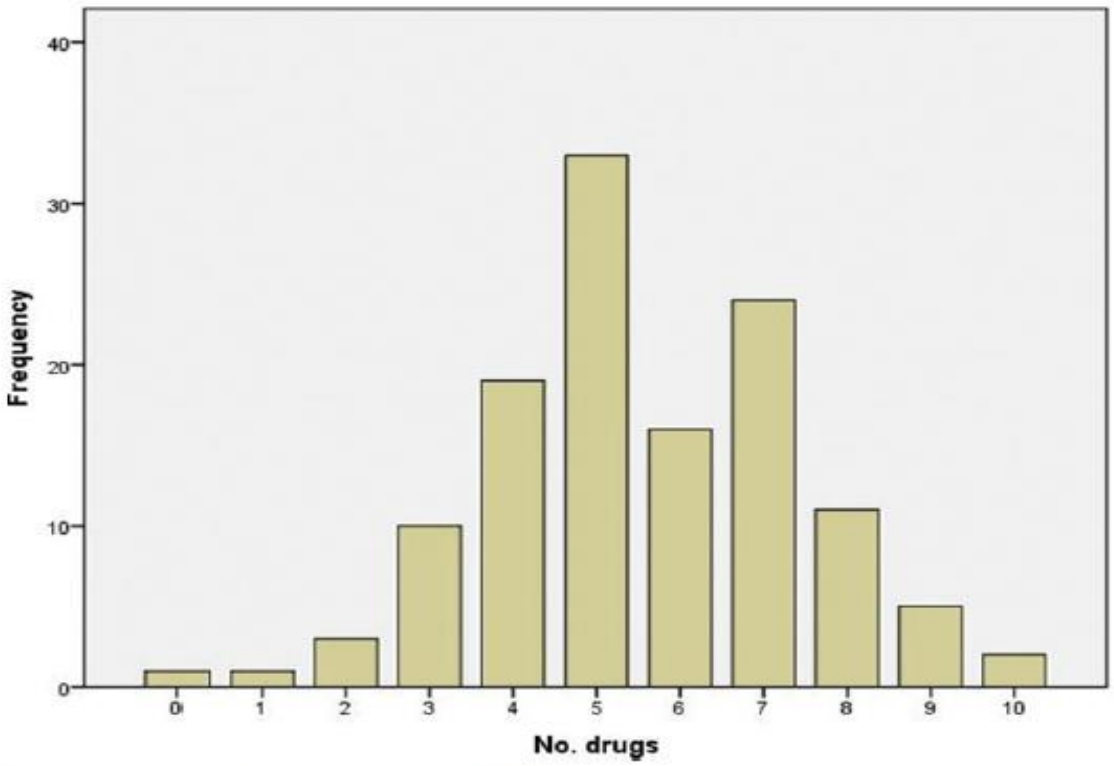

Fig. 2.2.6. Distribution of the number of drugs administrated per patient.

Discussion

The median survival in Mogensen et al. study (Mogensen et al 2011) was 20 months for patients >85 years and 50 months for the other age groups (<85 years) combined. In a study on octogenarians the in-hospital mortality was 10.9% and mean 12 and 24-months survival 62.3% and 48.2%. In a multivariate model, a significant association with the composite of all-cause mortality or cardiovascular hospitalization, or all-cause mortality alone was found for a higher NYHA class, higher uric acid level, lower body mass index, prior myocardial infarction, and larger left atrial (LA) dimension (Manzano et al 2011). In our study, the patient evolution (survival vs death) was correlated with presence of atrial fibrillation, NYHA class, smoker status, BUN, uric acid, serum sodium and brain natriuretic peptide (BNP) level, and with administration of dopamine and aspirin. Barsheshet et al. (2010) recently concluded that mortality risk in the older population is not decreased in those with preserved LVEF. In our study, the length-of stay and the number of drugs administrated was correlated with the left ventricle ejection fraction ($r = -0.331$, respectively $r=-0.321$).

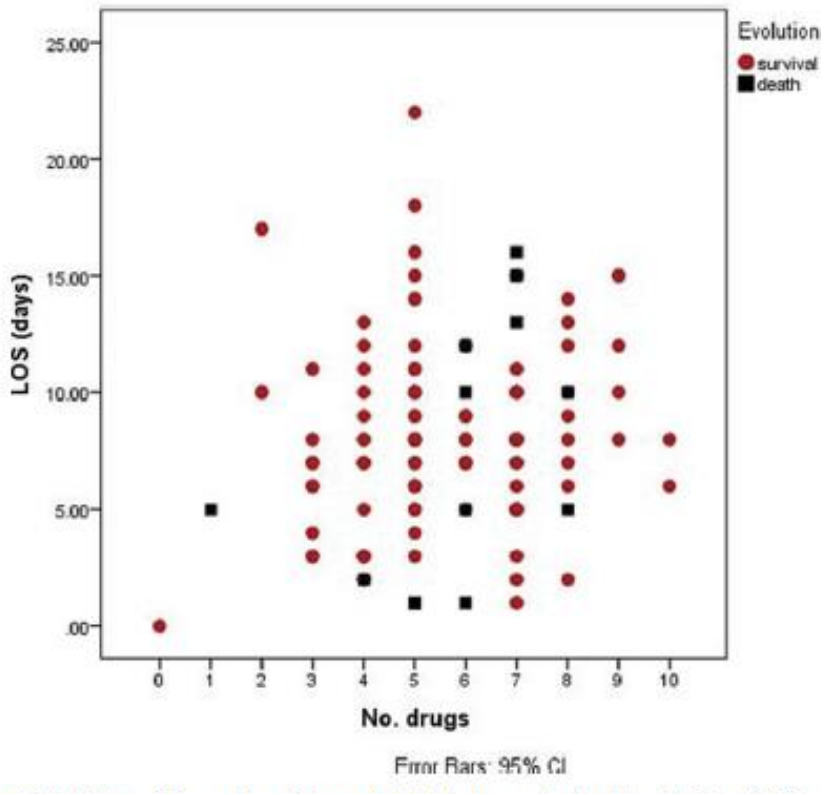

Fig. 2.2.7. Distribution of the number of drugs administrated per patient and length-of-stay (LOS) analyzed according S or D group

The contemporary Guidelines (McMurray et al 2012) mentions as established therapy of chronic left heart failure the following main classes of drugs: diuretics, angiotensin converting enzyme inhibitors (ACEi) /angiotensin receptor blockers (ARB), beta-blockers (BB), mineralocorticoid receptor antagonists (MRA) and digitalis (low doses). In Mahler study (Komajda et al 2005) the mean number of drugs per patient with congestive heart failure (at the first visit) differs depending on the analyzed country: 4.80 drugs in Germany, 4.86 drugs in Spain, 5.40 drugs in France, 5.51 drugs in UK, 5.54 drugs in Italy and 5.64 drugs in Nederland. Euro Heart Failure II notice in the elderly patients an underuse and underdosage of medications recommended for heart failure (Komajda et al 2009). The optimal treatment (association of ACEi/ARB+BB+MRA) was used in only 5% of patients at discharge in a cohort study in the French national healthcare insurance database (Vorilhon et al 2015). In our study they are 6.4% of patients treat with this drug triad. In the study cited above on multivariate analysis the associations ACEi/ARB+BB+MRA ($p = 0.01$) and ACEi/ARB+BB ($p < 0.001$) were associated with improved survival.

Conclusions

The number of drugs given to old old patients has not proven to reduce the length of stay, the rate of death and rehospitalization. Hyponatremia, anemia, increased BNP, blood urea nitrogen and serum uric acid are associated with increased mortality of old old patients.

There were significant differences between group S vs D in NYHA class, atrial fibrillation, hemoglobin level, blood urea nitrogen, and serum sodium (Petriș et al 2016).

Al Namat Razan, Mihai Constantin*, Ionela Larisa Miftode*, Andrei Manta, A. Petris, R. Miftode, A. D. Costache, D. Iliescu, **Irina Iuliana Costache**: **Biochemical Markers in Patients with Readmission for Congestive Heart Failure,** Rev.Chim. 2017; 69 (7): 1687-1691. **IF = 1.412.**

The aim of the study was to determine retrospectively the readmission rate in patients with congestive heart failure and also to identify factors related to an increased likelihood of early rehospitalization; also, the research tried to determine the percentage of readmissions that are potentially preventable or avoidable and also to identify remediable factors which contribute to early readmission.

Experimental part. Study population

The study group was 100 patients admitted to the the 1st Cardiology Clinic of the St. Spiridon Emergency Clinical Hospital Iasi during 2010-2017, aged 48-85 years, of which 75% were men and 78% of them came from rural areas. The inclusion criteria were: patients who have been readmitted in the Cardiology Clinic or who have been able to show readmissions in other clinics with the same profile have been selected. 35% of the readmitted patients were hospitalized in the Internal Medicine Clinic while 65% in the Cardiology Clinic. Regarding the readmissions, 90% were performed in emergency while 10% were scheduled. For every patient, it was performed a clinical examination, blood pressure, heart rate, echocardiography investigation at admission and subsequently repeated during hospitalization, focusing on the parameters that assess the systolic function of the left ventricle (estimated by the ejection fraction).

Laboratory investigations: these included analysis of blood urea and creatinine level, electrolytes (Na, K), total cholesterol, triglycerides, lipid profile, hepatic enzymes (alanine transaminase: ALT; aspartate transaminase: AST), myocardial enzymes (creatine kinase- heart isoenzyme: CK-MB).

Data collection: Informations on the general condition, medical history (hypertension, diabetes or coronary heart disease), personal history (smoking, alcohol ingestion) were obtained from all patients. Also, they were previously informed about the subject of the study, signing an informed consent (Al Namat et al 2018).

Statistical analysis was performed using the IBM SPSS 20.0 software (Statistical Package for the Social Sciences, Chicago, Illinois). Data were expressed as mean ± standard deviation or number of cases with percentage, for continuous and ordinal variables. Cross-tabulation and Pearson Chi-

Square test were used for describing the relationship between two categorical variables. The oneway analysis of variance (ANOVA) was used to determine the significant differences between the means of continuous variables and an independent categorical variable. For all data, a two-sided p value < 0.05 was considered statistically significant.

Results

The etiologies of heart failure, as shown in figure 2.2.8 , are as follows: ischemic dilated cardiomyopathy - 21 cases (11 men, 10 women); ethanolic cardiomyopathy - 43 cases (only men); mixed etiology (ischemic and ethanolic cardiomyopathy) -20 cases (13 men, 7 women); valvulopathy - 16 cases (8 men, 8 women).

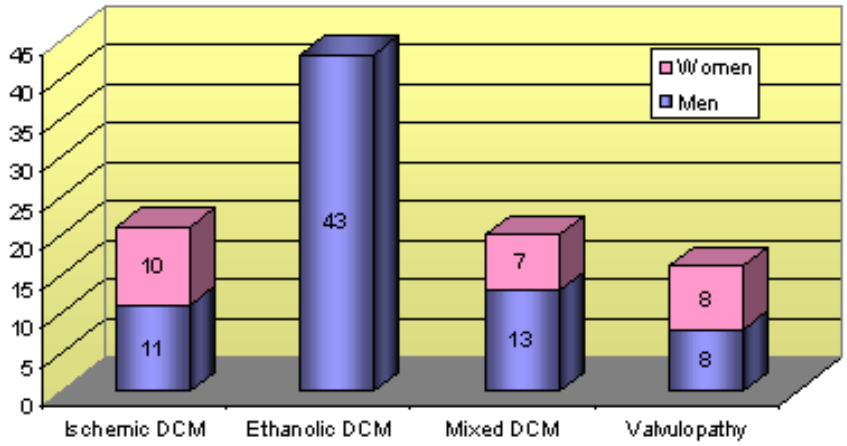

Fig. 2.2.8. Etiology of the heart failure

Number of readmissions per patient ranged from 3 to 8 per year. Among the readmission causes, patients presented with one or more complications (fig.2.2.9.): left heart decompensation – 78%; global cardiac decompensation - 60%; anticoagulation accident - 52%; side effects of medication - 34%; arrhythmias (ventricular extrasystole or atrial fibrilation) - 26%; pulmonary embolism - 21%; amiodarone induced thyroid dysfunction - 9%; angina pectoris -5% (Al Namat et al 2018).

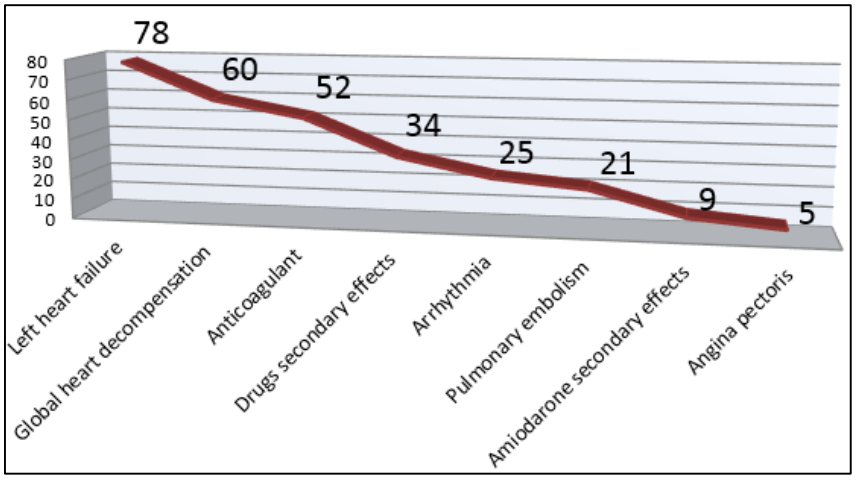

Fig. 2.2.9. Etiology of readmissions

In our study readmissions were more frequently met in patients from rural areas (78%) and, regarding the heart failure etiology, in the case of dilated cardiomyopathy compared to valvular etiology. Since men were predominant in the study group, we can not say that there were major differences between the two sexes with regard to readmission. No correlation between the age of the patients and the increased frequency of rehospitalisations could be established in our study, but we identified an inappropriate colaboration with family doctors especially for the patients from rural areas. **Discharge from a heart failure hospitalization is followed by a readmission within 30 days in ≈ 24% of cases**. **Recurrent heart failure and related cardiovascular conditions account for only about half of readmissions in patients with heart failure.** Although the proportion of noncardiovascular admissions is higher in those with preserved ejection fraction (EF), overall readmission rates for heart failure remain similar whether the heart failure occurs with reduced or preserved EF. **Another major cause for readmission was represented by the altered renal function, the further electrolyte imbalances (e.g. hyponatremia, hyperkalemia) being in the vast majority of cases a direct consequence of the diuretic medication**. These complications were more common in patients with NYHA III/IV heart failure (fig.2.2.10), not being related to the age of the assessed patients (fig. 2.2.11) (Al Namat et al 2018). The treatment and management of heart failure is associated with high mortality rates and treatment costs. Poor medication adherence is a major barrier towards an improved medical care while traditional interventions addressing non-adherence have not consistently demonstrated improvement for the outcome, translated in the number of readmissions. The reasons for nonadherence are complicated and illustrate the broader challenges patients face when managing a complex disease like heart failure. Studies have documented several factors contributing to hospital readmissions, including complications from inhospital treatment, inappropriate coordination of care or even the quality of medical services. Insufficient patient education and a poor follow-up should also be considered. The most beneficial strategy to reduce readmissions appears to be a coordinated care. Thereby, coordination between physicians and patient-centered approaches were far more successful in

reducing readmissions than single physician approaches with disease-centered management (Ather et al 2012, Bhatia et al 2006, Cintron et al 1993, Punnoose et al 2011). Dharmarajan et al (2013) reported that most readmissions among patients with CHF, pneumonia, and acute myocardial infarction were not attributed to the initial diagnosis. Although the main reason for 30-day readmission after CHF hospitalization was due to CHF, this accounted for only 35.2%, followed by renal disorders (8.11%), pneumonia (4.98%), arrhythmias and conduction disorders (4.04%), followed by septicemia and shock (3.55%) (Zile et al 2008, Stevenson et al 2010].

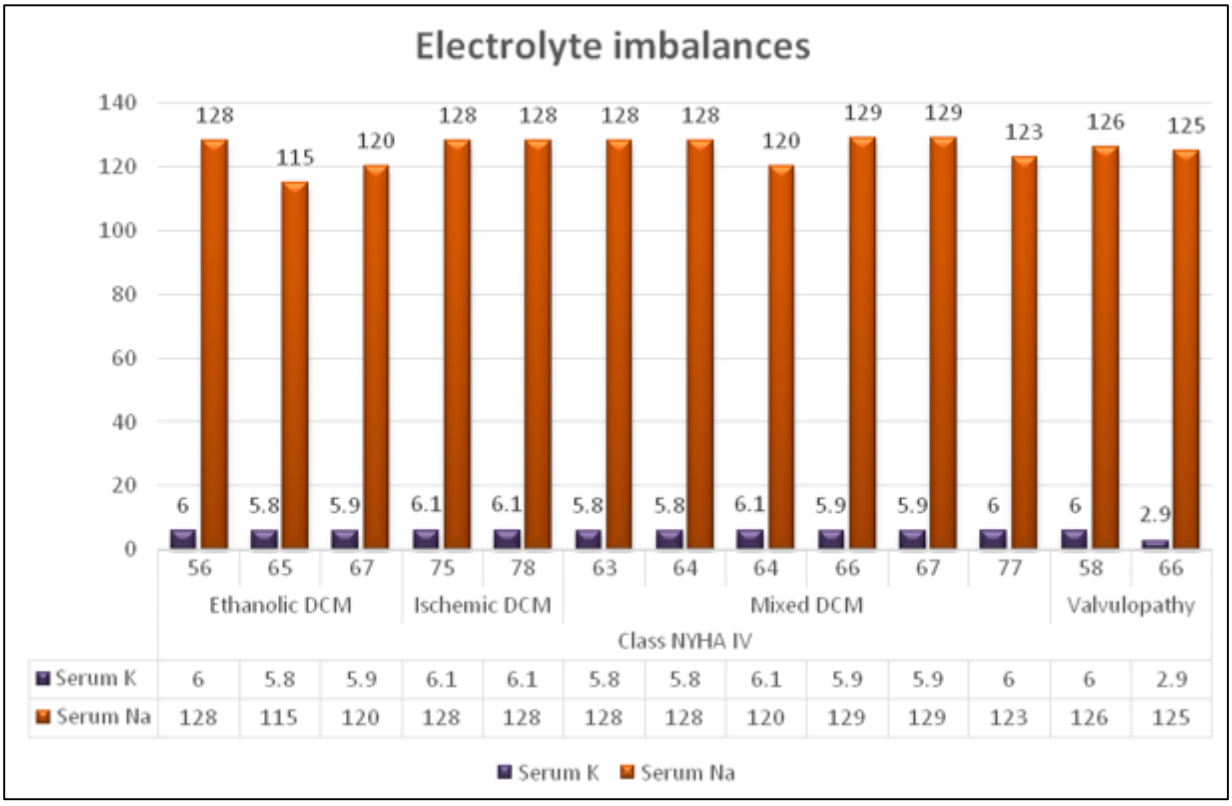

Fig. 2.2.10. Renal function in patients with NYHA Class IV Heart Failure dependent on etiology and age

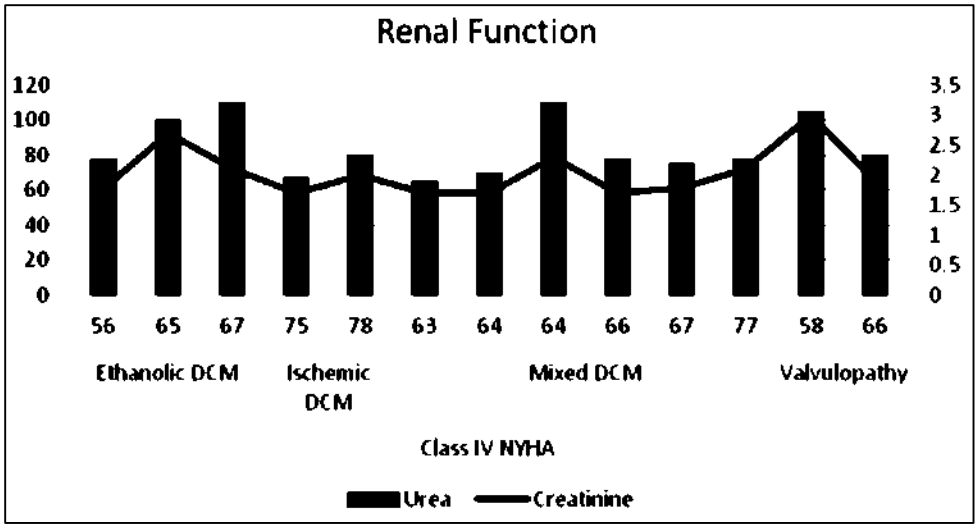

Fig. 2.2.11. Electrolyte imbalances in patients with NYHA Class IV Heart Failure dependent on etiology and age.

Discussions

Heart failure is the leading cause of hospitalization among adults > 75 years of age in the developed countries. Despite dramatic improvement in outcomes with medical therapy, readmission rates following heart failure hospitalization remain high, with ≥ 50% of patients being hospitalized within 6 months after discharge. Associated complications or comorbidities, including atrial fibrillation, ischemic heart disease or uncontrolled arterial hypertension, determine higher risk for cardiovascular readmission. Beyond clinical and laboratory parameters, the overall level of disability as reflected in measures of functional limitation, frailty and patient-reported quality of life seems to be a particularly important predictor of the overall readmission rate.

Effective and successful strategies for preventing CHF readmissions should include interventions across a full continuum of care from hospital to outpatient clinic and home. Enhancing initial risk stratification in order to safely increase the emergency department discharges, or transitioning low-risk patients to alternative treatment pathways in order to avoid excesive hospitalizations, is paramount to preserving health care resources (Soucier et al 2018, Talmor et al 2018, Chamberlain et al 2018, Lu et al 2016). Basoor et al (2013) conducted a prospective randomized controlled trial which involved 96 inpatients with CHF, of whom 48 patients were provided with a checklist at discharge that included relevant counseling, medication and dosage information, as well as follow-up instructions, while the other 48 patients were discharged without this checklist. Patients who received the CHF discharge checklist had higher rates of outpatient and medication compliance, as well as reduced 30-day (6 vs 19%, P>0.05) and 6-month (23 vs 46%, P=0.045) CHF readmission rates, compared to those who did not receive the above checklist (Kocher et al 2011). Phillips et al (2004) conducted a survey including 18 studies and 3,304 inpatients with CHF, evaluating the

use of comprehensive discharge planning and support, which included education and periodic follow-up visits. After an average observation period of 8 months, the use of comprehensive discharge planning was associated with an important reduction in readmission rates and a trend toward a reduction in all-cause with no significant difference in the length of hospitalization. The patients who received the comprehensive discharge plan also reported improved quality of life (25.7 vs 13.5%, P=0.01). Early readmissions after hospital discharge are often assumed to indicate an incomplete treatment in hospital, a deficit of coordination between services or poor communication of prescriptions at discharge. It can also imply inadequate access to medical care in early followup. The 30-day interval for readmission has increasingly compelled attention, emerging as a discrete time frame over which outcomes can be tracked or followed and potentially influenced by greater attention to improving inhospital heart failure treatment and care transitions. Goals during the heart failure readmission include monitorized decongestion and stabilization of fluid balance via oral diuretics, treatment of aggravating factors, and titration of neurohormonal antagonists for long-term benefit. Comprehensive discharge planning, including patient and caregiver education, nutritional advices like the limitation of sodium consumption and restriction of fluids, collaboration with visiting nurses and planned follow-up, are factors which may reduce early readmission rates by as much as 25%. Especially important may be the follow-up within 7 to 10 days, which was widely implemented after recognition that nearly half of heart failure readmissions occurred before the first ambulatory visit. Outcomes appear to be better when follow-up involves collaborative care between a cardiovascular specialist and the primary care physician (Dharmarajan et al, 2013, Scott et al 2014, Basoor et al 2013). Readmission rates are also higher when psychosocial and/or socioeconomic factors limit adherence, compliance and coordination with medication, self-monitoring, and follow-up. Patients with these risk factors tend to cluster according to the local geography of their hospitals. Resource limitations both for institutions and for individuals further influence the tendency for higher readmission in public hospitals, especially in those located in counties with low to medium income, with a limited number of cardiology specialists or with understaffed nursery. Local practice patterns also appear to be important, because hospitals with high overall rates of admissions tend to have higher rates of readmissions after heart failure hospitalization. Readmission rates are similar with and without heart transplant capability. This is due to the fact that transplant programs focus on younger populations with more severe cardiac disease but fewer comorbidities. High adherence to guidelines does not predict in a reliable way lower readmissions, because the highest performing centers may also receive a more complex referral case mix (Collins et al 2013, Phillips et al 2004).

Conclusions

Heart failure decompensation was the most common cause for readmission, being precipitated by an ischaemic episod, atrial fibrillation or medication side effects. HF hospitalization within one year prior to study enrollment, hyponatremia, renal dysfunction, hypotension and the degree of hyperkaliemia were particularly important risk factors for cardiovascular readmission (Al Namat et al 2018).

I. 2.2.2. Biochemical markers usefull in emergencies. Published papers in the field.

Stoica Alexandra, Victorita Sorodoc, Catalina Lionte, Irina M. Jaba, **Irina Costache**, Ecaterina Anisie, Cristina Tuchilus, Ovidiu Rusalim Petris,Oana Sırbu, Elisabeta Jaba, Alexandr Ceasovschih, Luminita Vata and Laurentiu Sorodoc. **" Acute cardiac dyspnea in the emergency department: diagnostic value of N-terminal prohormone of brain natriuretic peptide and galectin-3".** Journal of International Medical Research 2018: 1–14. **Impact Factor: 1.023**

Background

The majority of patients with acute heart failure (64% to 78%) are admitted through the Emergency Department (ED) (Searle et al 2016, Logeart et al 2013, Fonarow et al, 2004). In a "real life" context, patients with acute dyspnea and/or atypical manifestations of heart failure often require multidisciplinary evaluation in order to achieve an active and rapid approach to establishing the diagnosis of acute heart failure; specific treatment should be promptly initiated within an optimal time frame of 30 to 60 minutes after admission. (Mebazaa, et al 2015, 2016, Ponikowski et al, 2016, Arrigo et al, 2016). **Biomarkers are strongly objective when making medical decisions**. The diagnostic and prognostic implications of natriuretic peptides [brain natriuretic peptide (BNP) and N-terminal prohormone of brain natriuretic peptide (NT-proBNP)] in patients with heart failure has achieved worldwide agreement. The main limitation of these biomarkers is the different cutoff values established for the acute and chronic manifestations of heart failure and parameters such as age, sex, body mass index (BMI), high-output states (e.g., cirrhosis, sepsis), and renal function interfere with their diagnostic abilities. Novel therapies used in acute heart failure (e.g., neutral endopeptidase inhibitors) even further modify the natriuretic peptide levels, creating a divergent pattern: increased levels of BNP and decreased levels of NTproBNP. (Yancy et al 2017, Stokes et al 2016, Wettersten et al, 2016). Several biomarkers that reflect different physiopathological pathways have been proposed for the diagnosis, prognosis, and risk stratification of patients with acute heart failure when natriuretic peptide levels are not conclusive. **Galectin-3, a member of the lectin family,** is secreted by activated macrophages and is involved in biological processes such as inflammation, cardiac remodeling, and myofibroblast proliferation. **Galectin-3 was recently approved by the Food and Drug Administration for its prognosis utility in acute heart failure, fulfilling important criteria that make it a possible ideal biomarker** (early recognition of hypertrophic and fibrotic cardiac injuries, risk stratification, and potential therapeutic target as proven by experimental studies). (Wettersten et al 2016, Song et al 2015, AbouEzzeddine et al, 2015).

This prospective observational study focused on the difficulties that confront the clinician when assessing dyspnea of acute onset so it **aimed to prospectively investigate whether a dual biomarker approach using NT-proBNP and galectin-3 optimizes the diagnosis and risk stratification of acute cardiac dyspnea with a major impact on atypical clinical manifestations and overlapping pathologies.**

Material and methods. Study design and patient population.

The study was a prospective one and included 208 patients who presented to the ED of St Spiridon Hospital with sudden-onset or aggravated dyspnea requiring admission in the Department of Internal Medicine. The study was conducted from November 2016 to March 2018. All possible cardiac etiologies of acute dyspnea were accepted as inclusion criteria. Patients were excluded if they required admission to the cardiac intensive care unit or had associated active neoplasia, active liver disease (alanine aminotransferase level of >5 times the upper limit of normal), fibrotic pathologies (e.g., pulmonary fibrosis, collagenosis), or laboratory-based limitations for measurement of galectin-3 according to the manufacturer's instructions (serum cholesterol level of ≥ 500 mg/dL or serum creatinine level of >5 mg/dL). All procedures for obtaining and documenting written informed consent complied with the Good Clinical Practice and ethical principles for medical research involving human subjects stated in the Declaration of Helsinki.

The study was approved by the Ethics Committee of 'Sf. Spiridon' Emergency Hospital, Iasi, Romania and 'Gr. T. Popa' University of Medicine and Pharmacy, Iasi, Romania.

After completion of a standard evaluation (anamnesis, physical examination, laboratory tests, 12-lead electrocardiography, and chest radiography), additional investigations were performed as deemed necessary (abdominal ultrasound, vascular Doppler ultrasound, and computed tomography pulmonary angiography) (Stoica et al, 2018).

The NTproBNP and galectin-3 levels were measured upon admission. The galectin-3 level was determined from serum samples using a chemiluminescent microparticle immunoassay compatible with the ARCHITECT i System (Abbott Laboratories, Chicago, IL, USA). The specificity and sensitivity of NTproBNP are considered optimal when using age-related cut-offs. Hence, the following cut-off values were used for the study: 450 pg/mL for patients aged <50 years, 900 pg/mL for patients aged 50 to 75 years, and 1800 pg/mL for patients aged >75 years.6,10 Measurements above these values were defined as elevated values of NT-proBNP. Plasma galectin-3 levels were divided according to data obtained from clinical studies: low risk, <17.8 ng/mL; moderate risk, 17.8 to 25.9 ng/mL; high risk, >25.9 ng/mL. (Hrynchyshyna et al 2013, McCullough et al, 2002, 2011).

The following variables were recorded: age, sex, BMI, smoking habit, alcohol consumption, pathological antecedents, vital parameters, standard laboratory values, New York Heart Association (NYHA) functional class, glomerular filtration rate (GFR) calculated using the Modification of Diet in Renal Disease Equation, concomitant medication, length of hospital stay,

and discharge status. Transthoracic echocardiography was routinely performed at patient admission to evaluate the systolic and diastolic function of the left ventricle. Left ventricular systolic function was evaluated based on the following classification of the left ventricular ejection fraction (LVEF): reduced (LVEF of <40%), mid-range (LVEF of 40%–50%), preserved (LVEF of 50%–60%), or normal (LVEF of >60%). **The patients were divided into two groups according to the etiology of dyspnea: cardiac and non-cardiac**. (Stoica et al, 2018).

Statistical analysis

Continuous variables were tested for normal distribution using the Kolmogorov–Smirnov test and are presented either as mean ±standard deviation or median with 25th and 75th percentiles. Means between groups were compared using parametric tests (independent-sample t test, analysis of variance followed by the Bonferroni post-hoc test for multiple comparisons) or non-parametric tests (Kruskal–Wallis test, Mann–Whitney U test) as appropriate. In certain cases, logarithmic transformation of data was performed. Analysis of covariance (ANCOVA) was used to control for the effects that continuous variables such as age or GFR may have on the marker's output between patients with acute cardiac dyspnea and those with non-cardiac dyspnea. For correlations between variables, Pearson's test was used after logarithmic transformation. Measures of associations were studied using phi or Cramer's V (nominal by nominal) and eta (nominal by interval) coefficients. Receiver operating characteristic (ROC) analysis was used to ascertain the diagnostic performance of biomarker levels, and the areas under the curve (AUCs) were compared.

The diagnostic performance of NTproBNP and galectin-3 was ascertained for the entire group as well as for certain high-risk subsets such as patients with kidney failure (GFR of <60 mL/minute/ 1.73m2), age of >60 years, obesity (BMI of >30 kg/m2), rhythm disorders (atrial fibrillation/flutter), LVEF of <40%, arterial hypertension, and type 2 diabetes mellitus. ROC-optimized cut-off values as well as sensitivities and specificities were calculated. (Zweig et al, 1993). Data analysis was performed using IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY, USA) and MedCalc version 18.2.1 (MedCalc Software bvba, Ostend, Belgium). All tests were two tailed, and a p-value of <0.05 was considered statistically significant. (Stoica et al, 2018).

Results

This study included 208 patients ranging in age from 41 to 94 years and with a female:male ratio of 1.44. The patients' NT-proBNP level ranged from 12 to 30,000 pg/mL, and their galectin-3 level ranged from 7.5 to 86.6 ng/mL. The diagnostic criteria for acute cardiac dyspnea were fulfilled in 61.1% of the patients. The cardiac profile of the patients at the time of ED presentation showed that 76.0% were hypertensive and more than half (55.8%) had supraventricular rhythm disorders such as atrial fibrillation or atrial flutter. Chronic myocardial infarction was present in 5.3% of patients. Preserved left ventricular systolic function (50%– 60%) defined the largest proportion of patients (38.46%) (Figure 2.2.12). (Stoica et al, 2018).

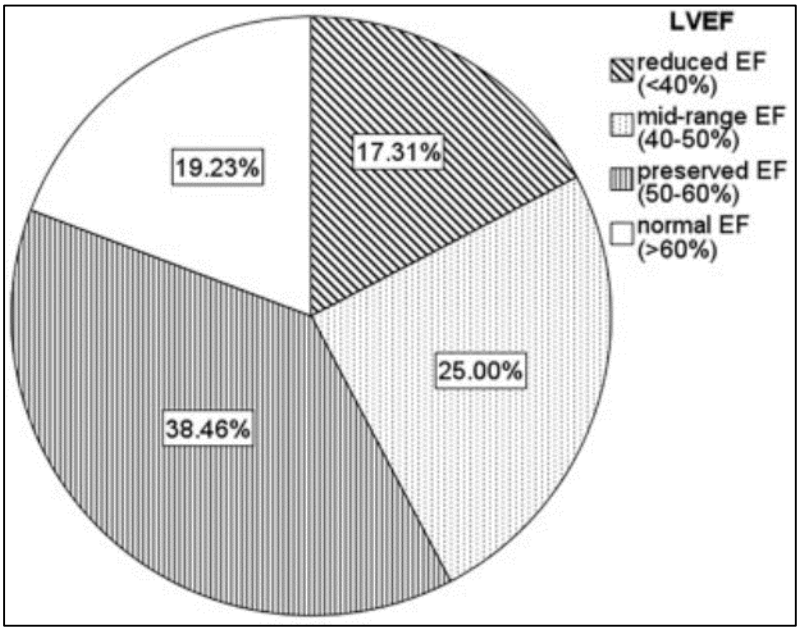

Figure 2.2.12. Distribution of patients according to the LVEF. LVEF, left ventricular ejection fraction.

Clinical manifestations compatible with NYHA functional class III to IV heart failure presented a balanced distribution when compared with the subgroup of patients with mild dyspnea (NYHA class I–II) (49.6% vs. 50.4%, respectively). One-third of the patients (30.3%) had associated acute bronchopulmonary manifestations, either community-acquired acute respiratory illness or an exacerbation of a previous chronic bronchopulmonary condition (chronic obstructive pulmonary disease or bronchial asthma). Anemia, chest wall syndromes (costochondritis, musculoskeletal pain), diseases of the digestive system (gastroesophageal reflux), and anxiety were encountered among other non-cardiac etiologies of acute dyspnea. Other comorbidities were obesity (BMI of >30 kg/m2; 30.3%), type 2 diabetes mellitus (28.4%), chronic stroke (18.6%), chronic kidney disease (15.4%), lower extremity peripheral arterial disease grade II to IV (14.4%), and chronic bronchopulmonary pathology (10.1%). (Stoica et al, 2018).

Associated medications at admission included: beta-blockers (49.5%), diuretics (42.3%), platelet anti-aggregants (32.7%), and angiotensin-converting enzyme inhibitors (27.4%). Alcohol consumption was classified as "yes," "no," and "former." Chronic alcohol consumption was present in 12.5% of the study group (n¼26) and was linked to an altered LVEF, an increased alanine aminotransferase level as an indicator of impaired hepatic function and a proinflammatory status as reflected by the serum C-reactive protein level. Additionally, higher estimated GFRs were noted in the subgroup of chronic alcohol consumers. Smoking habits were found in 20.2% (n¼42) of the patients but did not lead to any significant differences in the biomarker levels or LVEF between the cardiac and non-cardiac dyspnea groups. (Stoica et al, 2018).

Patients with acute cardiac dyspnea were significantly older (p¼0.000) and had a significantly longer in-hospital stay (p¼0.032). The heart rate was higher (p¼0.000) and the LVEF was lower (p¼0.000) in the cardiac than non-cardiac dyspnea group. Biochemical characteristics of the patients with acute cardiac dyspnea included increased serum levels of urea and uric acid (p¼0.000 for both) and significantly lower GFRs (p¼0.009) compared with the patients with non-cardiac dyspnea ($p<0.05$).

Both biomarkers followed in this study, NT-proBNP and galectin-3, were significantly higher in the patients with acute cardiac dyspnea than in those with acute noncardiac dyspnea (p¼0.000) (Figure 2.2.13.). (Stoica et al, 2018).

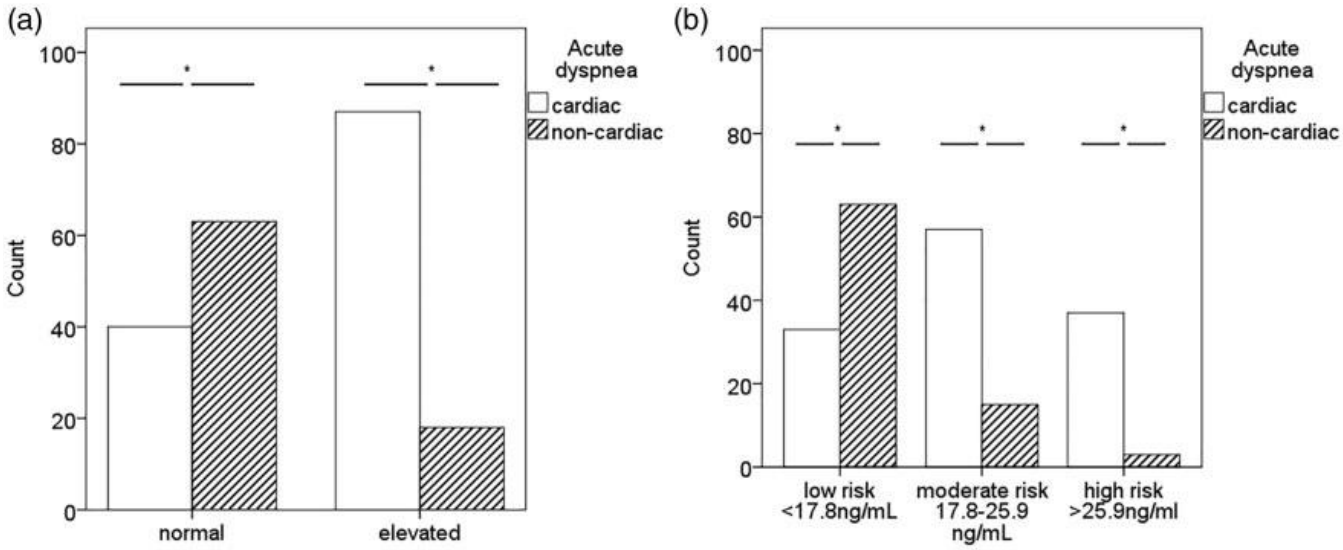

Figure 2.2.13. NT-proBNP and galectin-3 levels based upon acute dyspnea groups. (a) NT-proBNP. (b) Galectin-3. *$p<0.05$. NT-proBNP, N-terminal prohormone of brain natriuretic peptide.

Several parameters showed a significant association with the etiology of acute dyspnea: age, elevated systolic blood pressure, LVEF, presence of atrial fibrillation/flutter, and GFR. Additionally, both biomarkers had a strong and significant connection with the etiology of dyspnea (p¼0.000 for both). (Stoica et al, 2018).

ANCOVA was performed to determine whether the differences observed in the NT-proBNP and galectin-3 levels between patients with cardiac and non-cardiac dyspnea were indeed due to the type of dyspnea and not solely to the influences that factors such as older age or impaired GFR may exert on these markers. After controlling for the differences between patients with cardiac and non-cardiac dyspnea with respect to age (higher in patients with cardiac dyspnea) and GFR (impaired in patients with cardiac dyspnea), ANCOVA was performed to assess whether patients with cardiac dyspnea still had higher levels of NT-proBNP than patients with noncardiac dyspnea. The same analysis was performed for the second marker evaluated in this study (galectin-3).

The results indicated that after controlling for the differences in age and GFR between patients with cardiac and non-cardiac dyspnea, the differences in NT-proBNP (F¼16.81(1,204), p¼0.000) and galectin-3 (F¼22.45(1,204), p¼0.000) between the two groups remained significant. These findings indicate that the type of dyspnea significantly influences the levels of NT-proBNP and galectin-3. Among all patients enrolled in this study, 4.3% (n¼9) died before discharge from the hospital and 2.9% required transfer to the coronarography unit for acute coronary syndrome and revascularization procedures. In the subgroup of patients who died of cardiac causes (n¼8), the serum galectin-3 levels were significantly higher and ranged from 17.8 to 63.4 ng/mL [cardiac median, 29.2 ng/mL; interquartile range (IQR), 24.7–36.5 ng/mL and on-cardiac median, 20.1 ng/mL; IQR, 11.7–28.5 ng/mL; p¼0.038]. For NT-proBNP, these values ranged from 897 to and 30000 pg/mL (p¼0.003). (Stoica et al, 2018).

The patients were divided into three subgroups according to their outcome at discharge: deceased, aggravated, and improved.

Both NT-proBNP and galectin- 3 showed significant differences among these subgroups. The differences were very significant between the deceased subgroup [median galectin-3, 28.5 ng/mL (IQR, 21.05–36.55 ng/mL); median NT-proBNP, 9599 pg/mL (IQR, 4284–16235 pg/mL)] and improved subgroup [median galectin-3, 18.6 ng/mL (IQR, 15.2–23 ng/mL); median NT-proBNP, 1132 pg/mL (IQR, 432.5– 4184.5 pg/mL)] (NT-proBNP, p¼0.001; galectin-3, p¼0.012). (Stoica et al, 2018).

Statistically significant differences between the deceased and survivors (improved or aggravated) subgroups were observed for age (improved, 72.5±11.20 years; deceased, 79.78±10.09 years; p¼0.043), **the serum C-reactive protein level** (improved, 2.39 ±5.3mg/dL; deceased, 11.72±10.01mg/dL; p¼0.000), **and hyponatremia** (serum sodium level of <135mmol/L) (improved, 138.65 ±4.89mmol/L; deceased, 134.44 ±5.22mmol/L; p¼0.031). (Stoica et al, 2018).

The correlation between the NTproBNP and galectin-3 levels was direct, moderate to strong (r¼0.477), and significant (p¼0.000) (Figure 2.2.14.). (Stoica et al, 2018).

The diagnostic performance of both biomarkers was tested in all patients as well as in the subsets of patients with high risk and a potential for unclear interpretation of NT-proBNP and galectin-3.

These subsets of patients were those with kidney failure. (GFR of <60mL/minute/1.73m2), age of >60 years, obesity (BMI of >30 kg/m2), rhythm disorders (atrial fibrillation/flutter), LVEF of <40%, arterial hypertension, and type 2 diabetes mellitus.

Patients with acute cardiac dyspnea showed significantly higher serum concentrations of both NT-proBNP and galectin-3, both overall and in each particular subset of patients with these associated conditions (p<0.05 for all). (Stoica et al, 2018).

To test the diagnostic performance of NT-proBNP and galectin-3 for acute cardiac dyspnea, comparative accuracy was evaluated using ROC analysis. **Both biomarkers showed similar diagnostic abilities as indicated by the lack of statistically significant differences between the AUCs**. (Stoica et al, 2018).

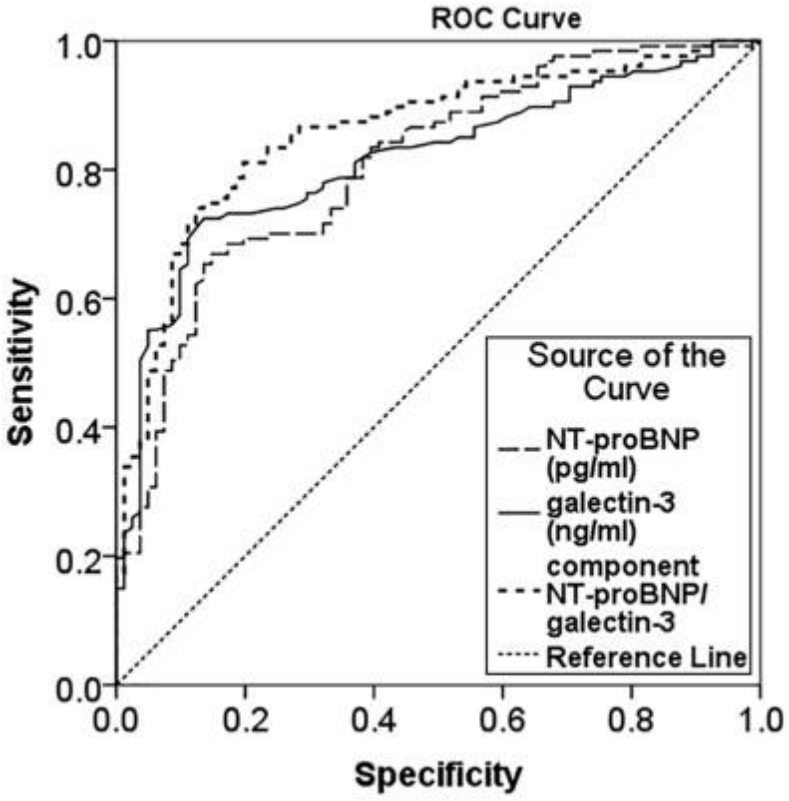

Figure 2.2.14. ROC curves for the diagnosis of acute cardiac dyspnea for NT-proBNP, galectin-3, and the component of the two markers. NT-proBNP, N-terminal prohormone of brain natriuretic peptide; ROC, receiver operating characteristic.

The diagnostic performance of the two biomarkers was also studied in subsets of particular interest, given the increased cardiovascular morbidity/mortality in the general population (age of >60 years, GFR of <60 mL/minute/1.73 m2, obesity as defined by a BMI of >30 kg/m2, impaired left ventricular function as defined by an LVEF of <40%, type 2 diabetes mellitus, and rhythm disorders). **There were no statistically significant differences between the AUCs for galectin-3 and NT-proBNP in any of the high-risk subsets.** ROC analysis also indicated that the optimal diagnostic cut-offs values were higher in certain high-risk subsets of patients such as those with impaired renal function, impaired cardiac function (reduced LVEF), rhythm disorders, and diabetes than in the overall study group. (Stoica et al, 2018).

Discussions

Although BNP and NT-proBNP have an established position regarding their diagnostic abilities in acute heart failure (Class I recommendation), **multiple interferences with different pathologies**

limit the utility of these biomarkers in emergency situations. (Yancy et al 2017, Darche et al, 2016). This category of patients represents a major concern regarding a clinician's diagnostic abilities because chronic therapy tends to mask the signs and symptoms characteristic of acute heart failure. **The most common coexistent cardiometabolic diseases on admission were arterial hypertension, supraventricular rhythm disorders (atrial fibrillation/atrial flutter), obesity, diabetes mellitus, chronic myocardial infarction, and lower extremity peripheral arterial disease.** (Stoica et al, 2018).

There were no significant sex-related differences between the two groups. The patients with cardiac dyspnea were older and had more advanced renal function decline. Moreover, prolonged hospitalizations were required for the subgroups of patients with acute dyspnea of cardiac origin. In this study, the distribution of patients according to their LVEF showed that most patients had a preserved LVEF. This is also the category of patients that is more likely to receive an incorrect diagnosis. (Stoica et al, 2018).

Assessment of the serum concentrations of both NT-proBNP and galectin-3 showed significantly higher levels in patients with acute cardiac dyspnea, suggesting an ability to identify patients with increased cardiovascular risk. The same observation was true for subsets of patients with higher risk such as those with kidney failure, advanced age, obesity, rhythm disorders, impaired left ventricular function, arterial hypertension, and type 2 diabetes mellitus. Concerns that the higher levels of NTproBNP and galectin-3 in patients with acute cardiac dyspnea were due solely to the influence of factors such as older age or impaired GFR were alleviated by controlling for the impact of these covariates. While this does not completely exclude the impact that older age or impaired GFR may have on these markers, it was important to prove **that the type of dyspnea significantly influenced the levels of NT-proBNP and galectin-3, making these markers useful indicators of the risk of acute cardiac dyspnea.** While the values for both markers were higher in the deceased group than in the improved outcome group, **galectin-3 had a tighter range than NT-proBNP in the deceased group.** This may imply that **galectin-3 has the potential to serve as a more specific prognostic factor. In patients expected to have a poor outcome, we found that more advanced age, an increased serum C-reactive protein level, and/or hyponatremia better identified patients with high cardiovascular risk. More advanced NYHA functional classes (III–IV) were also strong predictors of a negative outcome** (Stoica et al, 2018).

The present study findings confirm that **a significant association exists not only between NT-proBNP and acute cardiac dyspnea but also between galectin-3 and acute cardiac dyspnea.** The existence of a strong correlation between NT-proBNP and galectin-3 (figure 2.2.15) lends credibility to the utility of a dual-biomarker approach to the diagnosis of acute cardiac dyspnea. (Stoica et al, 2018).

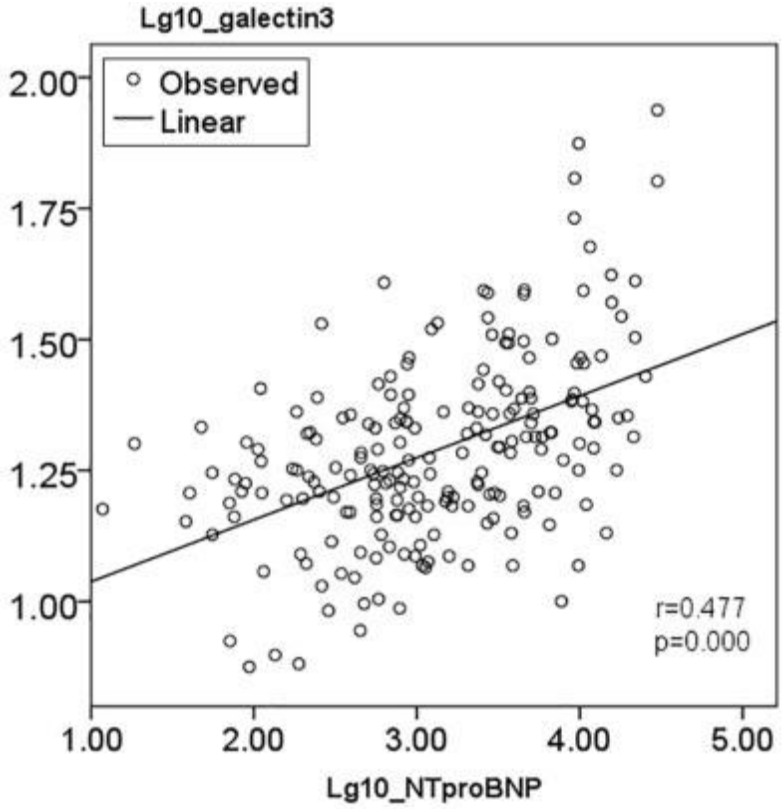

Figure 2.2.15. Pearson correlation between galectin-3 and NT-proBNP. A base-10 log scale is used for the x- and y-axes. NT-proBNP, N-terminal prohormone of brain natriuretic peptide.

This finding represented a good argument to further proceed with the ROC curve analysis in an effort to ascertain the diagnostic performance of both biomarkers. High diagnostic accuracy for NTproBNP and galectin-3 was demonstrated in the ROC analysis, indicating that **both biomarkers are reliable tools for the prediction of acute cardiac dyspnea.** The results were comparable when analyzing the entire study group as well as the subgroups of high-risk cardiovascular patients. **The associated comorbidities led to higher optimal diagnostic cut-off values but did not impair the diagnostic accuracy of either marker**. After the independent analysis of the two biomarkers confirmed their diagnostic performance in identifying acute cardiac patients, a component variable was proven to have even better predictive diagnostic ability. This indicates that serum determination of galectin-3 enhances the diagnosis of acute cardiac dyspnea when used in conjunction with NT-proBNP. (Stoica et al, 2018).

Study limitations

The patients came from a single tertiary center, and the population was representative of the northeastern region of Romania. Our study findings are not applicable to pediatric patients or non-Caucasian ethnic groups. Although the number of patients was limited, statistically significant

diagnostic accuracy of the two biomarkers in patients with acute cardiac dyspnea was demonstrated.

Conclusion.

1) These findings indicate that dual-biomarker analysis (NT-proBNP and galectin-3) represents an important practical approach that leads to early recognition of atypical manifestations of acute heart failure in patients with acute dyspnea presenting to the ED.

2) The combination of NT-proBNP and galectin-3 is superior to NT-proBNP alone. This may improve outcomes for patients in whom the determination of NT-proBNP does not reflect, for various reasons, the severity of the underlying cardiac condition.

3) These results have **immediate clinical applicability in identifying high-risk cardiovascular patients who require intensive care treatment.**

4) An accurate triage and early cardiovascular risk stratification of patients with acute dyspnea in emergency settings **reduces the economic impact of the disease and associated treatments** (Stoica et al, 2018).

I. 2.2.3. Experimental model with direct implications on Cardiovascular risk factors. New therapeutic perspectives.

Arterial hypertension is the most important risk factor for cardiovascular diseases. Oxidative stress leads to endothelial dysfunction and vascular remodeling, both processes being involved in the occurrence of arterial hypertension. Hypercholesterolemia is considered one of the key factor in the triggering and progress of inflammatory cardiovascular diseases such as atherosclerosis.

The human body protects itself from the harmful effects of oxidative stress by means of a series of enzymatic (superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase) and non-enzymatic (albumin, ceruloplasmin, ferritin, reduced glutathione, uric acid, lipoic acid, bilirubin, ascorbic acid, α-tocopherol, β-carotene) antioxidants. In physiological conditions, there is a balance between reactive oxygen species (ROS) production and the activity of the enzymatic and non-enzymatic antioxidant systems (Foncea et al, 2000). The determination of the level of oxidative stress and the activity of antioxidant systems is of particular importance since plasma markers of oxidative stress play a role in predicting coronary heart diseases. Many studies assess the total antioxidant status of plasma by determining the inhibitory capacity of an oxidative process at plasma level, whereas others determine the lipid peroxidation products (Levitan et al, 2009, Madamanchi et al, 2005). Intracellular and extracellular antioxidants, as well as cell membrane antioxidants, are designed to neutralize excess ROS and their formation.

Total antioxidant status in plasma consists of the net effect of different antioxidants and of the interactions between them, and may be determined by spectrophotometry.

There was a connection found between the initial antioxidant status in plasma and the independent risk factors for coronary heart diseases, and between the antioxidant status in plasma and specific oxidative stress markers, respectively (Ian et al, 2009, Quinones et al, 2013).

Clinical implications:

The new hypertension therapies which are thought to improve the mechanisms impairing the target organs in arterial hypertension (AHT) would have great practical value.

My concerns related with this subject are illustrated by the following research:

Păduraru Catrinel Florentina (Giurescu Bedreag), Nina Filip, Adriana Trifan, Sorin Dan Miron, Codruta Badescu*, **Irina Iuliana Costache***Razan Al Namat, Bogdan Diaconescu, Nicoleta Dumitrescu, Manuela Ciocoiu: **Evaluation of the Effects of a Pinus Brutia Bark Extract on Biochemical Parameters and Blood Pressure in an Experimental Arterial Hypertension**. Rev Chim 2018; 69 (7): 1718-1721. **IF = 1.412.**

The aim of our study was to investigate **the effects of Pinus brutia bark extract (EPb) on serum lipid profiles and oxidative stress in N(G)-Nitro-Larginine- methyl ester (L-NAME)-induced hypertension. The experiment was performed on the arterial hypertension model.**

Experimental model

Material and methods

The research was performed on Wistar white rats, with an average weight of 250-280 g, which were kept in individual cages, in a room maintained at 22 C with an alternating 12 h light-dark cycle and were divided into 4 groups of 12, namely: - Group W (martor) - control, normal animals, that did not receive natural polyphenols; - Group EPb, animals received solution of Pinus brutia extract (PbE) dosage of 22.85 mg/kbw/day), p.o. (by tube feeding), at every 2 days, for 8 weeks; - Group L-NAME, treated with N(G)-nitro-l-arginine-methyl ester (L-NAME) 40 mg/kbw/ day, i.p., at every 2 days, for 8 weeks; - Group EPb + LNAME, animals received simultaneously PbE+L-NAME in the dosage mentioned p.o, at every 2 days, for 8 weeks. The DL50 value of the EPb extract obtained from Pinus brutia bark was 228.51 mg/kg body weight, and the efficient dose used for later determinations was 1/10 of DL50 (22.85 mg/kgc). Serum total cholesterol, HDLcholesterol and triglycerides were measured by enzymatic colorimetric methods on a TECAN micro plate reader by commercially available kits (Audit Diagnostics Ireland). Non-HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol. The total antioxidant capacity was expressed in Trolox equivalents of testing animal serum using the colorimetric method developed by Rice-Evans and Miller. Blood pressure and heart rate were determined by non-invasive means, using a CODA4 device (Păduraru et al, 2018).

Results

The experiment demonstrated that PbE improved lipid profile and reduced pro-oxidative effects of L-NAME, thus suggesting a possible role of the extract in the management of AHT.

Systolic and diastolic blood pressure decrease was significant in the group undergoing simultaneous EPb extract and L-NAME therapy, as compared to the group that was administered only LNAME. The EPb extract increased the value of the total antioxidant status compared to the control (1.389 ± 0.04 mmol / L vs. 1.296 ± 0.1 mmol / L), **while LNAME caused its significant decrease** (1.133 mmol / L). When the extract was delivered in combination with LNAME, the decrease in the total antioxidant status was less significant (1.236 mmol / L). **This proves the ability of the EPb extract to reduce the pro-oxidant effects of LNAME, which confirms the remarkable antioxidant effects evidenced for this extract by in vitro studies (fig. 2.2.16.)** (Păduraru et al, 2018).

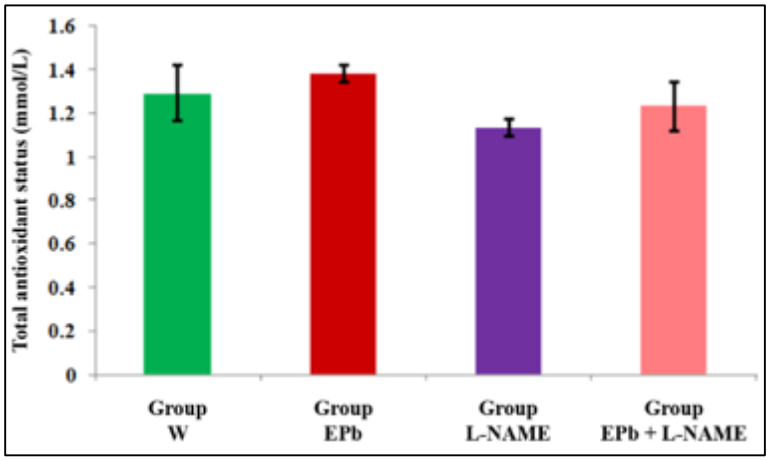

Fig. 2.2.16. Influence of EPb on the total antioxidant status

After 8 weeks of treatment, the EPb extract caused the serum cholesterol to decrease by 9.21% compared to the control group, while L-NAME produced a 26.22% increase. The EPb extract hypercholesterolemia-decreasing effect, showing a 23.53% decrease in total cholesterol in this group compared to the group receiving only L-NAME.

L-NAME has a strong influence on the lipid profile according with other studies which have shown that L-NAME increases total cholesterol and lowers HDL levels in plasma; moreover, it reduces plasma fibrinogen, shortens prothrombin times, increases arterial blood pressure and plasma levels of certain cardiac necrosis markers (creatine kinase, troponin C) (Cardoso et al, 2013, Yesil-Celiktos et al 2013, Crețu et al, 2013).

The cholesterol level was significantly lower in the EPb extract and L-NAME group compared to the LNAME group. The ability of the EPb extract to significantly reduce L-NAME-induced hypercholesterolemia is evident. It should be noted that the level of cholesterol in the group treated

with EPb extract and L-NAME (69.20 ± 5.88 mg /dL) was close to that of the control group (71.7 ± 3.77 mg/ dL) (table 2.2.12.). (Păduraru et al, 2018).

The LDL-cholesterol level in the L-NAME group was higher than that of the group treated with EPb extract and L-NAME. In contrast, the LDL-cholesterol levels increased significantly in the group treated with EPb extract and LNAME compared to the control (table 2.2.12.) (Păduraru et al, 2018).

The findings showed that after 8 weeks, LDL-cholesterol decreased by 3.54% in the group receiving EPb compared to the control group, while L-NAME produced a 102.52% increase. Administration of the extract concomitantly with L-NAME resulted in a decrease in the LDL-cholesterol level of about 11% as compared to the L-NAME group.

The HDL-cholesterol level decreased significantly in the LNAME group compared to the EPb and L-NAME group. A comparative analysis, with clear findings, may also be made between the control group (30.30 ± 1.12 mg / dL) and the group treated with EPb extract and L-NAME (24.20 ± 3.36 mg / dL). After 8 weeks of treatment, the group treated with EPb extract showed an increase in the HDLcholesterol level of 10.23% compared to the control group, while the administration of L-NAME produced a decrease of 38.29%. In the group treated with EPb extract and LNAME, the increase in HDL-cholesterol was 29.41% (table 2.2.12.) (Păduraru et al, 2018).

The hypotriglyceridemic effect is significant in the group treated with EPb extract and L-NAME as compared to the group treated only with L-NAME. However, the triglyceride level was higher in the EPb extract and L-NAME group compared to the control group. After 8 weeks of EPb extract treatment, triglycerides increased by 1.36% compared to the control group, while L-NAME produced an increase of 75.12% over the control group. When EPb extract was associated, the decrease was about 29%.

Table 2.2.12. Influence of epb extract on the lipid profile

Group	Total cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)
W	71.70± 3.77	19.80±4.98	30.30±4.98	80.40±6.05
Epb	65.10 ±5.08	19.10±3.54	33.40±4.52	81.50±8.01
L-NAME	90.5 ±5.1	40.10±3.31	18.70±4.39	140.80±17.66
Epb + L-NAME	69.20±5.88	35.70±7.88	24.20±3.35	100.20±7,25

Table 2.2.13. Influence of epb extract on arterial blood pressure and heart rate

Group	Diastolic blood pressure (mmHg)	Systolic blood pressure (mmHg)	Heart rate (beats/min)
W	88.6±6.8	121.05±4.97	311.4±36.51
Epb	87.8±5,6	123.75±6.75	315.9±34.1
L-NAME	105.75 ± 7.86	152.2±8.9	338.65±43.39
Epb + L-NAME	93.10 ±7.96	135.5±8.2	324.75±48.68

There were no statistically significant differences as concerns systolic and diastolic blood pressure between the EPb extract group and the control group. While L-NAME increased diastolic blood pressure by 19.35% compared to the control, when administered together with L-NAME, the EPb extract significantly decreased its hypertensive effect, lowering diastolic blood pressure by about 12%. Similarly, with regard to systolic blood pressure, as one may notice, when administered together with L-NAME, the EPb extract significantly reduced its hypertensive effect and systolic blood pressure by approximately 11%. The EPb extract did not reduce arterial blood pressure in normotensive animals, but only in animals with L-NAME-induced hypertension. (Păduraru et al, 2018).

This suggests that the antihypertensive action of the EPb extract could be due, at least in part, to its antioxidant properties. As concerns heart rate, the EPb extract did not produce significant changes (table 2.2.13.) (Păduraru et al, 2018).

The Pearson correlation proves an indirect correlation between total antioxidant status and total cholesterol levels. Thus, in 70% of the animals, the low total antioxidant status values are correlated with elevated total cholesterol values ($r = - 0.703$, $R2 = 0.494$, $p = 0.001$). A direct correlation is undeniable between total antioxidant status and HDL-cholesterol values, in which 53.8% of animals with elevated total antioxidant status values have elevated HDL-cholesterol values ($r = + 0.538$, $R2 = 0.2897$, $p = 0.014$). Another indirect correlation between total antioxidant status and LDL-cholesterol values occurs in 63.2% of animals, where the low total antioxidant status values are correlated with elevated LDL-cholesterol values ($r = -0.632$, $R2 = 0.399$, $p = 0.003$) (fig. 2.2.17.) (Păduraru et al, 2018).

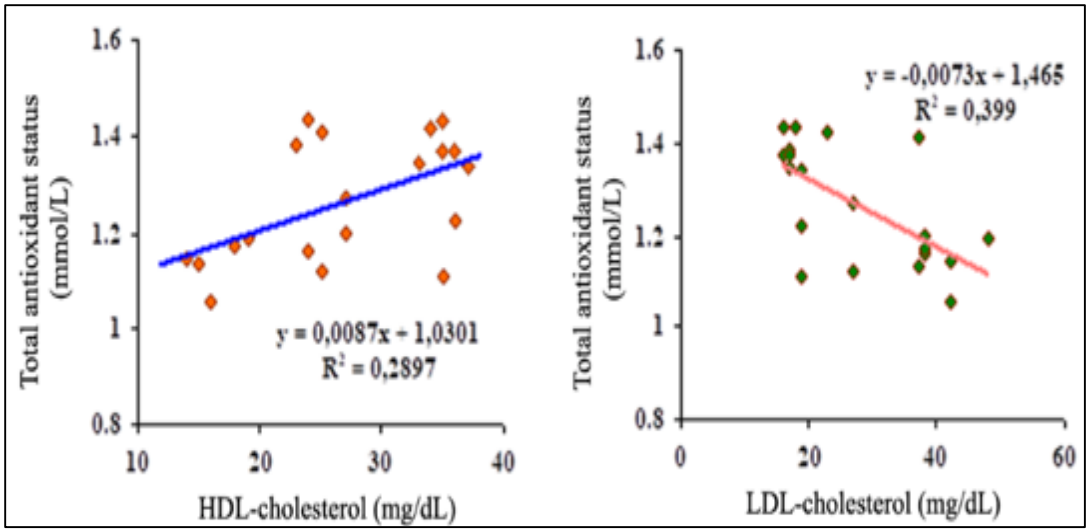

Fig. 2.2.17. Total antioxidant status in relation with lipid metabolism markers

We found an indirect correlation between total antioxidant capacity and diastolic blood pressure values in 58.4% of the testing animals (r = - 0.584, R2 = 0.3406; p = 0.007) **and systolic blood pressure values, respectively, in 59.7% of the testing animals** (r = - 0.597, R2 = 0.3568, p = 0.005) (fig. 2.2.18.). **There was no correlation between total antioxidant status and heart rate** (r = - 0.044, R2 = 0.002, p = 0.852) (Păduraru et al, 2018).

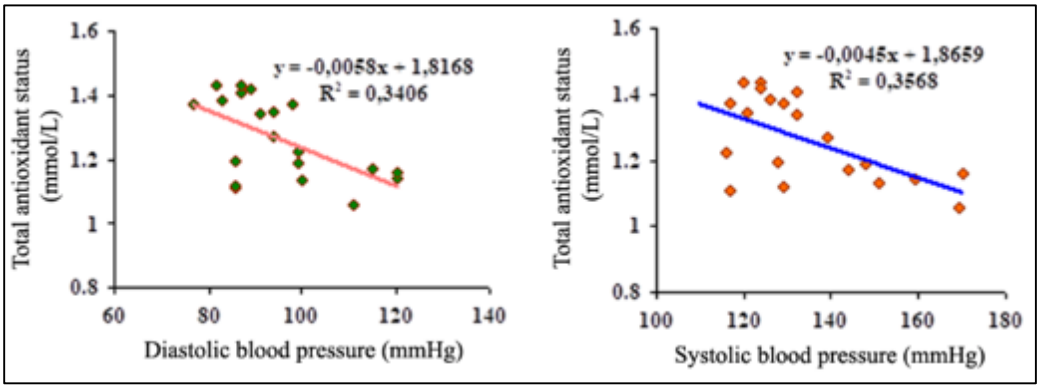

Fig. 2.2.18. Total antioxidant status in relation to blood pressure

Discussions.

L-NAME is a potent inhibitor of NO synthase and implicitly of NO• production. At the same time, L-NAME causes an increase in oxidative stress through various mechanisms: decrease of catalase and superoxide dismutase activity, intensification of lipid peroxidation processes (Cardoso et al 2013). The antihypertensive effects of polyphenols are mainly due to their antioxidant properties (Stephens et al 2009, Jozanov-Stankov et al 2009).

Polyphenols induce endothelial nitric oxide synthase expression, increase intracellular glutathione levels and inhibit the activity of certain pro-oxidant enzymes (NADPH oxidase, xanthine oxidase)

(Ince et al 2009, Rodrigo et al 2012, Mitu et al 2016). Many epidemiological studies have analyzed the correlation between the incidence of morbidity and mortality due to cardiovascular diseases and the high level of total cholesterol, LDLcholesterol and lipoproteins (a) (Yesil-Celiktas et al 2009, Kumar et al 2010).

The measurement of the cholesterol level and HDL-cholesterol and LDLcholesterol levels allows specialists to estimate the risk of cardiovascular conditions. Hypertriglyceridemia is also a risk factor for coronary heart disease by highlighting the presence of certain triglyceride-rich lipoproteins that undergo partial degradation on ver y low density lipoproteins, VLDL, which are also atherogenic. That is precisely the reason why the most important research directions are related to the investigation of protective capacity against ischemia-reperfusion injuries, as well as to the influence of polyphenolic extracts on the tone of the arterial wall; when a vasorelaxant action is detected, it would be useful to determine its type (endothelium-dependent or endothelium-independent relaxation).

Conclusions

Due to its effects, the **Pinus brutia bark extract may be used for the prophylaxis and as adjuvant therapy of cardiovascular conditions.** Our experiment demonstrated that **EPb improved lipid profile and reduced pro-oxidative effects of L-NAME,** thus suggesting a possible role of the extract in the management of AHT. **The in vivo study of the EPb extract revealed important antihypertensive effects in animals with induced hypertension.** The decrease of systolic and diastolic blood pressure was significant in the group treated with Epb extract and L-NAME, as compared to the group that was administered only L-NAME. The findings justify the continuation of research in this direction, namely on the possible uses of extracts obtained in the prophylaxis and adjuvant therapy of cardiovascular diseases. (Păduraru et al, 2018).

I. 3. Researches regarding cardiovascular diseases (CVD) in terms of comorbidities. Clinical implications.

I. 3. 1. Background:

Comorbidities are a cause of increased mortality, decreased quality of life and increased use of healthcare services. It is important particularly for physicians and other healthcare providers in primary care settings to evaluate these patients properly. Cardiovascular diseases (CVD) are the most common cause of death from non-communicable diseases worldwide and are characterized by a high level of comorbidities (Kendir et al, 2018). Previous researches confirmed that:

- 26.5% of patients had 1 CVD; 10.5% had 2 CVD diagnoses;.
- Each CVD was associated with other CVDs and many other comorbidities;
- In case of CVD, physicians should be alert for another CVD; these patients must be closely and carefully monitored for other chronic diseases (Kendir et al, 2018).

Cardiovascular diseases (CVDs) are the most common cause of death in the world. Globally, is estimated that 17.5 million people died from CVDs in 2012, representing 31% of all global deaths. CVDs are in the top three of most common causes of death in people older than 35 years (Kendir et al 2018). Weiner et al., (2004) found that patients with CVD who also have chronic kidney disease are 35% more vulnerable to recurrent CVD or mortality compared to CVD patients without comorbidity. Tripathy et al., (2017) found that the risk of mortality from CVD among patients who have no history of previous heart attack is higher for patients with diabetes than for those without diabetes.

I. 3. 2. Why it is important to evaluate CVD in the context of comorbidities?

It is important to manage patients with CVD in a way that takes comorbidity into account (Kendir et al 2018, Van den Akker et al 1998). Management of these patients in primary care settings is important since patients with comorbidity are characterized by increased mortality, decreased quality of life, and increased use of healthcare services compared to patients without comorbidity (Kendir et al 2018, Pati et al 2015; Salisbury et al, 2011, Farmer et al 2016). In addition, the occurrence of comorbidity influences medical decision making by physicians as regards prevention, treatment and utilization of services (Kendir et al 2018, Starfield et al 2003), and comorbidity increases the frequency of visits to general practitioners (GPs) and other medical specialists (Kendir et al 2018, Rasputina et al 2017). Previous studies have established relationships between CVDs and other chronic conditions, mainly focusing on specific age groups or chronic disease types (Aarts et al 2011, Bang et al 2016, Landwehr Johan et al 2000, Prados-Torres et al 2012), but CVD may be associated with a lot of acute clinical conditions that may worsen of the evolution. For exemple, it is a wellknown fact that anemia after bleeding or a surgical intervention may precipitate heart decompensation but the relationship can also work in the opposite direction: a heart condition can be an aggravating factor for other comorbidities and especially for some surgical interventions. The general practitioners should be alert for additional

diseases in patients with CVD, and these patients should be closely monitored. This can help to prevent comorbidity or slow down disease progression over time, and might result in higher quality of life and decreased mortality. (Starfield et al 2003).

A study conducted by Kendir et al (2018) analysed the associations between CVDs and other CVDs. For all CVD groups, positive and significant odds ratios were found for all combinations, which means that having one CVD always increased the risk of having another CVD. All CVDs showed associations with many of the comorbidities studied. **Coronary artery diseases had the strongest association with arrhythmias, diabetes mellitus and lipid metabolism disorder. Hypertension showed a strong association with arrhythmias, diabetes mellitus and lipid metabolism disorders**. **Heart failure showed the strongest association with arrhythmias**. In addition, it showed a **strong association with eye diseases, pulmonary circulatory disease, rheumatoid arthritis, Chronic obstructive pulmonary disease (COPD), thyroid disorders, diabetes mellitus and gout. The strongest association was found between heart failure and coronary artery disease.** Alternatively, heart failure showed a negative association with migraine and headache. **Cerebrovascular diseases showed a positive association with all chronic comorbidities,** the strongest associations being those with epilepsy and arrhythmias**. Peripheral artery diseases showed a strong association with peptic ulcer, COPD, diabetes mellitus and lipid metabolism disorder** (Kendir et al 2018).

Based on Kendir research (2018) we may conclude that in a large population of patients one from four patients suffered from at least one CVD. Having one CVD increased the risk of another, co-occurring CVD and a higher number of other chronic diseases. In addition, patients with peripheral artery disease were found to have a more than three-fold increased risk for coronary artery disease, heart failure and cerebrovascular disease compared to patients without peripheral artery disease. Diseases of the eyes, osteoarthritis, mood disorders and lipid metabolism disorders were the most prevalent comorbidities, and all showed a positive association with each of the CVDs. Another similar study was made by Landwehr Johan et al. (2000). Comparing the results of these two studies shows that the current study found **a higher number of comorbidities with a significant positive association with CVDs, suggesting that an earlier treatment may have better results in reducing the effects of these associations.** However, having more comorbidity compared to the previous study is also a result of aging population in Europe that has been shown on the dataset of Eurostat in comparison of years 2005 and 2015. Previous studies also found that patients with CVD were at increased risk for comorbidities compared to patients without such disease (Weiner et al 2004, Bruce et al 2016, Sarfati et al 2016, Dursunoglu et al 2016). In Kendir"s study (2018) all CVDs showed a positive association with all malignancies, peptic ulcer, eye diseases, ear diseases, arrhythmias, pulmonary circulatory diseases, osteoarthritis, rheumatoid arthritis, mood disorders, other mental disorders, COPD and bronchitis, thyroid disorders, diabetes mellitus, lipid metabolism disorders and gout. It was described a negative association between migraine and heart failure, but they found a significant positive association only between migraine and hypertension and cerebrovascular diseases. Another result of the present study which differed from previous

work regarded the association between asthma and CVDs. Asthma showed a statistically significant positive association only with hypertension, heart failure and cerebrovascular diseases, not with other CVDs. This might be due to lack of questioning of the predisposing and associated factors such as smoking habits, family history and environmental factors.

I. 3. 3. Clinical consequences:

The association between CVD and different comorbidities has some clinical implications:

- association may negatively influence the evolution of both types of disease; in some cases a disease may evoluate with cardiovascular complications (ex cirrhosis of the liver may be complicated with cirrhotic cardiomyopathy);

- medication administered for one of the conditions may have undesirable side effects on other co-morbidities.(ex anticoagulation in patients with cirrhosis of the liver may be a cause of bleeding);

- in other situations the concomitant administration of some medications may be accompanied by the reduction or even the elimination of the effect of one of them (ex concomitant administration of clopidogrel and proton pump inhibitors may cancel the antiaggregant effect).

Practical management:

1. GPs and other primary care healthcare providers should take the results of the present study into account in making decisions regarding the care for patients with CVDs and comorbidities. It is very important to know and to identify cardiovascular risk factors and also cardiovascular diseases in an earlier stage to be able to action with appropriate preventive or therapeutic measures.

2. Special guidelines for the management and coordination the care for these patients.Once a patient has a CVD, the healthcare provider should be particularly alert for another CVD, and must closely and carefully monitor these patients for other chronic diseases.

3. In addition, knowledge of the association between CVDs and comorbidities can guide health promotion workers in developing guidelines for the management of these patients in primary care.

4. Implications for health legislators who have to define priorities for future healthcare planning. Prevention, screening, early diagnosis and treatment of comorbid conditions in CVD patients will improve the health outcomes and quality of life for these patients.

5. Finding and choosing therapeutic solutions to avoid drug interactions.

I. 3. 4. CVD and endocrine/metabolic comorbidities.

Cardiovascular abnormalities associated with endocrine disorders are often frequent and due to complex relationships between endocrine glands and cardiovascular system. Endocrine pathology offers a large broadband of multiple interference and implications in cardiovascular pathologies.

The interrelations between endocrine and cardiovascular pathology are important, due to several reasons:

1. The cardiovascular symptoms may mask the endocrine disease; this is the major source of confusion and diagnostic errors.
2. After diagnosis the endocrine disease will request specific treatment.
3. Ignoring the cardiovascular events that may occur in the evolution of endocrine diseases may induce increased mortality due to cardiovascular complications.
4. Endocrine pathology occurred later than a heart disease may worse heart function.
5. There are also a lot of ecg changes caused by hormonal disorders that requires differential diagnosis and represents the source of erroneous diagnosis (Costache et al, 2013).

Electrocardiographic changes in endocrine disorders are quite frequent and may be provoked by: hormone excess (ex hyperthyroidism) or by secondary electrolites disturbances (ex hypokalemia associated with primary or secondary hyperaldosteronism). Sometimes these ecg changes may be related to other cardiac abnormalities such as: arterial hypertension, left ventricular hypertrophy often associated with acromegaly or pheochromocytoma. Ignoring the cardiovascular events that may occur in the evolution of endocrine disease may induce increased mortality due to cardiovascular complications (Costache et al, 2013, Costache et al, 2015).

In the majority of cases these ecg signs disappear together with the treatment of endocrine disease.

One of the most important problems concerning the relationship between cardiovascular pathology and endocrine disorders is represented by amiodarone induced thyroid disfunction.

Amiodarone is a class III antiarrythmic according the Classification of Vaughan and Williams who"s effectiveness in the prophylaxis and treatment of cardiac rhythm disorder is unanimously accepted.

Over time its beneficial effects have been far outweighed by the severe side effects occurred in various organs the most important of them being the fact that it affects the thyroid, the liver,the lungs, the eyes, the kidneys, the skyn and the nervous system.

As chemical structure, the amiodarone molecule contains a benzofuran cycle and a phenyl ring with 2 iodine atoms and 2 aliphatic radicals. Its action is that of blocking at the same time the sodium, potassium and calcium channels. It presents obvious structural similarities with thyroxine, a hormone produced and released by the thyroid gland (Gherasim, 2004, Stiuriuc).

Thyroid effects of amiodarone are due to its pharmacokinetic profile. Amiodarone together with its metabolite has a direct cytotoxic effect on the thyroid follicular cells, the result being a destructive thyroiditis and acts as a competitive antagonist of T3 at the heart level. Amiodarone-induced thyroid damage can take the clinical and biochemical picture of hypo-or hyperthyroidism.

An emergency might be in some cases only amiodarone induced hyperthyroidism which in its turn can be of 2 types:

- type 1 – due to iodine excess in amiodarone which causes excessive synthesis of thyroid hormones; it usually occurs in subjects with prior thyroid damage (subclinical or clinically manifested);

- type 2 – it appears as an inflammatory process followed by destruction in patients with previously normal thyroid.

A destructive thyroiditis appears with the release of hormones preformed from damaged thyroid follicular cells.

This manner of evolution requires an emergency specific therapeutic conduct that involves glucocorticoids.

Combined forms of thyroid damage may rarely appear. From a biochemical point of view amiodarone induced hyperthyroidism is characterized by very low levels of TSH and greatly increased FT4 and decreased FT3 during initial stages. (Daniels 2001, Gherasim 2004).

Causes and risk factors

The risk of developing thyroid damage under amiodarone treatment is independent of the daily or cumulative dose of amiodarone. There are studies that have shown that autoimmune thyroid damage is the main risk factor for developing hypothyroidism. At the same time obesity and family history regarding thyroid disease could be considered predisposing factors.

The presence of antithyroglobulin or thyroid antiperoxidase gives an estimated risk of 13% for developing hypothyroidism in women.

The clinical picture is variable – from the absence of any symptom (subclinical forms) up to forms with dramatic and life threatening clinical manifestations. As ”alarm” clinical signs for thyroid dysfunction which appeared under treatment with amiodarone can be included: signs and symptoms of cardiac decompensation, tachyarrythmias, worsening angina phenomena and unexplained fatigue. Thyrotoxicosis can occur both during treatment with amiodarone but also after several months of its discontinuation (Biondi, 2012, Libo 2012, Stiuriuc).

Laboratory data

Amiodarone induced thyroid damage must be confirmed through laboratory tests that basically consists of specific hormonal determinations.they may be modified even in the absence of a clearly contoured clinical picture (subclinical affecting).

Manifest or subclinical hyperthyroidism particularly the form with undetectable levels of TSH serum is correlated with a substantial increase in cardiovascular mortality (up to 20%). Factors associated with the increase of mortality in these situations are: arrhythmias (atrial fibrillation), embolic stroke (systemic), the aggravation of cardiac insufficiency, hypertension, pre-existing coronary disease. Also, treatment with radioactive iodine may increase cardiovascular mortality. In hyperthyroidism a status of hypercoagulability and hypofibrinolisis were described increasing the risk of thrombotic events (especially brain).

Therapeutic aspects

For patients who associates cardiac damage collaboration between cardiologist and endocrinologist is essential. In the case of thyreotoxicosis making the differences between the two types is mandatory for the choice of treatment. The decision to interrupt or not the treatment with amiodarone, ussualy belongs to the endocrinologist but he will take into account the severity of concomitant cardiac damage which could highly obscure the prognosis (Biondi B 2012, Libo 2012, Wocher 1992).

I.3.4.1. The principal contributions in this field are reflected in the following publications:

1. Costache Irina Iuliana, Maria Cristina Ungureanu, D.Iliescu, A.Petriș, Gina Botnariu: **"Electrocardiographic changes in endocrine disorders",** Rev Med Chir , 2015, 119 (1): 18-22. IF = 0,21.

2. Preda Cristina, Ana Clara Aprotosoaie, A.Petriș, **Irina-Iuliana Costache, "Amiodarone – induced thyroid dysfunction – clinical picture. Study on 215 cases"** Rev Med Chir 2014, 118 (2): 359-363.

3. Costache Irina Iuliana, Voichița Mogoș, Cristina Preda, Carmen Vulpoi, Cristina Ungureanu. **"Therapeutic particularities in amiodarone induced thyroid disorder in patients with underlying cardiac condition"** Rev Med Chir 2014, 118 (4): 944-949.

4. Costache Irina Iuliana, Clara Aprotosoaie, **„Clinical and therapeutic aspects of amiodarone induced thyroid dysfunction**" Rev Med Chir 2013, 117 (2):375 -379.

The **aims of this studies** was:
1) to investigate the prevalence and the clinical-evolutive implications of thyroid damage in patients treated with amiodarone in the Cardiology Clinical from St Spiridon University Hospital of Iași.
2) to analyse the therapeutic approach in patients with basic heart condition and amiodarone-induced thyroid dysfunction in order to establish some correlations with the evolution and prognosis.

Material and methods

The study included a group of 215 patients 90 men (41,86%) and 125 women (58,13%) (fig 3.1.) with ages between 35-87 years, hospitalized in the Cardiology Clinic between 2004-2014 who received amiodarone treatment in most cases for prophylaxia of various arrhythmias both supra- and ventricular. Basic cardiac pathology was represented by arterial hypertension (76 cases – 35,34%), dilatative cardiomyopathy (49 cases 22,79%), acute myocardial infarction (7 cases - 7,44%) and other types of ischaemic heart disease (11 cases – 5,11%), acute viral myocarditis (2 cases – 0,93%) (fig. 3.2.). Amiodarone was administrated for treatment of different rhythm

troubles (supra-ventricular - especially atrial fibrillation and ventricular arrythmias). In 60 patients (27,9%) it was performed electrical cardioversion to convert atrial fibrillation to sinus rhythm and amiodarone was administered pre- and postcardioversion using a standard protocol. In all patients the thyroid function was investigated prior to the administration of amiodarone (TSH, FT3, FT4) levels that were within normal limits. Duration of treatment until the time of discovery the thyroid damage was between 6 month and 8 years. (Costache et al, 2014, Preda et al, 2014).

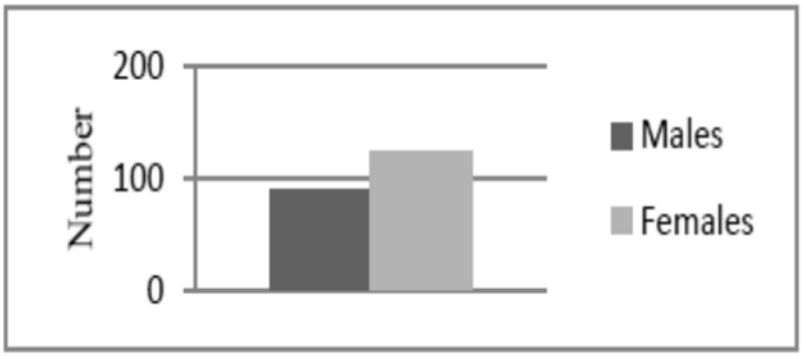

Figure 3.1. Gender distribution of patients

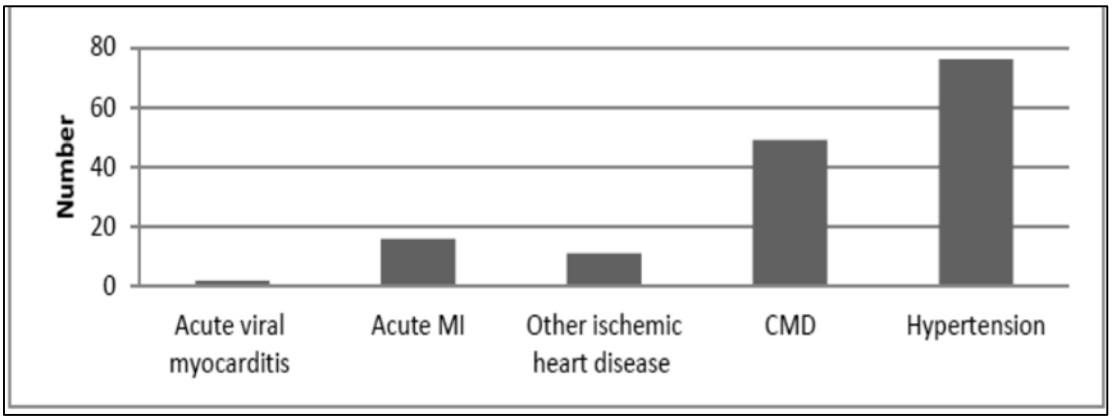

Figure 3.2. Basic cardiac pathology of the study group

Results

In 27,8% of the patients the assessment of the thyroid function was imposed by the appearance of clinical picture characteristic of hypo or hyperthyroidism and for 72,19% it was carried out as a routine examination. Amiodarone –induced hypothyroidism was clinically diagnosed in 20,85% of the patients. Hyperthyroidism occurring during the treatment with Cordarone was found in 6,95% of the patients.the confirmation of the diagnosis of amiodaron –induced thyroid dysfunction was based on hormonal dosages (TSH, fT4,fT3), on the endocrinological clinical examination and on imaging study – ie thyroid echography (fig.3.3.).

Evaluation of thyroid function after starting the treatment occurred in 187 patients (86,97%). Diagnosis of amiodarone induced thyroid disfunction was based on laboratory data.

Amiodarone induced hypothyroidism has been diagnosed clinically in 39 patients (20,85%)and confirmed by elevated levels of TSH and low levels of FT4. Thyrotoxicosis occurred in 13 patients (6,95%). In all cases TSH level was low wuth FT4 normal or increased. In 7 patients with thyrotoxicosis ultrasound examination showed a hypervascularization of thyroid (thyrotoxicosis type 1) and in 6 cases the vascularization was decreased or absent (thyrotoxicosis type B). (Costache et al, 2014, Preda et al, 2014).

All patients with amiodarone induced thyroid dysfunction were monitored initially in hospital for a period depending on the particularities of each case. The treatment was initiated if necessary in the hospital. The first evaluation was performed after one month and at 3, 6, 12 month. In patients receiving Methimazole monthly blood counts were indicated. Also, in patients receiving prednisone the we associateblood glucose and lipid profile were monitored. For patients who confirmed the diagnosis of hypothyroidism we still maintain amiodarone in the treatment plan and we associated Levothyroxine in gradually increasing doses. Evaluation at 3 and 6 month showed normal levels of thyroid hormones and we continued the initial dose of Levothyroxine. All patients maintained normal thyroid function at 12 month under a minimum dose of Levothyroxine. No patient required more than 50 ug/day. (Costache et al, 2014, Preda et al, 2014).

For patients diagnosed with amiodarone induced thyreotoxicosis it was necessary to replace amiodarone with another antiarrythmic drug and to start treatment with Methimazol (doses between 10-40 mg/day) or/plus Prednisone. The evolution of these patients was unpredictable in 25% of cases after a variable period of time (3-6 month) they developed hypothyroidism (TSH greatly increased) which imposes a temporary elimination of antithyroid drugs. After a short period of hypothyroidism (3 month) 3 patients presented thyreotoxicosis relapse which led to the resuming of treatment with antithyroid drugs. Only one patient in this group needed thyroidectomy. Amiodarone induced thyreotoxicosis was more common in male patients which presented an evolution and a very unfavorable prognosis. 2 patients had atrial fibrillation and flutter with rapid ventricular rate resistant to antiarrythmic treatment and another 2 patients died due to worsening heart failure phenomena evolving to cardiogenic shock and refractory acute renal failure. (Costache et al, 2014, Preda et al, 2014).

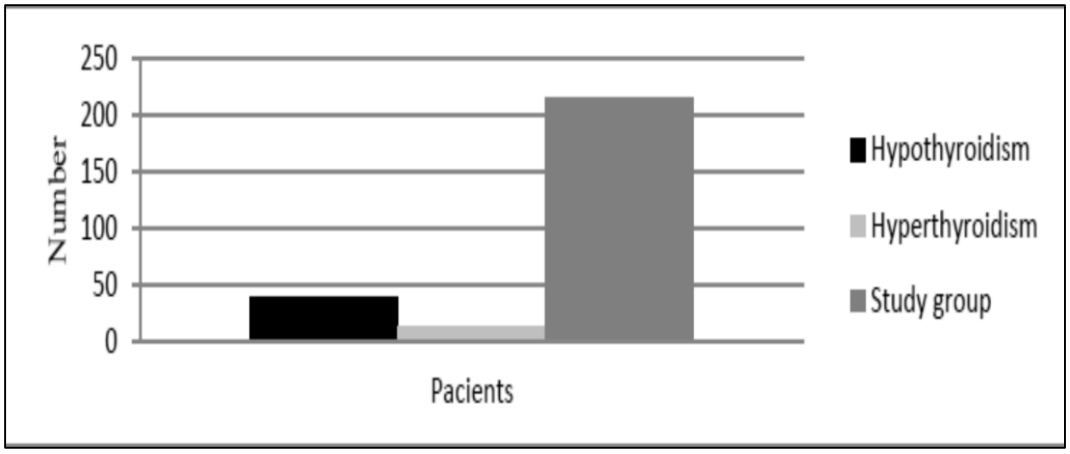

Figure 3.3. Patients with amiodarone thyroid dysfunction

Patients diagnosed with hypothyroidism had a somewhat favorable evolution after hormone replacement. Levothyroxine therapy improved the clinical picture of hypothyroidism in all patients without requiring discontinuation of amiodarone in any of the cases.

The side effects related to the medication in our study group were the following:

- 3 patients treated with Methimazole presented leucopenia;
- 5 patients who received Levothyroxine presented increased heart rate unjustified by any other causes;

in 2 patients who received combination of Methimazole and Prednisone high levels of blood glucose were observed and one patient presented high blood pressure values. (Costache et al, 2014, Preda et al, 2014).

Conclusions

1) Amiodarone-induced thyroid dysfunction is relatively rare compared with the number of patients treated with this antiarrythmic drug (27,8%) from the group under study.

2) Thyroid dysfunction regarless of the type (hypo or hyperthyroidism) represents a negative element in the evolution of the patients with pre-existing cardiovascular diseases not only by aggravating the clinical picture of the basic illness by also by the necessity of permanently reviewing the therapeutic scheme imposed also by the association of thyroid disfunction medication according to the case.

3) Amiodarone induced thyroid dysfunction requires immediate therapy. Our study showed that amiodarone induced hypothyroidism had a better prognosis after hormone replacement therapy and did not require removal af antiarrythmic regimen.

4) Amiodaron induced hyperthyroidism proved to be a severe clinical presentation worsening heart failure by maintaining a high heart rate generally resistant to antiarrythmic drugs. It usually require the removal of amiodarone treatment and antithyroid drugs.in all cases the evolution was slow and unpredictable sometimes being fatal. We consider thyroid dysfunction (either hypo or hyperthyroidism) a negative element in the evolution of the patients with pre-existing heart disease not only by the worsening of the underlying heart disease but also by the need to permanently

review the cardiovascular treatment and the possible association with the appropriate medication for the thyroid dysfunction. It is also necessary to consider the side effects of thyroid dysfunction medication including corticosteroid treatment. (Costache et al, 2014, Preda et al, 2014).

Diabetes mellitus and cardiovascular diseases

Background:

Metabolic syndrome is a frequent association in hypertensive patients because insulin-resistance is the main trigger in the metabolic abnormalities and is related to the lifestyle characteristics. High blood pressure is associated with primary lifestyle risk factors and induces a large range of injuries in cardiovascular and renal system since the earliest stages (NCEP,Adult Treatment Panel III 2001).

Metabolic syndrome is acknowledged as the clustering of risk factors (obesity, insulin resistance, dyslipidaemia and hypertension) associated with the subsequent development of CVD and type 2 Diabetes (McMurray et al 2014).

The metabolic syndrome is a known risk factor for CV morbidity and mortality (NCEP, Adult Treatment Panel III 2001). Moreover, some of the antihypertensive drugs such as betablockers and diuretics have metabolic side effects that could interfere and worsen the preexistent anomalies. Diabetes mellitus (DM) and congestive heart failure (HF) are frequent associated.

The presence of DM in HF patients determines an increased number of adverse events compared with patients without DM. Recent guidelines regarding glycemic control emphasized the importance of the individualization of therapy and targets depending on patients diseases and the risks associated with hypoglycemia.

This balance in establishing therapeutic targets may be particularly relevant in patient with DM and HF.

The concerns about this subject are supported by the following research:

Botnariu Gina, AOPetriș, OR Petriș, Alina Popa, **Irina-Iuliana Costache "Associated factors of ejection fraction in insulin –treated patients with type 2 diabetes".** Rev Med Chir, 2014; 118 (4): 950- 956.

The aim of the study was to evaluate the association between ejection fraction (EF), diabetes characteristics and cardiovascular risk factors.

Material and methods

We carried out a cross sectional study in 171 patients with insulin treated type 2 diabetes hospitalized at St Spiridon Emergency Clinical Hospital Iași. All patients were evaluated for asymptomatic organ damage and CV risk factor of arterial hypertension and diabetes metabolic control. Global EF was evaluated by transthoracic echocardiography. The main characteristics of the patients are illustrated in table 3.1. (Botnariu et al, 2014).

Table 3.1. Background characteristics of the studied patients

	Mean
Age (mean ± SD)	58.40±11.052 y.o
Gender - males (N, %)	84 (49.1)
Smokers (N, %)	21 (12.3)
Diabetes duration (mean ± SD)	9.56±6.52 years
High blood pressure (N, %)	141 (82.5)
Myocardial infarction (N, %)	42 (24.6)
Ejection fraction (mean ± SD)	54.82±12.50
HB A1c % (mean ± SD)	8.6409 ±2.46
LDLc mg/dl (mean ± SD)	156.64±53.99
HDL c mg/dl (mean ± SD)	38.68 ±10.93
Microalbuminuria mg/dl (mean ± SD)	63.43 ± 36.87
Polineuropathie (N,%)	123 (71.9)
Cardiac failure (N. %)	60 (35.1)
HB A1c > 7% (N, %)	126 (73.7)
LDLc > 100 mg/dl (N, %)	135 (78.9)
HipoHDLc (N,%)	147 (87)

Results and discussions

In the studied group the EF had significant negative correlations with the duration of the disease (p=0.007) and the presence of microalbuminuria (p=0.001)(fig 3.4.). There were some differences between the categories realized by grouping the patients according to the presence of hypertension and/or previous myocardial infarction (MI). In patients without personal history of CV disease EF was correlated only with LDLc levels, in the hypertensive patients without MI it was correlated with diabetes duration, HbA1c and LDLc. In those patients with both conditions EF had a significant correlation with HbA1Cc and microalbuminuria (table 3.2., 3.3.). (Botnariu et al, 2014).

Correlation: r = 2617

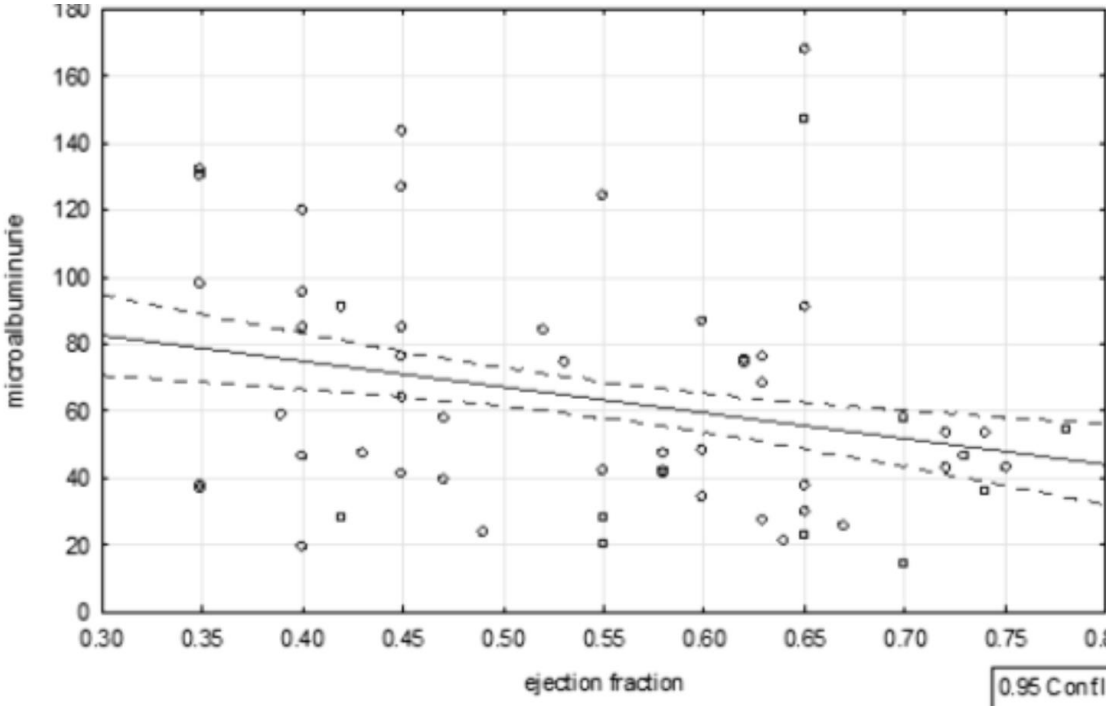

Fig 3.4. Association between ejection fraction and microalbuminuria

Table 3.2. Significant correlations between the ejection fractions and characteristics of diabetes

Ejection fraction		Diabetes duration	Age	Hb A1c	LDLc	HDLc	Micro albuminuria
Total sample	r	**-.207****	-.08	.087	.010	.02	**-.262****
	p.	**.007**	.27	.256	.900	.79	**.001**
Patients without high BP and MI (N=30)	r	-.118	-.05	.200	**-.47****	-.04	-.221
	p.	.533	.78	.288	**.008**	.83	.240
Patients with high BP and without MI (N=99)	r	**-.338****	-.09	**.232***	**-.218***	-.04	-.194
	p.	**.001**	.36	**.021**	**.030**	.63	.054
Patients with high BP and with MI (N=42)	r	-.110	.24	**-.467****	.260	.27	**-.392***
	p.	.487	.11	**.002**	.096	.07	**.010**

Table 3.3. Multiple linear regression coefficients and associated p-values for ejection and specific characteristics.

		Standardized Coefficients	p.	95% Confidence Interval for B	
		Beta		Lower Bound	Upper Bound
Total sample					
1	(Constant)		.000	.568	.641
	microalbuminuria	-.262	.001	-.001	.000
2	(Constant)		.000	.598	.687
	microalbuminuria	-.262	.000	-.001	.000
	diabetes duration	-.207	.005	-.007	-.001
Patients without high BP and MI (N=30)					
1	(Constant)		.000	.170	.533
	LDL	.476	.008	.000	.003
Patients with high BP and with MI (N=42)					
1	(Constant)		.000	.552	.629
	microalbuminuria	-.256	.002	-.001	.000
2	(Constant)		.000	.573	.673
	microalbuminuria	-.257	.002	-.001	.000
	diabetes duration	-.163	.047	-.006	.000
Patients with high BP and without MI (N=99)					
1	(Constant)		.000	.693	1.155
	Hb A1c	-.467	.002	-.064	-.016
2	(Constant)		.000	.680	1.083
	Hb A1c	-.641	.000	-.077	-.032
	LDL	.488	.001	.001	.002

Conclusions. These results emphasized that there would be some differences between determinants associated with heart failure in type 2 insulin-treated diabetics according to the presence of high blood pressure and myocardial infarction (Botnariu et al, 2014).

I.3.5. Cardiovascular and hepatic comorbidities.

It has been known for many years that the heart and the liver are intimately related. There is a close connection and interaction between the function of the heart and the liver and a large spectrum of acute and chronic conditions that affect both the heart and the liver.
These can be classified into:

a. heart diseases affecting the liver,

b. liver diseases affecting the heart, (including here hepatopulmonary syndrome and portopulmonary arterial hypertension) and

c. conditions affecting the heart and the liver at the same time caused by common etiological factors.

These entities also add:

d. **coagulation changes** to the cirrhotic patient with impact on cardiovascular medication and

e. **drug interference** (especially in patients with viral hepatitis C following interferon free treatment or drug"induced hepatotoxicity or side effects of cardiovascular medication).

a. Heart diseases affecting the liver

The fundamental mechanisms underlying cardiac hepatopathy are: reduced arterial perfusion (associated with left sided heart failure) whose deleterious effects are amplified by concomitant hypoxia and passive congestion secondary to increased systemic venous pressure (associated with right sided heart failure). Arterial hypoperfusion predominates in acute heart failure leading to hypoxic hepatitis, while passive congestion prevails in congestive hepatopathy secondary to chronic heart failure. However, these factors often coexist and potentiate each other. Hepatic steatosis, which is frequent in patients with cardiac hepatopathy because of comorbid conditions such as diabetes, obesity, and hyperlipidaemia, increases liver susceptibility to the ischaemia/reperfusion injury (Møller et al 2013). Cardiac hepatopathy is associated with systemic haemodynamic changes that accompany heart failure, including increased right atrial and inferior caval venous pressures. Prothrombin time international normalized ratio (INR) greater than two is considered an independent risk factor for all-cause mortality (Møller et al 2013).

b. Liver diseases affecting the heart

The hyperdynamic circulation in patients with cirrhosis is a well-known fact. In patients with advanced cirrhosis, the cardiac performance is reduced with systolic and diastolic dysfunction and electrophysical abnormalities named **cirrhotic cardiomyopathy.** Electrophysiological abnormalities include prolonged QT interval, chronotropic incompetence, and electromechanical uncoupling (Møller et al, 2013). Systolic dysfunction is estimated by ejection fraction and it may be implicated in the subsequent development of renal failure in advanced stages of the disease. Diastolic dysfunction in cirrhosis may reflect ventricular hypertrophy, altered collagen structure, and it seems related to prognosis. The electrocardiographic QT interval (QT) interval is prolonged

in about half of the cirrhotic patients and may be related to different factors including electrolyte disturbances.

Despite the characteristic high cardiac output, systolic dysfunction is included in the working definition of the cirrhotic cardiomyopathy and relates to the inability of the heart to meet its demands with respect to generation of an adequate arterial blood pressure.

Liver diseases frequent associated with cardiovascular diseases

Nonalcoholic Fatty Liver Disease (NAFLD) is a very common cause of chronic liver disease. A strong association between NAFLD and cardiovascular disease has been long suspected and recent studies have confirmed that cardiovascular disease is the single most important cause of mortality in these patient population. There is a suggestion that NAFLD may rise cardiovascular risk that is conferred by traditional cardiovascular risk factors (e.g., dyslipidemia, diabetes and smoking) (Musso et al, 2010, Corey et al 2012, Vuppalanchi et al 2009). Health care providers managing patients with NAFLD should recognize this increased cardiovascular risk and should undertake early aggressive risk factor modification (Misra et al 2009). The majority of patients with NAFLD exhibit features of metabolic syndrome. Therefore, clinicians must have a high index of suspicion and actively screen for metabolic syndrome.

Dyslipidemia is an important risk factor for CVD and is highly prevalent in NAFLD patients. It is characterized by elevated low-density lipoprotein (LDL) levels as well as atherogenic dyslipidemia, which consists of hypertriglyceridemia, low HDL levels, and elevated small, dense LDL levels. There has long been a reluctance in starting treatment with statins in patients with liver disease because of hepatotoxicity. However, the incidence of serious hepatotoxicity with statins in patients with NAFLD is exceedingly low, and statins are safely used in patients with liver disease.(Vuppalanchi et al 2009) Although the role of statins in the treatment of NAFLD itself remains unclear, the role of statins in the primary and secondary prevention of CVD is well established.

Statins are the initial treatment of choice for NAFLD patients. However, patients with NAFLD may demonstrate persistent atherogenic dyslipidemia, which may require additional treatment. Omega 3 fatty acids are an attractive option for NAFLD patients because of their minimal side-effect profile. Early data suggest that omega 3 fatty acids may also improve steatohepatitis and clinical trials are ongoing. Niacin and fibrates can be used for the treatment of isolated hypertriglyceridemia and can be added to statin therapy. Although NAFLD is associated with an increased risk of CVD, the target lipid goals for patients with NAFLD are not well established. Is recommended the Adult Treatment Panel III guidelines of the National Cholesterol Education Program for target LDL and atherogenic dyslipidemia goals (Table 3.4.).

Tabel 3.4. Treatment targtes for patients with NAFLD (adapted after Adult Treatment Panel III guidelines of the National Cholesterol Education Program)

Patient risk factor	Treatment target	Recommended treatment

CVD or risk equivalents*	LDL < 100 mg/dl	Lifestyle intervention and statin initiation
≥ 2 CV risk factors **	LDL < 130 mg/dl	Lifestyle intervention and statin initiation
≤ 1 CV risk factor	LDL < 160 mg/dl	Lifestyle intervention and if needed statin initiation
Atherogenic dyslipidemia	HDL > 40 mg/dl and triglyceride < 150 mg/dl	Omega 3 fatty acids, nicotinic acid and fibrates
Hypertension	<140/90 mmHg (< 130/80 mmHg for diabetes or renal diseases)	Angiotensin converting enzyme inhibitors/sartans

- risk equivalents include: peripheral vascular disease, carotid artery disease, diabetes mellitus and abdominal aortic aneurysms.

** Cardiovascular risk factors include: tobacco use, a family history of premature heart disease, hypertension and low HDL levels.

Nondiabetic NAFLD patients often have underlying insulin resistance, impaired fasting glucose, or impaired glucose tolerance, which may contribute to the increased risk of CVD. **Diabetes mellitus** is frequently associated with NAFLD.

Arterial Hypertension should be treated to prevent the development of CVD in NAFLD patients. Both sartans and angiotensin-converting enzyme inhibitors have been shown in experimental model to reduce fibrosis progression liver disease. Although these results have not been confirmed in randomized controlled trials, if a patient requires treatment for hypertension, these agents are preferentially recommended (Musso et al, 2010).

Patients with NAFLD diagnosed with CVD should be managed similarly to those without liver disease. The presence of NAFLD should not contraindicate any necessary procedures, including cardiac catheterization and coronary artery bypass surgery. The presence of cirrhosis impose an evaluation of the functional status and the Child class by a hepatologist, but for patients without end-stage liver disease, no further evaluation is needed before cardiac procedures. Patients with CVD are often prescribed antiplatelet agents, including aspirin and clopidogrel. In patients with cirrhosis and known varices, a risk-benefit analysis should be undertaken between hepatologist and cardiologist, but the use of such agents is generally safe, and secondary measures can be taken to reduce the risk of gastrointestinal bleeding (Musso et al, 2010).

Portopulmonary hypertension (HTPP) and hepatopulmonary syndrome (HPS) are pulmonary vascular changes associated with cirrhosis.

Hepatopulmonary syndrome is defined by the triad:

• the presence of liver disease;

• impairing pulmonary gas changes (PaO2 <80 mm Hg or P (A-a)> 15 mm Hg in atmospheric air);

• objectifying of pulmonary vasodilatation (by contrast echocardiography) (Droc G, , Rodriguez-Roisin et al 2008, Raval Z et al, 2011).

The degree of severity is assessed in terms of impaired oxygenation and it is very important to assess the survival and risks associated with liver transplantation (Naschitz et al 2000, Droc, Ripoll et al 2011, Swanson et al, 2008, Rodriguez-Roisin et al 2008, Raval et al, 2011).

Pathogenesis is still unclear and several mechanisms are incriminated: nitric oxide (NO) causing excessive pulmonary vasodilatation, ventilation-perfusion (V / Q) disorders, intrapulmonary arteriovenous shunts, and oxygen diffusion limitation. The mean survival in patients with hepatic cirrhosis and hepatopulmonary syndrome is less than 12 months. Severe form is the best predictor of perioperative mortality. This condition is reversible and therefore has an indication of emergency liver transplantation. Patients with PaO2 <60 mmHg in athmospheric air, in the absence of other lung disease, may be included on the list of transplant priorities over the next three months (Rugina et al, 2012).

Portopulmonary hypertension (HTPP) is a form of hepatic pulmonary hypertension characterized by increased pulmonary vascular resistance (RVP) as a consequence of vasoconstriction and remodeling in the pulmonary vasculature. By definition, cirrhotic patients develop in their evolution porto-pulmonary hypertension (Droc, Ripoll et al 2011, Swanson et al, 2008, Rodriguez-Roisin et al 2008, Raval et al, 2011, Simonneau, 2009). Prevalence is 2-14% of patients with cirrhosis of the liver.

Diagnosis is done by catheterization of the right heart cavities and determination of cardiac output (DC) and pulmonary vascular resistance (RVP).

HTPP is defined as the increase in pulmonary artery average pressure (mPAP) above 25 mmHg, associated with a value less than 15 mmHg of pulsed capillary pressure (PCWP) and a pulmonary circulation resistance (RVP) above 120 dynes / s / cm -5, in patients with liver cirrhosis and portal hypertension (Droc, Raval et al, 2011, Simonneau, 2009).

A mPAP value between 35 and 50 mmHg in the preoperative period is associated with an increase in mortality by 50% after liver transplantation (Simonneau, 2009).

Another study reports a 100% mortality in patients with mPAP greater than 50 mmHg (Swanson, 2008). Patients with moderate and severe forms have increased postoperative mortality. For this reason, liver transplantation is indicated in these situations only to those responding to vasodilatory drug therapy, the aim of which is to decrease mPAP below 35mmHg and RVP below 400 dynes / s / cm-5 (Rugina et al, 2012).

In the transplanted patient, pulmonary hypertension is resolved within 4-6 months, at which point medication may be discontinued. In severe form, mortality is 42% at 9 months and 71% at 36 months (Droc, Raval et al, 2011, Simonneau 2009, Swanson et al, 2008).

Cardiovascular Comorbidities in cirrhotic patients

Cirrhosis patients' comorbidities are represented by other diseases than cirrhosis (Jepsen 2014, Ording et al 2013, Feinstein1970). Comorbidities increase mortality and are therefore clinically relevant (Jepsen et al, 2014, 2008). The presence of comorbidity may also be an important source of diagnosis errors and also therapeutic difficulties and should be accounted for in epidemiologic studies of cirrhotic patients.

Comorbidities must be distinguished from complications such as ascites, variceal bleeding, and hepatic encephalopathy. Complications are at least to some extent a consequence of the portal hypertension and loss of liver function resulting from cirrhosis, whereas comorbidities are neither causes nor consequences of cirrhosis (Ording et al 2013).

Studies of individual comorbidities' effect on the clinical course of cirrhosis can provide insight into the pathophysiology of cirrhosis.

Comorbidity scoring systems have been developed as tools to measure the cirrhosis patient's total burden of comorbidity, and they are useful in the clinic and for epidemiologic studies.

A comorbidity scoring system should reflect the combined effects of all a patient's comorbidities. This might be complex, but for purposes of mortality prediction it appears that there is no need to consider more than two diseases for each patient (Jepsen et al, 2014).

Two comorbidity scores have been validated as predictors of mortality among cirrhosis patients: The Charlson comorbidity index and the CirCom score (Jepsen P et al, 2014, 2008). The Charlson comorbidity index and a modified version thereof, the CCI-OLT, have also been shown to predict mortality among liver transplant recipients (Volk et al 2007, Grosso et al 2012).

The recently developed **CirCom score** is the only comorbidity scoring system developed specifically for cirrhosis patients, and it may be preferred over the older, generic, and more complex than previous indexes. **The CirCom score** is based on nine diseases of which at most two count towards a patient's CirCom score and it was slightly better at predicting mortality: chronic obstructive pulmonary disease, acute myocardial infarction, peripheral artery disease, epilepsy, substance abuse other than alchohol, heart failure, cancer, chronic kidney disease etc.

Cardiovascular diseases and cirrhosis

The hyperdynamic circulation in cirrhosis provides some protection against atherosclerosis, ischemic events, and overt heart failure but acute myocardial infarction, peripheral arterial disease, and heart failure were all strong predictors of mortality in the CirCom study. Other cardiovascular diseases were weaker predictors (Jepsen et al 2014]. Coronary disease, defined by acute myocardial infarction or coronary disease on angiography, was also a predictor of mortality among liver transplant recipients (Volk et al 2007). The reasons for these associations are unclear. From the risk factors, smoking has also been identified as an adverse prognostic factor in patients with cirrhosis (Pessione et al 2003), but this association is unexplained, too (Altamirano et al 2010).

Venous thromboembolism

In the CirCom cohort, venous thromboembolism manifested as deep venous thrombosis or pulmonary embolism increased mortality 1.20-fold after adjustment for gender and age (Jepsen et al 2014). Coagulation in liver disease is complex (Northup et al 2006), and it remains unclear whether venous thromboembolism is a marker of severe liver function loss.

Several studies reported a incidence of non-portal VTE in patients with chronic liver disease in a range between 0.5% and 6.3%. (Northup et al 2006, García-Fuster et al 2008, Dabbagh et al 2008)

Two retrospective studies by Northup et al.(2006) and Garcia- Fuster et al. (2008), showed that the risk of developing VTE increases in patients with low serum albumin, but is independent from

elevated INR or low platelet count. Serum albumin is a marker of liver synthetic function, and hence, indirectly, of the imbalance of serum levels of anticoagulant factors as antithrombin III, protein C and protein S: this explains the increased risk of VTE in these patients. A retrospective study by Dabbagh et al.(2010) confirmed that an elevated INR (even >2.2) does not protect patients with chronic liver disease against the risk of VTE. Other studies showed that patients with cirrhosis are not naturally auto-anticoagulated, as initially thought, but also they seem to have an increased relative risk of venous thromboembolism (both deep vein thrombosis and pulmonary embolism) compared with controls. (Dabbagh et al 2010, Gulley et al 2008, Søgaard et al 2009)

Wu et al. (2010) assessed the incidence of VTE in patients with compensated or decompensated cirrhosis and a random sample of control patients. Interestingly, results indicate that patients younger than 45 years have an increased risk of VTE independently from being compensated or decompensated. After 45 years of age, patients with compensated cirrhosis had a lower risk of VTE, while those with decompensated disease had a similar risk compared with controls. These results may be explained with age-related factors not correlated with liver disease; in younger patients, who normally have low baseline risk of VTE, presence of cirrhosis imply an increased risk of VTE compared with noncirrhotic patients, whereas in older patients extrahepatic factors seem to outweigh liver-related risk factors.

Portal vein thrombosis (PVT), the obstruction of portal vein and/or tributaries, is a relatively frequent event in cirrhotic patients; its incidence varying from 7.4% to 17.9% in different studies. (Francoz et al 2005, Villa et al 2012, Abdel-Razik et al 2015) The development of PVT in cirrhosis is directly proportional to disease severity, being more frequent in individuals with decompensated cirrhosis. A lot of situations are known risk factors for PVT in patients with cirrhosis: endoscopic treatment of varices, abdominal surgery, injury to the portal venous system, and paradoxically also low platelets count, as well as bacterial infections. All these events are able to trigger the intrinsic pathway of coagulation leading to blood clot formation. The pathogenesis of PVT includes both "systemic factors", (coagulation abnormalities or presence of antiphospholipid antibodies), and "local factors", as peri-portal lymphangitis and fibrosis that lead to alteration of liver cytoarchitecture with consequent flow reduction and endothelial activation. (Leonardi et al 2017)

Clinical presentation of PVT varies from asymptomatic to a medical emergency, depending on the extension and the rapidity of thrombus formation: "acute PVT" may be characterized by intestinal congestion and ischemia to peritonitis, shock and death. "Chronic PVT" may be asymptomatic, but when discovered, the presence of varices and ascites should be investigated. Ultrasonography and Doppler ultrasound are usually first-line imaging method for the diagnosis of PVT, with a sensitivity and specificity variable from 66% to 100%.21 Computed Tomography and Magnetic Resonance Imaging are however better for determining presence and extent of thrombosis, with a sensitivity and specificity near 100% (Leonardi et al 2017).

Studies of individual comorbidities can provide insight into the interactions between cirrhosis and other diseases. (Jepsen et al, 2014).

Diabetes is the best studied comorbidity to cirrhosis. Among the 12976 Danish cirrhosis patients included in the CirCom study, diabetes without complications was unassociated with mortality whereas diabetes with complications did increase mortality (Jepsen et al 2014). A study from the Netherlands including 226 patients diagnosed with cirrhosis in 2001-2011 found that diabetes was unassociated with all-cause and liver-related mortality (Wlazlo et al 2013), and a smaller Mexican study found that the reduced survival for cirrhosis patients with diabetes was due to confounding by cirrhosis severity and renal impairment (Quintana et al 2011). Earlier studies have been reviewed by Garcia-Compean et al (2009) who concluded that diabetes mellitus does increase mortality in cirrhosis, and that the excess mortality in diabetes patients is due to hepatocellular failure, not to diabetes (Bianchi et al 1994). More detailed studies are needed to clarify the interactions between cirrhosis and diabetes.

c. Conditions affecting the heart and the liver at the same time

Numerous conditions affect both the heart and the liver such as infections, inflammatory and systemic diseases, and chronic alcoholism. The risk and prevalence of coronary artery disease are increasing in cirrhotic patients and since the perioperative mortality is high, a careful cardiac evaluation of such patients is required prior to orthotopic liver transplantation

d. **Coagulation changes** to the cirrhotic patient.

Another important phenomenon to be considered in the patient associating concomitant cardiovascular and hepatic impairment is represented by **coagulation disorders** characteristic of severe hepatic impairment.

The liver plays a crucial role in coagulation cascade. Global hemostatic process is profoundly influenced by the presence of liver disease and its complications. Patients with cirrhosis have impaired synthesis of most of the factors involved in coagulation and fibrinolysis process due to a reduced liver function and altered platelet count secondary to portal hypertension. Altered routine tests and thrombocytopenia were considered in the past as associated with increased risk of bleeding. These concepts explain why these patients were considered "auto-anticoagulated". New recent evidences show that patients with liver cirrhosis have a more complex hemostatic alteration. Despite the presence of altered levels of factors involved in primary hemostasis, coagulation and fibrinolysis, patients with stable cirrhosis have a **rebalanced hemostatic**, which however can easily be altered by decompensation or infection, both in hemorrhagic or thrombotic direction. Patients with cirrhosis have an increased risk of venous thrombotic events (namely portal vein thrombosis) while bleeding seems to be related to the grade of portal hypertension rather than to a hemostatic imbalance. The use of anticoagulants both as treatment or prophylaxis is safe, reduces the rate of portal vein thrombosis and decompensation, and improves survival. Standard laboratory coagulation tests are unable to predict bleeding and are inadequate for the assessment of hemostatic status in these patients, hence more comprehensive tests are required to guide the management of thrombotic and bleeding complications (Jepsen 2014).

Anticoagulation in cirrhosis

No guidelines reported the management of PVT in patients with cirrhosis. Also, there are no definitive consensus publications for prevention, treatment and monitoring of PVT in these patients.

Anticoagulation is usually the first-line therapy, allowing a good chance of complete recanalization, a reduced incidence of portal hypertension complications and a decreased rate of thrombosis progression. (Leonardi et al 2017). Several studies are available indicating that anticoagulation, either through low molecular weight heparins (LMWH) or vitamin K antagonists' administration, is both safe and effective in treating PVT (Leonardi et al 2017). Importantly, increased hemorrhagic events are not reported, and chronic administration of LMWH does not affect the outcome of gastro-intestinal bleeding following varices rupture: in a retrospective multicentric trial published in 2015, anticoagulation treatment for PVT or cardiovascular disorders did not increase neither the rate of treatment failure following varices rupture nor the 6 months mortality rate (Cerini et al 2015). Anticoagulation should be initiated with subcutaneous LMWH, which is as effective as intravenous heparin, does not require laboratory monitoring, has a more predictable dose response relationship, and has a lower risk of adverse events (i.e., heparin-induced thrombocytopenia). Lifelong anticoagulation should be decided on an individualized case-to-case basis. It is advisable, before starting anticoagulation, to evaluate the presence of varices, and, in case of large varices or previous history of gastro-intestinal bleeding, start prophylaxis, either with beta-blockers or endoscopic band ligation. Transjugular Intrahepatic Porto-systemic Shunt (TIPS) results a valid alternative when anticoagulation fails, with a reported complete recanalization of portal venous system in 57% of patients with cirrhosis, and a decrease in thrombosis in 30% of patients, but with the limitation that it could be considered only in experienced centers. (Luca et al, 2011, Leonardi et al 2017).

Prophylaxis of PVT

Data from a prospective randomized study (Leonardi et al 2017, Villa et al 2012) showed that anticoagulation not only prevented the occurrence of portal vein thrombosis but, most importantly, decreased the occurrence of decompensation and improved survival. Two other studies with anticoagulants and with the same endpoints are currently ongoing (Childbenox, NCT02271295, and Cirroxaban, NCT02643212).

e. Drug interferences. Virus C (HCV) infected patients often take multiple co-medications to treat other co-morbidities. Drug–drug interactions associated with this polypharmacy are related especially to the inhibition of the cytochrome P450 (CYP) 3A iso-enzyme so, knowledge and awareness of drug–drug interactions have become a cornerstone in the evaluation of patients starting and continuing HCV combination therapy. An overview of conducted drug–drug interaction studies and a list of contraindicated medications are essential for the clinical management of those patients. Knowledge of pharmacokinetic profiles is also beneficial and

providing information how to manage these interactions (e.g., dose adjustments, safe alternatives and therapeutic drug monitoring) (Burger et al, 2013).

I.3.5.1. The concerns about this subject are supported by the following researches:

ISI

1. Costache Irina Iuliana, Irina Garleanu, O. Mitu, Adriana Ion, Amalia Darie, Razan Al Namat*, Radu Stefan Miftode, Dan Alexandru Costache*, Dan Iliescu. **Correlations Between Biochemical Profile and Echocardiographic Parameters in Patients with Cirrhosis of the Liver Without Previous Cardiovascular Abnormalities.** Rev Chim 2018; 69 (8): 2213-2216. **IF= 1.412.**

BDI

2. Costache Irina Iuliana, Adriana Ion, O. Mitu, Amalia Darie*,Irina Gîrleanu, R. Miftode, A. D. Costache, A. O. Petriș, **"The therapeutic approach to deep venous thrombosis in the patient with cirrhosis of the liver",** Rev. Med. Chir. 2018; 122 (3): 438-443.

3. Miftode RS, Larisa Miftode*, A. Vata, Anca Trifan, **Irina Costache,** S. Toader, M. Hurmuzache, Claudia Plesca, Egidia Miftode: **"Impact of hepatic steatosis on disease course in patients with compensated hepatitis c virus-related cirrhosis receiving interferon-free therapy (paritaprevir, ritonavir, ombitasvir dasabuvir and ribavirina)".** Rev. Med. Chir. 2018; 122 (1): 51-58.

The aim of this studies was:

1) to investigate echocardiographic parameters: left ventricle dimensions, wall thickness (left ventricle posterior wall thickness + interventricular septum thickness) and also diastolic function (E wave, A wave, E/A ratio, deceleration time of E wave) and systolic function (ejection fraction) by using transthoracic echocardiography along with biochemical variables in patients previously diagnosed with end-stage liver disease in order to establish a possible correlation between these parameters and biochemical data.

2) to determine the frequency of porto-pulmonary hypertension (POPH) in patients with end-stage liver cirrhosis, explore and analyze the biochemical profile of each patient, in order to identify some correlations between biological factors, the severity of the disease and porto-pulmonary hypertension.

3) to appreciate the incidence and the best therapeutic approach for deep venous thrombosis associated with cirrhosis of the liver starting from the existing data in the literature.
4) to evaluate the impact of advanced steatosis grade on virological, hematological and biochemical parameters in patients with HCV cirrhosis treated with Ombitasvir + Paritaprevir + Ritonavir + Dasabuvir and Ribavirin. The need for such an assessment is justified by the existence of regional particularities regarding comorbidities, diet, age at the time of treatment, previous use of an antiviral drug and the response to it.
5) to evaluate the comorbidities associated in patients with viral C hepatitis who will be treated with interferon free medication.

Material and methods:

One of the study included 60 cirrhotic patients admitted in the Institute of Gastroenterology and Hepatology and the Cardiology Department of "St. Spiridon Emergency Hospital" Iasi from June 1, 2016 to June 1, 2018, for liver transplant evaluation. The only inclusion criteria was the presence of liver cirrhosis. Patients with other conditions which could lead to portal hypertension (non-cirrhotic portal vein thrombosis, Budd Chiari syndrome), patients with any pre-existing cardiovascular abnormalities or other cause of pulmonary hypertension (such as chronic obstructive pulmonary disease, left cardiac disease, atrial fibrillation, pulmonary embolism, idiopathic) were excluded from the study.
The study was conducted as a cross-sectional analysis for a period of two years. A written informed consent was obtained from all the patients. The past medical history, clinical examination and laboratory details were obtained from the medical file.
Echocardiography was the most commonly used modality for assessing systolic and diastolic function of left ventricle in cirrhotic patients (Benjaminov et al, 2003, Wong 1999). Measurements were made in M mode. The following parameters were determined: diastolic diameter (Dd), systolic diameter (Ds), thickness of the walls, left ventricle volume using the formula: end diastolic volume (EDV) = Dd3, end systolic volume (ESV) = Ds3. Ejection fraction (EF) was the most widely used parameter of global left ventricular systolic function. It was calculated using end-systolic and enddiastolic volumes, by the formula: $EF = (Dd^3 – Ds^3) \times 100 / Dd^3$ and we considered normal value EF = 60-80%. Shortening fraction (SF) was also appreciated using a formula: (Dd-Ds) x 100/Dd; normal range between 20- 40%. The visual estimation of the EF (eye balling) was used in parallel with the determinations using the diameters without significant differences (Costache et al, 2018).
Diastolic function was appreciated in our study in 2D Echo (dimensions and function of left atrium -LA and left ventricle -LV), in M Mode (dimensions of LA, LV, LV – Wall thickness, the pattern of the interventricular septum (IVS) movement). Non-invasive assessment of DD was classically based on the echocardiographic analysis of mitral inflow pattern using pulsed-wave Doppler. In the presence of DD, early diastolic filling is decreased as a consequence of delayed LV relaxation and atrial contraction becomes a more important contributor to left ventricular filling. This

impaired relaxation pattern is characterized by a decrease in E wave velocity, prolongation of E-wave deceleration time, and an increase in A wave velocity resulting in an inverted E/A ratio (< 1) (Valeriano et al 2000, Ceolotto et al 2008, Dorosz et al 2012, Finucci et al 1996, Torregrosa et al 2005).

2) Diagnosis of POPH

Pulmonary vascular resistance is an essential parameter of the pulmonary hypertension. This is measured traditionally by right heart catheterization (Wong et al 1999). However, the bleeding complications are high among cirrhotic patients. Doppler echocardiography is an accepted method to evaluate the pulmonary pressure with comparable results. For cirrhotic patients undergoing liver transplantation, it decreases the requirement for repeated invasive measurements (Gassanov et al 2010). In our study, pulmonary pressure was measured by the peak systolic velocity of the tricuspid regurgitation flow and the acceleration time of the pulmonary systolic flow. All cardiac cavities and volumes were measured according to the current guidelines. All echocardiographic measurements were repeated three times. The patients were considered to have pulmonary hypertension by a sPAP (pulmonary artery systolic pressure) > 40 mmHg. Mean PAP was calculated from sPAP (mPAP = 0.61 sPAP + 2mmHg). (Costache et al, 2018).

Abdominal ultrasound abdomen and upper gastrointestinal endoscopy were performed in all patients.

The diagnosis of liver cirrhosis was established based on clinical manifestations and biological, endoscopical, and ultrasound changes suggestive for advanced liver disease and portal hypertension. LC severity was evaluated using the MELD score and Child–Pugh class.

Biochemical investigations included complete blood count, serum bilirubin, albumin, total protein, prothrombin time/international normalized ratio, liver enzymes (alkaline phosphatase, aspartate transaminase, alanine transaminase, gamma glutamyl transpeptidase), lipid panel, renal function, inflammatory marks such as C reactive protein and fibrinogen. Child – Pugh and MELD scores were calculated in order to appreciate the severity of cirrhosis (Alexopoulou et al 2012).

3) Related to the incidence of deep venous thrombosis in patients with cirrhosis of the liver, starting from the existing data in the literature so far, we studied during the 2014-2018 period, 8 male patients previously known with cirrhosis were hospitalized with deep vein thrombosis (DVT) in the lower limbs in the Cardiology Clinic. The diagnosis of liver cirrhosis had been previously confirmed and justified by the presence of medical records at admission, and the diagnosis of DVT was based on clinical symptoms of DVT (unilateral swollen or painful leg) and confirmed using Duplex Doppler ultrasonography criteria for DVT. They are the following: no flow signal, direct clot visualization, the absence of spontaneous flow, and the absence of respiration-modulated phasicity of the evaluated veins. Valvular competence was assessed using the Valsalva maneuver. (Costache et al, 2018).

4) This retrospective study included 113 patients with compensated liver cirrhosis with C genotype 1b treated with interferon-free therapy (PR-OD R) evaluated and clinically-biological monitored

at the Iași Hospital of Infectious Diseases between November 2015-May 2017. The patients were assessed from cardiovascular point of view in order to appreciate the eventual drug interactions or contraindications to interferon free treatment. (Miftode et al, 2018).

Statistical analysis

Statistical analysis was carried out using the SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables are expressed as mean ± SD, and categorical variables as percentages. Biochemical parameters in cirrhotic patients with and without POPH were compared. Quantitative variables with normal distribution were compared using the Student's t test. For non-normal data, we have benefited from nonparametric methods like Mann-Whitney test. To check the normality of the data distributions the Kolmogorov-Smirnov test was used. Chi square test (Fisher exact test for small samples) for categorical data was exploited. Spearman's correlation coefficient was used to investigate possible correlations between the POPH and different clinical and biological parameters. A two-tailed P-value ≤ 0.05 was considered to indicate a statistically significant difference.

Results

In the first study we included 60 cirrhotic patients evaluated for liver transplant, mean age 50.55±8.3 years (range 26-63 years), most of them males 83.3%. The first three etiologies of LC were viral B infection (45%), viral C infection (26.7%) and alcoholic (16.7%). All the patients diagnosed with alcoholic LC had more than 6 months of alcohol abstinence. The majority of the patients were classified as Child-Pugh class B (43.3%), with a mean MELD score of 16.9±5.5.

Left ventricular hypertrophy was found during the echocardiographical examination in 15 patients with a moderate degree (left ventricular posterior wall between 12-13 mm and interventricular septum between 12-14 mm). Echocardiographic changes of the left ventricular hypertrophy were correlated with electrocardiographic changes in only 10 patients. In most cases the electrocardiogram had a normal morphology. Only 4 patients with alcoholic cirrhosis showed ectopic ventricular beats on the electrocardiogram and left bundle branch block was present in 2 male patients who associated dilated cardiomyopathy having the same alcoholic etiology.

In our study, diastolic dysfunction was found in 15 patients (36.58%). Of these 15, 7 were CPS Grade B and 6 were Grade C. In our study patients with diastolic dysfunction had more diminished values of serum albumin ($p = 0.156$) (fig. 3.5.) and diastolic dysfunction was not significantly different depending on the Child class ($p = 0.637$). (Costache et al, 2018).

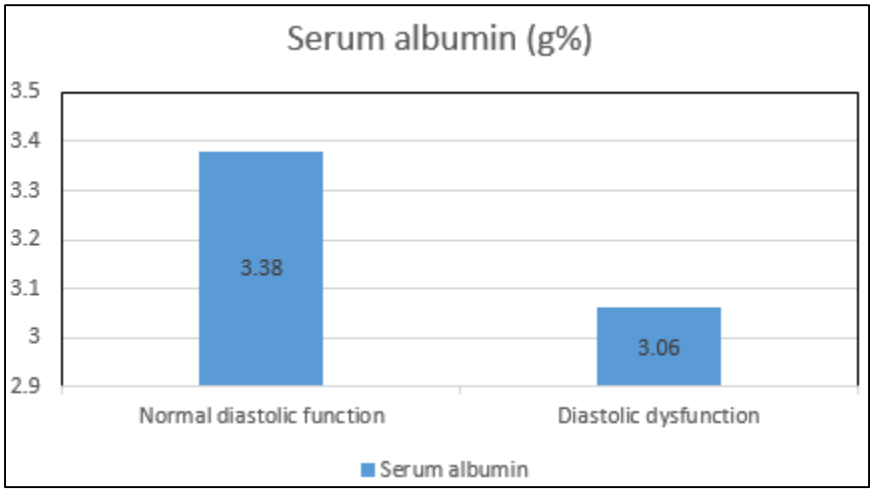

Fig. 3.5. Diastolic function difference according to serum albumin

Table 3.5. Descriptive data of the study population

Parameter	Value
Age (years)	51.20±8.02
MELD Score	17.58±5.71
AST (U/L)	84.39±60.43
ALT (U/L)	59.86±55.00
GGT (U/L)	84.05±70.07
Total bilirubin (mg/dl)	3.43±3.58
Total cholesterol (mg/dl)	127.63±38.05
Seric albumin (g/l)	3.23±0.70
Urea (mg/dl)	54.07±48.78
Creatinine (mg/dl)	1.02±0.62
EFLV (%)	62.93±5.35

Depending on the etiology, only the MELD score was significantly different ($p = 0.006$), while the other parameters did not reach the statistical significance. Based on gender, no echocardiographic parameter was statistically significant. Only the MELD score has been associated with some biochemical parameters (bilirubin, albumin, urea, creatinine) (table 3.6). (Costache et al, 2018).

Table 3.6. Associations of MELD score with biochemical parameters

Parameter	r	p
AST	0.130	0.424
ALT	0.039	0.813
GGT	0.021	0.900
Total bilirubin	0.438	0.005
Total cholesterol	-0.150	0.357
Tryglicerides	-0.110	0.499
Total proteins	-0.224	0.164
Seric albumine	-0.497	0.001
Urea	0.377	0.016
Creatinine	0.411	0.008

No significant association was found between echocardiographic changes and CPS in patients with cirrhosis of liver. However, advising an echocardiogram for patients with cirrhosis (CPS - B and C) may prove beneficial for early screening. This needs further evaluation by larger studies, with echocardiographic modalities that can detect subclinical changes in left ventricular function. The correlation between DD and the prognosis of cirrhotic patients also remains controversial, with conflicting results reported in different studies (Bosch et al 2000, NAZAR et al 2013).

Of the 60 patients evaluated, 5 (8.3%) of them were diagnosed with porto-pulmonary hypertension. In the group of patients with POHP there were 3 males (60%), viral B infection and autoimmune hepatitis being the most frequent etiologies, and the severity of liver disease was classified in Child-Pugh class B or with mean MELD score of 19.9±1.8. The baseline characteristics of the patients included in the study are presented in Table 3.7. POPH was not observed in alcoholic cirrhosis patients. Among the three POPH patients, PASP values were 42 to 55 mmHg. (Costache et al, 2018).

Table 3.7. Baseline characteristics of the study groups

Parameter	All patients n=60	POPH group n=5	Control group n=55	p-value
Gender, male, n (%)	50 (83.3)	3 (60)	47 (85.4)	0.144
Age, years, mean±SD	50.55±8.35	54.4±6.22	50.20±8.47	0.641
Etiology of cirrhosis, n (%) HBV	27 (45)	2 (40)	25 (45.4)	0.246

HCV Alcohol Autoimune Other	16 (26.7) 10 (16.7) 4 (6.7) 3 (5)	0 (0) 1 (20) 2 (40) 0 (0)	16 (29.1) 9 (16.3) 2 (3.6) 3 (5.6)	
Child-Pugh class A/B/C, n	12/26/22	0/3/2	12/23/20	0.483
Child-Pugh score, mean±SD	8.68±2.11	9.40±0.54	8.62±2.19	**0.015**
MELD score, mean±SD	16.96±5.5	19.0±1.82	16.8±0.78	0.360
Creatinine (mg/dl), mean±SD	0.97±0.54	0.71±0.08	0.99±0.56	0.202
Albumin (g/l), mean±SD	3.24±0.74	3.33±0.61	3.23±0.75	0.457
Total Bilirubine (mg/dl), mean±SD	3.37±3.29	3.66±1.47	3.35±3.43	0.365
INR, mean±SD	1.54±0.74	1.50±0.09	1.54±0.78	0.286
Fibrinogen (mg/dl), mean±SD	271.8±92.1	341.2±93.2	264.7±89.9	0.077
ALT (UI/L), mean±SD	59.6±51.3	34.0±13.6	62.2±53.1	0.090
AST (UI/L), mean±SD	81.9±55.1	74.6±44.9	82.7±56.3	0.418
GGT (UI/L), mean±SD	96.9±84.4	81.2±71.9	98.6±86.1	0.518
Ascites, n (%)	39 (65)	5 (100)	34 (61.8)	0.033
HRS, n (%)	7 (11.7)	1 (20)	6 (10.9)	0.396
Encephalopathy, n (%)	15 (25)	2 (40)	13 (23.6)	0.418
Hepatocellular carcinoma, n (%)	15 (25)	0 (0)	15 (27.3)	0.173

There was a statistical significant difference between sPAP in women compared with males (24.08±7.76 vs 27.33±13.7, p=0.18), although this difference has not reach the statistical significance for calculated mPAP (16.91±5.29 vs 19.42±8.1, p=0.140). (Costache et al, 2018).
Regarding ascites, there has been found a direct correlation between the presence of ascites and the severity of pulmonary hypertension (p=0.033, figure 3. 6.). There was no difference between the LC etiology and the risk of POPH, although mPAP was higher in patients with autoimmune LC (figure 3.7.). (Costache et al, 2018).

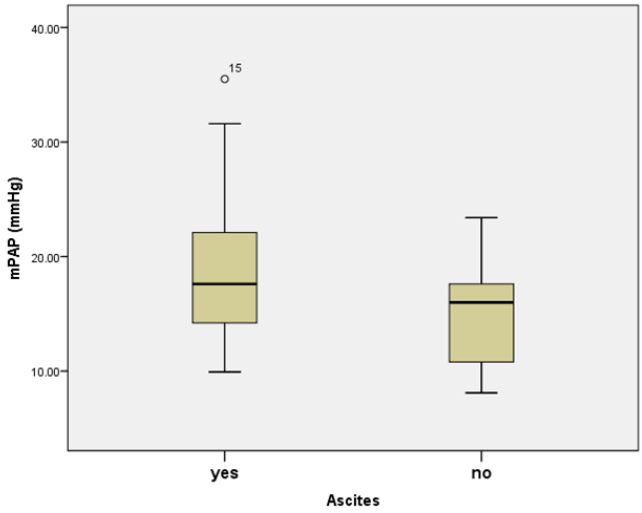

Figure 3.6. The relation between ascites and mPAP.

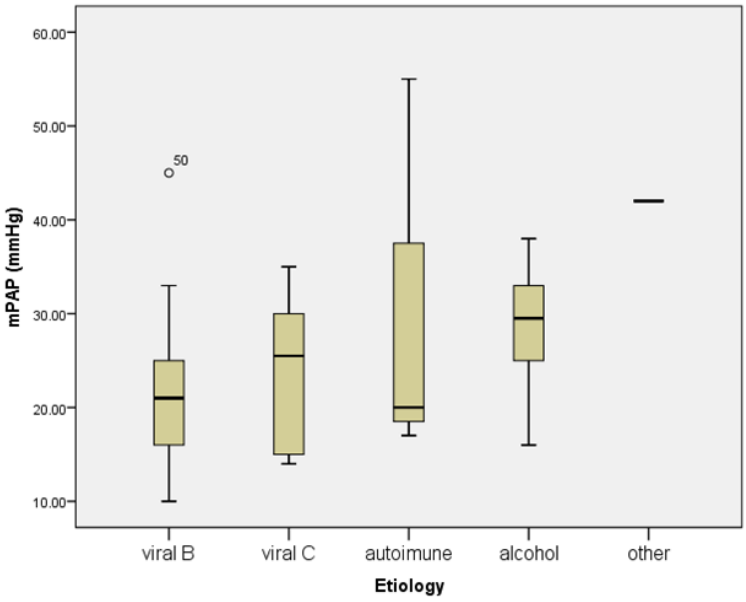

Figure 3.7. The relation between liver cirrhosis etiology and mPAP.

The Child-Pugh functional classification was used to assess the severity of hepatic cirrhosis. In Child-Pugh class A were 19,7 % of the patients with cirrhosis, in class B 39.3 % and in class C 36.1 %. A correlation between Child-Pugh class and severity of pulmonary hypertension (reflected by the value of sPAP) was investigated. The results revealed that the sPAP values were not significantly correlated with Child-Pugh class (p = 0.84).

The levels of blood count, serum bilirubin, albumin, total protein, international normalized ratio, liver enzymes (alkaline phosphatase, aspartate transaminase, alanine transaminase, GGT), lipid panel, renal function, inflammatory marks, were investigated in the study to establish some correlations with the severity of the pulmonary hypertension. We found a significant direct correlation between mPAP and MELD score (r=0.832, p=0.030) (figure 3.8.), fibrinogen level (r=0.887, p=0.021) (figure 3.9.) and bilirubine level (r=0.758, p=0.045). (Costache et al, 2018).

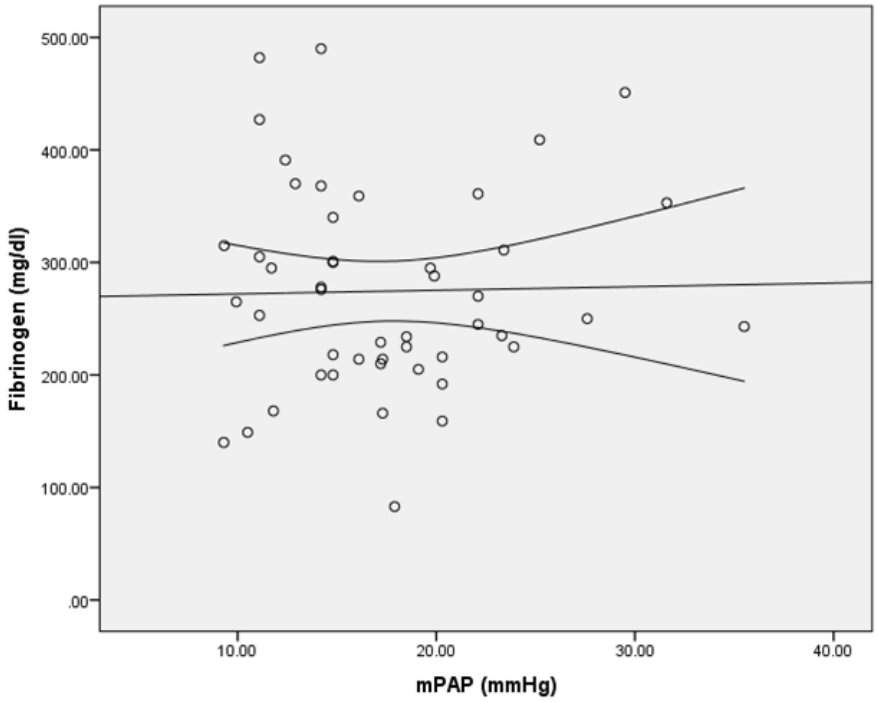

Figure 3.8. Correlation between fibrinogen level and mPAP.

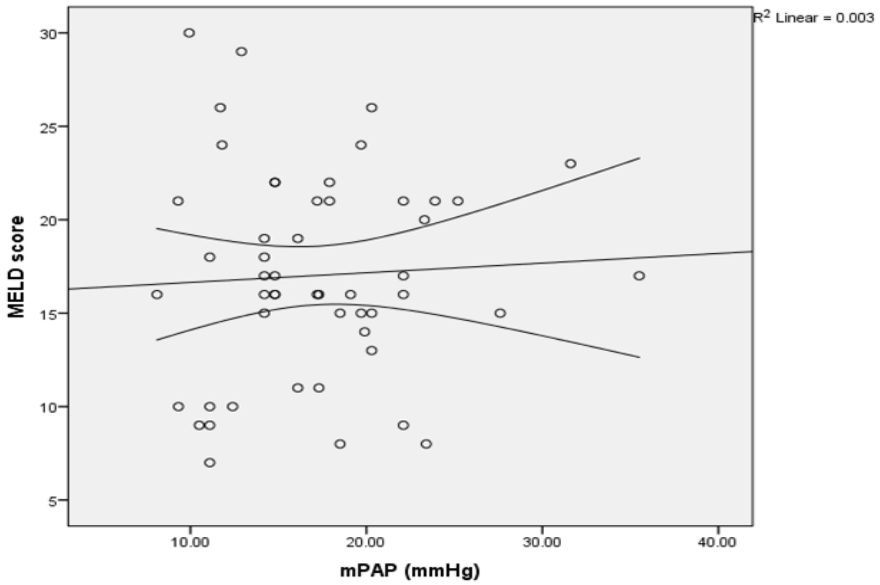

Figure 3.9. Correlation between MELD level and mPAP.

During the follow-up period 20 patients (33.3%) died and 10 patients (16.7%) received a liver transplant. Increased mPAP was not a risk factor for mortality on the awaiting liver transplant list.

3) Related to the association between thrombotic events in patients with cirrhosis of the liver, only 8 patients were diagnosed with DVT; patients were aged between 33-63 years and all of them were males. Five of them developed DVT during hospitalization for cirrhosis, which was confirmed clinically by the observation of swollen calf with rash and tenderness, together with Duplex Doppler ultrasonography. Three of the patients were admitted in the Emergency Department. Regarding the location of DVT in 5 cases it was at the level of the popliteal vein (right in 3 cases and left in 1 case), and in 3 cases it was a high DVT of the left ilio-femoral vein. There was no relation between the localization of thrombosis and the biochemical profile in our patients. The platelet count was between 95.000 and 125.000/ mm3 and no active bleedings were present at the admission. (Costache et al, 2018).

All patients were treated initially with low-molecular-weight heparin (for 7-10 days) and then with Acenocumarol to maintain INR between 2 and 3. Three of the patients showed ascites on admission and no patient with hepatic encephalopathy was found. One patient was previously diagnosed with diabetes mellitus and he was under insulin therapy. Also, no signifi-cant variations in platelet counts and no bleedings occurred during anticoagulation therapy.

Other risk factors, such as smoking, obesity, dyslipidemia, or status of inherited hypercoagulability, were not known. Viral etiology was predominant in the group of cirrhotic patients with DVT (6 patients out of 8), but with no statistical significance. Diabetes mellitus was the only variable that was significantly associated with the presence of DVT. Subsequent

multivariate analysis confirmed diabetes mellitus as an independent risk factor for developing DVT in patients with liver cirrhosis with a high odds ratio. However, serum albumin level of less than 3 mg/dL tended to be a risk factor. These results coincide with those reported in the studied literature. (Costache et al, 2018).

4) In the studied group we found an advanced steatosis (grade ≥2) in 54% of patients. A higher rate of the patients with cardiovascular, kidney and metabolic (diabetes mellitus) were in the advanced steatosis group compared with the early steatosis group (19.5% versus 8% and 10.6% vs. 0.9%, respectively) (p = 0.02 and p = 0.003, respectively). (Table 3.8., Table 3.9.) (Miftode et al, 2018).
Nearly half of the patients had thrombocytopenia (<150,000 / mm3) at baseline and was identified in a slightly higher rate in the patients with steatosis ≥2 compared to those with steatosis <2 (28% vs. 22%, p = 0.64). (Miftode et al, 2018).

Table 3.8. Response to previous treatment with interferon+ribavirin and the presence of comorbidities by steatosis grade.

	Steatosis<2 (no=52)		Steatosis >2 (no=61)		Total (no=113)		p
	No	%	No	%	No	%	
Previous interferon-based regimen (no=75)							
Non-responder	27	36	27	36	54	47.8	0.85
Relapse	11	14.7	10	13.3	21	18.6	
Treatment naïve (no=38)	14	12.4	24	21.3	38	33.6	0.16
With cardiovascular or kidney disease (no=31)	9	8	22	19.5	31	27.4	0.02
With diabetes mellitus (no=13)	1	0.89	12	10.6	13	11.5	0.003

Table 3.9. Hematologic and biochemical parameters at the initiation of therapy PrOD-R by steatosis grade

Parameters at the initiation of therapy	Steatosis<2 (no=52)		Steatosis >2 (no=61)		Total (no=113)		p
	No	%	No	%	No	%	
Anemia (Hb < 11g/dl)	1	0.9	3	27	4	3.5	0.39
Thrombocytopenia (< 150.000 /mmc)	25	22	32	28.3	57	50.4	0.64
Increased ALT level > 30 UI in women, > 40 UI in men	45	39.8	60	53.1	105	92.9	**0.014**
Increased bilirubin level (> 1.2 mg/dl)	11	9.7	9	8	20	17.7	0.11
Increased GGT level (> 65 UI/L)	35	31	59	52.2	94	83.2	**0.0003**
Increased AFP level (> 20 UI)	11	9.7	14	11.5	25	22.1	0.81

In most patients the hepatic cytolysis syndrome was identified before the initiation of therapy (93%) with a significant difference between patients with advanced vslow grade steatosis (53% vs 40%, respectively p=0,014). (Miftode et al, 2018).

In patients with steatosis grade ≥2 GGT levels above the normal range were detected in 52,2% cases and in the group with steatosis grade <2 in 35% (p=0,0003). (table 3.10.).
At the time of treatment initiationonly 3,5% of patients presented various grade of anemia but at the end of therapy 3,8% had different grades of anemia, this anomaly being more common in patients with advanced steatosis (23% vs 15% respectively p=0,27) (table 3.11.)
At the end of therapy only 14,2% still had higher than normal ALT levels most of them in the group with advanced steatosis (11,5% vs 2,6% respectively p= 0,018). (Miftode et al, 2018).

Table 3.10. Mean levels of viral load, ALT, GGT and AFP at baseline by steatosis grade

Mean levels	Steatosis S0 (n=19)	Steatosis S3 (n=34)	p
Mean viral load (UI/ml)	582,450	1,613,050	0.048

Mean ALT	71 UI	172 UI	0.002
Mean GGT	42.3 UI	162.4 UI	0.0003
Mean AFP	5.37 UI/ml	30.82 UI/ml	0.018

Table 3.11. Hematological and biochemical parameters at the end of PrOD-R therapy by steatosis grade.

Parameters at the initiation of therapy	Steatosis<2 (no=52)		Steatosis >2 (no=61)		Total (no=113)		p
	No	%	No	%	No	%	
Anemia (Hb < 12g/dl)	17	15	26	23	43	38	0.27
Thrombocytopenia (< 150.000 /mmc)	19	16.8	19	16.8	38	33.6	0.54
Increased ALT level > 30 UI in women, > 40 UI in men	3	2.7	13	11.5	16	14.2	**0.018**
Increased bilirubin level (> 1.2 mg/dl)	20	17.7	15	13.3	35	31	0.11
Increased GGT level (> 65 UI/L)	4	3.5	15	13.3	19	16.8	**0.016**
Increased INR level (> 1.2)	44	38.9	53	46.9	97	85.8	0.73

Discussions

Different studies did not show significant difference in echocardiographic parameters among the subgroups of CPS10, which was in agreement with our results (Moller et al 2013, Swanson et al 2005). Echocardiographic parameters may be significant in determining prognosis in cirrhosis and help in early diagnosis and treatment of co-existing cardiac abnormalities. Subclinical diastolic and systolic impairments that are missed by conventional echocardiography may be picked up by echo parameters such as strain, strain rate from tissue-Doppler and speckle tracking echocardiography (Swanson et al 2005). Thus, future studies are needed to appreciate the subclinical cardiovascular involvement among patients with cirrhosis.

Porto-pulmonary hypertension is a recognized subtype of pulmonary arterial hypertension and a complication of liver cirrhosis. Not all patients with hepatic cirrhosis associate pulmonary hypertension. In fact, this pulmonary complication depends on the presence of portal hypertension, but even in this case not all patients will develop this complication (Iwao et al 1997).

In our study, 8.3% of the patients with portal hypertension developed porto-pulmonary hypertension. This is within the range of prevalence reported in the literature. (Farzaneh-Far et al 2008).

Although, in our study there were more males with cirrhosis that developed porto-pulmonary hypertension (POPH), no significant difference was observed in the incidence of POPH across the gender (p=0.144). In a study performed in the United States published in 2008 (Swanson et al) a greater incidence of pulmonary hypertension in cirrhotic women was reported. We found no association between gender, age, etiology of the hepatic disease and the appearance of pulmonary hypertension. As a matter of fact other studies showed similar results (Rodriguez-Roisin et al 2008, Rugină et al 2012). Our result is in contradiction with results from other studies, that suggested that females are at higher risk of developing POPH (Carey et al 1995).

The severity of the hepatic disease could be a determining factor, and our results demonstrated that the severity of porto-pulmonary hypertension is related to liver function, as we found that the patients with POPH had an higher Child-Pugh class, and we demonstrated a direct positive correlation between the mPAP and the MELD score.

Ascites is a marker of severity of portal hypertension (Bosch et al 2000). In our study, ascites was present in 65% of the patients and a direct relation was observed between the presence of the ascites and the severity of the pulmonary hypertension (p=0.033). Some other studies showed no correlation but some suggest that porto-pulmonary hypertension is more common in patients with cirrhosis and refractory ascites, possibly due to excess endothelin 1 in the pulmonary circulation (Costache et al 2018).

Regarding the female patient with POPH and autoimmune cirrhosis, 1 week after the liver transplant the pulmonary pressure normalized, remaining normal at 1 month also, suggesting a good outcome and prognosis. The mechanism of these findings is not fully understood. In many studies, a strong association has been found between portal hypertension and pulmonary hypertension, whether or not liver disease is present. Also, the severity of the liver disease did not correlate with the pulmonary hypertension. This leads to the idea that normalizing the portal pressure could help lower the pulmonary pressure. Findings in other studies are in concordance with this idea, showing improvements and even normalization of the pulmonary pressure after liver transplant and normalization of the portal pressure.The histological abnormalities in POPH are almost the same as in idiopathic PAH. The main pathological abnormalities include proliferate arteriopathy, obliteration of the vascular lumen by endothelial and smooth-muscle cells, formation of plexiform lesions, necrotizing arteritis, fibrinoid necrosis and in-situ thrombi secondary to a systemic inflammation. High portal pressure leads to portosystemic shunts which may allow the shunting of the vasoactive substances including endothelin 1 (ET-1), vasoactive intestinal peptide, serotonin, thromboxane A2, interleukin 1, glucagon, and secretin from the splanchnic circulation to the pulmonary circulation, allowing these vasoactive mediators to bypass the liver metabolism and causing substantial effects on the pulmonary vasculature (Rodríguez-Roisin et al 2004). We demonstrated a direct strong correlation between fibrinogen level as a marker of systemic

inflammation and the mPAP. These mechanisms suggest that reversing the portal hypertension could lead to a decrease in pulmonary pressure. (Costache et al, 2018).
Among the laboratory parameters examined in our study, we found a direct correlation between mPAP and the severity of liver disease assessed by MELD score, the fibrinogen level a s a marker of systemic inflammation and the total bilirubine level as a marker of hepatic dysfunction. Other studies suggest a correlation between hemoglobin and porto-pulmonary hypertension, with a lower value of hemoglobin in the POPH group than in the non-POPH group (Benjaminov et al 2003). This study suggests that hemoglobin level is an independent risk factor and plays a role in the development of POPH. A decreased haemoglobin leads to a significant increase in cardiac output and exacerbated hyperdynamic splanchnic circulation, which is known to be a major contributor to portal hypertension (Torregrossa et al, 2005). However, in our study no correlation was found between the hemoglobin value and pulmonary / porto-pulmonary hypertension.
Our study has some strengths and also several limitations. Thus, it is one of the few studies examining the incidence of POPH in liver transplant patients. Our study has several limitations such as having relatively small sample size and lack of long-term evaluation.

3) Venous thromboembolism is considered a very rare event in patients with liver cirrhosis, with unpredictable course and un-clear mechanism. Viral hepatitis was the predominant cause of cirrhosis in our study (5 cases out of 6) compared with alcoholic and nonalcoholic steatohepatitis in Northup's study (Northup et al, 2006). Diabetes mellitus was present only in one case and, as we know, it is a risk factor for the occurrence of DVT.
We did not find any association between the clinical factors, such as sex, age, and laboratory findings, and the presence of DVT in liver cirrhotic patients. Because of the small number of patients, no laboratory marker could be used as a predictor for prothrombotic state, including serum albumin levels. Most of our patients came already with low serum albumin levels (less than the normal cutoff point of 3.0 mg/dL). This data explains why serum albumin levels failed to show significant association with the presence of DVT. Low albumin level may indicate an advanced liver disease in most of our study subjects. In addition, it may be used as an indirect marker for the decreased synthesis of other proteins produced by the liver (Swanson et al, 2008, Rugina et al 2012, Raval et al, 2011, Rodrigez-Roisin et al, 2008). There are other factors, such as protein C, protein S, and antithrombin III, that are involved as natural anticoagulants. During acute or chronic liver disease, their concentrations decrease concomitantly with other coagula-tion factors, but usually are not lower than 20% of the normal values. Decreased natural anticoagulants, therefore, could be another risk factor for developing DVT.
We identified diabetes mellitus as an independent risk factor for developing DVT. This observation was not surprising, since the risk of VTE is increased in diabetic patients (Pilatis et al 2000). Other features of endo-crinological disturbances, such as obesity and dyslipidemia, might be risk factors of DVT; however, a recent study concluded that symptoms of metabolic syndrome were not clinically important risk factors for VTE (). Further studies are needed to confirm whether

metabolic abnormalities, especially those with hepatitis C infection, could be the risk factors of DVT in patients with liver cirrhosis.

There are many risk factors and conditions predisposing to VTE such as age above 40 years, cancer with or without ongoing chemotherapy treatment, history of VTE, prolonged bed immobilization, surgery, trauma, obesity, smoking, and inherited hypercoagulable states (e.g., antithrombin deficiency, protein C and/or protein S deficiency, factor V Leiden, pro-thrombin gene mutation). Besides, decreased synthesis of the natural anticoagulants, hypercoagulation in liver disease, could also be related to poor flow and vasculopathy associated with a chronic inflammatory state. The potential disease state favoring DVT or PE in patients with liver cirrhosis could be an imbalance in the clotting cascade favoring coagulation, immobilization associated with of end-stage liver disease, infection, and systemic inflammation (Gulley et al 2008, Sogaard et al 2009, Wu et al 2010). Our study showed that the age of the patients was between 33-63 years and most of them had chronic hepatic viral infection. Patients with this profile often need hospitalization due to the cirrhosis complications especially ascites. No patient of the group had encephalopathy. Immobilization during hospital stay is known as a risk factor for venous thrombosis due to the stasis of blood flow in the venous system. Therefore, patients with cirrhosis may share the same risk factors of VTE as other hospitalized patients. We did analyze the patients' length of stay before the occurrence of DVT symptoms and we concluded that the risk increased when the length of hospital stay was more than 6 days. No patient of the studied group presented electrocardiographic signs of pulmonary embolism or other arrhythmias. As we mentioned, no active bleedings were present at admission, no bleeding during anticoagulation therapy and no significant variations in platelet counts as we would have expected. Cancer alone is a known risk factor of thrombosis. We also analyzed the presence of hepatocellular carcinoma (HCC) and its association with DVT, but the presence of HCC apparently was not a risk factor for developing DVT in our study subjects because HCC was not discovered. The treatment unanimously accepted so far, initially with low-molecular-weight heparin (for 7-10 days) continued with Acenocumarol is adequate, with a low risk of bleeding. (Costache et al, 2018).

Conclusions

1) A large number of parameters derived from different imaging modalities are currently available for the assessment of left ventricular function but echocardiography is a widely available method allowing a rapid and detailed evaluation of myocardial function, improving the diagnostic accuracy in patients with different comorbidities. Echocardiography parameters are needed to evaluate cardiac performance in patients with cirrhosis of the liver and, more important, to orientate the clinical management of this specific group of patients. In our study no significant association was found between echocardiographic changes and biochemical parameters in patients with cirrhosis of liver. (Costache et al, 2018).

2) Echocardiography is a useful and easy method for pulmonary hypertension screening in patients with portal hypertension.
3) Pulmonary hypertension is relatively frequent in these patients and not related to the etiology of the hepatic disease.

4) We found no association between gender, age, etiology of the hepatic disease and the development of pulmonary hypertension. (Costache et al, 2018).

5) The severity of the hepatic disease, evaluated by Child-Pugh score and MELD score, fibrinogen level and total bilirubine level are in correlation with mPAP in cirrhotic patients. (Costache et al, 2018).

6) Refractory ascites in end-stage cirrhosis patients could be associated with a higher risk of pulmonary hypertension. Further larger studies on POPH incidence in liver cirrhosis are warranted.
7) The precise mechanisms of hypercoagulability and thrombosis in cirrhosis are not well known and need further investigation. The treatment unanimously accepted so far, initially with low-molecular-weight heparin (for 7-10 days) continued with Acenocumarol is adequate, with a low risk of bleeding. (Costache et al, 2018).

8) A higher rate of the patients with cardiovascular, kidney and metabolic (diabetes mellitus) were in the advanced steatosis group compared with the early steatosis group in patients with C viral hepatitis. (Miftode et al, 2018).
9) We found a significant correlation between the advanced grade of hepatic steatosis and the levels of hepatic cytolysis enzymes and GGT both before initiation of therapy and at its completion in patients with advanced steatosis. (Miftode et al, 2018).

SECTION II. FUTURE EVOLUTION AND DEVELOPMENT PLANS

The elaboration of the habilitation thesis offered a good opportunity for me to analyze the main positive elements that led to the development of my career in the three fields of activity: **professional, academic and scientific.**

Starting from my previous experience, the development plan in the future will have to maintain the continuity between the main achievements and future projects.

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

I consider an essential target to continue improving my professional and research performances in order to participate in national and international competitions that would help me to obtain and update the necessary research skills in the future.

I also intend to contribute to expanding the prestige of the University by publishing the research results in prestigious ISI Thomson Reuters-indexed journals and by participation in Congresses and International Conferences.

Therefore I intend to extend my collaboration with scientists from other Universities and research institutes and initiate and develop the necessary collaborative networks. Within the domain I have already approached in this thesis, some of the directions I wish to contribute and develop in my future research are summarized above.

II.1. Perspectives in professional activity

Professionally, I intend to improve the management and care of patients with cardiovascular disease. In this regard, my main objectives are:

- to acquire new skills and competences;
- to create research teams capable to get access to funding in open competitions for modernization of infrastructure and equipment endowment;
- to participate in the development of primary and secondary prevention programs for patients with cardiovascular diseases;
- to cooperate with other different specialties aimed to improve the complex approach of patients with cardiovascular disease-associated co-morbidities;
- to continuate the activity within professional associations and join new national and international societies;
- to acquire and respect professional ethics and deontology rules in relation with patients and implicitly to respect their rights regarding informed consent, applicated in daily practice.

II.2. Perspectives in academic activity

For the future academic development, I intend to maintain and to develop the relationship between the existing research field and the achieved professional and scientific objectives taking into account the greater complexity and new requirements offered by academic life. That is why I want to integrate my research into the climate of progress of our university in order to create oportunities to attract students, residents and PhD students in many practical activities and projects.

Proposals related to future didactic work with students:

• Continue to include in the notions of the concept of "evidence-based medicine", present in current practice in the form of guides of medical practice, a concept that students need to know and learn to use from college;

• Completing applications for obtaining additional funding for the recognition of Romanian medical education internationally;

• Encouraging and coordinating students and residents to dedicate themselves and persevere in scientific activity, promoting the current rigors of science paper presentation;

* Supporting and involving students in the process of education and research and providing an information exchange at national and international level.

• Continuation of activities within the academic community and other committees for the evaluation or promotion of our activity at the Iasi "Grigore T. Popa" University of Medicine and Pharmacy;

• Participation in other national and international exchange programs.

• Continuing to guide students with creative and research skills and engage in writing works for national and international student congresses.

Proposals related to future didactic work with resident doctors:

• Involvement of the most valuable residents in research programs.

• As in the case of students, feed-back is very important, which is why I consider it important to organize regular meetings with resident doctors under direct guidance, for their discussions / opinions and to help them in order to fulfilling professional aspirations of their own.

Proposals related to post-graduate teaching:

• Attracting to the clinical research activity the doctors who have motivation and abilities in this respect and encouraging their enrollment in the doctoral school and later among the teaching staff of the University.

II.3. Perspectives in scientific activity

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

From the point of view of the approached themes, I will consider the continuation of the main topics debated so far, but also the development of new research themes.
As my areas of interest are very extensive (as evidenced by the work done and the collaboration so far), one of my main objectives is **to continue the researches started** with the aim of identifying new aspects and implicitly results that could be based the development of new scientific papers. In this respect, further collaboration through the organization of regular meetings with those involved in the above-mentioned research, namely the Department of Genetics, the Hospital Immunology Laboratory, the Discipline of Gastroenterology, are essential for assessing the current state of research as a starting point for future research.
An important research direction seems to me to be the exploitation of the results of research grants to which I have participated either as a director or as a memnber in order to carry out scientific work and possibly extend the collaboration with the related specialties.
Related to the research started in the Grant I intend to continue with:
- the optimization the Protocol Assessment for patients with ACS in terms of response to antiplatelet therapies;
- the introduction of modern tests that provide quality precise, results (which are able to indicate a specific therapy), but that can be achieved with existing lab an affordable cost;
- the prediction since the diagnosis, of the patient's evolution under the treatment with clopidogrel, according to the presence of genetic abnormalities that can lead to defects in the prodrug metabolism;
- the initiation of a collection of DNA enabling application and other molecular methods of investigation when the first test provides unsatisfactory results; thus avoiding excess stress on patients by repeated medical visits and the possibility to optimize laboratory testing.
- the creation of a starting point for future projects (more research-related and other polymorphisms in the cytochrome study or superfamily of CYP450 that can cause resistance to anticoagulant therapy, use of data / methods obtained for evaluation / explanation other drug resistance using the same metabolic line - antipsychotics, antiepileptics, etc, and the interactions of these drugs), and premises in order to become part of similar European projects.

One area of interest for future research is to collaborate with Interventional Cardiology to extend the research started in the Grant I was directing to in order to detect resistance to clopidogrel in patients who have supported an angioplasty and in whom clopidogrel resistance may be a cause of intrastent thrombosis and implicitly of unfavorable evolution.
Expanding the research on the patients with liver pathology could have a practical implication, helping to identify patients at risk of bleeding or of intrastent early thrombosis and implicitly unfavorable evolution postangioplasty.
The research would certainly have practical implications for the particularities of anticoagulant and antiaggregant treatment in patients with chronic hepatopathy and implicitly the finding of effective and risk-free therapeutical solutions.

2) Fields of my actual scientific interest refer especially **to cardiovascular evaluation of patients with other different pathologies, especially those with end - stage liver diseases**. The complex cardiovascular evaluation means – risk factors evaluation, clinical examination, echocardiographic complete examination in order to appreciate cardiac function in patients with liver diseases, biochemical parameters in order to identify those risk factors that may be eliminated/treated for prognosis improvement. The evaluation of cardiovascular comorbidities in this population could be beneficial both for the assessment of prognosis but especially for the establishment of appropriate therapeutic behavior to avoid adverse drug interactions.

This implies to maintain the collaboration in very good conditions with doctors of various specialties, both related to cardiology (diabetologists), as well as those from other specialties (gastroenterologists, surgeons, family doctors).

I intend **to expand the research theme of cardiovascular risk factors** by trying to establish some relationships between these factors and genetic mechanisms and their implications in different comorbidities. As we know, the most common cause of Lower extremity artery disease (LEAD) is atherosclerotic vascular disease. LEAD is relatively highly prevalent and is a significant public health problem that impairs quality of life, as well as a major cause of cardiovascular morbidity and mortality (Eraso et al 2014, Popa et al 2017). Genetic investigation of LEAD is an evolving concept useful for understanding its pathogenesis and for identification of new therapeutic targets (Costache et al, 2017, Popa et al, 2017, Leeper et al 2012). A lot of cardiovascular risk factors, including metabolic disorders, negatively affect endothelial function by increasing inflammation and oxidative stress (Chao et al 2016, Cipollone et al 2005). PCSK5 is a precursor protein with 1860 amino acids (206.942 Da) and two isoforms, and is encoded by PCSK5 gene located on chromosome 9 (78505560-78977255 bp from p-terminal end (9q21.13-31)) (Chao et al 2016). In patients with impaired HDL-c levels seven variants of PCSK5 gene were detected, but none of these variants was a missense mutation (Seidah et al 2012). The study of PCSK5 gene is justified by its role in HDL-c metabolism since it was suggested that some polymorphisms of PCSK5 gene could be associated with a variable expression that generates mild forms of atherosclerotic disease (Liu et al 2010). Some experimental and clinical studies have identified PCSK5 in the atherosclerotic plaque and suggested that PCSK5 modulates the inflammation in atherosclerosis. It has been shown that PCSK5 activates matrix metalloproteinases and integrins, while the levels of PCSK expression increase during macrophage differentiation (Jin et al 2007). **These findings provide insights into potential new mechanisms, whereby PCSK5 in endothelial cells may participate in cardiovascular pathophysiology and may provide new targets for cardiovascular therapy** (Sluijter et al 2005).

3) Another direction of research is represented **by the new biomarkers useful in assessing the prognosis of patients with heart failure and concomitant cirrhosis of the liver, such as syndecan-1 and Galectin 3**. The last one has elevated levels in liver cirrhosis and is involved in myocardial fibrosis (Pateron et al, 1999, Wanninger et al, 2011).

Both cardiac dysfunction and inflammation precipitate hepatic decompensation in cirrhosis, with the occurrence of complications that contribute significantly to mortality and prognosis. Thus, a number of cardiac biomarkers (BNP, NT-proBNP, ANP, proANP, Troponin T-hs) and inflammatory (high sensitive reactive C-protein CRPhs, soluble urokinase plasminogen activator-suPAR) be useful in estimating the severity of liver disease, the degree of portal hypertension and long-term survival. (Wiese et al, 2014, Elnegoulyet al, 2018)

Heart failure (HF) represents the main cause of hospitalization throughout the world, with limited therapeutical options and high mortality rates (Joffe et al, 2013). HF is the consequence of myocardial injury of various etiologies, fibrosis and subsequent remodellation further affecting the cardiac function. Recent studies have demonstrated that syndecan-1 is a main component of the glycocalyx, a high blood concentration of syndecan-1 indicating a serious endothelial dysfunction (Kim et al 2017, Neves et al 2015) and is involved in both inflammation and fibrosis after myocardial injury (Lei et al 2012, Schellings et al 2010).

In patients with ischemic heart disease or heart failure, elevation of serum syndecan-1 has been associated with a degradation not only of cardiac, but also renal function, justifying further studies on this molecule (Neves et al 2015, Tromp et al 2014) Also, syndecan-1 was associated with poor prognosis in HF patients with preserved ejection fraction, suggesting the future use of syndecan-1 as a possible marker of cardiac fibrosis (Tromp et al 2014).

Syndecan-1 is a type-I transmembrane heparan sulfate proteoglycan and is a member of the syndecan proteoglycan family, which consists of four transmembrane heparan sulfate proteoglycans mainly present on the cell surface, mediating cell adhesion, cell signaling, endocytosis and the structural layout of the cytoskeleton (Kim et al 2017). Syndecan-1 has been associated with the development of cardiac fibrosis and a poor outcome in patients with HF. . In addition, patients with high syndecan-1 levels had also elevated serum NT-proBNP, fibrosis markers, and a worse renal function. (Tromp et al 2014). Male sex was also a predictor of a high serum syndecan-1 in patients with HF ($P=0.029$). An increase in syndecan-1 levels represented a much stronger increase in risk and poor clinical outcome for patients with preserved ejection fraction ($EF>40\%$) than in patients with reduced ejection fraction ($EF<40\%$) (Tromp et al 2014).

The determination of these biomarkers in patients with hepatic cirrhosis and subclinical heart failure could be useful in early detection of cirrhotic patients who will subsequently develop cardiac damage (Miftode et al, 2018).

All these ideas may be a starting point for further research which will need to:

• Develop the logistics base necessary to associate clinical research with the molecular approach of some etiopathogenic mechanisms of the studied diseases.

• Creating patient databases by type of disease to facilitate data processing for scientific purposes. Such databases already exist for patients with acute coronary syndromes who received clopidogrel, for patients with heart failure and for the patients with end stage cirrhosis of the liver that are on the waiting list for liver transplantation. Participation in interdisciplinary collaborations is essential as well as the involvement of students and resident doctors in creating these databases.

• Participation in the appropriate coordination / management of the research activity in the clinic, favoring the participation in grants obtained through the competition, as well as in national and international multicentre studies.

• Publication of the results of the studies carried out, both through preliminary communications at the main national and international congresses, and especially through original articles in extenso in specialized journals.

• Encouraging the most talented and motivated young researchers to start doctoral studies and to choose a topic of common interest for the doctoral thesis, as well as their direct support for the successful completion of the doctorate.

• Ensure a rigorous selection of young researchers, able to perform a doctoral training program.

• Promoting the dissemination and visibility of research results by publishing the results in journals indexed and cited by international databases and communicating them at congresses.

• Development of interdisciplinary and interdepartmental collaboration within the "Grigore T. Popa" University of Medicine and Pharmacy Iaşi and collaboration with other medical universities in the country. I will sustain the development of the university and of the medical structures by implementing the collaboration with university centres and research institutes at national and international level.

• Collaboration and involvement of family doctors in the elaboration of scientific papers on the basis of the data provided by them regarding the incidence of cardiovascular risk factors among the adult population and intervention with preventive measures (fact already materialized in 3 ISI papers).

• Obtaining funding through participation in national and international competitions for optimal performance of the scientific projects considered and the possibility to support and motivate young researchers;

• Identify ways to ensure adequate research provision by acquiring equipment from funding obtained by winning national and international grants.

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