GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

Habilitation Thesis

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GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

INSIGHTS INTO MODERN RESEARCH IN METABOLIC AND NUTRITION-RELATED DISEASES

- Habilitation Thesis -

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Abstract

This habilitation thesis describes my scientific activity in the time period since my doctoral degree, extending from 2007 to the present moment, and represents the support needed to obtain the habilitation to coordinate Ph.D. students. Description of a set of forthcoming research directions towards which I plan to commit my efforts in the near future has also been included in this thesis.

The present manuscript has been divided in three main sections, according to the criteria recommended and approved by The National Council for the Attestation of University Titles, Diplomas and Certificates:

Section I – Scientific achievements over the postdoctoral period

Section II – Forthcoming projects and development in the scientific field

Section III – References

The first section of my habilitation thesis, titled "Scientific achievements over the postdoctoral period", consists of 4 chapters.

Previous to these chapters, this section is introduced by a short review of my academic, professional and scientific activity, during which I have described the studies I followed and the main direction upon which I have built my professional career from graduation day to the present moment. I have herein described the main details concerning my teaching activities for students and resident physicians, activities I have fulfilled within the academic community and the professional societies I belong to, projects of continuous medical education I have been involved in, either as a coordinator or a speaker, clinical studies in which I have taken part, first as a sub-investigator, then as a principal investigator, as well as the main publications to which I have contributed.

I have emphasized here the importance I have granted, throughout my whole career, to my research activity and to scientific publications, among which I mention here 3 books and 34 book chapters, 33 ISI and ISI proceeding papers rated by Clarivate Analytics Web of Science Core Collection, 32 papers indexed by other international databases, other 13 papers in various publications, 36 abstracts rated by Clarivate Analytics Web of Science Core Collection, as well as 60 other abstracts and multiple scientific works presented at international and national congresses or conferences. These articles have drawn a total of 164 citations in Clarivate Analytics Web of Science Core Collection publications and 311 citations in Google Scholar, thus generating a Hirsch-index of 7 according to Clarivate Analytics.

The four chapters I have included in Section I review the original contribution I have brought in the field of my two main scientific preoccupations: complications and comorbidities related to diabetes mellitus and clinical nutrition. They review my previous preoccupations and efforts on the topics of the epidemiology of diabetes mellitus and its complications and comorbidities, as well as the study of its main cardiovascular and digestive consequences.

As member in the Directing Committee of the Romanian Society of Diabetes, Nutrition and Metabolic Diseases, and later as vice-president of the same society, I took part in the design and coordination of two national *epidemiological studies*, PREDATORR and MENTOR, that aimed to identify the prevalence of diabetes mellitus and its complications, of the other

metabolic diseases (obesity, dyslipidaemia, hyperuricemia) and of the renal impairment in the adult population of Romania.

In the chapter dedicated to the *cardiovascular complications of diabetes mellitus*, I have presented the results of my research in the field of heart disease, peripheral artery disease and cerebrovascular disease in diabetes patients, as well as on the influence the antihyperglycemic medication can exert on these diseases. I have dedicated a distinct subchapter to the cardiovascular disease in subjects with various forms of prediabetes, the first step in the hyperglycaemic continuum, given the high risk these patients have to subsequently progress to diabetes mellitus.

Another chapter presents the research I have performed along with my professional team in the field of *digestive diseases associated with diabetes mellitus*: the gastrointestinal motility, the metabolic fatty liver, the effects of the antiviral drugs on individuals at high metabolic risk.

The final chapter in this section has been dedicated to the research on topics of clinical nutrition, covering *ethical problems of the artificial nutrition*, the study of *healthy dietary patterns* such as the Mediterranean diet and the topic of *nutrition in the inflammatory bowel diseases*.

Section II includes the description of several specific strategies on which I intend to uphold the development of all three professional domains fundamental in my career: the academic activity, the medical activity and the scientific research activity. I intend to focus my forthcoming scientific activity on two novel fields, the glycaemic variability and the blood pressure variability, taking into account the potential influences exerted by our therapeutic intervention they may be submitted to.

Section III includes a number of 353 references used for the preparation of this thesis and the elaboration of all papers included here.

Rezumat

Prezenta teză de abilitare descrie activitatea mea științifică în perioada post-doctorală (din anul 2007 până în prezent), constituind suportul necesar pentru obținerea abilitării de a coordona doctoranzi. Am inclus în această lucrare și descrierea unei serii de direcții viitoare de cercetare cărora îmi doresc să le dedic eforturile mele în viitorul apropiat.

Manuscrisul de față este structurat în trei secțiuni principale, conform criteriilor recomandate și aprobate de către Consiliul Naţional de Atestare a Titlurilor, Diplomelor si Certificatelor Universitare (CNATDCU):

Secțiunea I – Realizări științifice din perioada postdoctorală;

Secțiunea II – Proiecte viitoare în activitatea științifică;

Secțiunea III – Referințe.

Prima secțiune a tezei de abilitare, intitulată „Realizări științifice din perioada postdoctorală", cuprinde 4 capitole.

Această secțiune are o scurtă introducere în care am trecut în revistă activitatea mea didactică, profesională și științifică, cu menționarea studiilor urmate și a direcțiilor în care mi-am construit cariera profesională de la absolvirea facultății până în prezent. Am descris aici activitatea mea didactică cu studenții și cu medicii rezidenți, activitatea în cadrul comunității academice și a societăților profesionale din care fac parte, proiectele de educație medicală continuă în care sunt implicat în calitate de coordonator și lector, studiile clinice în care am participat în poziția de coinvestigator, iar ulterior de investigator principal, precum și principalele publicații la care mi-am adus contribuția.

Am subliniat aici importanța activității de cercetare și a realizării de publicații în decursul întregii mele cariere, dintre care menționez un număr de 3 cărți și 34 capitole de carte, 33 articole in extenso publicate în reviste ISI și ISI proceedings indexate în Clarivate Analytics Web of Science Core Collection, 32 articole in extenso indexate în alte baze de date internaționale, alte 13 articole in extenso apărute în diverse publicații, 36 de rezumate publicate în reviste ISI indexate în Clarivate Analytics Web of Science Core Collection, precum și alte 60 de rezumate în diverse publicații științifice și numeroase lucrări prezentate la congrese și conferințe interne și internaționale. Toate aceste lucrări au atras un număr de 164 citări în Clarivate Analytics Web of Science Core Collection și 311 citări în Google Scholar, care au generat un indice Hirsch de 7 conform Clarivate Analytics.

Cele patru capitole din Secțiunea I detaliază contribuţia mea originală în domeniul celor două teme principale de studiu: complicațiile și comorbiditățile legate de diabetul zaharat și nutriția clinică. Am detaliat aici preocupările și eforturile mele anterioare în ceea ce privește epidemiologia diabetului zaharat și a complicațiilor și comorbidităților acestuia, precum și studiul principalelor sale complicații cardiovasculare și digestive.

Ca membru în Comitetul Director al Societății Române de Diabet, Nutriție și Boli Metabolice, și ulterior ca vicepreşedinte al acestei societăți, am participat la proiectarea și coordonarea a două *studii epidemiologice naționale*, PREDATORR și MENTOR, studii care și-au propus să stabilească prevalența diabetului zaharat și a complicațiilor sale, precum și a celorlalte boli metabolice (obezitate, dislipidemie, hiperuricemie) și a afectării renale în populația adultă a României.

În capitolul dedicat *complicațiilor cardiovasculare ale diabetului zaharat*, am prezentat rezultatul cercetărilor mele în domeniul bolilor cardiace, bolilor arterelor periferice și al bolii cerebrovasculare la pacienții cu diabet zaharat, precum și referitoare la modul în care medicația antidiabetică poate influența aceste afecțiuni. Un subcapitol aparte este dedicat afectării cardiovasculare la persoanele cu diverse forme de prediabet, primul pas din continuumul hiperglicemic, știut fiind riscul mare al acestor pacienți de a evolua ulterior spre diabet zaharat.

Alt capitol prezintă cercetările efectuate împreună cu echipa mea de colaboratori în domeniul *complicațiilor digestive ale diabetului zaharat*: motilitatea gastrointestinală, ficatul gras de cauză metabolică, precum și efectele medicației antivirale asupra persoanelor cu risc crescut metabolic.

Ultimul capitol este dedicat cercetărilor în domeniul nutriției clinice, incluzând probleme de *etică a nutriției artificiale* și *studiul unor pattern-uri alimentare* considerate sănătoase (dieta mediteraneană). Într-un alt subcapitol am prezentat principalele lucrări în domeniul *nutriției în bolile inflamatorii intestinale*.

Secțiunea a II-a include descrierea unei serii de strategii specifice pe baza cărora îmi propun să îmi dezvolt fiecare dintre cele trei domenii profesionale importante pentru mine: activitatea academică, activitatea medicală și activitatea de cercetare științifică. Îmi propun ca în cercetarea științifică să mă concentrez pe două domenii de mare actualitate, variabilitatea glicemică și variabilitatea tensională, studiind și modul în care acestea ar putea fi influențate de intervenția noastră terapeutică.

Secțiunea a III-a include un număr de 353 referințe bibliografice utilizate pentru această teză și pentru articolele incluse.

SECTION I
SCIENTIFIC ACHIEVEMENTS OVER THE POSTDOCTORAL PERIOD

OVERVIEW OF ACADEMIC, PROFESSIONAL AND SCIENTIFIC ACHIEVEMENTS

Academic and Professional activity

I graduated the "Grigore T. Popa" University of Medicine and Pharmacy in Iași, Romania in 1992, at the Faculty of General Medicine (licence diploma no. 137 from September 28, 1992), with the overall mean of 9.99 (personal scholar record, scholar registry excerpt no. 9767, volume 53). During the same year, I took the national internship contest and I became an intern at the Second Medical Clinic – Gastroenterology in the Clinical Emergency Hospital "Sf. Spiridon", Iași. In 1993 I became a resident physician in Internal Medicine in the Third Medical Clinic of the same hospital. During the residency period, I was more and more fascinated by the complexity of the Internal Medicine specialty and I realized the study of internal medicine could offer me a comprehensive perspective on the patient and a more complex approach to his pathologies. In 1998 I was confirmed as a specialist physician in Internal Medicine by Order of the Health Ministry no. 1011 from December 30, 1998 (general mean 9.37).

In 1999 I became by contest an Assistant Professor in the Discipline of Diabetes, Nutrition and Metabolic Diseases of the "Grigore T. Popa" University of Medicine and Pharmacy, Iași. In the same year I began the second residency in the field of Diabetes, Nutrition and Metabolic Diseases. I finished it in 2002 and I became a specialist physician in Diabetes, Nutrition and Metabolic Diseases, confirmed by Order of the Health and Family Ministry no. 1024 from December 17, 2002 (general mean 10.00). In 2003 I became a senior physician in Internal Medicine, confirmed by Order of the Health Ministry no. 846 from September 12, 2003 (general mean 9.22), and in 2004 I became a senior physician Diabetes, Nutrition and Metabolic Diseases, confirmed by Order of the Health Ministry no. 1067 from August 25, 2004 (general mean 9.88). In 2006 I became by contest a Lecturer in the Discipline of Diabetes, Nutrition and Metabolic Diseases of the "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, and in 2014 I became an Associate Professor in the same discipline.

During all these years I tried to combine the medical and academic activities. During lectures and practical works, I did my best to offer my students and residents a complex view on the patient with diabetes mellitus, comprising all the problems manifested by such cases. As a metabolic disease, diabetes mellitus involves the whole body, leading to specific complications. It is only by a thorough knowledge of internal medicine that we are able to understand the pathophysiology of these complications, to detect them early enough and to efficiently treat them.

Given the complex implications of diabetes mellitus on the human body, a wide range of post-academic lectures, both in the country and abroad, proved very useful for my professional development:

- 1996 – Immunology lecture organized by the "Centre de formation des enseignants dans le domaine des sciences de la vie, espace européen de la diffusion des connaissances et de reflexions prospectives"
- 2000 – "Enhancing the Scope of Primary Care Practice" lecture, organized by The United States Agency for International Development, the US Department for Health and Human Services and World Learning
- 2001 – The Sixth "Module d'Enseignement Francophone": "Actualités en Endocrinologie et Pharmacovigilance"

From 2012 I am a member in the Directing Committee of the Romanian Society of Diabetes, Nutrition and Metabolic Diseases, and from 2015 to the present moment I am a vice-president of this professional organization. In these positions, I became involved from 2012 in the organization of the annual national congresses of this society and of the Romanian Federation of Diabetes, Nutrition and Metabolic Diseases, as well as in the INTERDIAB ("International Conference on Interdisciplinary Management of Diabetes Mellitus and its Complications") international conferences, taking place each year in Bucharest. In my quality of vice-president of the Romanian Society of Diabetes, Nutrition and Metabolic Diseases, I organize 4 or 5 times a year the DIAMOND (Diabetes, Metabolic Syndrome, and Other Nutrition-related Diseases) lectures in a broad range of large cities in the country, as well as the National Campaign "Control Your Diabetes". The DIAMOND lectures aim to offer up-to-date information about the prevention, early detection and therapy of metabolic diseases, as well as of their complications, to physicians from various specialties, in order to support the efficacious management of these disorders. The National Campaign "Control Your Diabetes" is a project involving physicians, authorities and mass-media in the spreading of alarm messages on diabetes-related problems. We endeavour to involve Romanian authorities in spreading alarm messages on the high incidence of diabetes cases in Romania, as well as on the complex medical dimensions of diabetes cases, to inform and educate the general public about the importance of prevention and early detection of diabetes, to determine an efficacious control of diabetes and lifestyle improvement, and to facilitate the continuous medical education for physicians involved in the management of persons with diabetes mellitus, in order to increase the quality of medical care.

At the same time, I have been, from the very moment of its establishment in 2005, the secretary of the Nutrition Society in Romania; in this quality, I am involved each year in the organization of a scientific event: a congress every two years and yearly courses of nutritional education, which bore the name of "Nutrition Summer School" in the last years and received a warm welcome from the medical public. Together with the other colleagues in the discipline, we elaborated the "Healthy Eating Guide", which delivered the main information about a healthy eating pattern to the general population and was chosen by the Health Ministry for its site.

I am author of 3 books and 34 book chapters.

Beginning with 2014, I served as Head of the Discipline of Diabetes, Nutrition and Metabolic Diseases of the "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi and

as local coordinator of the Diabetes, Nutrition and Metabolic Diseases residency programme. I have been a member of the Council of Second Medical Department from 2016 and I served as a member of the Council of the Faculty of Medicine between 2016 and 2020. Beside the student lectures included in my didactic duties of associate professor, I hold as very important the residency training programme, both with resident physicians in the Diabetes, Nutrition and Metabolic Diseases specialty and with residents in other medical specialties.

I have coordinated or I have participated as an invited speaker to a broad range of post-academic lectures organized by the "Grigore T. Popa" University of Medicine and Pharmacy, Iași Medical College or other professional societies and associations. I am often invited to speak and share my clinical and academic experience during various medical congresses in the country and abroad, serving the interests of both my specialty and other medical fields (internal medicine, nephrology, cardiology, surgery, geriatrics, genetics, infectious diseases, endocrinology, gynaecology, etc.)

I am a member of multiple professional societies:

- Vice-president of the Romanian Society of Diabetes, Nutrition and Metabolic Diseases
- Secretary of the Nutrition Society of Romania
- Member of the Romanian Federation of Diabetes, Nutrition and Metabolic Diseases
- Member of the European Association for the Study of Diabetes
- Member of the Society of Physicians and Naturalists Iași

Scientific activity

In order to develop my scientific research abilities, I took a high number of lectures, in the country and abroad, where I learned the Good Clinical Practice rules and notions of medical statistics. The most important of these lectures are:

- 2001 – "Study Site GCP" lecture, organized by the Brookwood International Academy of Healthcare Research
- 2004 – "Introduction to Good Clinical Practice" lecture, organized by Vienna School of Clinical Research (completion certificate no. 12 from June 08, 2004)
- 2014 – "Statistics and Data Interpretation, Medical Writing" lecture in Warsaw

I also took part, first as a co-investigator, and then as a principal investigator in more than 25 clinical studies, some of them remaining as benchmark trials in the history of modern Diabetology (DIRECT, RECORD, LEAD, etc.). Participation in these studies taught me once more rigour and discipline needed to coordinate and finalize them.

I completed my PhD thesis, named **"Diagnosis and treatment of diabetic nephropathy – the value of some biochemical markers in estimation of nephropathy in type 1 diabetic patients"**, under the coordination of Professor Maria Covic, MD, PhD and I publicly presented its final results in 2007. The above-mentioned PhD thesis approached a modern, interdisciplinary issue situated between the domains of diabetology and nephrology, namely diabetic nephropathy in patients with type 1 diabetes mellitus. This PhD research performed the assessment of the prevalence and characteristics of diabetic nephropathy, the study of its risk factors and the analysis of some novel biochemical markers in the evaluation of diabetic nephropathy in patients with type 1 diabetes mellitus. The PhD thesis was based on a descriptive transversal study on two groups of patients with type 1 diabetes mellitus

aimed at reflecting both the classical risk factors for diabetic nephropathy and the correlations between the renal function and two new markers, adiponectin and cystatin C. Many of the conclusions in the thesis can be regarded as reference values in Romania and alert factors for enhancing the quality of type 1 diabetic patients monitoring.

In the years after the completion of my PhD thesis, my research interests have progressively widened. I have kept preoccupied by the same two fundamental directions wherein I built up my medical and academic training, the medical care of diabetes mellitus and clinical nutrition, but I gave them new dimensions through my research. I have been actively involved in the design and coordination of two national epidemiological studies aiming to determine the prevalence of diabetes mellitus, of its complications, of the other metabolic diseases and of the renal impairment in the adult population of Romania. Part of this epidemiological research is still ongoing. I am very interested in the analysis of cardiovascular disease in patients with diabetes mellitus, with a special emphasis on the study of specific clinical forms such as heart failure, peripheral artery disease and cerebrovascular disease. I was also involved in a research project aiming to study subclinical cardiovascular disease in subjects with prediabetes, which can be considered the first step in the hyperglycaemic continuum and a risk factor for the subsequent development of diabetes mellitus. Another direction of my scientific development was the interactive field of digestive diseases (anomalies of gastrointestinal motility, the metabolic fatty liver and the chronic hepatitis C) associated with diabetes mellitus and other metabolic diseases. Last but not least, I am continuously expressing a special interest in the field of clinical nutrition, which can be seen in my personal scientific achievements seen on the topics of specific ethical problems, healthy dietary patterns and of nutrition in some digestive diseases.

I published the results of my scientific research activity in articles Web of Science Core Collection indexed articles, as well as in papers belonging to other international databases. I have also disseminated my scientific results at local, national and international congresses, as well as various conferences, seminars or workshops. Up until now, the results of my scientific research activity have represented the base for 33 papers rated by Clarivate Analytics Web of Science Core Collection, 32 papers indexed by other international databases, other 13 papers in various publications, 36 abstracts rated by Clarivate Analytics Web of Science Core Collection and 60 other abstracts. These articles have drawn a total of 164 citations in Clarivate Analytics Web of Science Core Collection publications and 311 citations in Google Scholar. I have participated at multiple international and national congresses or conferences.

I was part of the teams of 3 different research projects:

- Internal research grant of the "Grigore T. Popa" University of Medicine and Pharmacy, Iași, 2010-2011: *Metabolic markers in patients with early glucose intolerance – association with incipient cardiovascular disease* – **project director** – financing contract no. 17077 from September 30, 2010

- *SIMOPAC* Project ("*Integrated informatic system for patients' identification and monitoring")*, PN II Programme, 4th Programme „Partnerships in priority domains": financing contract no. 11-011 from September 18, 2007, PC 3071, project director for the 2nd partner – Professor Mariana Graur, MD, PhD

- SaIN project (Santé Instruction Nutrition): an educational grant financed by contest by the Agence Universitaire de la Francophonie – **project director;** this grant was developed in

multiple directions: nutritional education in schools, research on the population health status in relationship with the nutritional features in regions of Romania, Bulgaria and Moldavia, results dissemination by conferences inviting all partners in this consortium and publication in French language journals. A wide range of educational brochures to support nutritional education in schools was developed and printed, having different contents for three school ages (6-10 years, 11-14 years, and 15-18 years), based on their understanding abilities. We also developed nutrition education guides in Romanian and French. The colleagues from our discipline used these materials during educational school meetings in Iași and neighbouring settlements, where their presentations on the topic of healthy eating were welcomed by students and teachers in these schools. The research on the population health status in relationship with the nutritional features in regions of Romania, Bulgaria and Moldavia is currently under way.

For my professional and scientific activity, I received several awards:

- The Youth Award conferred by the Romanian Society of Diabetes, Nutrition and Metabolic Diseases “for the scientific activity and sustained involvement in the medical care diabetes mellitus persons” – 2005

- The Annual Award conferred by the Romanian Society of Diabetes, Nutrition and Metabolic Diseases – 2015

- Multiple awards for the results of the scientific activity of my team.

CHAPTER 1. THE NATIONAL EPIDEMIOLOGICAL PROFILE IN METABOLIC AND RENAL DISEASES

1.1. State of the Art

Diabetes mellitus is a metabolic disease with a continuously increasing prevalence, especially in developing countries. This increase is mostly due to the alarmingly outgrowing number of cases of type 2 diabetes, originating in an unhealthy lifestyle and an increased prevalence of obesity (*International Diabetes Federation, 2019*). According to the most recent available data, more than 463 million persons with diabetes existed worldwide in 2019, and their number is predicted to outrun 700 million in 2045 (*International Diabetes Federation, 2019*). Two-thirds of people with diabetes live in urban areas and three out of four are adults of active age (20 to 64 years) (*International Diabetes Federation, 2019*).

Given the chronic complications it may induce, diabetes mellitus is a major public health problem. Diabetes is a major risk factor for cardiovascular disease and, if not efficiently treated, it may lead to blindness, renal disease or lower limb amputations. Moreover, diabetes predisposes to severe infections such as tuberculosis or HIV infection and AIDS (*International Diabetes Federation, 2019*). Fortunately, the risk for these complications is considerably lower if diabetes is diagnosed early and treated correctly. On the other hand, type 2 diabetes evolves silently and without symptoms in most cases, and therefore pre-diagnostic hyperglycemia is often prolonged and many patients present with chronic complications the time diabetes is discovered (*American Diabetes Association, 2020*).

The reported prevalences for diabetes mellitus and prediabetes display large fluctuations from one country to the other, both in Europe and worldwide. These differences are due to the methodology used to select study populations, to the various sets of criteria used for diabetes diagnostic in the last decades, as well as to the uneven prevalences of diabetes risk factors in the studied populations (*Sociedade Portuguesa de Diabetologia, 2014*; *Soriguer et al, 2012*; *DECODE Study Group, 2003*). Moreover, many countries do not hold yet their own studies to assess diabetes prevalence in their own territories, in spite of the obvious importance of such endeavours, given the marked increase in the prevalences of obesity and metabolic syndrome. Thus, the only European countries having nation-wide studies to assess diabetes and prediabetes in 2015 were Portugal, Spain and Iceland (*Valdés et al, 2007*; *Núñez García et al, 2006*; *Boronat et al, 2006*; *Catalá Bauset et al, 2006, Gardete-Correia et al, 2010*).

The metabolic syndrome is defined as a complex of risk factors for cardiovascular disease: abnormal values of serum glucose levels, high blood pressure, increased triglyceride levels, low HDL-cholesterol levels and abdominal obesity. Even though this association between cardiovascular risk factors was postulated years ago, the pathogenesis of the metabolic syndrome is still unclear; insulin resistance was progressively attributed a more and more important role in the determinism of the metabolic syndrome during the last years. The increasing prevalence of obesity and the sedentary lifestyle induce a continuously increasing prevalence of the metabolic syndrome, which thus becomes a clinical and public health problem. Subjects with metabolic syndrome exhibit atherogenic dyslipidemia, high blood pressure and serum glucose levels, as well as a prothrombotic și proinflammatory status. The

atherogenic dyslipidemia is a term describing several lipoprotein anomalies: increased levels of triglycerides and apoprotein B, a high number of small dense LDL-cholesterol particles and low levels of the HDL-cholesterol. Most individuals with metabolic syndrome depict abdominal obesity and insulin resistance (*Alberti et al, 2009*). The metabolic syndrome is not an absolute risk factor, since it does not comprise many of the factors producing this absolute risk, such as age, gender, smoking status or the LDL-cholesterol level. Nevertheless, subjects with metabolic syndrome have a double risk for developing cardiovascular disease in the next 5 to 10 years, when compared with individuals without metabolic syndrome (*Alberti et al, 2009*). Moreover, the existence of the metabolic syndrome determines a 5-fold increase in the risk for type 2 diabetes (*Alberti et al, 2009*). An exhaustive analysis of the whole range of cardiovascular risk factors, including chronic kidney disease or smoking, should also be performed in all diabetic patients, in order to identify the subjects at high or very high cardiovascular risk and treat them intensively and in a multifactorial approach.

Intensive glycemic control in diabetes mellitus is already acknowledged to reduce the risk of microvascular complications and, to a lesser extent, the one of macrovascular complications (*Stratton et al, 2000*). A recent meta-analysis demonstrated that HbA1c target achievement is low, with a pooled average of 43% worldwide (*Khunti et al, 2018*). Level of education may influence the therapeutical success, as higher education degrees seem to be associated with more positive health behaviors, such as a higher adherence to medication and to lifestyle optimization, once diabetes and other metabolic diseases are diagnosed (*Borrell et al, 2006*). A complete gap of evidence exists on the topic of diabetes-targeted drug therapies in Romania. Even though international guidelines (*American Diabetes Association, 2020; Davies et al, 2018; Garber et al, 2019*) substantially changed their recommendations in the last years, in order to prevent cardiovascular complications, a lag in the practice translation of these guidelines is often seen worldwide.

My preoccupations in this research field were materialized in the following papers:

Published papers in this field

1. Mota M, Popa SG, Mota E, Mitrea A, Catrinoiu D, Cheta DM, Guja C, Hancu N, Ionescu-Tirgoviste C, Lichiardopol R, ***Mihai BM***, Popa AR, Zetu C, Bala CG, Roman G, Serafinceanu C, Serban V, Timar R, Veresiu IA, Vlad AR. Prevalence of diabetes mellitus and prediabetes in the adult Romanian population: PREDATORR study. *J Diabetes* 2016; 8(3): 336-344.
2. Popa S, Moţa M, Popa A, Moţa E, Serafinceanu C, Guja C, Catrinoiu D, Hâncu N, Lichiardopol R, Bala C, Popa A, Roman G, Radulian G, Timar R, ***Mihai B***. Prevalence of overweight/obesity, abdominal obesity and metabolic syndrome and atypical cardiometabolic phenotypes in the adult Romanian population: PREDATORR study. *J Endocrinol Invest* 2016; 39(9): 1045-1053.

3. Moţa E, Popa SG, Moţa M, Mitrea A, Penescu M, Tuţă L, Serafinceanu C, Hâncu N, Gârneaţă L, Verzan C, Lichiardopol R, Zetu C, Căpuşă C, Vlăduţiu D, Guja C, Catrinoiu D, Bala C, Roman G, Radulian G, Timar R, ***Mihai B***. Prevalence of chronic kidney disease and its association with cardio-metabolic risk factors in the adult Romanian population: the PREDATORR study. *Int Urol Nephrol* 2015; 47(11): 1831-1838.
4. Popa SG, Moţa M, Mihălţan FD, Popa A, Munteanu I, Moţa E, Serafinceanu C, Guja C, Hâncu N, Catrinoiu D, Lichiardopol R, Bala C, ***Mihai B***, Radulian G, Roman G, Timar R. Associations of smoking with cardiometabolic profile and renal function in a Romanian population-based sample from the PREDATORR cross-sectional study. *Eur J Gen Pract* 2017; 23(1): 164-170.
5. Serafinceanu C, Elian V, Catrinoiu D, Guja C, ***Mihai B***, Moţa M, Timar R. Clinical and therapeutic characteristics of patients with type 2 diabetes mellitus in Romania – MENTOR Study. *Rom J Diabetes Nutr Metab Dis* 2018; 25(4): 409-418.

1.2. The PREDATORR study

1.2.1. Rationale of the PREDATORR study

Before 2014, Romania did not hold exact estimates for the prevalence of diabetes mellitus and other metabolic diseases. The International Diabetes Federation (IDF) estimated a 9.3% prevalence of diabetes mellitus in Romania (*Guariguata et al, 2014*). A 2010 indirect estimate, based on data from the National Programme for Diabetes, a national health insurance programme granting free pharmacological therapy to patients with diabetes, revealed that 803,489 individuals were beneficiaries of this programme; this number was the equivalent of a prevalence of 4.2% (*Mota et al, 2013*). All these data were incomplete and correct estimates for the number of undiagnosed or unreported cases of diabetes were not possible.

Previous data on the prevalences of obesity and metabolic syndrome were at an even worse level, as information on this topic was limited and sporadic in Romania. Nation-representative figures on the prevalences of overweight, overall obesity, abdominal obesity and the metabolic syndrome were not available in the adult population of Romania before 2014. Similarly, Romania had very few data reflecting the prevalence of the chronic kidney disease previous to 2014. The only existing informations issued from the National Health Evaluation Program, which was performed by the Romanian government between the years 2007-2008, but these data were flawed by their limitation to both a single region of the country and to the isolated evaluation of the serum creatinine and an urine sample in individuals considered to have an increased risk. This study showed a 6.69% prevalence when the Modification of Diet in Renal Disease (MDRD) Study formula for the estimation of the glomerular filtration rate was used and a 7.32% prevalence when the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used (*Cepoi et al, 2012*).

Based on the uncertain epidemiological resources existing in Romania before 2014, I joined colleagues from all other research centers in the country in order to conduct a national representative study (PREvalence of DiAbeTes mellitus, prediabetes, overweight, Obesity,

dyslipidemia, hyperuricemia and chRonic kidney disease in Romania – PREDATORR) including participants from the entire country, which aimed to estimate in a cross-sectional analysis, for the first time in the adult population of Romania, the prevalences of diabetes mellitus, prediabetes, overweight and obesity, dyslipidemia, hyperuricemia and chronic kidney disease.

1.2.2. Materials and methods

The PREDATORR study took place between December 2012 and February 2014. The public database of the National Health Insurance Agency was used to randomly select 101 general practitioners, equally distributed in each of the eight historical regions of Romania. Patients participating in the study were also randomly selected, using the practice databases of the physicians included in the PREDATORR study. The overall number of adults aged 20 to 79 years was 2728, which we considered to be statistically significant and representative for Romanian population, based on the 2002 Romanian Census. The inclusion criteria were: age of 20 to 79 years, individuals born and having their residence in Romania, mostly living for the past ten years on the territory of Romania, being included on a general practitioner's list, not pregnant, and not lactating. Each of these persons signed an informed consent before any study-related procedure took place. The study was conducted according to the International Conference on Harmonisation/Good Clinical Practice standards and was approved by the Romanian National Ethics Committee. The PREDATORR study potentially included four visits to the general practitioner's office for each of the eligible participants.

During the first visit, socio-demographic data (age, gender, marital status, education level), lifestyle and physical activity-related data, family and personal history (diabetes mellitus in the subject or his/her family, arterial hypertension, dyslipidemia, obesity, concomitant medication, smoking status) were collected, and the clinical examination (height, weight, waist circumference, systolic and diastolic blood pressure) was performed. On the second visit, fasting blood samples and urine samples were taken. The third visit took place only in subjects known with diabetes or when diabetes diagnostic was not possible during the second visit; in such cases, fasting glycemic samples were taken, HbA_{1c} was determined and an oral glucose tolerance test with 75 grams of glucose was performed. The fourth visit took place three months after the second visit and was performed only in individuals not previously known with chronic kidney disease where this diagnostic was not set during the second visit; during this fourth visit, serum creatinine was determined, in order to calculate the estimated glomerular filtration rate (eGFR), and spot urine samples were taken in order to determine the urinary albumin:creatinine ratio (ACR) (*Moța et al, 2016*; *Moța et al, 2015*).

In line with international definitions, the term of overweight was assigned to BMI values of 25 to 29.9 kg/m^2, obesity – to BMI values over 30 kg/m^2 and abdominal obesity – to waist circumference values of 80 cm or more in women and 94 cm or more in men. We used the current definition for the metabolic syndrome which was issued in 2009 by the International Diabetes Federation (IDF) and the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI). The metabolic syndrome diagnostic is possible when 3 out of the next five criteria are met (*Alberti et al, 2009*):

- High values of waist circumference (population-specific and country-specific definitions apply for waist circumference; for instance, abnormal values for adult Europid population are 94 cm or more in men and 80 cm or more in women)
- High values of serum triglycerides (150 mg/dL or more); drug therapy already initiated for high triglycerides values constitutes an alternate indicator
- Low values of HDL-cholesterol (40 mg/dL or less in men and 50 mg/dL or less in women); drug therapy already initiated for low HDL-cholesterol values constitutes an alternate indicator
- High values of blood pressure (systolic blood pressure of 130 mmHg or more and/or diastolic blood pressure of 85 mm Hg or more); blood pressure-lowering drug therapy already initiated in a patient with a history of hypertension constitutes an alternate indicator
- High values of fasting plasma glucose (100 mg/dL or more); drug therapy already initiated for high plasma glucose levels constitutes an alternate indicator.

The methodology we used in the PREDATORR study divided the investigated subjects in four distinct cardiometabolic phenotypes, based on their BMI values and the presence of the metabolic syndrome (Table 1).

Table 1. Cardiometabolic phenotypes of obesity in the PREDATORR study

	BMI<25 kg/m^2	BMI≥25 kg/m^2
Absence of the metabolic syndrome	MHL	MHO
Presence of the metabolic syndrome	MUHL	MUHO

MHL=metabolically healthy lean (absence of the metabolic syndrome and BMI<25 kg/m^2),
MUHL=metabolically unhealthy lean (presence of the metabolic syndrome and BMI<25 kg/m^2),
MHO=metabolically healthy obese (absence of the metabolic syndrome and BMI≥25 kg/m^2),
MUHO=metabolically unhealthy obese (presence of the metabolic syndrome and BMI≥25 kg/m^2).

The definition of the chronic kidney disease we used was in line with the 2012 Kidney Disease Improving Global Outcomes (KDIGO) criteria, reflected by an estimated glomerular filtration rate less than 60 mL/min/1.73 m^2 and/or a urinary albumin-to-creatinine ratio of 30 mg/g or more (*KDIGO, 2013*).

1.2.3. Results

1.2.3.1. Epidemiology of glucose homeostasis disorders in Romania

The results of the PREDATORR study showed a 11.6% prevalence of diabetes mellitus in the Romanian population aged 20 to 79 years (95% CI 9.6%-13.6%). Among the total number of diabetes cases, only 9.2% have been previously diagnosed with diabetes, while the other 2,4% were not aware of having diabetes. The prevalence of diabetes mellitus (either previously known or not) significantly increased with age, having the highest value in the age group of 60 to 79 years (Table 2). The prevalence of prediabetes was 16.5% (95% CI 14.8%-18.2%), the highest value in the age group of 60 to 79 years. Diabetes prevalence was higher in men, while prediabetes had a higher prevalence in women.

Table 2. Prevalence (%) of diabetes mellitus and prediabetes in the Romanian population aged 20–79 years, adjusted by population distribution

	Age group (years)			
	20–39	**40–59**	**60–79**	**Overall**
Total population				
NGT	90.2 (88.9–91.6)	67.8 (65.7–69.9)	50.2 (47.9–52.5)	71.9 (69.9–73.9)
Prediabetes	6.9 (5.8–8.1)	18.9 (17.1–20.7)	27.8 (25.8–29.8)	16.5 (14.8–18.2)
Known diabetes	2.7 (2.0–3.4)	10.1 (8.7–11.5)	17.7 (16.0–19.4)	9.2 (7.9–10.5)
Unknown diabetes	0.2 (0.0–0.4)	3.2 (2.4–4)	4.3 (3.4–5.2)	2.4 (1.7–3.1)
Men				
NGT	88.4 (86.7–90.1)	64.5 (63.1–65.9)	49.7 (48.6–50.8)	70.1 (68.0–72.2)
Prediabetes	8 (7.5–8.6)	18.5 (17.7–19.3)	26.1 (25.3–26.9)	16.3 (14.6–18.0)
Known diabetes	3.5 (3.1–3.9)	12.1 (11.5–12.8)	19.7 (19.0–20.4)	10.7 (9.3–12.1)
Unknown diabetes	0.0	4.9 (4.5–5.3)	4.6 (4.3–4.9)	2.9 (2.1–3.7)
Women				
NGT	91.9 (90.2–93.6)	70.6 (69.0–72.2)	50.7 (50.6–51.8)	73.5 (71.5–75.5)
Prediabetes	5.7 (5.2–6.2)	19.2 (18.3–20.1)	29.3 (28.4–30.2)	16.7 (15.0–18.4)
Known diabetes	1.9 (1.6–2.2)	8.4 (7.8–9.0)	15.9 (15.3–16.6)	7.9 (6.7–9.1)
Unknown diabetes	0.5 (0.4–0.6)	1.8 (1.5–2.1)	4.1 (3.8–4.4)	1.9 (1.3–2.5)

Data show adjusted percentages, with 95% confidence intervals in parentheses. NGT, normal glucose tolerance.

Analysis of regional prevalences in historical regions of Romania found the highest prevalence in the region of Muntenia (13.39%), with București-Ilfov region on second rank (12.79%) and Moldova region on third rank (12.38%); we found the lowest prevalence in the Banat region (8.2%).

Univariate analysis found the presence of known diabetes to be significantly associated with overweight, obesity, dyslipidemia and family history of diabetes. Presence of previously undiagnosed diabetes or prediabetes was also significantly associated with overweight, obesity and dyslipidemia; beside this, undiagnosed diabetes was also significantly associated with the male gender, the divorced status, and a family history positive for diabetes. When data were submitted to multivariate logistic regression analysis, the male gender, overweight, overall and abdominal obesity, high levels of serum triglycerides, a widowed status, and a family history of diabetes were all found to be independent predictors for the presence of already diagnosed diabetes; on the contrary, only body mass index (BMI) values of more than 25 kg/m^2, abdominal obesity, and a family history of DM were found to be independent predictors for undiagnosed diabetes (Table 3) (*Moța et al, 2016*). The multivariate logistic regression analysis found overweight, overall and abdominal obesity, and the lower degrees of education to be the only items independently associated with prediabetes (Table 3) (*Moța et al, 2016*).

Table 3. Predictive factors for diabetes and prediabetes (multivariate logistic regression)

	Prediabetes	**Known DM**	**Unknown DM**
Male gender	1.0 (0.8–1.3)	1.5 (1.1–2.1)*	1.7 (0.9–3.1)
Age	1.0 (0.9–1.0)	1.0 (0.9–1.0)	0.6 (0.3–1.1)
BMI (kg/m^2)			
25–29.99	2.3 (1.6–3.2)***	2.9 (1.8–4.7)***	3.9 (1.3–11.5)*
≥30	3.1 (2.2–4.3)***	4.8 (3.0–7.7)***	8.0 (2.8–23.4)***
Abdominal obesity	1.4 (1.1–1.8)*	2.4 (1.6–3.6)***	4.4 (1.6–12.0)**
Hypertension	1.4 (0.9–1.8)	1.3 (0.9–1.8)	1.1 (0.6–2.1)
Hyper-LDL cholesterolemia	1.3 (0.9–1.7)	0.4 (0.3–0.5)***	0.5 (0.3–0.8)
Hypertriglyceridemia	1.3 (1.0–1.6)	1.7 (1.2–2.3)**	1.5 (0.9–2.6)
Hypo-HDL cholesterolemia	1.0 (0.8–1.3)	0.9 (0.7–1.3)	0.9 (0.5–1.6)
Smoking status			
Current smoker	0.8 (0.6–1.0)	0.8 (0.6–1.2)	1.0 (0.5–1.8)
Former smoker	0.8 (0.6–1.1)	0.4 (0.2–0.6)***	1.1 (0.5–2.2)
Sedentary	1.1 (0.9–1.5)	0.9 (0.6–1.2)	1.1 (0.6–2.1)
Marital status			
Widowed	1.1 (0.8–1.6)	2.2 (1.5–3.3)***	1.2 (0.5–2.8)
Divorced	0.7 (0.4–1.1)	0.6 (0.3–1.3)	1.7 (0.7–4.2)
Single	0.5 (0.3–0.9)*	0.6 (0.3–1.2)	1.0 (0.4–2.9)
Low education level	1.7 (1.2–2.3)**	1.1 (0.8–1.7)	1.5 (0.7–3.1)
Family history of diabetes (yes)	0.8 (0.6–1.1)	3.3 (2.5–4.4)***	1.8 (1.03–3.1)*

Data show odds ratios, with 95% confidence intervals in parentheses. Normal glucose tolerance was considered the reference category. *p<0.05, **p<0.01, ***p<0.001. BMI, body mass index; DM, diabetes mellitus; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

1.2.3.2. Epidemiology of other metabolic diseases in Romania

The prevalence of obesity (BMI values of 30 kg/m^2 or more) in the adult population of Romania was 31.90% (95% CI 30.44-33.36%), while the prevalence of overweight (BMI values of 25 to 29.9 kg/m^2) was 34.70 % (95% CI 33.24-36.16%). The highest rates of obesity were found in men and in the age group of 40 to 59 years. The highest rates of obesity were found in women and in the age group of 60 to 79 years. Underweight (BMI values of 18.5 kg/m^2 or less) was present in only 2.20% (95% CI 0.74–3.66%) of the adult population of Romania, with young female subjects in most cases (*Popa et al, 2016*).

The prevalence of abdominal obesity was 73.90% (95% CI 72.44–75.36%) and the prevalence of metabolic syndrome was 38.50% (95% CI 37.04–39.96%), with the highest rates in the age group of 60 to 79 years. Abdominal obesity was more frequent in women, while metabolic syndrome was more frequent among men (*Popa et al, 2016*) (Table 4).

Table 4. Prevalence of metabolic syndrome, overweight/obesity and abdominal obesity in Romanian population aged 20–79 years

	Age groups			Overall
	20–39 years	**40–59 years**	**60–79 years**	
Total population				
MetS	20.00 (18.54–21.46)	45.20 (43.74–46.66)	56.60 (55.14–58.06)	38.50 (37.04–39.96)
Men				
MetS	26.50 (25.04–27.96)	52.00 (50.54–53.46)	56.70 (55.24–58.16)	43.20 (41.74–44.66)
Women				
MetS	13.80 (12.34–15.26)	39.40 (37.94–40.86)	56.40 (54.94–57.86)	34.20 (32.74–35.66)
Total population				
Underweight	4.40 (2.94–5.86)	1.59 (0.13–3.05)	1.47 (0.01–2.93)	2.20 (0.74–3.66)
Overweight	27.20 (25.74–28.66)	36.60 (35.14–38.06)	43.10 (41.64–44.56)	34.70 (33.24–36.16)
Obesity	20.90 (19.44–22.36)	39.40 (37.94–40.86)	37.40 (35.94–38.86)	31.90 (30.44–33.36)
Men				
Underweight	1.49 (0.03–2.95)	1.47 (0.01–2.93)	1.53 (0.07–2.99)	1.51 (0.05–2.97)
Overweight	40.20 (38.74–41.66)	45.00 (43.54–46.46)	47.50 (46.04–48.96)	43.70 (42.24–45.16)
Obesity	20.70 (19.24–22.16)	37.70 (36.24–39.16)	31.10 (29.64–32.56)	29.40 (27.94–30.86)
Women				
Underweight	7.60 (6.14–9.06)	1.70 (0.24–3.16)	1.54 (0.08–3.00)	3.50 (2.04–4.96)
Overweight	14.80 (13.34–16.26)	29.40 (27.94–30.86)	39.20 (37.74–40.66)	26.40 (24.94–27.86)
Obesity	21.10 (19.64–22.56)	40.90 (39.44–42.36)	43.10 (41.64–44.56)	34.10 (32.64–35.56)
Total population				
Abdominal obesity	52.10 (50.64–53.56)	84.10 (82.64–85.56)	91.00 (89.54–92.46)	73.90 (72.44–75.36)
Men				
Abdominal obesity	47.30 (45.84–48.76)	82.80 (81.34–84.26)	82.60 (81.14–84.06)	68.90 (67.44–70.36)
Women				
Abdominal obesity	52.10 (50.64–53.56)	84.10 (82.64–85.56)	91.00 (89.54–92.46)	73.90 (72.44–75.36)

Data show adjusted percentages, with 95% CI in parentheses
MetS, metabolic syndrome (Harmonization definition 2009). Abdominal obesity: waist circumference ≥80 cm in women and ≥94 cm in men. Underweight: BMI<25 kg/m^2, Overweight: BMI=25-29.99 kg/m^2, Obesity: BMI≥30 kg/m^2

In the PREDATORR study, the MHO phenotype of obesity was found at a rate of 31.60% (95% CI 30.14–33.06%), with the highest prevalences seen in the age group of 60 to 79 years. The prevalence of the MUHL phenotype was 3.90% (95%CI 2.44–5.36%). Comparison of MUHL and MHL phenotypes by multivariate logistic regression analysis showed that the former had a stronger association with the cardiovascular risk, prediabetes, diabetes, low levels of HDL-cholesterol and high values of serum triglycerides. MHO phenotype had a stronger association with prediabetes and with low HDL-cholesterol levels. Abdominal obesity was an independent predictor for the existence of MUHL and MHO phenotypes (*Popa et al, 2016*) (Table 5).

Table 5. Factors associated with MUHL and MHO phenotypes (Multivariate multinomial logistic regression)

Variables	MUHL (105 participants) OR (95 %CI)	MHO (847 participants) OR (95 %CI)
Framingham 10–year CVD risk≥10 %	5.8 (2.3–14.9)*	1.3 (0.8–2.1)
Maximum BMI≥25 kg/m^2	1.5 (0.6–3.6)	77.1 (29.9–198.7)*
Abdominal obesity	94.7 (35.9–249.5)*	16.8 (10.6–26.6)*
Prediabetes	2.8 (1.2–6.4)*	2.9 (1.7–5.2)*
Known diabetes	26 (2.4–279.9)*	0.4 (0.1–4.2)
Unknown diabetes	44.7 (12.7–157.3)*	0.9 (0.3–2.8)
Hypertension	0.9 (0.5–1.7)	1.2 (0.8–1.7)
Hypercholesterolemia	2.4 (0.9–6)	1.3 (0.8–2)
Hypertriglyceridemia	59.6 (26.9–132)*	1.3 (0.7–2.3)
Hypo–HDL cholesterolemia	43.2 (18.3–59.5)*	3.1 (1.5–6.5)*
Hyper–LDL cholesterolemia	0.4 (0.2–1.1)	1.2 (0.6–2.1)
Hyperuricemia	0.9 (0.4–2.3)	1.3 (0.7–2.3)
CKD	0.8 (0.2–2.4)	0.8 (0.4–1.7)

The regression analysis was adjusted for covariates (age, sex, educational level, marital status, alcohol drinking, sedentariness, sleep duration). MHL was considered reference category
MUHL metabolically unhealthy lean (MetS present and BMI<25 kg/m^2), *MHO* metabolically healthy obese (MetS absent and BMI≥25 kg/m^2), *OR* odds ratio, *CI* confidence interval, *BMI* body mass index, *CVD* cardiovascular disease, *CKD* chronic kidney disease, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein
* p<0.05

1.2.3.3. Epidemiology of chronic kidney disease in Romania

In the data issued from the PREDATORR study, the prevalence of the chronic kidney disease according to the CKD-EPI equation was 6.73% (95% CI 5.60–7.87) in men and 6.75% (95% CI 5.61–7.89) in women (*Moţa et al, 2015*) (Table 6). When estimated by the MDRD equation, the adjusted overall prevalence of the chronic kidney disease in the adult population of Romania was 7.66% (95% CI 6.45–8.87). The adjusted overall prevalences of the reduced kidney function (an estimated glomerular filtration rate less than 60 mL/min/1.73 m^2) associated with an albumin-to-creatinine ratio of less than 30 mg/g and of albuminuria (albumin-to-creatinine ratio of 30 mg/g or more) associated with normal kidney function (an

estimated glomerular filtration rate of 60 mL/min/1.73 m^2 or more) were 3.31% (95% CI 2.50–4.13) and 2.98% (95% CI 2.21–3.76), respectively (Table 6). A limited proportion of 0.45% (95% CI 0.14–0.74) of the subjects had both albuminuria and reduced kidney function (Table 6).

Table 6. Prevalence of CKD, reduced kidney function and albuminuria in the Romanian population aged 20–79 years

	Age (years)			Overall
	20–39	**40–59**	**60–79**	
Total population				
CKD present (%)	3.69 (2.32–5.05)	4.76 (3.18–6.34)	14.35 (11.14–17.56)	6.74 (5.60–7.88)
eGFR < 60 mL/min/1.73 m^2 and ACR < 30 mg/g (%)	0.78 (0.00–1.70)	1.58 (0.75–2.43)	9.76 (7.28–12.24)	3.31 (2.50–4.13)
eGFR ≥ 60 mL/min/1.73 m^2 and ACR ≥ 30 mg/g (%)	2.91 (1.88–3.94)	2.78 (1.42–4.13)	3.40 (1.48–5.32)	2.98 (2.21–3.76)
eGFR < 60 mL/min/1.73 m^2 and ACR ≥ 30 mg/g (%)	0.00	0.40 (0.40–0.41)	1.19 (0.00–2.41)	0.45 (0.14–0.74)
Men				
CKD present (%)	3.58 (1.62–5.54)	4.71 (2.39–7.03)	14.60 (9.94–19.25)	6.73 (5.60–7.87)
eGFR < 60 mL/min/1.73 m^2 and ACR < 30 mg/g (%)	1.59 (0.27–2.91)	1.28 (0.05–2.52)	8.07 (4.48–11.67)	3.10 (2.31–3.88)
eGFR ≥ 60 mL/min/1.73 m^2 and ACR ≥ 30 mg/g (%)	1.99 (0.52–3.46)	3.43 (1.43–5.42)	4.66 (1.88–7.44)	3.17 (2.38–3.97)
eGFR < 60 mL/min/1.73 m^2 and ACR ≥ 30 mg/g (%)	0.00	0.00	1.87 (0.08–3.65)	0.46 (0.16–0.77)
Women				
CKD present (%)	3.79 (1.82–5.75)	4.81 (2.63–6.98)	14.12 (9.74–18.50)	6.75 (5.61–7.89)
eGFR < 60 mL/min/1.73 m^2 and ACR < 30 mg/g (%)	0.00	1.85 (0.48–3.22)	11.30 (7.32–15.28)	3.51 (2.68–4.35)
eGFR ≥ 60 mL/min/1.73 m^2 and ACR ≥ 30 mg/g (%)	3.79 (1.82–5.75)	2.22 (0.72–3.72)	2.26 (0.39–4.13)	2.81 (2.06–3.56)
eGFR < 60 mL/min/1.73 m^2 and ACR ≥ 30 mg/g	0.00	0.74 (0.00–1.61)	0.56 (0.00–1.51)	0.43 (0.13–0.72)

Data show adjusted percentages, with 95 % confidence intervals in parentheses. CKD was defined as eGFR<60 mL/min/1.73 m^2 and/or ACR≥30 mg/g; reduced kidney function as eGFR<60 mL/min/1.73 m^2 and ACR<30 mg/g and albuminuria as ACR≥30 mg/g and eGFR≥60 mL/min/1.73 m^2
CKD chronic kidney disease, *eGFR* estimated glomerular filtration rate, *ACR* albumin-to-creatinine ratio

In most individuals aged 20 to 59 years, the diagnosis of chronic kidney disease was established based on the albuminuria criterion, while in most subjects older than 60, the diagnosis of chronic kidney disease was based on the reduced kidney function criterion. In both genders, age and cardio-metabolic features such as BMI, waist circumference and systolic blood pressure values, fasting plasma glucose, HbA_{1c}, serum triglycerides and uric

acid levels were significantly higher in the chronic kidney disease group compared to subjects without chronic kidney disease (p<0.01).

Table 7. Clinical and biological characteristics by CKD presence and gender

Variable	**Male**		**Female**	
	CKD present	**CKD absent**	**CKD present**	**CKD absent**
Age (years) (mean, SD)	58.73 (15.26)***	46.62 (14.81)	57.53 (16.19)***	47.05 (14.79)
Marital status (%)				
Widowed	8.0	2.3	21.6	11.1
Divorced	4.6	4.3	4.1	6.4
Single	5.7	16.6	7.2	12.6
Married	81.6	76.9	67	69.9
High education (%)	16.1**	6.8	22.9**	12.5
Sedentary (%)	70.9*	81.3	72.9	80.2
Alcohol drinking (yes) (%)	69.8	78.1	22.9*	37.0
Body mass index (Kg/m^2) (mean, SD)	29.43 (4.42)**	27.86 (4.86)	30.79 (6.88)***	27.39 (6.16)
Waist circumference (cm) (mean, SD)	104.89 (12.88)**	100.04 (14.52)	98.36 (14.59)***	90.38 (15.82)
Fasting glycaemia (mg/dL) (mean, SD)	111.32 (51.07)***	88.91 (29.22)	100.95 (45.08)**	86.01 (25.47)
HbA_{1c} (%) (mean, SD)	6.45 (1.52)***	5.55 (0.84)	5.96 (1.27)***	5.49 (0.79)
HOMA-IR (mean, SD)	4.16 (3.53)***	2.67 (4.15)	4.69 (10.71)*	2.35 (2.38)
Uric acid (mg/dL) (mean, SD)	6.28 (1.79)**	5.72 (1.36)	5.81 (3.06)***	4.40 (1.51)
Systolic blood pressure (mmHg) (mean, SD)	144.57 (23.75)***	133.66 (17.31)	135.48 (20.15)***	127.39 (21.92)
Diastolic blood pressure (mmHg) (mean, SD)	80.95 (14.78)	79.17 (11.44)	79.27 (11.93)*	76.89 (11.37)
Total cholesterol (mg/dL) (mean, SD)	210.80 (52.62)	202.44 (45.09)	218.29 (64.37)	207.85 (76.07)
Triglycerides (mg/dL) (mean, SD)	217.96 (169.59)**	157.77 (123.99)	155.70 (125.98)**	113.77 (68.79)
HDL cholesterol (mg/dL) (mean, SD)	47.35 (16.25)	48.97 (14.73)	57.79 (15.37)	58.47 (15.05)
LDL cholesterol (mg/dL) (mean, SD)	122.28 (45.75)	123.20 (38.31)	129.12 (56.01)	126.92 (73.01)
eGFR (mL/min/1.73 m^2) (mean, SD)	74.45 (27.74)***	100.22 (16.09)	73.30 (32.09)***	100.54 (16.96)
ACR (mg/g) (mean, SD)	121.29 (95.61)**	5.54 (10.51)	190.45 (136.37)**	4.99 (6.18)

HOMA-IR homeostasis model assessment for insulin resistance, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *eGFR* estimated glomerular filtration rate, *ACR* albumin-to-creatinine ratio, *CKD* chronic kidney disease, *SD* standard deviation
* p<0.05; ** p<0.01; *** p<0.001

At univariate analysis, age, diabetes and prediabetes, hyperuricemia, overweight and obesity, abdominal obesity, high serum triglycerides levels, the metabolic syndrome and a family history of kidney diseases were significantly associated with the existence of chronic kidney disease (p<0.001) (*Moța et al, 2015*). Hyperuricemia and hypertriglyceridemia were both found to be significantly associated with the existence of chronic kidney disease (Table 7).

1.2.3.4. Correlations between smoking status, cardio-metabolic features and renal function

Based on the data we collected in the PREDATORR study, a secondary analysis aimed to evaluate the association between smoking status, cardio-metabolic features and renal function in the subjects we investigated.

In the PREDATORR study, 18% of all patients were current smokers, while 30.8% were former smokers. The smoking rates were higher in men, among whom 22.1% of subjects were current smokers and 43.4% were former smokers, than in women, among whom 14.3% of subjects were current smokers and 19.3% were former smokers. Females currently smoking had a longer smoking duration (27.4±11.8 years versus 18.7±12 years, p<0.001) and had started to smoke at younger ages (21.9±6.7 versus 22.9±6.5 years, p=0.04) as compared with former female smokers. Males currently smoking also had a longer smoking duration (30.5±14.2 years versus 23.2±13.4 years, p<0.001) compared with former smokers, but exhibited lower rates of daily tobacco consumption (13.8±7.5 cigarettes/day versus 16.8±10.7 cigarettes/day, p<0.001) (*Popa et al, 2017*).

Females currently smoking displayed higher levels of total cholesterol and LDL-cholesterol compared to non-smoking female subjects, while currently smoking men displayed higher values of serum triglycerides and lower values of HDL-cholesterol compared to male non-smoking subjects. Male former smokers also displayed higher BMI values, a higher prevalence of abdominal obesity, hypertension and hypertriglyceridemia compared to non-smokers. Both male and female subjects that were currently smoking displayed higher values of the estimated glomerular filtration rate than non-smoking and formerly smoking subjects (Table 8).

Subjects in the PREDATORR study displayed lower BMI values in current smokers than in non-smokers and former smokers. The currently smoking subjects had lower values of waist circumference compared to non-smoking subjects. Smoking cessation was found to be an independent predictor for hypertension, but lower blood pressure values were also seen in currently smoking subjects than in non-smoking and formerly smoking subjects (Table 9).

Table 8. Clinical and biological characteristics by smoking status

	Female			Male		
	Non-smokers	**Former smokers**	**Current smokers**	**Non-smokers**	**Former smokers**	**Current smokers**
FPG (mg/dl), mean (SD)	91.4 (29.1)	91.8 (28.5)	86 (26)#&	94.8 (30.9)	97 (35.9)	89.5 (34.9)#&
HbA1c (%), mean (SD)	5.8 (2.6)	5.7 (0.8)	5.5 (0.8)#&	5.8 (0.9)	5.8 (1.1)	5.6 (0.9)#&
HOMA-IR, mean (SD)	4.5 (2.9)	2.7 (2.4)	2.6 (2.3)#&	4.7 (3)	3.1 (2.8)*	2.5 (2.4)#&
HOMA %B, mean (SD)	408.3 (254)	386 (238.5)	419.1 (301.6)	370.8 (242)	458.3 (252.8)	395.2 (244.1)

	Female			Male		
	Non-smokers	**Former smokers**	**Current smokers**	**Non-smokers**	**Former smokers**	**Current smokers**
IGR, %	37.8	35.9	26.2#&	40.5	42.1	30.2#&
SBP (mmHg), mean (SD)	135.5 (23.2)	129.2 (20.9)*	129 (19.7)#	140.1 (19.3)	140.2 (19.6)	134.7 (18.6)#&
DBP (mmHg), mean (SD)	79.2 (12.2)	77.6 (11.7)	77.5 (11.2)	81.3 (11.5)	81.1 (12.6)	79 (11.8)#&
Hypertension, %	63.8	65.9	63.4	56.4	64.0*	63.7
TC (mg/dl), mean (SD)	213.9 (86.8)	214 (46.5)	220.6 (50.4)#	201.9 (43.5)	205.2 (48.2)	205.5 (45.3)
Hypercholesterolaemia, %	63.2	65.4	72.1#	60.0	64.3	62.8
TG (mg/dl), mean (SD)	125.5 (74)	129.4 (80.4)	132.2 (86)	144.3 (94.2)	166.6 (124.6)*	170.7 (129.8)#
Hypertriglyceridaemia, %	29.8	30.6	28.5	35.6	43.6*	41.4
HDL-C (mg/dl), mean (SD)	58.6 (18.3)	59.1 (15.2)	57 (16)	50.4 (14.1)	50 (15.1)	48 (15.5)#&
Low HDL-C, %	33.3	32.0	33.3	24.3	26.8	33.1#
LDL-C (mg/dl), mean (SD)	130.7 (83.7)	129.3 (41.1)	137.8 (45.2)#&	123.6 (37.6)	123.1 (40.6)	124.6 (39.3)
High LDL-C, %	76.9	80.9	84.8#	78.9	75.8	79.5
eGFR (ml/min/1.73 m^2), mean (SD)	90.7 (19.1)	91.4 (19.7)	96.9 (16.8)#&	90.3 (18)	90.4 (18.2)	97.6 (17)#&
CKD, %	9.5	9.5	6.4	10.1	10.9	4.9 #&
IVD, %	30.5	29.0	20.8#	30.9	40.5*	26.7&

* p<0.05 for former smokers versus non-smokers; # p<0.05 for current smokers versus non-smokers; & p<0.05 for current smokers versus former smokers; %: percentage of participants; SD: standard deviation; FPG: fasting plasma glucose; HbA1c: glycated haemoglobin; HOMA-IR: insulin resistance; HOMA%B: insulin secretion; IGR: impaired glucose regulation; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; eGFR: estimated glomerular filtration rate; CKD: chronic kidney disease; CKD-EPI: chronic kidney disease epidemiology; IVD: ischaemic vascular diseases.

Table 9. Factors associated with smoking status (multinomial logistic regression)

	Former smokers OR (95% CI)	**Current smokers OR (95% CI)**
Age	0.98 (0.97–0.99)**	0.96 (0.95–0.98)###
Overweight/obesity	0.97 (0.72–1.31)	0.67 (0.48–0.94)#
Abdominal obesity	1.05 (0.75–1.48)	0.97 (0.67–1.40)
IGR	1.01 (0.80–1.27)	0.99 (0.75–1.33)
Hypertension	1.26 (1.03–1.56)*	1.15 (0.89–1.47)
Hypercholesterolaemia	1.26 (0.95–1.69)	1.40 (1.01–1.96)#
Hypertriglyceridaemia	1.10 (0.84–1.44)	1.14 (0.82–1.59)
Low HDL-C	0.97 (0.74–1.26)	1.39 (1.01–1.91)#
High LDL-C	0.81 (0.58–1.12)	1.07 (0.72–1.58)
CKD	1.23 (0.87–1.74)	0.96 (0.59–1.55)
IVD	1.48 (1.18–1.86)***	1.07 (0.79–1.43)

The analysis was adjusted for covariates (sex, educational level, marital status, alcohol drinking, and sedentariness). 'Non-smokers' was considered the reference category.
* $p<0.05$
** $p<0.01$
*** $p<0.001$ for former smokers versus non-smokers.
# $p<0.05$
$p<0.01$
$p<0.001$ for current smokers versus non-smokers.
OR: odds ratio; CI: confidence interval; HDL: high-density lipoprotein; LDL: low-density lipoprotein; IGR: impaired glucose regulation; IVD: ischaemic vascular disease; CKD: chronic kidney disease.

1.2.4. Discussion

1.2.4.1. Significance of epidemiological data on glucose homeostasis disorders

Overall, 28.1% of the investigated subjects had either a diabetes or a prediabetes diagnosis, and therefore we concluded that almost one third of the Romanian population has some degree of a disordered glucose homeostasis (*Moța et al, 2016*).

The total prevalence of diabetes mellitus in the adult population of Romania was lower than estimates found in earlier studies which used a similar methodology and performed similar research in Spain (13.8%) (*Soriguer et al, 2012*) or Portugal (13.0%) (*Sociedade Portuguesa de Diabetologia, 2014*). On the other hand, these estimates fall within the same range reported by some studies showing moderate to low prevalences of glucose homeostasis disorders (10%-20%) throughout European countries (*DECODE Study Group, 2003*). Nevertheless, prevalence of diabetes mellitus in Romania was superior to values published in 2015 by the IDF for the entire world (8.8%) and for the whole European continent (6.7%) (*International Diabetes Federation, 2015*). Diabetes prevalence in Romania was also higher than the similar estimates in the United States (9.4%) (*Centers for Disease Control and Prevention, 2017*).

PREDATORR study identified cases with unknown diabetes in 2.4% of the population, with 20.6% (one out of five individuals) being newly diagnosed with diabetes and 79.4% having already been diagnosed with diabetes; this detection rate is superior to the rate reported worldwide (50.0%) (*International Diabetes Federation, 2015*). Cases with previously unknown diabetes exerted a lower rate than the rates reported by researchers from Spain (6.0%) and Portugal (5.7%) (*Sociedade Portuguesa de Diabetologia, 2014*; *Soriguer et al, 2012*). The higher detection rate we found in the Romanian population may be interpreted as a positive consequence of the "Prevention and Control in Diabetes Mellitus and Other Nutrition-Related Diseases" National Health Program, which is active in Romania since 2005 (*National Health Insurance House, 2005*).

The overall prevalence of the two prediabetic states, impaired fasting glucose and impaired glucose tolerance, in the adult population of Romania was 16.5%, higher than the 14.8% prevalence of prediabetes reported in the Spanish study (*Soriguer et al, 2012*), but lower than the 27% prevalence of prediabetes reported by the Portuguese researchers (*Sociedade Portuguesa de Diabetologia, 2014*) or the 37% prevalence reported in the United States population (*Centers for Disease Control and Prevention, 2017*).

By applying these estimates to the total adult population of Romania, we can infer that there are approximately two million people with either diagnosed or undiagnosed diabetes currently live in Romania. We must add to this number another more than three million people with prediabetes, which are therefore at high risk of developing diabetes in the next years. These estimates are consistent with numbers already reported for some other European countries such as UK (2.9 million people living with diabetes), Bulgaria (889,000 individuals living with diabetes) or Poland (2.6 million people living with diabetes) (*Diabetes UK, 2011; Doničová et al, 2011*). The results we obtained in the PREDATORR study can be viewed as exerting major practical consequences within the future public health strategies of resource planning and diabetes prevention.

The education status was the only autonomous item we found to be associated with the presence of diabetes. Univariate analysis pointed to the existence of a direct association

between lower degrees in education and the presence of prediabetes and undiagnosed diabetes; however, this association was maintained only for prediabetes after multivariate analysis was applied. Other researchers controlling for the effect of education also found that subjects with lower levels of education expressed a higher risk for developing diabetes than subjects with higher educational levels (*International Diabetes Federation, 2015; Borrell et al, 2006; Guizé et al, 2008*). For instance, individuals having a high school diploma or less showed in the National Health Interview Survey (1997–2002) a 60% higher risk of developing diabetes than subjects having at least a Bachelor degree (*Borrell et al, 2006*). Higher educational levels were suggested to contribute to the overall adherence to behaviors included in a healthy lifestyle, and therefore to be able to prevent diabetes. However, we may describe the effects of higher levels of education as being, most probably, indirect; behaviors belonging to a healthy lifestyle are probably able to prevent weight gain, which may intuitively connect education to a positive effect of preventing diabetes. More than this, education may also promote some supplementary positive health behaviors, such as a higher adherence to medication and to lifestyle optimization, once diabetes is diagnosed (*Borrell et al, 2006*).

Based on these analyses and results, we were able to consider the PREDATORR study as the first national study – and the only one, up to this date – which was able to systematically estimate diabetes and prediabetes prevalences adult population of Romania.

1.2.4.2. Significance of epidemiological data on other metabolic diseases

The PREDATORR study showed high prevalences of overweight and obesity in the adult population of Romania. After age adjustments, the total prevalence of obesity in the Romanian adult population was higher in the PREDATORR study than rates originating from global reports (13%) (*World Health Organization, 2014*) or from overall European reports (16.7%) (*Organisation for Economic Co-operation and Development, 2014*). On the other hand, our results were inferior to rates of obesity prevalence estimated in the United States (34.9%) (*Ogden et al, 2013*).

Previous analyses in the Romanian adult population reported obesity rates of 24% in the SEPHAR study and 26.3% in the CARDIO-Zone study; these estimates were based on measured height and weight (*Cinteza et al, 2007; Dorobantu et al, 2008*). The differences between our results in the PREDATORR study and previous reports may be generated by the use of self-reported information in most of the previous studies, originating in population-based health surveys and subsequent estimates of anthropometric data. However, these types of procedures may underestimate the rates of overweight and obesity. In the PREDATORR study, BMI values were calculated using objective anthropometric data measured during the physical examination of the subjects, thus ensuring optimal quality of results.

The prevalence of abdominal obesity in the PREDATORR study was similar to the results reported by the international IDEA Study; the latter used the same criteria to define and select cases with abdominal obesity among 18 to 80 years old subjects from 63 countries and issued an overall prevalence of abdominal obesity of 56% in men and 71% in women (*Balkau et al, 2007*). As for the prevalence of the metabolic syndrome, the corresponding rates in the adult population of Romania, as reflected by the results of the PREDATORR study, were higher than the approximately 25% rates reported for the metabolic syndrome

prevalence in European adults, (*Grundy, 2008*), and resembling to the rates reported in the United States population, where the prevalence of the metabolic syndrome is estimated at 38.5% (*Ford et al, 2010*).

Subjects with metabolic syndrome exhibit atherogenic dyslipidemia, high blood pressure and serum glucose levels, as well as a prothrombotic și proinflammatory status. The atherogenic dyslipidemia is a term describing several lipoprotein anomalies: increased levels of triglycerides and apoprotein B, a high number of small dense LDL-cholesterol particles and low levels of the HDL-cholesterol. Most individuals with metabolic syndrome depict abdominal obesity and insulin resistence (*Alberti et al, 2009*). Subjects with metabolic syndrome need an early diagnostic, as multifactorial intervention on each of the existing risk factors is the only efficient posibility to prevent cardiovascular diseases. The prevalence of the MUHL phenotype in the PREDATORR study was lower than those reported by the National Health and Nutrition Examination Survey (5%) (*Kaur, 2014*) or by the Finnish type 2 diabetes survey (7.2%) (*Pajunen et al, 2011*), but higher than the rates seen in the Framingham Offspring Study (2.6%) (*Meigs et al, 2006*).

In the PREDATORR study, MUHL subjects had higher values of the BMI and of the waist circumference compared to MHL individuals. It is thus plausible that pre-existing overall excess weight and excessive visceral adipose tissue might explain, at least partly, the existence of metabolically harmful features among the lean participants (*Kramer et al, 2011*). When the prevalence of chronic kidney disease and the 10-year Framingham risk score for cardiovascular disease were analyzed, each of these values were higher in subjects with an abnormal metabolic profile than in individuals with isolated overweight or obesity. These results are similar with findings from previous prospective, observational studies (*Hinnouho et al, 2015; Chen et al, 2014; Seo et al, 2014*), and may suggest that an increased risk for chronic kidney disease and for cardiovascular disease is rather due to metabolic changes rather than to simple, isolated excess weight.

Since the cross-sectional design of the PREDATORR study was one of the main limitations of our research, we were not able to evaluate the subsequent evolution of the cardiometabolic profile, of the renal function or of the cardiovascular risk levels on subjects with MUHL or MHO phenotypes. Nevertheless, these results brought a great value for the health authorities, as they revealed the need to initiate and implement prevention programs aiming to reduce the overall, health and economic burden of obesity in Romanian population. We think that MUHL phenotype should be actively screened for and identified, in order to act with best results on the cardiometabolic profile and on the cardiovascular risk of apparently lean and low-risk individuals (*Popa et al, 2016*).

1.2.4.3. Significance of epidemiological data on chronic kidney disease

A distinct major objective of the PREDATORR study was to evaluate the prevalence of chronic kidney disease in the adult population of Romania and to determine its associations with various cardiometabolic, sociodemographic or life style risk factors. The worldwide prevalence of the chronic kidney disease continuously increased during the last years to overreaching 10% and to become therefore a major public health problem, given the high costs of therapies directed towards end-stage kidney disease (*Eckardt et al, 2013*). In the United States, the prevalence of the chronic kidney disease increased from 10.00% between

the years 1988-1994 to 13.07% in the interval 1999 to 2004; this increase was seen both in males and females (*Coresh et al, 2007*). This escalating trend is to be maintained and continued, given both the population ageing and the increasing incidences of diabetes mellitus (*Meguid El Nahas et al, 2005*).

In the PREDATORR study, we found the prevalence of chronic kidney disease in the adult population of Romania to be inferior to the rates seen in other European and non-European countries, including the United States. In the case of the latter, the most thorough assessment of the prevalence of chronic kidney disease was performed in the Third National Health and Nutrition Examination Survey (NHANES III), where it corresponded to a rate of 11 % (*Coresh et al, 2003*).

As expected, the PREDATORR study found the prevalence of chronic kidney disease to increase with age, from a rate of 3.69% in the 20 to 39 years old age group to a rate of 14.35% in the 60 to 79 years old age group. It is largely acknowledged that increasing age is a major risk factor for chronic kidney disease development. The underlying pathway of this fact subsists in the functional and structural renal changes concerning both the renal cortex and the renal medulla tissue that are induced by the aging process (*Zhou et al, 2008*). Even though other studies found the prevalence of chronic kidney disease to be higher in women than in men, which was suggested to be owed to the more rapid decline with age of the glomerular filtration rate in women (*Zhou et al, 2009*), the prevalence of chronic kidney disease in the adult population of Romania found by the PREDATORR study was similar in both genders in the most vulnerable age group of 60 to 79 years old subjects (14.60 vs. 14.12 %).

Subjects of both genders in our study that displayed chronic kidney disease had higher rates of metabolic syndrome components than subjects without chronic kidney disease. This finding is similar to other results in the medical literature, which showed individuals with metabolic syndrome to have a higher risk for developing chronic kidney disease; at this moment, it is not entirely clear whether the simple existence of the metabolic syndrome is sufficient as to be associated with a higher risk for renal damage, or the latter is induced by the combined action of multiple risk factors (*Gluba et al, 2013*).

Arterial hypertension was found not to be associated with a higher risk for chronic kidney disease in the PREDATORR study. This unexpected lack of association between hypertension and chronic kidney disease might be underlain by the fact that blood pressure values were satisfactorily controlled in most subjects in the PREDATORR study, with systolic and diastolic blood pressure values nearly reaching the targets in most patients. As uncontrolled blood pressure values, and not the simple hypertension diagnostic, are the major risk factor for chronic kidney disease development and for its rapid progression, this fact is able to explain our results (*Segura et al, 2011*).

In the PREDATORR study, both hyperuricemia and hypertriglyceridemia were found to be significantly associated with the existence of chronic kidney disease. High serum values of the uric acid were recently recognized as another potential risk factor for chronic kidney disease (*Johnson et al, 2013*). Data supporting the role of hyperuricemia in the development of chronic kidney disease in humans are provided by a meta-analysis published by Wang et al. (*Wang et al, 2013*). Two different subsequent metaanalyses, including fewer studies this time, confirmed this association (*Kanji et al, 2015; Bose et al, 2014*). High values of serum

triglycerides were also proven to be associated with an increased incidence of chronic kidney disease, independently of the co-existence of hypertension and diabetes (*Ryu et al, 2009*).

1.2.4.4. Significance of correlations found between smoking status, cardio-metabolic features and renal function

Smoking is a major risk factor for both cardio-metabolic diseases and chronic kidney disease. Even though the detrimental effects of tobacco consumption on morbidity and mortality are well acknowledged, smoking prevalence is still high in Europe. A 2014 European set of data reveals prevalence amounts between 38% in Greece and 11% in Sweden, with a Romanian prevalence estimated at 27% (*Popa et al, 2017*). Recent evolutions on the territory of Romania are showing a decreasing smoking prevalence in men, but an initial decrease followed by a steady-state in women (*Thun et al, 2012*). However, before the PREDATORR study, effective, nationwide, recent data related to smoking prevalence or to its associations with cardio-metabolic diseases and chronic kidney disease were not available. The PREDATORR study was the first nationwide research to investigate these associations.

The prevalence rate we identified in the PREDATORR study for current smokers was lower than the rates reported by other studies carried on Romanian territory (27% in SEPHAR, 2008; 27% in the Eurobarometer survey, 2014) (*Dorobantu et al, 2008; Bunescu et al, 2008*). A potential explanation for these differences is the distinct structure of the population samples included in each of these studies.

In both genders, smoking subjects were younger, had lower BMI and waist circumference values, better levels of serum glucose and of the estimated glomerular filtration rate when compared to both non-smokers and former smokers (*Popa et al, 2017*). Given the younger age and the lower values of weight and waist circumference, the association of current smoking status with the high values of total cholesterol and the low values of HDL-cholesterol suggests a direct negative impact of smoking on the lipid profile, similar to results seen in other studies (*Naisargi et al, 2013; Slagter et al, 2013*).

Subjects in the PREDATORR study displayed lower BMI values in current smokers than in non-smokers and former smokers, similar to the results obtained by other researchers. Potential explanations for this observation might be the younger age seen in current smokers, but also the negative effects of nicotine on appetite and its positive effects on energy expenditure (*Chiolero et al, 2008*). A finding distinct from ones seen in other studies, the currently smoking subjects in the PREDATORR study had lower values of waist circumference compared to non-smoking subjects. This result may be explained by the younger age of current smokers, as it is acknowledged that a more advanced age is associated with a redistribution of fat tissue from the subcutaneous region to the visceral depots, with no distinguishable change in the BMI values (*Stevens et al, 2010*).

In the PREDATORR study, we found smoking cessation to be an independent predictor for hypertension, but lower blood pressure values were also seen in currently smoking subjects than in non-smoking and formerly smoking subjects. A potential explanation for this finding might be the weight gain following smoking cessation, which might favour increased blood pressure values in former smokers. An alternative explanation might be the younger age and the lower weight of current smokers (*Slagter et al, 2013*). As to the influence of smoking on blood pressure levels, data in medical literature are heterogenous, with some results

showing that chronic smoking associates with lower blood pressure values compared to non-smoking status (*Slagter et al, 2013*), while others show that blood pressure values tend to be higher in male current smokers compared with non-smoking subjects, but the independent effect exerted by chronic smoking on blood pressure is relatively small (*Leone, 2011*).

Both male and female subjects in the PREDATORR study that were currently smoking displayed higher values of the estimated glomerular filtration rate than non-smoking and formerly smoking subjects; this finding might be underlain by the intraglomerular hypertension induced by the direct effect of nicotine, followed by glomerular hyperfiltration (*Noborisaka et al, 2013*).

The high prevalence of current smokers and former smokers in the adult population of Romania compels for a systematic education intervention within the entire population. An essential role in this direction must be attributed to general practitioners, since smoking is particularly associated to an unfavourable cardio-metabolic profile.

1.3. The MENTOR study

1.3.1. Rationale of the MENTOR study

Another nationwide epidemiological study, wherein I had the role of regional coordinator for the North-East region of Romania, is the MENTOR study. MENTOR is the first major, nationwide study trying to assess the most important features of type 2 diabetes: chronic complications, evolution, and therapy options. The MENTOR study was designed as an observational, multicenter, prospective study that aims to evaluate the quality of the metabolic control of patients with T2DM, the therapeutic options used for these patients, as well as the microvascular and macrovascular complications prevalence in order to achieve a better management for Romanian patients with T2DM.

1.3.2. Materials and methods

Study population was selected from T2DM patients attending diabetologists from all over Romania (all the 41 districts and Bucharest) between Oct 2016 and Apr 2018. The sample was estimated to be representative for T2DM population of Romania, taking into consideration the estimate of Romanian population from 2011 Census and diabetes prevalence reported by the PREDATORR Study (*Mota et al, 2016*).

Inclusion criteria were: subjects that meet the criteria for T2DM diagnosis, age between 45 and 75 years old, given informed consent to participate to the study. Main exclusion criteria are presence of type 1 diabetes mellitus, neoplasia, and diseases that can affect life expectancy in the next 3 years. Subjects were randomly screened from the patients that came to the periodical evaluation in each investigator practice, using the criteria of “first arrived” that matched the requirements. The subjects enrolled were stratified by: 2 age groups (45-60 years old and 60-75 years old), 3 diabetes duration groups (<5 years, 5-15 years, >15years) and gender. 1300 subjects were enrolled in this study, with the intention of obtaining valid data for a sample of at least 1050 subjects.

Data from 67 sites from all around Romania was collected in an electronic case report form where all the investigators could enter their patients’ characteristics: age, gender, medical history, concomitant medication, living conditions, education. Physical examination included measurement of weight, height, waist circumference, mid arm circumference, blood

pressure and heart rate. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2).

Blood samples were collected after the patients fasted for over 8 hours, with standard evaluation methods in local labs: fasting blood glucose (mg/dL), complete blood count, HbA_{1c} (%), urea (mg/dL), serum creatinine (mg/dL), uric acid (mg/dL), AST (U/l), ALT (U/l), GGT (U/l), total bilirubin (mg/dL), direct bilirubin (mg/dL), total cholesterol (mg/dL), serum triglycerides (mg/dL), HDL-cholesterol (mg/dL), and albumin-to-creatinine ratio. The co-existence of retinopathy was determined according to the fundoscopy examination performed by an ophthalmologist; findings were classified in non-proliferative and proliferative, based on the presence of new vessels formation. Neuropathy was diagnosed after neurological exam or sensitivity and autonomic testing and was classified as somatic and/or autonomic. Chronic kidney disease was defined according to the KDIGO 2012 criteria as an estimated glomerular filtration rate <60 mL/min/1.73m^2 and/or urinary albumin-to-creatinine ratio (ACR) ACR 30-300 mg/g for more than 3 months (*KDIGO, 2013*).

Study primary endpoints are: death from any cause, major acute cardiovascular events and microvascular events. Secondary endpoints comprise hyperglycemic complications, hypoglycemia, chronic complications incidence, glycemic control, lipids control, renal function and blood pressure control.

1.3.3. Results

The study is still ongoing. The following pages include the results obtained so far by our team. Further data will be collected over a 3 years period in order to have a better image on micro and macrovascular complications evolution, as well as on treatment dynamics in T2DM patients.

1.3.3.1. Baseline data in the MENTOR study

A preliminary analysis of the MENTOR study analyzed a sample of 1093 patients from the total of 1300 patients enrolled, considered representative for the T2DM population from Romania, with most of the patients in the 50-70 years age group. Age distribution was: under 50 years old – 9.42%, between 51-60 – 37.52%, between 61-70 – 47.12%, over 70 – 5.94%. Most of the patients included lived in urban areas (72.18%); 19.85% had university degrees, 59.74% high school degree and only 20,41% primary school.

The mean duration of diabetes was 9.90 years. Both females and males had an average BMI over 30 kg/m^2, with a decreasing trend over the age. 37.85% of males were overweight and 53.84% were obese, and 27.87% of females were overweight and 64.94% were obese. 69% of patients have been already diagnosed with hypertension.

1.3.3.2. Quality of glycemic control in the MENTOR study

60% of patients had their HbA_{1c} over the threshold of 7%, and mean HbA_{1c} varied between 7% and 8%. Metabolic control was better with a diabetes evolution of short duration; females in the 0-9 years diabetes duration group had a mean HbA_{1c} of 7.72%, while those with a diabetes duration for more than 20 years had a mean HbA_{1c} of 8.49%. For males, there were minor differences in HbA_{1c} according to diabetes duration. Still, fasting glycemia was

over target value in all patients' groups, and with an increasing value in parallel with diabetes duration (Table 10).

Table 10. Glycemic control of study population according to gender and diabetes duration

Gender	Diabetes Duration (years)	No.%	FPG (mg/dl)	HbA1c (%)	Hb (g/dl)
Women	0 – 9	53.42%	162 ± 64.12	7.72 ± 1.67	13.24 ± 1.48
	10 – 19	38.90%	177 ± 64.21	8.13 ± 1.67	13.17 ± 1.49
	>20	7.68%	196 ± 64.57	8.49 ± 1.67	13.09 ± 1.49
	Total	100%	171 ± 64.21	7.95 ± 1.67	13.2 ± 1.48
Men	0 – 9	53.64%	165 ± 64.32	7.67 ± 1.66	14.46 ± 1.49
	10 – 19	41.50%	170 ± 64.25	7.77 ± 1.67	14.23 ± 1.49
	>20	4.86%	192 ± 65.61	7.73 ± 1.70	13.13 ± 1.5
	Total	100%	169 ± 64.25	7.72 ± 1.67	14.3 ± 1.49

FPG fasting plasma glucose, HbA_{1c} glycated hemoglobin, Hb hemoglobin

1.3.3.3. Chronic complications of diabetes mellitus in the MENTOR study

All patients included in our study were screened for diabetes complications. 76.66% of the enrolled T2DM subjects had at least one diagnosed chronic complication. A total of 26.16% of subjects had diabetic eye diseases, with the most frequent condition being non-proliferative retinopathy (22.32%). From the patients having proliferative retinopathy, 56% had undergone at least one session of laser therapy. Diabetic neuropathy affected 62.85% of all T2DM patients, with 35.71% of newly diagnosed already having sensitive neuropathy and a percentage increasing to 85.71% in patients with more than 20 years of disease evolution. Diabetic kidney disease was encountered in about 25.89% of the patients (Table 11).

Table 11. Diabetes microvascular complications according to diabetes duration

Duration of Diabetes (years)	No.	No. (%)	Diabetic Retinopathy			Diabetic Kidney Diseases eRFG<60 and/or ACR > 30 mg/g
			NDR	PDR	Laser	
0 – 9	585	53.52	15.90%	2.91%	1.20%	23.25%
10 – 19	438	40.07	27.85%	6.85%	3.65%	26.48%
>20	70	6.41	41.43%	15.71%	14.29%	44.29%
Total	1,093	100.00	22.32%	5.31%	3.02%	25.89%

NDR, non-proliferative retinopathy; PDR, proliferative retinopathy; SN, sensitive neuropathy; AN, autonomic neuropathy; MN, mononeuropathy; eGFR, estimated glomerular filtration rate; ACR, albumin to creatinine ratio

1.3.3.4. Therapy of diabetes mellitus in the MENTOR study

Most of the patients were treated with an association of 2 antidiabetic drugs (44.37%), followed as frequency by monotherapy 28.36%. Overall, the proportion of patients on diet and lifestyle intervention was 2.84%, 55.44% of patients were on oral therapy, 12.99% were on insulin only therapy and 22.51% were on a combination of oral and insulin therapy. Considering patients treated with metformin (69.53% from all study population), 27.50%

were on metformin monotherapy, 13.76% on metformin and sulfonylureas, 8.4% on metformin and dipeptidylpeptidase-4 (DPP-4) inhibitors, 5.5% on metformin and GLP-1 receptor agonists (GLP-1 RA), 20% on metformin and another 2 antidiabetics, 12.24% on metformin and basal insulin, and 6.88% on metformin and multiple insulin injection therapy (Table 12).

Table 12. Treatment options in T2DM patients divided by gender and diabetes duration

Duration of Diabetes (years)	No.	No. (%)	Diet (%)	NA (%)	OAD (% from each population group)				OAD+Insulin (% from each population group)			
					1 Drug	2 Drugs	3 Drugs	Total	2 Drugs	3 Drugs	4 Drugs	Total
0 – 9	585	53.52	2.73	6.83	34.02	28.89	5.30	68.21	8.55	5.98	0.34	14.87
10 – 19	438	40.07	2.74	5.25	12.10	23.29	8.45	43.84	12.56	15.75	0.91	29.22
>20	70	6.40	4.28	7.14	5.71	12.86	2.86	21.43	17.14	24.29	2.86	44.29
Total	1,093	100.00	2.84	6.22	23.42	25.62	6.40	55.44	10.70	11.07	0.73	22.51

No, number; NA, no available data; OAD, oral antidiabetic treatment

From the patients treated with insulin alone or in combination with oral antidiabetics, 52.83% were on basal insulin analogs (detemir or glargine), 28.33% on basal-bolus regimen, 2.31% on basal plus therapy, 2.83% on basal plus-plus therapy and 12.62% on premixed insulin or on a combination of rapid and premixed insulin and 1.08% on NPH (Table 13).

Table 13. Insulin regimens in T2DM patients divided by gender and diabetes duration

Duration of Diabetes (years)	No.	No. (%)	Insulin (% from each population group)						
			Basal	Basal +	Basal ++	Basal bolus	Premix	NPH	Total
0 – 9	585	53.52	14.52	0.51	0.34	4.10	2.39	0.17	22.03
10 – 19	438	40.07	23.05	0.68	2.05	15.06	7.07	0.22	48.13
>20	70	6.40	27.14	4.28	0.00	28.57	5.71	1.42	67.12
Total	1,093	100.00	18.75	0.82	1.01	10.06	4.48	0.27	35.39

Basal, Basal insulin analog; Basal +, Basal insulin analog plus one shot of prandial insulin; Basal ++, Basal insulin analog plus two shots of prandial insulin

1.3.4. Discussion

1.3.4.1. Significance of baseline data in the MENTOR study

The population included in this study has an age and gender distribution and a diabetes duration in accordance with the structure of the population from the latest national census reports and results of the PREDATORR trial (*Popa et al, 2016*; *Serafinceanu et al, 2018*). Based on that, we can consider our data relevant for the Romanian T2DM population. Age distribution was consistent with the normal age distribution for patients with T2DM. As expected, diabetes duration increases with patients' age group and this confirms that nowadays survival with diabetes is much increased due to improved management (*Serafinceanu et al, 2018*). The prevalence of obesity among Romanian adult population was 32% and there were 34.7% overweight subjects in the PREDATORR study (*Popa et al, 2016*). Obesity and overweight are currently on a rising trend. The prevalence of obesity in

our study was higher among women than men, with a significant decrease in BMI with age increase. The decreasing trend of average BMI over the age was probably due to a decrease in muscle mass. However, no change or minor changes were seen in waist circumference and mid arm circumference, supporting our intention to perform bioimpedance measurements in our selected patients.

Mean blood pressure in our group, of whom 69% of patients have been already diagnosed with hypertension, was in the recommended target area for both men and women. No matter how high the hypertension incidence, the surveillance of both general practitioners and diabetologist apparently results in a good control of blood pressure values. By comparison, 72% of US patients with diabetes achieved the targets of blood pressure control (*Stark Casagrande et al, 2013*) and 78% in an Iranian population of T2DM patients (*Esteghamati et al, 2017*). A further analysis should be performed, involving the association of high blood pressure with other cardiovascular complications, in order to identify the patients that are at risk for poor blood pressure control.

1.3.4.2. Significance of data on glycemic control in the MENTOR study

Most of our patients had their HbA_{1c} over the threshold of 7%. Intensive glycemic control of T2DM reduces the risk of microvascular complications and, to a lesser extent, the risk of cardiovascular complications (*Stratton et al, 2000*). In our study, as well as in other observational studies, mean HbA_{1c} varied between 7% and 8%. We observed a higher HbA1c in patients with a longer duration of diabetes and/or older, this being a possible indicator of compliance with guidelines recommendations that suggest looser HbA1c targets in the elderly population. Still, the mean HbA1c for all analyzed groups was above the recommended target. A recent meta-analysis demonstrated that HbA_{1c} target achievement is low, with a pooled average of 43% worldwide (*Khunti et al, 2018*). If we take into account fasting plasma glucose, we identify some trend, with a low percentage of patients reaching targets. A further analysis will be performed in order to determine what were the main factors that play a role in low target achievement. Cardiovascular risk remains high in DM patients and a modified lipid profile as we have encountered also in our group with high LDL and non-HDL and low HDL cholesterol can increase the incidence of cardiovascular events. Hyperlipidemia treatment should be intensified to achieve targets recommended by present guidelines [9], as the main reason for high lipids remains low treatment adherence (Banach et al, 2016).

1.3.4.3. Significance of data on chronic complications of diabetes mellitus in the MENTOR study

All patients included in our study were screened for diabetes complications, so we have a real estimate of complications in this patient population.

From the IDF estimates, one in three people living with diabetes have some degree of diabetic retinopathy and one in ten will develop a vision threatening form of the disease. Diabetic eye disease (DED) was affecting around 30.65% of our T2D patients with 27.64% having various degrees of retinopathy. An intensive treatment of both hyperglycemia and hypertension such as that achieved in the UKPDS trial indicated that a 1% decrement in HbA1c equated to a 31% reduction in retinopathy and a 10-mmHg decrement in systolic blood pressure equated to an 11% reduction in photocoagulation or vitreous hemorrhage

(*Kohner, 2008*). It is important to implement these strategies to limit an already increased diabetic eye diseases prevalence.

In terms of neuropathy, a divergence in the reported prevalence between studies was observed (*Hicks et al, 2019*; *Iqbal et al, 2019*; *Gregg et al, 2007*; *Boulton, 2014*). In our study, as expected, neuropathic complications' prevalence increased with diabetes duration, with no gender differences.

Chronic kidney disease prevalence was 25.89%, similar to that reported in European countries (*International Diabetes Federation, 2017*) but lower than data from US reports in CDC's Chronic Kidney Disease Surveillance System reveal (*United States National Diabetes Statistics Report, 2017*). After adjusting for age, the overall prevalence of complications significantly increased with disease duration.

1.3.4.4. Significance of data on diabetes mellitus therapy in the MENTOR study

Although international guidelines such as the American Diabetes Association (ADA) Standards of Care (*American Diabetes Association, 2020*), ADA/European Association for the Study of Diabetes (EASD) Consensus on the Management of Hyperglycemia (*Davies, 2018*), American Association of Clinical Endocrinologists (AACE)/American College of Endocrinology (ACE) Algorithm 2019 (*Garber et al, 2019*) recommend metformin as first line of therapy even from diagnosis, 2.84% of our patients are on diet only, with no glucose-lowering drug.

In Romanian population, 35.39% of T2DM patients are insulin treated, proportion that is largely consistent with national diabetes reports from other countries that show a prevalence of 15% for insulin monotherapy and 19% for the combination of insulin therapy with oral therapy among people with T2DM in the United Kingdom (*Holden et al, 2014*) and a total insulin treated T2D population of 28% in the USA (*Centers for Disease Control and Prevention, 2017*). Access to the latest insulin molecules substantially diminished human insulin and premixed insulin use. Nowadays in Romania only 12.6% of T2DM patients use premixed insulin. A possible explanation for the high percentage of insulin treated patients in Romania is the fact that insulin treatment is free of charge, so all patients can afford it. In addition, modern therapies such as GLP-1 RA and sodium-glucose co-transporter type 2 (SGLT-2) inhibitors were only recently reimbursed.

This cross-sectional analysis of a Romanian representative T2DM population sample enrolled in MENTOR Study showed a high prevalence of diabetes complications and metabolic alterations (obesity, hypertension, dyslipidemia, etc.). Age, male gender and diabetes duration were associated with a higher risk for poor metabolic control and diabetes complications. Diabetes treatment was comprised of oral medication in over a half of the patients and insulin regimens in over a third.

CHAPTER 2. NOVEL APPROACH IN CARDIOVASCULAR DISEASE ASSOCIATED WITH DIABETES MELLITUS

2.1. State of the Art

Type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) are connected by a bidirectional relationship acting as a vicious circle: the former increases the risk for the latter, while the latter is an important mortality cause for the former (*Kenny et al, 2019*). In the last years, prevalences of both entities are increasing worldwide. According to recent research, approximately one third of type 2 diabetic patients have some clinical form of CVD, and CVD is the identifiable cause of death in half of the patients (*Coutiño-Castelán et al, 2019*).

With other risk factors (aging, obesity, metabolic syndrome, hypertension) either present or missing the picture, diabetes is able to distort heart function by itself and to generate potentially serious cardiac complications such as heart failure. In daily clinical practice, where all the wide spectrum of diabetes-related complications must be managed simultaneously, some clinicians may not consider the early echocardiographic detection of diastolic dysfunction as significant enough, or may lack logistic resources. Such practice-related concerns about the poor cost-benefit ratio of such programs could be outweighed by future extension of scientific evidence to prove the long-term benefits of early detection and prevention. There is also a gap of evidence, or at least scarce proof, of the association of diabetes mellitus (and potentially of its therapies) with some specific forms of cardiovascular disease that are not usually the main outcome of cardiovascular safety trials, such as stroke, atrial fibrillation or peripheral artery disease. Among clinical forms of diabetes-associated atherosclerotic artery disease, cerebrovascular disease is a serious event, able to determine major disabilities and a shorter duration of life. In a large meta-analysis of 102 prospective studies, diabetes mellitus was associated with a 2.27-fold increase in the risk for ischemic stroke when compared with a non-diabetic status (*Emerging Risk Factors Collaboration, 2010*). In some of the most prestigious statistics in the United States, based on the National Health and Nutrition Examination Survey data, peripheral arterial disease prevalence comes as high as 20% to 30% among the diabetes-related population, and duration of diabetes is directly linked with a higher risk of developing peripheral artery disease (*Cheun et al, 2019*). Some clinical evidence also indicates a connection between diabetes mellitus and an increased risk for atrial fibrillation (*Bell et al, 2019*).

Determinism of cardiovascular disease in type 2 diabetes patient is multifactorial, including a wide range of anomalies, which contribute to an accelerated atherosclerosis pathway. These include endothelium, smooth muscle cells, lipoproteins, platelets, and coagulation abnormalities. Even though it has been postulated for an already long time that the full understanding of the pathogenesis of cardiovascular disease can help developing more effective preventive therapeutic approaches, partial and somewhat limited progress has been made until now. This progress includes significant advances in the field of knowledge related to the pathophysiology of the endothelial function, to the role of inflammation, lipoproteins, and glucose metabolism. Nevertheless, these theoretical notions have begun to produce tangible results in the practical approach of cardiovascular risk reduction; for example, use of GLP-1 receptor agonists or SGLT-2 inhibitors has been shown to lower the rates of

cardiovascular morbidity and mortality, compared to other types of antihyperglycemic therapies (*Coutiño-Castelán et al, 2019*). It must be alleged that the increased cardiovascular risk seen in all diabetic patients, and especially in type 2 diabetes individuals, cannot be mitigated with only a monofactorial intervention on plasma glucose control, but it requires a multifactorial, simultaneous action on all cardiovascular risk factors (*Rawshani et al, 2018*). Some of the newer classes of antihyperglycemic drugs have the potential to improve other risk factors except plasma glucose levels, and to offer this way a partial explanation for their protective abilities against major cardiovascular events. In the last years, the existence of cardiovascular disease has become one of the key factors the international guidelines use to counsel decisions on the choice of the second-line antihyperglycemic medication after metformin (*Davies et al, 2018*).

Last but not least, a critical issue that must be approached by current research is the incipient portion of the metabolic continuum, which includes the first subclinical part of the glucose dysregulation disorders. Modern medicine witnesses an alarming increase in the number of patients with prediabetes (*International Diabetes Federation, 2019*), induced by the increased incidence of obesity in the global population. Prediabetes is defined as incipient forms of glycemic abnormalities, including impaired fasting glycemia (IFG) and impaired glucose tolerance (IGT).

Prediabetes can be considered a transition period between normal glucose levels and values which are diagnostic for type 2 diabetes. The same as with overt type 2 diabetes, the synergism between a relatively impaired β-cell function and an increased insulin resistance is considered the main combined pathological pathway that leads to prediabetes. The increased insulin resistance is usually the first to develop, most probably years before prediabetes and then diabetes (*Tabák et al, 2012*). Insulin resistance usually develops first in the skeletal muscle and can be considered as a triggering factor for the impairment seen later in the β-cell function (*DeFronzo et al, 2009*), which is manifested as a decreased glucose-induced insulin release from the pancreatic β-cells (*Khan et al, 2019*). The latter process seems to be biphasic, as a significant increase in the β-cell function seems to occur first, 3 to 4 years before diabetes is diagnosed, then followed by a steep decrease (*Tabák et al, 2012*). As the insulin resistance continues to increase, plasma glucose levels tend to become more and more unregulated, and prediabetes is then succeeded by overt type 2 diabetes.

My preoccupations in this research field have been directed towards the following three important axes:

- Heart disease and diabetes mellitus
- Peripheral artery disease and diabetes mellitus
- Cardiac involvement and its metabolic substrate in prediabetes

The results of my research have been materialized in the following papers:

Published papers in this field

- Heart disease and diabetes mellitus

1. Grigorescu ED, Lacatusu CM, Floria M, ***Mihai BM***, Cretu I, Sorodoc L. Left ventricular diastolic dysfunction in type 2 diabetes-progress and perspectives. *Diagnostics* 2019; 9(3): 121. DOI 10.3390/diagnostics9030121.
2. Grigorescu E-D, ***Mihai BM***, Lăcătuşu CM, Floria M, Popa AD, Botnariu EG, Onofriescu A, Șorodoc L. Analysis of diastolic dysfunction in type 2 diabetic patients with asymptomatic cardiovascular disease. *Acta Diabetol Rom* 2017; 43: 156-157.
3. Grigorescu E-D, ***Mihai BM***, Lăcătuşu CM, Floria M, Popa AD, Botnariu EG, Onofriescu A, Șorodoc L. Relationships between insulin resistance, subclinical inflammation and diastolic dysfunction in type 2 diabetic patients**.** *Acta Diabetol Rom* 2018; 44: 109-111.
4. Grigorescu E-D, ***Mihai BM***, Lăcătuşu CM, Floria M, Onofriescu A, Jaba IM, Șorodoc V, Ceasovschih A, Șorodoc L. Subclinical inflammation as a predictive factor for mitral annular calcification in type 2 diabetic patients without established cardiovascular disease. *Acta Diabetol Rom* 2019; 45: 29.
5. Lăcătușu CM, Grigorescu ED, Stătescu C, Sascău RA, Onofriescu A, ***Mihai BM***. Association of antihyperglycemic therapy with risk of atrial fibrillation and stroke in diabetic patients. *Medicina-Lithuania* 2019; 55(9): 592. DOI 10.3390/medicina55090592.

- Peripheral artery disease and diabetes mellitus

1. Ceasovschih A, Sorodoc V, Aursulesei V, Tesloianu D, Jaba IM, Cozma C, ***Mihai B***, Statescu C, Lionte C, Sirbu O, Stoica A, Vata LG, Coman A, Bologa C, Haliga R, Puha G, Dumitrescu G, Constantin M, Simionov L, Sorodoc L. Study of cardiovascular risk factors in patients with peripheral artery disease. *Atherosclerosis* 2019; 287: E190.
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3. Ceasovschih A, Șorodoc V, Aursulesei V, Tesloianu D, Jaba IM, Dima Cozma C, ***Mihai B***, Stătescu C, Sîrbu O, Stoica A, Tuchiluș C, Anisie E, Grigorescu ED, Lilia Simionov, Obreja M, Șorodoc L. Beyond the arteries in peripheral artery disease. *Internal Medicine* 2018; 15(3): 17-25.

- Cardiac involvement and metabolic substrate in prediabetes

1. ***Mihai B***, Ungureanu D, Petriş A, Lăcătuşu C. Are there any substantial differences in the anthropometric and metabolic profile between patients with impaired fasting glycemia and impaired glucose tolerance? *Rom J Diabetes Nutr Metab Dis* 2013; 20(3): 267-277.
2. ***Mihai BM***, Petriş AO, Ungureanu DA, Lăcătuşu CM. Insulin resistance and adipokine levels correlate with early atherosclerosis - a study in prediabetic patients. *Open Med (Wars)* 2014; 10(1): 14-24.

2.2. Echocardiographic assessment of left ventricular diastolic disfunction in type 2 diabetes mellitus – benefits and limits

2.2.1. Rationale of the analysis of echocardiografic assessment in type 2 diabetes

Type 2 diabetic patients may develop CVD with atypical signs and symptoms, thus compromising proper diagnosis and therapy. Even more so, subclinical manifestations are thus difficult to identify and report, turning the real scale of the problem into an unsolved facet of an otherwise well-studied associated pathology. Researchers have proposed lately an appropriate terminology, and they also have stated the need for screening tools able to identify subclinical disease in diabetic subjects without cardiovascular symptoms (*Schernthaner et al, 2018*). Among these new concepts, the "unrecognized diabetic cardiac impairment" is an expression being used today not only to describe the silent manifestations identified on the rest electrocardiogram (ECG), but also the left ventricular (LV) diastolic dysfunction (DD). Identification of diastolic dysfunction needs a thorough imagistic investigation. If not detected in due time, LVDD can develop into a life-threatening clinical condition of heart failure with preserved, and then reduced ejection fraction. Therefore, early screening tools, which could develop into an efficient, widely used and limited-cost standard practice, are a focus of recent guidelines (*American Diabetes Association, 2020*). LVDD is the most easily identifiable change among the wide range of early subclinical cardiac alterations potentially seen in type 2 diabetes patients. The early diagnosis of LVDD by echocardiography may exert a substantial practical significance in these patients.

At present, the existence of subclinical heart dysfunction can be detected using various techniques (biomarkers and imagistic methods), before the patients reach the more advanced, clinical stages of heart failure. The previously described mechanisms are involved in the development of a restrictive phenotype of diabetic cardiomyopathy, which is also consistent with the imagistic pattern of heart failure with preserved ejection fraction (HFpEF). Within this phenotype, the ejection fraction commonly used to identify heart failure is not a reliable tool to indicate cardiomyopathy in type 2 diabetes, as it may still have normal values even after the LVDD has already set in. In addition to the usual E and A waves' ratio (E/A) (Figure 1), more elaborate imagistic measurements are needed in these patients to adequately evaluate diastolic function and to detect potential heart misfunctions. Such measurements do not exert

any technical difficulty and should become a standard evaluation if the diabetic patient is in sinus rhythm (*Seferovic et al, 2015*).

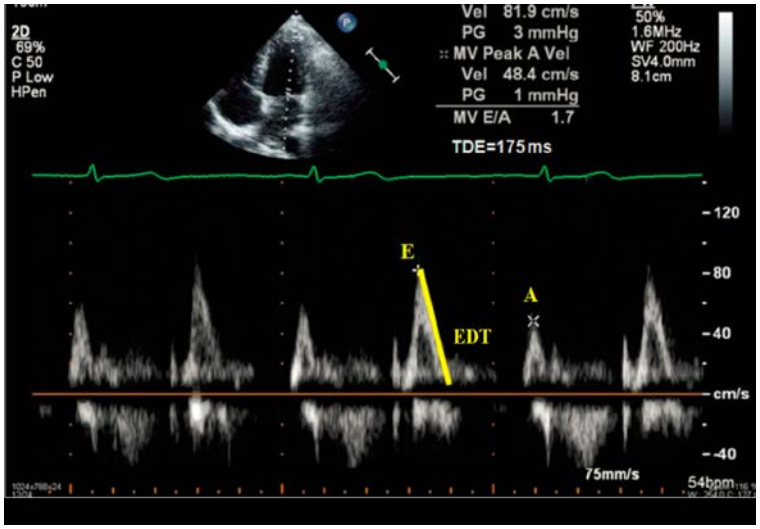

Figure 1. Echocardiographic assessment of mitral flow with pulsed Doppler in a patient in sinus rhythm. A: A wave velocity (cm/s); E: E wave velocity; EDT = E wave deceleration time

2.2.2. Materials and methods

Paper selection

The most well-known, discussed and widely applied set of recommendations for the assessment of diastolic function is the 2009 guideline for the echocardiographic evaluation of left ventricular diastolic function (*Nagueh et al, 2009*). However, a revised version has been published in 2016, which aims to improve effectiveness in clinical practice (*Nagueh et al, 2016*). In our literature search, we mainly focused on the identification of studies which analyzed the patient data in accordance with these latest 2016 recommendations on LVDD. We used a protocol recommended for medical reviews to survey the four major international databases of Medline, Scopus, Science Direct, and Clarivate Analytics Web of Science (*Lang et al, 2006*); the corresponding flow chart is presented in Figure 2.

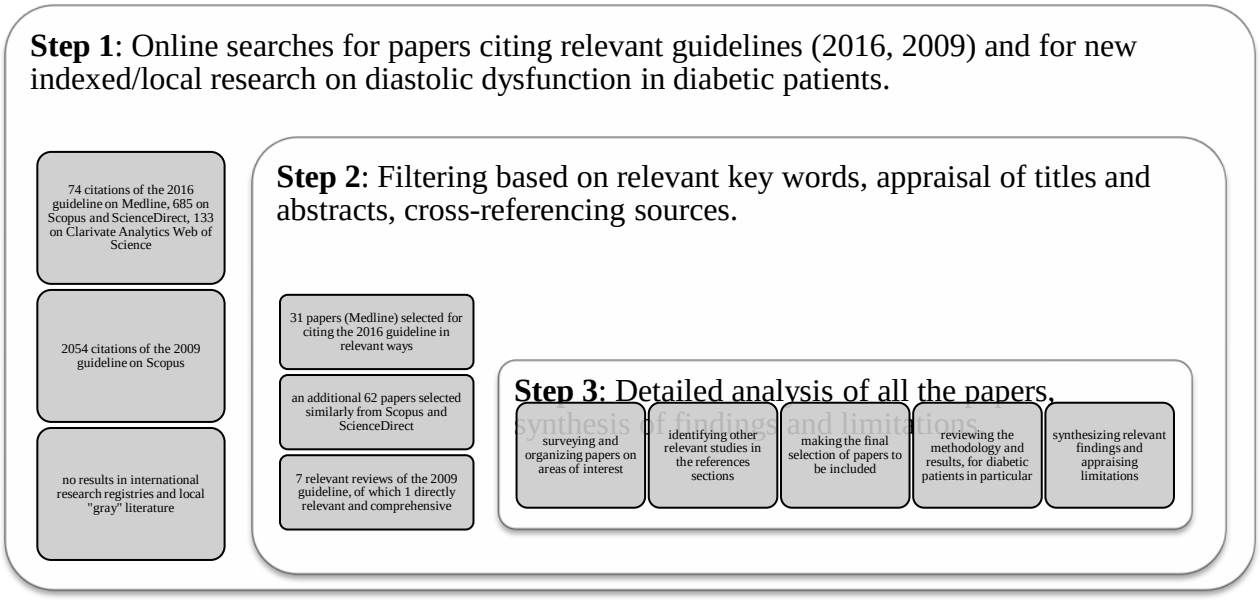

Figure 2. Flow chart of the study

We started from the text of the 2016 recommendations and proceeded to identify online all its Medline citations up to mid-March 2019. We found seventy-four English-written papers citing the new guidelines from various medical perspectives. We screened the titles and abstracts of these papers to select all publications relevant for LVDD diagnosis, classification, or therapy. Based on their use and/or analysis of the new algorithm, on the proportion of diabetic subjects, and on their implications for clinical practice, thirty-one papers were selected in the end. To note, only one study was found to specifically address LVDD in T2DM subjects based on this selecting procedure (*Zoppini et al, 2018*). However, when the other selected papers were analyzed, we found one more publication having had included data from diabetic patients (*Almeida et al, 2017*). In the next step, we reviewed the full text of all papers exerting a partial interest, in order to find mentions of LVDD in patients with diabetes mellitus; by extending our research to the subsequent bibliographic references, we identified 12 additional papers. The same approach was then repeated for Scopus and Science Direct, resulting in a total of 685 citations. These were first filtered down to 187 based on the keywords "diastolic dysfunction" and "diabetes mellitus". Among these, a similar analysis on their titles and abstracts led to a further reduction to 62 papers other than those previously indexed on Medline. In the end, the 133 citations available through the Clarivate Analytics Web of Science search engine were submitted to the same procedure, with no additional results.

Echocardiographic assessment

In our research approach, we searched the papers previously selected for review, looking for any mention of the clinical use of these imagistic parameters within the whole text. Enforcement of these measurement tools was analyzed afterwards based on the correspondence with one edition or other of the guidelines (*Grigorescu et al, 2019*).

2.2.3. Results

When need and opportunity for preventive screening was searched for in the reviewed papers, we found that such programs were not recommended for patients with diabetes until recently. Nevertheless, a recent prospective study with a five-year follow-up and using the 2016 guidelines identified the existence of diastolic dysfunction in 47% of 219 diabetic subjects without cardiovascular complications. In this study, a predictive model based on clinical, ECG, and echocardiographic data was advanced. When diastolic dysfunction was assessed and computed into the model, the predictive power for the risk of adverse events improved (*Gori et al, 2017*). According to these data, screening of asymptomatic diabetic subjects who meet certain criteria, but do not yet express the clinical symptoms of heart failure may be warranted, in order to prevent hospitalization and other disease-related incidents. Assessment of cost-effectiveness of such programs, as well as allocation of the needed resources are difficulties yet to overcome.

The potentially aggravating influence of diabetes on the diastolic function of the patient, taken either *per se* or in association with other factors, was also considered. Increased BMI values, corresponding to overweight and obesity, were found to independently exert deleterious effects on both systolic and diastolic functions. Likewise, some research results suggest that diabetes may act in a similar fashion, and that the coexistence of both may induce

cumulating negative effects. Diabetes was also found to impair diastolic function both in coexistence with and independently from an associated hypertension, which is an already well-known factor to interfere with the normal functioning of the left ventricle (*Ng et al, 2018*).

Mean values of the E/e' ratio were found in several studies to be higher in diabetic patients, including subjects without any overt cardiovascular manifestations (*Zoppini et al, 2018*).

2.2.4. Discussion

Due to its non-invasiveness, transthoracic echocardiography is the standard tool to assess heart function. According to both editions of the 2009 and 2016 guidelines (*Nagueh et al, 2009*; *Nagueh et al, 2016*), estimation of left ventricular relaxation and subsequent filling pressures needs measurement of multiple parameters:

- Peak of passive filling (E wave), peak of active filling (A wave), E/A ratio, deceleration time of E wave (EDT) with pulsed Doppler (Figure 1);
- Isovolumetric relaxation time (IVRT) (Figure 3), septal, lateral, and average early diastolic annular velocities (E'), septal, lateral, and average late diastolic annular velocities (A'), both via tissue Doppler imaging (TDI), which measures myocardial tissue velocities during the cardiac cycle;
- Tricuspid regurgitation peak velocity (TRpV; Figure 4) withCW (continuous Doppler) on tricuspid regurgitation jet;
- S and D wave peak velocity on right superior pulmonary vein flow in pulsed Doppler (Figure 5);
- Left atrial size assessed by area, or better by indexed left atrium volume (LAVi; Figure 6).

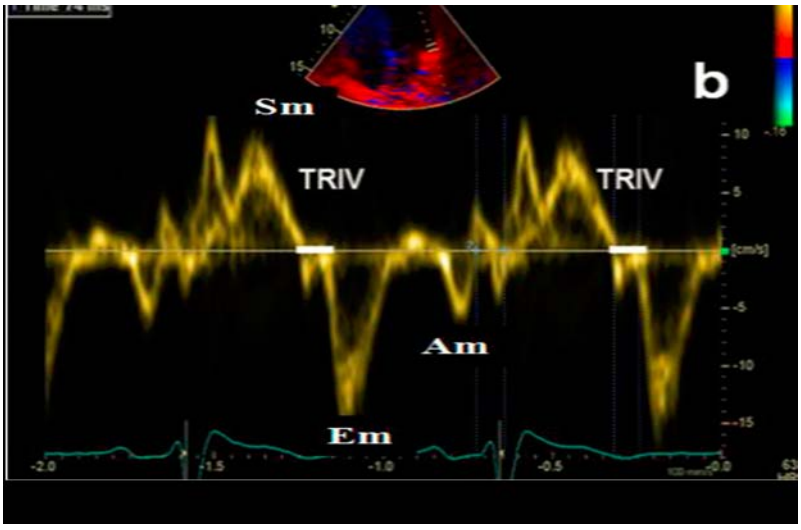

Figure 3. Echocardiographic assessment of isovolumetric relaxation time (IVRT) by Tissue Doppler Imaging (TDI). Am (or A'): septal, lateral, and average late diastolic annular velocity; Em (or E'): septal, lateral, and average early diastolic annular velocity; Sm: systolic annular velocity; TRIV: isovolumetric relaxation

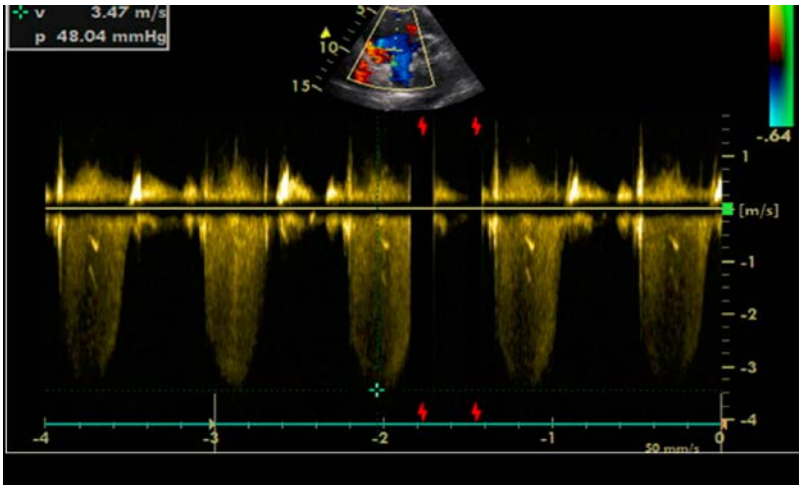

Figure 4. Echocardiographic assessment of tricuspid regurgitation peak velocity (TRpV) by continuous Doppler (CW) on tricuspid regurgitation jet. V: velocity; P: gradient (is V: specific echocardiographic parameter (it does not mean a measurement data)

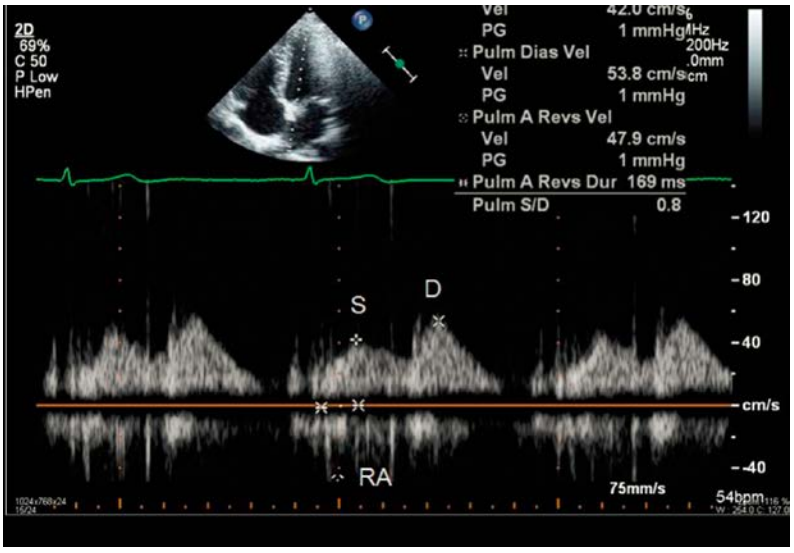

Figure 5. Echocardiographic measurement of S and D wave peak velocity by pulsed Doppler on right superior pulmonary vein flow. D: peak velocity of diastolic wave: RA: peak velocity of reverse atrial wave; peak S: peak velocity of systolic wave

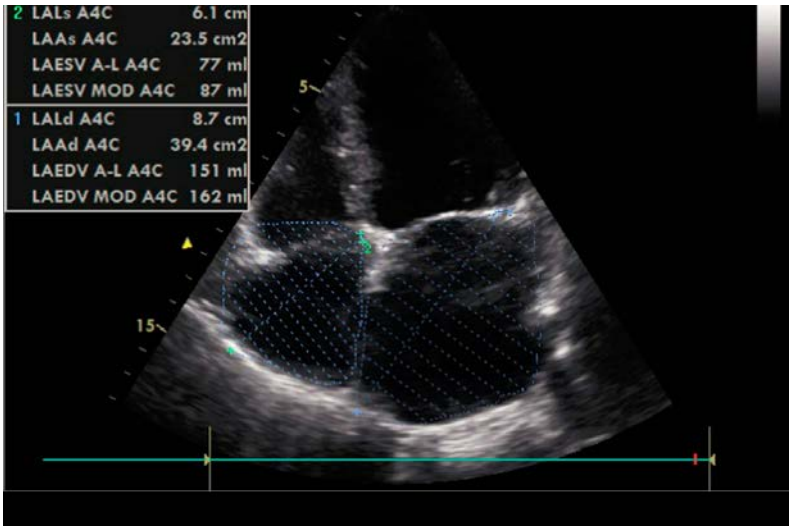

Figure 6. Echocardiographic measurement of left (and right) atrial volume by disks method

The 2016 guidelines revised and extended the 2009 edition, offering more details on the use of these parameters to differentiate between patterns of diastolic function when the left ventricular ejection fraction is normal (Figure 6). In 2019, the guidelines were further expanded, as to facilitate a wider scale understanding and application in routine clinical reasoning and decision-making (*Silbiger, 2019*).

The restrictive definition of systolic dysfunction by low values of the left ventricular ejection fraction has proven inaccurate for the heart failure diagnostic, as symptoms may also occur in subjects with mid-range, or even preserved left ventricular ejection fractions (recently redefined as values of 40-50%, and ≥50% respectively) (*Ponikowski et al, 2016*). Recent evidence proves that diastolic dysfunction is a more valuable indicator for early heart failure, where the patient does not experience any symptoms, and standard assessment reveals normal ejection fraction values (*Ponikowski et al, 2016*). Moreover, assessment of diastolic dysfunction increases the accuracy of heart diseases prognosis, as it is able to reveal the structural alterations within the myocardial cells and matrix. At cellular level, the myocardial stiffness resulting from collagen damage, interstitial fibrosis, and inflammation delays the relaxation period, thus casting negative consequences on the further chain of diastolic filling pressure (*Frati et al, 2017*). Early identification of metabolic and structural changes in the heart, followed by adequate measures, may therefore help avoiding the progressive impairment of cardiac structure and function leading up to irreversible heart failure. Multiple pathways have already been identified as involved in early cardiomyocyte damage and subsequent clinical aggravation: metabolic anomalies (decreased glucose oxidation, increased free fatty acids), impaired intracellular organite functions (inadequate calcium circuits, high levels of oxidative stress and advanced glycation end-product, mitochondrial dysfunction), structural alterations (cardiomyocyte hypertrophy), direct effects of the activation of renin-angiotensin-aldosterone system (fibrosis, cardiomyocyte stiffness), and cardiac autonomic neuropathy. These pathways seem to be triggered by hyperglycemic, glucotoxic and lipotoxic anomalies related to insulin resistance (*Riehle et al, 2019*). A special mention is needed about the difference between these alterations and the classical features included in the old concept of diabetic cardiomyopathy. Since diabetes-related cardiomyopathy was mostly studied in

patients with a long duration of diabetes, the currently circulating definition of cardiomyopathy does not address the previously described pathophysiological anomalies, which tend to be early, insidious, and subclinical. As a matter of fact, it is difficult to single out the individual contributions of hyperglycemia or hyperinsulinism (a consequence of insulin resistance, rather specific for type 2 diabetes) from other risk factors such as hypertension, dyslipidemia, obesity, or coronary ischemic heart disease, as most diabetic subjects in clinical trials feature a combination of these comorbidities (*Jia et al, 2018*).

The question rises as whether a high E/e' index is an indicator, or even a distinctive mark, of early diastolic alterations in diabetes-related cardiomyopathy. However, research assessing early anomalies, which may occur even before the onset of diastolic disfunction, is not yet conclusive. A more comprehensive picture of diabetes-induced heart disfunction, which is in fact a slow phenomenon, may be better developed by the simultaneous assessment of both E/e' ratio and LAVi. A volumetric parameter, LAVi is less susceptible to the variations induced by the subject's cardiac physiology at the time of measurement, because it is a consequence of chronic diastolic dysfunction (*Cameli et al, 2015*).

As the diagnosis and grading of diastolic function are concerned, the latest, 2016 guidelines appear to serve best in identifying the more severe cases. Echocardiographic parameters which are subject to early impairment, such as the diastolic E/e' index, were found to serve as predictors of adverse cardiovascular events. Such tools, while still unable to completely evaluate the subclinical domain, may be useful to select cases classified as "indeterminate", to screen for silent cardiovascular disease and to warrant subsequent monitoring.

The review of existing studies, together with the analysis of other reviews, editorials, and commentaries in the same interval of time, led us to become aware of the limits of thorough investigations and accurate interpretations: equipment model and availability, heterogeneity of studies' methodologies, selection and matching of patients, inter-observer variability, and clinician experience and expertise. Second, specific demographic and anthropological features underlying the study population profile, as well as associated comorbidities, may further interfere with interpretation and results. All these factors have contributed to generate a significant heterogeneity of the selected and analysed literature, from the perspective of scientific outcomes. Even though retrospective, comparative studies may bring some supplementary information, its value is limited by both the methodology itself, and the small number of cases where databases were approached and analysed using updated parameters and formulae.

As few of the existing studies focused entirely on diabetic patients, choosing instead to include only a limited number of T2DM subjects in their cohorts, further studies focusing exclusively on diabetic patients and using robust methodologies of matching active and control groups are needed, in order to increase the level of proof. Moreover, dedicated, prospective research is also needed to identify the slow progression, stagnation, or regression of subclinical cardiac disfunctions over longer amounts of time. Such studies would obviously require the same rigorous design of methodology in order to account for inherently confounding factors and avoid bias. An inter-disciplinary, multi-centric, semi-automated approach of data collection, followed by a thorough statistical processing and interpretation of data, would reduce variability and heterogeneity of results, and also advance our

understanding of asymptomatic cardiovascular disease in type 2 diabetes enough as to enable preventative screening.

2.3. Asymptomatic heart disease in type 2 diabetes mellitus

2.3.1. Rationale for the study of asymptomatic heart disease in type 2 diabetes

I am part of a multidisciplinary team set to investigate asymptomatic heart disease in type 2 diabetes mellitus patients without any clinical signs of cardiovascular disease. For this purpose, we designed a prospective study which enrolled asymptomatic type 2 diabetes patients with inadequate glycemic control from the Clinical Centre of Diabetes, Nutrition and Metabolic Diseases Iași and searched for subclinical heart disfunction and metabolic anomalies in these subjects. The study finished the patients' enrollment period and is currently ongoing with next-scheduled visits. Data presented below belong to interim analyses based on partial collection of information from initial visits and part of the patients.

2.3.2. Materials and methods

Asymptomatic type 2 diabetes patients with inadequate glycemic control willing to participate in this prospective study were enrolled. All patients signed a written informed consent previous to any procedure in the study. Evaluation of subjects included direct anthropometric measurements (weight, height), derived anthropometric measurements (waist circumference – WC, body mass index – BMI), determination of biological parameters (fasting glycemia, HbA_{1c}, lipid profile, uric acid, C-peptide, high-sensitivity C-reactive protein – hsCRP, tumour necrosis factor-α – TNF-α, N-terminal-prohormone brain natriuretic peptide – NT-proBNP), calculation of visceral adiposity index (VAI), the homeostatic model assessment of insulin resistance index (HOMA-IR), the homeostatic model assessment C-peptide-based index (HOMA-C-peptide) and the CHA2DS2-VASc score, ultrasound assessment of mitral annular calcification (MAC), aortic valve sclerosis (AVS) and of parameters estimating diastolic (E/A index, E/Ea index, deceleration time – DT, isovolumic relaxation time – IVRT, left atrial area – LAA, left atrial diameter – LAD, left atrial volume index – LAVi, pulmonary capillary wedge pressure – PCWP) and systolic function (ejection fraction of the left ventricle – LVEF by Simpson biplan).

The database was compiled electronically and, apart from the general descriptive statistics, the following statistical analyses were conducted in SPSS 17.0 for Windows (SPSS Inc., Chicago): the t-test, Spearman correlations, linear regression, logistic regression and area under the ROC curve, at $p<0.05$ statistical significance thresholds.

2.3.3. Results

A first interim analysis on baseline data originating from 70 subjects showed us an overweight and obese population, with 45.7% men, a mean age of 57.96±8.98 years, a mean BMI of 33.31±5.98 kg/m^2 and a mean duration of type 2 diabetes at study entry of 6.47±4.66 years. The mean values of HbA_{1c}, hsCRP and VAI were 8.05±0.97%, 10.35±10.3 mg/L and 6.91±3.85, respectively. The mean values of the echocardiographic parameters were 1.08±0.42 for E/A, 3.45±1.14 for E/Ea, 104.54±20.59 for IVRT, 187.13±45.65 msec for DT and 66.94±9.58% for LVEF. 35% of patients had type 1 diastolic dysfunction. Significant correlations were identified between hsCRP and BMI (r=0.433, p=0.001), hsCRP and DT (r=-

0.397, p=0.002), IVRT and WC (r=0.267, p=0.039), IVRT and HbA_{1c} (r=-0.345, p=0.007), E/A and HDL-cholesterol (r=-0.330, p=0.010) (Table 14). No other statistically significant associations between anthropometric measurements, VAI or hsCRP and other diastolic dysfunction parameters were identified.

Table 14. Analysis of correlations between biochemical, anthropometric and echocardiographic parameters

			hsCRP	**IVRT**	**E/A**	**BMI**	**DT**	**HbA_{1c}**	**HDL**
Spearman's rho	hsCRP	Correlation Coefficient					-		
		Sig. (2-tailed)	1.000	-0.040	0.177	**0.433**	**0.397**	0.042	-0.129
	IVRT	Correlation Coefficient	-0.040	1.000	0.218	0.168	0.016	**-0.345**	-0.059
		Sig. (2-tailed)	0.763	.	0.092	0.196	0.903	**0.007**	0.665
	E/A	Correlation Coefficient	0.177	0.218	1.000	0.070	- **0.351**	-0.181	**-0.330**
		Sig. (2-tailed)	0.169	0.092	.	0.585	**0.005**	0.152	**0.010**

Significance of all abbreviations is explained in the text.

Another more extended interim analysis, using baseline data originating from 106 subjects, revealed a proportion of 44.3% men, a mean age of 57.96±8.98 years, a mean BMI of 33.31±5.98 kg/m^2 and a mean duration of type 2 diabetes at study entry of 6.47±4.66 years. 67.9% of the analysed subjects were obese, 65% had associated hypertension and 70% were dyslipidemic. The mean values of the metabolic parameters and inflammatory markers were: 8±1% for HbA_{1c}, 174.9±43.3 mg/dL for plasma glucose levels, 193.9±47.1 mg/dL for total cholesterol, 103.9±41.4 mg/dL for LDL-cholesterol, 179.5±89.9 mg/dL for serum triglycerides, 5.42±1.37 mg/dL for the uric acid levels, 11.91±15.63 mg/L for hsCRP, and 9.6±17.3 pg/mL for TNF-α. Mean values of insulin resistance indexes were 6.28±4.01 for HOMA-IR and 4.25±1.0 for HOMA-C-peptide. Mean values of echocardiographic parameters were 67.09±9.29% for LVEF, 3.28±0.95 for E/Ea, 1.05±0.40 for E/A, 191.42±42.97 msec for DT, 105.05±18.62 msec for IVRT, 24.48±4.35 cm^2 for LAA, and 43.81±11.83 ml/m^2 for LAVi.

37.7% of patients were identified with MAC and 13.2% with AVS. Prevalence of diastolic disfunction was 38.7%, with the predominance of type 1. Significant correlations were found between HOMA-IR and LAD (r=0.22, p=0.03), HOMA-IR and E/Ea index (r=0.22, p =0.02) (Table 15).

No significant differences depending on the existence of MAC were found, except for HDL-cholesterol (54.48 mg/dL vs 60.2 mg/dL, p=0.029) (Tabel 16). TNF-α was the only inflammatory marker expressing a statistical behaviour of predictive factor for MAC (ROC=0.614, p=0.049, CI=0.503-0.726) (Figure 7).

Table 15. Correlations between insulin resistence and diastolic disfunction

Pearson Correlation Sig. (2-tailed)		**Insulin**	**C-peptide**	**HOMA-IR**	**HOMA-C peptide**	**E/A**	**DT**	**IVRT**	**E/Ea**
Insulin	r	1	**0.73**	**0.90**	**0.60**	0.09	0.07	0.16	**0.24**
	p		0.00	0.00	0.00	0.33	0.42	0.09	0.01
C-peptide	r	**0.73**	1	**0.68**	**0.86**	0.03	0.00	0.17	0.08
	p	0.00		0.00	0.00	0.73	0.95	0.07	0.36
HOMA-IR	r	**0.90**	**0.68**	1	**0.76**	0.12	0.06	0.13	**0.22**
	p	0.00	0.00		0.00	0.20	0.49	0.18	0.02
HOMA-C peptide	r	**0.60**	**0.86**	**0.76**	1	0.02	0.03	0.11	0.07
	p	0.00	0.00	0.00		0.80	0.76	0.23	0.46
E/A	r	0.09	0.03	0.12	0.02	1	**0.29**	0.10	0.08
	p	0.33	0.73	0.20	0.80		0.00	0.28	0.38
DT	r	0.07	0.00	0.06	0.03	**0.29**	1	0.00	0.00
	p	0.42	0.95	0.49	0.76	0.00		0.99	0.92
IVRT	r	0.16	0.17	0.13	0.11	0.10	0.00	1	0.16
	p	0.09	0.07	0.18	0.23	0.28	0.99		0.10
E/Ea	r	**0.24**	0.08	**0.22**	0.07	0.08	0.00	0.16	1
	p	0.01	0.36	0.02	0.46	0.38	0.92	0.10	

Significance of all abbreviations is explained in the text.

Table 16. Baseline features for subjects with and without mitral annular calcification

Parameter	**No MAC**	**MAC**	
	mean	mean	p
Age (years)	57.24	59.13	0.430
DM duration (years)	6.08	5.98	0.796
HbA1c (%)	7.99	8.29	0,177
BMI (kg/m^2)	32.69	33.66	0.397
WC (cm)	109.83	111	0.855
TNF-alfa (pg/mL)	9.84	9.29	0.550
hsCRP (mg/L)	13.49	9.3	0.287
Cholesterol (mg/dL)	194.09	193.58	0.945
HDL-chol (mg/dL)	54.48	60.02	**0.026**
LDL-chol (mg/dL)	107.07	98.73	0.336
Triglycerides (mg/dL)	206.18	190.2	0.265
Uric acid (mg/dL)	5.4	5.45	0.504

Significance of all abbreviations is explained in the text.

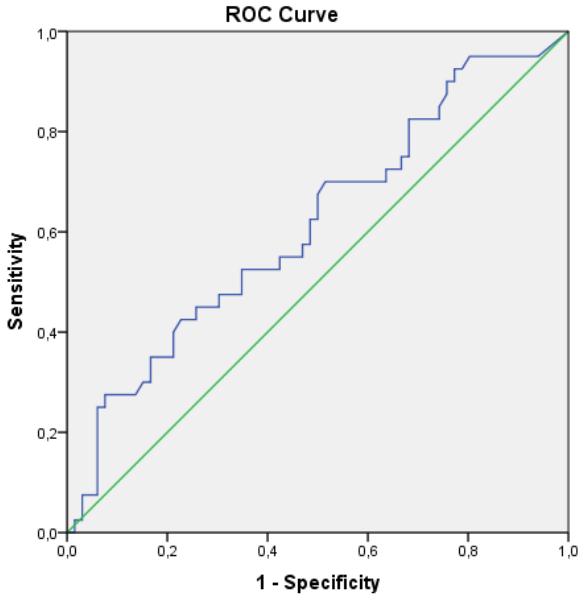

Figure 7. Predictive power of TNF-α for mitral annular calcification

In a third partial analysis on baseline data from 120 patients, the percentage of male subjects was the same (44.3%). The mean age was 58.16±8.72 years, the mean values of BMI were 32.75±5.65 kg/m^2 and the mean duration of type 2 diabetes at study entry was 6.04±4.63 years. The mean values of CHA2DS2-VASc score were 2.73±0.96 and the mean values for NT-proBNP were 106.08±125.41 pg/mL. The mean values of the metabolic parameters and inflammatory markers were: 8.03±0.95% for HbA_{1c}, 103.9±40.68 mg/dL for LDL-cholesterol, 205.19±93.4 mg/dL for serum triglycerides, and 5.41±1.35 mg/dL for the uric acid levels. Values of the CHA2DS2-VASc score equal or higher than 3 were seen in 68 subjects, predicted by type 2 diabetes duration (ROC=0.735, p=0.003), but not by the severity of uncontrolled hyperglycemia as reflected in the HbA_{1c} values. Diastolic disfunction was predominantly of type 1 and was identified in 56.7% of the subjects, with a statistically significant correlation between the E/Ea index and the CHA2DS2-VASc score values (r=0.4, p<0.0001) (Table 17). MAC was identified in 37.5% of patients, but the mean values of the CHA2DS2-VASc score (2.82 vs. 2.68) did not reach statistically significant differences between patients with and without MAC.

The largest interim analysis already performed for this study included the baseline data originating from 138 patients. We found a 47.9% proportion of male subjects, a mean age of 57.86±8.82 years, a mean BMI of 32.65±5.50 kg/m^2 and a mean duration of type 2 diabetes at study entry of 6.16±4.73 years. The mean values of the metabolic parameters and inflammatory markers were: 8.06±0.99% for HbA_{1c}, 103.12±38.96 mg/dl for LDL-cholesterol, 202.57±90.46 mg/dL for serum triglycerides, and 5.74±3.87 for HOMA-IR levels. Mean values of echocardiographic parameters were 67.14±9.35% for LVEF, 6.54±1.84 for E/Ea, 1.09±0.46 for E/A, 192.88±42.76 msec for DT, 104.23±18.74 msec for IVRT, 43.79±11.84 mL/m^2 for LAVi, and 10.01±2.28 for PCWP. 50.4% of patients had type 1 diastolic disfunction.

Table 17. Baseline features for subjects with a CHA2DS2-VASc score below and above 3

Parameter	CHA2DS2-VASc mean	CHA2DS2-VASc mean	p
DM duration (years)	4.98	7.23	0.006
BMI (kg/m^2)	31.5	33.71	0.080
WC (cm)	108.74	110.04	0.850
HbA1c %	7.88	8.14	0.159
Glycemia (mg/L)	169.73	173.03	0.680
E/A	1.23	0.93	0.000
E/Ea	2.91	3.55	0.000
Cholesterol (mg/dL)	194.37	195.53	0.765
HDL-chol (mg/dL)	51.84	60.90	0.001
LDL-chol (mg/dL)	107.99	99.94	0.372
Triglycerides (mg/dL)	206.96	203.84	0.557
Uric acid (mg/dL)	5.39	5.43	0.870

Significance of all abbreviations is explained in the text.

2.3.4. Discussion

Our first preliminary data from this limited number of type 2 diabetic patients revealed a lower prevalence of diastolic dysfunction compared to previous results from other studies. According to current literature, left ventricle diastolic dysfunction is the most frequent, classical early functional anomaly of the left ventricle in type 2 diabetic patients T2DM patients (*Fang et al, 2004*). Subclinical diastolic dysfunction has been identified by other authors in approximately 75% of type 2 diabetes patients without hypertension and overt coronary artery disease (*Boyer et al, 2004*).

Some of the echocardiographic markers correlated with subclinical inflammation, abdominal distribution of the adipose tissue or the quality of glycemic control. Hyperglycemia may determine structural alterations of the myocardial tissue, which includes classical macrovascular and autonomic neuropathy modifications, but also the remodeling of the microvascular network and myocardial fibrosis. Suboptimal plasma glucose control and reduced insulin sensitivity have shown direct correlations with an increased risk for the development of heart failure or for increased mortality levels in patients already displaying cardiac dysfunction (*Yokota et al, 2019*). On the other hand, the increased risk for hypoglycemia often associated with an intensive glycemic control may impair the potential benefits of the latter, thus indicating a paradoxically J-shaped association between HbA_{1c} and heart failure outcomes (*Tomova et al, 2012*; *Eshaghian et al, 2006*). At this point, it is desirable but not yet proven that early diagnosis of subclinical diastolic dysfunction may facilitate therapeutic control and thus contribute to the prevention of progression towards heart failure in type 2 diabetic patients.

Based on our data, we support the hypothesis of a predominance of type 1 diastolic disfunction in our subjects and to the correlations existing between the ultrasonographic parameters expressing diastolic disfunction and the insulin resistance indexes. The prevalence of MAC in our subjects was also higher than that in previous findings published by other researchers, and TNF-α was identified as a marker of low-grade inflammation seemingly predicting the existence of MAC. As the thromboembolic risk is usually higher in diabetic

patients, and as it may also be augmented when MAC coexists, follow-up of these data on the whole period of time stipulated in the prospective design of our study may provide interesting results.

We identified statistically significant differences ($p<0.05$) for age, HDL-cholesterol levels, E/Ea, LAVi, PCWP when we compared patients without diastolic disfunction vs. patients with diastolic disfunction of indeterminate degree, and for LAVi only when different degrees of diastolic disfunction were compared. No statistically significant differences were identified when the associations between metabolic parameters and the diastolic disfunction of indeterminate degree were compared to those in patients with altered diastolic function. The cardiovascular risk profile for type 2 diabetic patients with diastolic disfunction of indeterminate degree or of various severity degrees seems therefore to be the same. Some other studies found the deterioration of the diastolic function to be independently associated with age, retinopathy, and the increase in systolic blood pressure values, but not to the lipid profile or to the rest of metabolic parameters we chose to test (*Adameova et al, 2014*). As tissue Doppler parameters are concerned, high values of the E/e' ratio were found in a large cohort study to predict a higher risk for heart failure and mortality (*Adameova et al, 2014*; *Cheung et al, 2008*).

According to current data in the literature, heart failure patients exert a risk of similar magnitude no matter if their ejection fraction is normal or low, as there is currently no efficient pharmacological therapy for patients with heart failure with preserved ejection fraction. Hence, an increasing interest exists to detect patients with diastolic dysfunction, where lowering of the ejection fraction had not yet been observed, in hope that implementation of preventative measures in such clinically healthy patients may delay the development of an altered systolic function (*Yokota et al, 2019*).

2.4. Antihyperglycemic therapies in specific forms of cardiovascular disease associated with diabetes mellitus

2.4.1. Rationale for the study of antihyperglycemic therapies in relation to specific forms of cardiovascular disease in diabetic subjects

Among all potential clinical forms of diabetes-associated obstructive artery disease, cerebrovascular disease is a serious condition, inducing major disabilities and a shortened life span. In a large meta-analysis of 102 prospective studies, diabetes mellitus was associated with a 2.27-fold increase in the risk for ischemic stroke when compared with a non-diabetic status (*Emerging Risk Factors Collaboration, 2010*).

Accumulating clinical evidence also seems to connect diabetes mellitus with an increased risk for atrial fibrillation (AF). Diabetes mellitus may induce structural and electrical alterations of the left atrium (deposition of advanced end-glycation products and connexin-mediated fibrosis), and stimulate the production of pro-coagulant factors (von Willibrand factor, soluble P-selectin, and other molecules exerting pro-inflammatory and pro-oxidative actions or favoring platelet activation and aggregation). All these changes promote clotting in the left atrial appendage and subsequent thromboembolism. In a turning point in diabetes-related clinical research, several older or newer drugs used to control glycemic values in diabetic patients were recently shown – mostly in observational studies, post-hoc analyses of the major trials, or various meta-analyses – to display different levels of risk for

either AF or stroke. Such evidence exists for all classes of antidiabetic drugs included in the major international guidelines (*Bell et al, 2019*).

Stroke episodes in AF patients frequently have a thromboembolic nature; hence, the question arises as to whether a specific risk for AF in one or the other of the antidiabetic drugs would reflect an accordingly modified risk for cerebral thromboembolism, and thus stroke. Unfortunately, the major clinical trials have not yet distinguished between the ischemic or hemorrhagic nature of stroke episodes, and least of all, between the atherothrombotic or thromboembolic etiology of ischemic strokes (*Chiao et al, 2018*). In the absence of dedicated studies using electrocardiogram (ECG) technologies to monitor the heart rhythm, a high number of asymptomatic AF and/or paroxysmal, recurrent episodes of AF may go unrecognized; this may underlie the inconstant associations between diabetes and incidences of AF or stroke seen in clinical studies, especially those not reporting AF as a specific outcome.

2.4.2. Materials and methods

We searched Medline and Scopus databases using the logical string “atrial fibrillation” OR “stroke” AND “antihyperglycemic AND “diabetes” to identify these key terms in the title or abstract of English-written articles published before June 2019. Clinical studies or trials, meta-analyses, and systematic reviews focusing on human subjects were selected. After eliminating duplicates, this initial search returned 14 results. We screened all titles and abstracts to select papers that could be considered relevant to the aim of our review. This operation led to a further reduction to only 11 titles. A second search using the same algorithm and replacing the key term of “antihyperglycemic“ with “insulin“ OR “metformin” OR “sulfonylurea (SU)” OR ”thiazolidindione (TZD)” OR ”dipeptidyl peptidase-4 (DPP-4) inhibitor” OR ”glucagon-like peptide-1 (GLP-1) receptor agonist” OR ”sodium-glucose cotransporter-2 (SGLT-2) inhibitor” issued 28 supplementary papers, which were also included in our analysis (*Lăcătuşu et al, 2019*).

2.4.3. Results

Insulin

In a case-control study on Taiwan registries, insulin therapy was associated with a higher risk of new-onset AF in diabetic patients than with other antihyperglycemic medications. Among patients in the PREvention oF thromboembolic events – European Registry in Atrial Fibrillation (PREFER in AF) registry, insulin users, but not diabetic patients treated with non-insulin antihyperglycemic drugs, were shown to have a higher risk of stroke compared with non-diabetic individuals. In a Medicare analysis on 798,592 AF patients, insulin-requiring diabetic subjects had a higher risk of stroke than diabetic patients not requiring insulin therapy or non-diabetic individuals (*Liou et al, 2018*).

Metformin

In a cohort study on 645,710 Taiwan patients, monotherapy with metformin was associated with a 19% reduction in the risk of AF compared with the use of other antihyperglycemic medications during a 13-year follow-up. The results of the United Kingdom Prospective Diabetes Study (UKPDS) suggested that intensive blood glucose

control with metformin, compared with the use of sulfonylureas or insulin, significantly reduced the risk of stroke (*Homan et al, 2019*).

Sulfonylureas

The previously mentioned Taiwan case-control study found SUs to not be associated with an increased risk of new-onset AF. A meta-analysis of 27,705 diabetic patients from 17 trials found SUs to be associated with a higher relative risk for stroke than other antihyperglycemic drugs administered for glycemic control (*Castilla-Guerra et al, 2018*).

Thiazolidinediones

In an observational study on 12,605 patients with insulin-naïve type 2 diabetes, the risk of developing AF was reduced by 31% after a five-year follow-up in patients treated with TZD. A better recovery to sinus rhythm was reported in isolated cases of patients with paroxysmal AF and diabetes who received rosiglitazone. A large cohort study of 108,624 diabetic, AF-free Danish patients, treated with either metformin or sulfonylureas as first-line antihyperglycemic therapy, showed a 24% risk reduction in the incidence of AF when TZDs were used as a second-line drug for glycemic control, compared with other antidiabetic drugs. Post hoc analyses on the incidence of AF in the PROactive (PROspective pioglitAzone Clinical Trial In macroVascular Events) and BARI 2D (Bypass Angioplasty Revascularization Investigation 2 Diabetes) trials did not show significant differences in the number of patients developing AF. In another sub-analysis of the PROactive study, the risk for fatal or non-fatal stroke was significantly reduced with pioglitazone in type 2 diabetes patients with a history of previous stroke, but not in those without a history of cerebrovascular events. In the Insulin Resistance Intervention after Stroke (IRIS) trial, pioglitazone was able to lower the risk for recurrent stroke or myocardial infarction compared with placebo therapy (*Zhang et al, 2017*).

DPP-4 Inhibitors

In a cohort study on 90,880 patients with type 2 diabetes previously treated with metformin as a first-line antihyperglycemic drug, the add-on of DPP-4 inhibitors (mostly sitagliptin) as a second-line therapy was found to be associated with a lower risk of AF development than the use of other drugs (mainly SUs) as the second antidiabetic medication. The use of DPP-4 inhibitors was associated with neither an increased nor a decreased risk of new-onset AF in the case-control study on Taiwan registries that was previously mentioned. In another longitudinal observational Taiwan study on 123,050 type 2 diabetes patients that were newly initiated on oral antidiabetic drugs, the use of DPP-4 inhibitors was associated with a lower risk for ischemic stroke compared with meglitinides or insulin. None of the cardiovascular outcome trials with DPP-4 inhibitors identified a reduced risk for stroke with any of these medications.

GLP-1 Receptor Agonists

A pooled analysis of the phase 2b and phase 3 trials in the Albiglutide and cardiovascular outcomes in patients with type 2 diabetes and cardiovascular disease (Harmony Outcomes) program with albiglutide showed a statistically significant increase in the AF incidence with this drug. The cardiovascular outcome trials with lixisenatide, liraglutide, or semaglutide found no differences in the AF incidence between any of the active drugs and the placebo comparator. A meta-analysis of all trials available in 2017 with GLP-1 receptor agonists showed no increase in the risk of AF with these drugs. In the major

cardiovascular outcome trials, liraglutide and albiglutide demonstrated non-significant differences opposite to the placebo in terms of the risk of stroke, whereas injectable semaglutide showed a significant 39% reduction, and dulaglutide was associated with a 24% reduction in the calculated risks for non-fatal stroke. In a previously mentioned meta-analysis, including the four cardiovascular outcome trials with GLP-1 receptor agonists, this class of drugs was associated with a 13% reduction in the risk for non-fatal stroke (*Monami et al, 2017*).

SGLT-2 Inhibitors

Currently, no research on the risk of AF development with any of the SGLT-2 inhibitors has been published. A sub-analysis of the Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients-Removing Excess Glucose (EMPA-REG OUTCOME) acknowledged a slightly increased incidence of stroke in the empagliflozin treatment group, even though not reaching statistical significance. A meta-analysis of 57 studies using seven different approved or unapproved SGLT-2 inhibitors reported a 30% higher risk of non-fatal stroke. Another meta-analysis of trials with SGLT-2 inhibitors did not confirm an increased risk of stroke. The above-mentioned pooled analysis, including the three available cardiovascular outcome trials with SGLT-2 inhibitors, revealed no supplementary risk of stroke with SGLT-2 inhibitors compared with placebo comparators (*Usman et al, 2018*).

2.4.4. Discussion

Insulin

In the cited Medicare analysis on 798,592 AF patients, insulin-requiring diabetic subjects expressed a higher risk of stroke than diabetic patients not requiring insulin therapy or non-diabetic individuals; use of insulin therapy was associated in this registry study with an attenuation in the efficacy of anticoagulant drugs. However, the association between insulin therapy and this pro-arrhythmic status may be biased by the longer duration of type 2 diabetes usually seen in patients treated with insulin. Such subjects may have experienced years of suboptimal glycemic control on other non-insulin therapies, and may have had the time to develop significant comorbidities (*Liou et al, 2018*).

Metformin

In the large cohort study on 645,710 Taiwan patients where monotherapy with metformin was associated with a 19% reduction in the risk of AF, metformin users had the lowest AF incidence rates in the first two years after diagnosis, but the protective effect tended to fade afterward. Possible explanations accounting for the favorable effect of metformin include its actions on adenosine monophosphate-activated kinase, and the drug-induced reduction of the oxidative stress and the myolysis in the atrial tissue. The loss of its protective effect over time may be underlain by the progressive deterioration of β-cell function typically observed in type 2 diabetes, which may lead to a worsened glycemic control, or by the gradual remodeling of the atrial wall. Current evidence suggests that metformin also has a protective effect against ischemic stroke, even though specific outcome studies analyzing a potential cause-effect relationship between the protective role of metformin against AF development and the rate of thromboembolic events are lacking in the medical literature. Results of the United Kingdom Prospective Diabetes Study (UKPDS) may be quoted to demonstrate that metformin significantly reduced the risk of stroke, compared

with sulfonylureas or insulin, when used to obtain intensive glycemic control (*Homan et al, 2019*).

Sulfonylureas

Most researchers analyzing the risk of AF development have considered SU therapy as only a control to report comparative AF outcomes of other antidiabetic medications. The few studies making an exception include the previously mentioned Taiwan case-control study where SUs were not associated with an increased risk of new-onset AF. This class of hypoglycemiant drugs acts on the SU receptor (SUR) unit of the ATP-sensitive potassium channels. In normal conditions, these ionic channels may play a protective role against neuronal ischemia. SUs were therefore feared by some authors to inhibit this neuroprotective mechanism, and thus to increase the risk of stroke, which seems to be confirmed by the results of the cited meta-analysis of 27,705 diabetic patients from 17 trials (*Castilla-Guerra et al, 2018*).

Thiazolidinediones

Thiazolidinediones are insulin sensitizers acting primarily on the peroxisome proliferator-activated receptor (PPAR)- and, in the case of pioglitazone, also exerting a weak agonist activity on PPAR-γ. Reports of an increased risk of hydro-saline retention, heart failure, and cardiovascular events seen with rosiglitazone drastically limited their use in diabetic patients. As a direct effect of these reports, regulatory agencies subsequently requested proof of cardiovascular safety for the newer generations of antihyperglycemic drugs by means of dedicated trials. Paradoxically, the few existing studies found a reduced incidence of AF in TZD-treated patients. The only exception exists in the post hoc analyses on the incidence of AF in the PROactive and BARI 2D trials, where no significant differences in the number of patients developing AF were found between TZD and placebo. However, neither of these two randomized studies were designed to include AF between their specific endpoints, so they did not systematically search for its existence using any ECG-monitoring device. The number of patients receiving TZDs who developed AF was lower than their counterparts in both studies. The other quoted sub-analysis of the PROactive study indicates a real possibility that pioglitazone has the ability to protect diabetic patients with a history of cerebrovascular events against stroke development. The same hypothesis is supported by the IRIS trial, which was performed in non-diabetic but insulin-resistant patients with a history of stroke or transient ischemic attack (*Zhang et al, 2017*).

DPP-4 Inhibitors

The previously quoted evidence of positive or neutral results of DPP-4 inhibitors on AF risk raised the question of potentially protective effects of DPP-4 inhibitors against stroke. This hypothesis is supported by the large longitudinal observational Taiwan study on 123,050 type 2 diabetes patients, where the use of DPP-4 inhibitors was associated with a lower risk for ischemic stroke compared with meglitinides or insulin; however, their risk for stroke was comparable to that observed in metformin users, and higher than the risk observed in patients treated with pioglitazone. Similar to the case with other drugs, none of the cardiovascular outcome trials or meta-analyses with DPP-4 inhibitors published so far have differentiated between stroke events of hemorrhagic or ischemic origin, least of all between atherothrombotic or thromboembolic events (*Barkas et al, 2017*).

GLP-1 Receptor Agonists

A side effect of GLP-1 receptor agonists includes a moderate increase in heart rate, which may be due to either an effect of the direct stimulation of the GLP-1 receptor found on sino-atrial cells, or a compensatory response to the relative lowering of blood pressure levels seen with GLP-1 receptor agonists. Acknowledgement of this effect on the heart rate led to concerns that GLP-1 receptor agonists may be associated with a higher risk for AF, which seemed justified by the results of the Harmony Outcomes program with albiglutide, where a statistically significant increase in the AF incidence with this drug was observed. However, such a result was not found in the cardiovascular outcome trials with lixisenatide, liraglutide, or semaglutide. As cardiovascular outcome trials are specifically designed to follow major cardiovascular events, it is plausible to think that an AF episode should be more recognized in such studies than in trials with metabolic outcomes, to therefore offer a better statistical accuracy. Since these three cardiovascular outcome trials included patients with pre-existing cardiovascular disease, it is also presumable that such subjects would be treated with β-blockers, thus reducing the probability of AF occurrence and reducing the number of cases below the limit of statistical significance. The same result is found in the 2017 meta-analysis of trials available with GLP-1 receptor agonists (including studies with albiglutide, but also with exenatide, lixisenatide, liraglutide, dulaglutide, and semaglutide), where no increase in the risk of AF with these drugs was noted. However, aside from speculations about the risks of AF, GLP-1 receptor agonists are definitely not associated with a higher risk for stroke. All GLP-1 receptor agonists developed from the human GLP-1 backbone (liraglutide, injectable semaglutide, albiglutide, and dulaglutide) are able to lower the risk for the composite outcome of major cardiovascular events (cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke). The separate analysis of the stroke endpoint included in the composite cardiovascular outcome of the dedicated safety trials also indicated some benefits may be seen, with the strongest results features by the injectable semaglutide, displaying a significant 39% reduction. The previously mentioned meta-analysis, including, in this case, the four cardiovascular outcome trials with GLP-1 receptor agonists available at the end of 2018, revealed a 13% reduction in the risk for non-fatal stroke in this class of drugs, even if atherothrombotic, thromboembolic, and/or hemorrhagic events were not differentiated (*Monami et al, 2017*).

SGLT-2 Inhibitors

SGLT-2 inhibitors exert their actions by inhibiting the active reabsorption performed by this specific co-transporter of sodium and glucose at the level of the proximal convoluted tubule. As a result, glucose, sodium, and water are lost in the final urine, lowering blood pressure and blood glucose levels, and creating a negative energy balance that induces weight loss. Based on these direct effects on multiple cardiovascular risk factors, but also on other adjunctive metabolic actions, SGLT-2 inhibitors seem able to lower the cardiovascular risk in diabetic patients. In the dedicated cardiovascular outcome trials, empagliflozin and canagliflozin were shown to reduce the progression to the composite outcome of major cardiovascular events, whereas dapagliflozin reduced the risk for the composite outcome of cardiovascular death and hospitalization for heart failure. Currently, no research on the risk of AF development with any of the SGLT-2 inhibitors has been published. The sub-analysis of the EMPA-REG OUTCOME, suggesting a slightly increased, but insignificant, incidence of

stroke with empagliflozin was not confirmed by the forthcoming results of other trials, but found an isolated support of this hypothesis in the meta-analysis of 57 studies using seven different approved or unapproved SGLT-2 inhibitors, where a 30% higher risk of non-fatal stroke was noted. Hypothetical explanations attribute this negative effect either to chance or to the relative increase in hematocrit, leading to a higher blood viscosity, as these agents exert an effect of osmotic diuresis. However, the other meta-analysis of trials with SGLT-2 inhibitors, this time including studies lasting at least 24 weeks and reporting at least one cardiovascular outcome, did not confirm an increased risk of stroke, thus assuring a reasonable level of cerebrovascular safety with this class of drugs. The same type of results was seen in the pooled analysis that included all three available cardiovascular outcome trials with SGLT-2 inhibitors, where no supplementary risk of stroke with SGLT-2 inhibitors was found (*Usman et al, 2018*).

In conclusion, current evidence supports the existence of a relationship between diabetes mellitus and an increased risk for atrial fibrillation and stroke. In these high-risk patients, several reports linking antidiabetic medications to modified risks for atrial fibrillation, stroke, or both, have been published in the last years. The most relevant of these results are summarized in Table 18.

Table 18. Summary of the main current evidence on the association of current antihyperglycemic drugs with risks of atrial fibrillation (AF) and stroke

Drug	**Risk for AF**	**Risk for Stroke**
Insulin	Increased	Increased
Metformin	Reduced	Reduced
Sulfonylureas	Unchanged	Reduced, unchanged, or increased
Thiazolidinediones	Reduced or unchanged	Reduced
DPP-4 inhibitors	Reduced or unchanged	Reduced or unchanged
GLP-1 receptor agonists	Increased with albiglutide, unchanged with semaglutide, liraglutide, and dulaglutide, or in meta-analyses	Reduced in meta-analyses and with semaglutide, unchanged with liraglutide, albiglutide, and dulaglutide
SGLT-2 inhibitors	Data not available	Increased in some meta-analyses, unchanged in others

The cause-effect relationship between the modified risk for atrial fibrillation of these drugs and cerebrovascular disease due to thromboembolic events has not yet been analyzed in studies with dedicated outcomes. However, depicting the ability of some specific antihyperglycemic therapies in reducing the risks for both atrial fibrillation and stroke as completely separate mechanisms would mean allowing the existence of slightly too much coincidental evidence. Trials searching for a potentially causal triangular relationship between antidiabetic drugs, risks for atrial fibrillation, and cerebral thromboembolism are needed to fill in a gap in evidence, and to potentially supplement the adaptation of the recommendations of current guidelines to prevent the negative outcomes of cardiovascular disease in diabetic patients as much as possible.

2.5. Peripheral artery disease and diabetes mellitus

2.5.1. Rationale for the study of peripheral artery disease in diabetes mellitus

Peripheral arterial disease (PAD), defined as the partial or complete occlusion of peripheral arteries, is usually determined by atherosclerosis. Diagnostic of PAD is subject to a variety of recommendations; however, the most common of all objective definitions requires an ankle-brachial index (ABI) below 0.9. PAD may determine a broad range of manifestations, varying between completely asymptomatic disease, intermittent claudication, rest limb pain, tissue loss, and gangrene. The existence of PAD is considered a coronary artery disease equivalent, associated with a high risk for coronary and cerebrovascular complications and mortality. PAD shares many risk factors with diabetes, and the latter increases the severity and the progression speed of the former. Diabetic patients are most vulnerable to the development of PAD, and they end up by expressing the highest number of complications and the worst outcomes, which makes early detection of PAD and multifactorial approach of all risk factors to become paramount in their therapy. Diabetes also induces a higher risk for developing critical limb ischemia (*Thiruvoipati et al, 2015*). The magnitude of hyperglycemia has also been shown to correlate with the risk for developing PAD, as a United Kingdom research team demonstrated a 28% increase in the risk of developing PAD with every 1% increase in the values of glycosylated hemoglobin (HbA_{1c}) (*Cheun et al, 2019*). Paradoxically at first sight, but nevertheless a logical consequence, diabetic patients with already diagnosed vascular disease were found to express a better management of their cardiovascular risk factors when compared to diabetic subjects with only occult, subclinical PAD, thus proving the paramount importance of early diagnostic and multifactorial intervention (*Cheun et al, 2019*). Besides diabetes, other risk factors for PAD development include smoking, advanced age, male sex, hypertension, and hyperhomocysteinemia; some of these risk factors are frequent features of persons with diabetes (*Selvin et al, 2004*).

I am part of a multidisciplinary team set to investigate the range of cardiovascular risk factors in patients with PAD from our region. For this purpose, we designed a prospective study which enrolled all the patients admitted in the last years in the 2nd Department of Internal Medicine and the Department of Cardiology of the Emergency Clinical Hospital "Sfântul Spiridon" Iasi and searched for overt cardiovascular disease and cardiovascular risk factors in these subjects. The study finished the patients' enrollment period and is currently ongoing with next-scheduled visits. Results presented below belong to interim analyses based on partial collection of data from the initial visits of the patients (*Ceasovschih et al, 2019*).

2.5.2. Materials and methods

All patients signed a written informed consent previous to any procedure in the study. Evaluation of subjects included collection of demographic and clinical data (age, gender, current smoking status, presence of diabetes mellitus, dyslipidemia and hypertension) and determination of the ankle-brachial index (ABI). Diagnosis of peripheral arterial disease (PAD) was based on ABI values of less than 0.9. Patients were then divided into two groups, with and without PAD. In patients with PAD we studied the psychological profile and the life quality by completing two dedicated questionnaires: the DASS 21-R (Depression, Anxiety and Stress Scales) and Quality of Life Inventory (QLI), respectively.

2.5.3. Results

A first interim analysis on baseline data was performed on 3,430 patients that were enrolled in the study between August 2016 and April 2017. 46.8% of them were male and 53.2% were female, with a mean age of 66.17 years. Peripheral artery disease was detected in 4.8% of cases. When we compared the proportion of cardiovascular risk factors in patients with and without PAD, we found mean ages of 70.0 vs. 65.3 years ($p<0.072$), a proportion of the male gender of 77.4% vs. 45.3%, the proportion of currently smoking status of 87.8% vs. 33.4% ($p<0.0001$), dyslipidemia in 100% vs. 43.3% of patients ($p<0.0001$) and hypertension in 90.9% vs. 52.1% of patients ($p<0.0001$). Diabetes mellitus was present in 36.6% vs. 15.8% of cases ($p<0.0001$) (Figure 8).

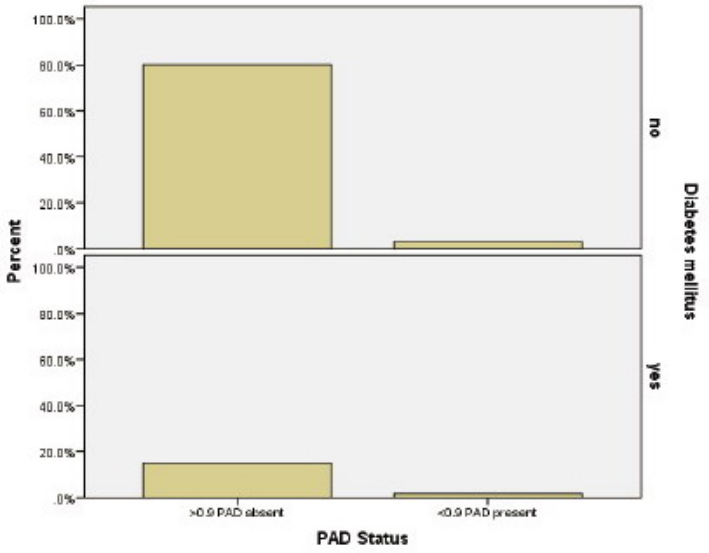

Figure 8. Proportion of diabetes mellitus in patients with and without PAD

In another interim analysis we included data from 216 PAD patients consecutively enrolled between January 2017 and February 2018, with a mean age of 69.03 years. This subgroup included 78.2% male and 17.8% female patients. Men were more likely to smoke compared to women (100% versus 30%, $p<0.0001$). No statistically significant differences were seen when these PAD patients were divided by gender and life quality ($p<0.909$), depression ($p<0.432$) and anxiety ($p<0.282$) were compared in the two subgroups. The total stress scores were higher for women than for men ($p<0.031$).

2.5.4. Discussion

At this point in the research, we can confirm that diabetes mellitus accounts among the major risk factors for PAD, next to dyslipidemia, hypertension and cigarette smoking, which is similar to previous data existing in the literature. The regional hierarchy of PAD risk factors is not yet established at present, but we could consider it a useful outcome that would reflect in better local prevention strategies in the future.

Also at this point, we can assert that a low quality of life, depression and anxiety are equally common among PAD patients of both genders, most probably due to the high disability and poor prognosis associated with PAD. As men and women seem to react differently to stress, a distinct approach of this factor may perhaps prove useful in the

generation of different management algorithms and distinct holistic approaches of the PAD patients.

The clinical diagnostic of symptomatic PAD is based on various history and physical exam findings, including claudication, low temperature in the extremities, distal hair loss, nail thickening, dependent rubor, diminished or absent pulses, or femoral bruit. Pulse examination may raise some limits in diabetic patients, as a diminished pulse amplitude may in fact mirror the mere calcification in the wall of the arterial vessel, without any flow-limiting stenosis. On the other hand, palpation distal pulse does not exclude the possibility of a more proximal flow-limiting stenosis. Vascular claudication is defined as the sensation of muscular pain, cramping, fatigue, or heaviness which is triggered by walking, relieved by rest, and reproducible (*Gerhard-Herman et al, 2016*). History of symptoms associated with PAD in diabetics may also raise some limits in diabetic patients, as the coexistence of diabetic hyposensitive neuropathy may mask claudication symptoms, and motor neuropathy may limit mobility enough as to never trigger the claudication. Hence, diagnostic studies become of certain importance in patients where diabetes mellitus and peripheral arterial disease coexist.

In the stage next to the clinical diagnostic, PAD is confirmed by various investigations, which, beside ABI, include duplex ultrasonography, continuous wave Doppler examination, computed tomography angiography, magnetic resonance angiography, or at least conventional arteriography. ABI has gained a broad use, due to its simplicity, low cost, noninvasiveness, and reproducibility.

Management of PAD includes lifestyle optimization, simultaneous approach of all risk factors, and revascularization treatment algorithms in advanced cases, where PAD continues to develop and induce clinical manifestations. Even though diabetes mellitus is confirmed as one of the strongest predictors of the development and severity of PAD, intensive glycemic control was not able to prove improved outcomes or survival, perhaps due to the fact that macrovascular disease has a multifactorial determinism, and therefore requires the correction of all risk factors. Smoking has also already been confirmed as a major risk factor for PAD, its progression to amputation, cardiovascular non-fatal events, and cardiovascular death, and it is still considered as the strongest and most preventable risk factor for PAD development. Even though hypertension is also an independent risk factor for PAD, this association is not as strong as those of PAD with smoking and diabetes. Pharmacological reduction of high low-density lipoprotein (LDL) cholesterol levels has also proven to slow down the progression and the symptoms of PAD. Obesity has not been demonstrated to represent a risk factor for PAD or for adverse lower extremity outcomes; however, weight loss in obese PAD patients is able to reduce weight and stress on the lower extremities, thus improving claudication-related symptoms. As patients may self-limit their physical activity levels in the effort of avoiding claudication, the risk for sedentary lifestyle obviously increases. Nevertheless, the latter has been shown to associate with overall poorer outcomes, a decreased walking distance and a lower quality of life. Antiplatelet therapy is a first-class recommendation to reduce the risk of both fatal and non-fatal cardiovascular events in patients with symptomatic PAD. Treatment with cilostazol, but not with pentoxifylline, has been shown to exert positive effects on the claudication and walking distance. Under these interventions, most patients with symptomatic PAD are generally able to stabilize, or even decline, their disease, together with the slow and gradual improvement in their risk factors. However, 20% to 30% of them will progress to

lifestyle-limiting or limb-threatening manifestations of PAD. Such patients require invasive revascularization management (*Cheun et al, 2019; Society for Vascular Surgery Lower Extremity Guidelines Writing Group, 2015*). Specific revascularization indications for PAD are the development of lifestyle-limiting claudication or that of critical limb ischemia despite best pharmacological therapy.

2.6. Cardiovascular disease over the glucose continuum

2.6.1. Rationale for the study of cardiovascular disease in prediabetic subjects

Chronic hyperglycaemia induced by insulin resistance (either prediabetes or type 2 diabetes mellitus) display an epidemic trend in the present world. Insulin resistance is also believed to increase the risk for atherosclerotic cardiovascular disease, besides the classical cardiovascular risk factors. The association between insulin resistance-determined hyperglycemia and the increased level of the cardiovascular risk is well confirmed in type 2 diabetes mellitus (*Danaei et al, 2006*). However, the risk of atherosclerosis development in prediabetes is controversial, as it is acknowledged as double by some authors and only moderately increased by others (*Ford et al, 2010*).

Excess adipose tissue is associated with abnormal anthropometric data and loss of insulin sensitivity. The "golden standard" protocol for studying insulin resistance features a high level of technical complexity and may therefore prove difficult to apply in the clinical practice. Hence, surrogate assessment methods, which evaluate insulin resistance indexes based on more accessible serum parameters, became available and widely used by practitioners. Metabolic anomalies in insulin resistant individuals are multiple and complex. Major changes occur, for example, in the adipose tissue, which is acknowledged today to be an active producer of hormones, generally designated as adipokines. The moment the insulin sensitivity decreases, changes in adipokine secretion occur (*Yadav et al, 2013*). Up until now, a large number of molecules secreted by the adipose cells were identified, but only several of them are well-known in terms of role and functions. Adiponectin and leptin are two of the most studied adipokines, and both seem to correlate with overt cardiovascular disease (*van de Voorde et al, 2013*), even though studies trying to identify a quantitative relationship have previously provided contradictory results. Most data on this issue (*Kim et al, 2013*; *Bidulescu et al, 2013*) usually focus on high-risk populations, such as subjects with metabolic syndrome, type 2 diabetes mellitus, obesity, polycystic ovary syndrome, preexisting cardiovascular disease or ethnic predisposition. On the opposite, scarce data about a potential relationship between the adipokine secretory profile and early atherosclerosis are available; moreover, the existing results are often contradictory (*Watanabe et al, 2012*).

The diagnostic of clinical manifestations of atherosclerotic cardiovascular disease needs various methods, among which contrast arteriography is often considered as a gold standard. Incipient atherosclerosis is less evaluated in clinical practice and may need other diagnostic methods, as contrast arteriography may provide false-negative results. Some non-invasive methods developed in the last years, such as the study of arterial intima-media thickness (IMT), allow a reliable estimation of any degree of atherosclerotic lesions. The few studies that have previously evaluated the relationship between insulin resistance, adipokines and IMT (*Juonala et al, 2011*) did not examine subjects with only incipient hyperglycemia and no clinical signs of atherosclerosis.

Therefore, we developed a project intending to research two directions that have been investigated very scarcely in the existing literature. First, we aimed to identify the potential differences in anthropometric data, the lipid profile, some insulin resistance surrogate parameters (HOMA-IR and QUICKI) and two of the most known adipokines, adiponectin and leptin, between patients with isolated IFG and those with IGT associated with IFG. Second, we aimed to analyze the associations that might exist between reduced insulin sensitivity, adipokines and incipient atherosclerosis (estimated by IMT) in prediabetic subjects, whose cardiovascular risk profile is not yet fully defined.

2.6.2. Materials and methods

Between November 2010 and July 2011, we screened all the patients aged 30 to 70 years that had been diagnosed with prediabetes (IFG and IGT) during the previous 6 months in the Clinical Centre of Diabetes, Nutrition and Metabolic Diseases of Iaşi. The study was approved by the Ethics Committee of our university and all procedures were performed in accordance with the Declaration of Helsinki. All subjects gave their written informed consent to participate in the study. The diagnostic was based on the most recent IDF criteria. IFG was defined as a fasting blood glucose (FBG) level of 110 to 125 mg/dL and a 2-hour blood glucose level during the OGTT below 140 mg/dL. IGT was defined as an FBG level below 126 mg/dL and a 2-hour blood glucose level during the OGTT of 140 to 199 mg/dL. The exclusion criteria we used were: previously existing diabetes mellitus, diagnosed according to IDF/WHO criteria; cardiovascular disease (other than arterial hypertension); severe liver or kidney disease; endocrine diseases; treatment with biguanides, thiazolidinediones or lipid-lowering drugs; patients who did not signed the informed consent.

All participants underwent general clinical examination. Height and weight in light clothing were measured following standardized procedures. Body mass index (BMI) was calculated as body weight (in kilograms) divided by square of the height (in meters). The waist circumference (WC) was measured midway between the lower rib margin and the iliac crest and the hip circumference was measured at the widest circumference over the trochanter in standing subjects after normal expiration. Waist-to-hip ratio (WHR) was calculated.

Venous blood samples were collected after an overnight fast. Routine biochemistry tests were performed in the same day: fasting glycemia, total cholesterol, triglycerides, high-density lipoprotein cholesterol – HDL-cholesterol, urea, creatinine, uric acid, transaminases; a part of serum was stored at −80°C for immunological measurements: insulin, adiponectin and leptin. Based on these measurements, the following derived parameters were calculated: low-density lipoprotein cholesterol (LDL-cholesterol) = total cholesterol – HDL-cholesterol – triglycerides/5 (in cases with triglycerides under 400 mg/dL); non-HDL-cholesterol = total cholesterol – HDL-cholesterol; total cholesterol-to-HDL ratio; triglycerides-to-HDL ratio; leptin-to-adiponectin ratio (LAR). The following cut-off points were used for biochemical variables: total cholesterol – over 200 mg/dL, HDL-cholesterol – below 40 mg/dL in men and 50 mg/dL in women, triglycerides – over 150 mg/dL, uric acid – over 7 mg/dL in men and 6 mg/dL in women.

Insulin resistance was evaluated by the homeostasis model assessment (HOMA-IR), calculated as fasting insulin (FI) (microunits/mL) × FPG (mg/dL) / 405, as well as by the values of QUICKI index, calculated as 1 / (log FI + log FPG).

All participants underwent resting electrocardiogram and echocardiography in order to exclude cases with previously unknown cardiovascular disease. Intima-media thickness (IMT) was measured using a full digital ultrasound system. All measurements were carried out by the same investigator, who was blinded to the cardiovascular risk factors of the patients. The far wall of both the right and left common carotid and femoral arteries was used, because of higher reproducibility and possible overestimation of the IMT of the near wall. The mean readings of the left and right arteries were then averaged to obtain a single mean value at carotid and femoral levels for each participant.

All previously mentioned data were centralized in a database using Microsoft Excel. Statistical analysis was performed using SPSS version 16.0. For descriptive statistics, discrete and continuous variables were expressed as frequencies and percentages, and means and standard deviations, respectively. Student t-test (for continuous variables) and $\chi 2$-test (for categorical variables) were used to compare differences between subjects with isolated IFG and IFG+IGT; the degrees of freedom (DF) are mentioned in each case. Pearson and Spearman correlations were used to analyse associations between variables, and Bonferroni's corrections were applied on the results in order to minimize the probability of a type I statistical error. Because some parameters may vary differently between males and females, analysis was sometimes stratified by gender. Due to close matching of the groups with isolated IFG and with IFG+IGT, data are presented unadjusted. When appropriate, multiple linear regression analysis was performed to disclose independent contributions between variables. A two-sided p-value below 0.05 was considered as statistically significant (*Mihai et al, 2013*; *Mihai et al, 2014*).

2.6.3. Results

2.6.3.1. Results of study 1: Comparison between the patients with isolated IFG and with IFG+IGT

A total of 154 subjects were initially selected. Among them, 32 (20.77%) were excluded based on laboratory and imagistic results (abnormal clinical, biological, electrocardiogram or ultrasonography findings that identified the existence of previously unknown diabetes mellitus, cardiovascular, renal or hepatic disease). Therefore, we performed our analysis on the remaining 122 subjects. Patients remaining in the study (50 men and 72 women) were aged between 30 and 70. 52.5% of them had isolated IFG (64 patients) and 47.5% had IFG+IGT (58 patients).

Sex ratio was similar in the group with isolated IFG (42.2% men and 57.8% women) and the group with IFG+IGT (39.7% men and 60.3% women), without any statistically significant difference ($\chi 2=0.01$; DF=1; p=0.921). Mean age was not different in subjects with isolated IFG and with IFG+IGT (59.06 ± 11.23 years vs. 58.91 ± 10.43 years; t=0.08; DF=120; p>0.05).

BMI values ranged from 19.3 to 43.8 kg/m^2; excess weight (BMI ≥ 25 kg/m^2) was present in 82.8% of subjects. Patients with first- and second-degree obesity were the most numerous (32% with BMI between 30-34.9 kg/m^2 and 16.4% with BMI between 35-39.9 kg/m^2). Mean BMI values were not significantly different between subjects with isolated IFG and with IFG+IGT (30.09 ± 5.63 kg/m^2 vs. 31.73 ± 4.96 kg/m^2; t=1.71; DF=120; p>0.05). Similar data were found when analyzing the distribution of excess fat. Both groups with

isolated IFG and with IFG+IGT displayed very high prevalences of abnormal WC and WHR values, with no differences between males and females (Table 19). Mean values of WC were not different between the two groups (isolated IFG and IFG+IGT). Mean values of WHR showed statistical differences between subjects with isolated IFG and with IFG+IGT only in women, but not in men. Mean values of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and uric acid were not significantly different between subjects with isolated IFG and those with IFG+IGT; this finding was true both in males and females (Table 20).

Table 19. Prevalences of abnormal WC and WHR values in subjects with isolated IFG and with IFG+IGT

		Elevated WC		**Elevated WHR**	
IFG	Men	88.88% (n = 27)	χ^2=0.07; DF=1;	77.77% (n = 27)	χ^2=0.03; DF=1;
	Women	83.33% (n = 36)	p=0.795	81.25% (n = 32)	p=0.853
IFG+IGT	Men	90.47% (n = 21)	χ^2=0.16; DF=1;	66.66% (n = 18)	χ^2=1.46; DF=1;
	Women	91.17% (n = 34)	p=0.693	85.29% (n = 34)	p=0.227
Men	IFG	88.88% (n = 27)	χ^2=0.09; DF=1;	77.77% (n = 27)	χ^2=0.23; DF=1;
	IFG+IGT	90.47% (n = 21)	p=0.766	66.66% (n = 18)	p=0.630
Women	IFG	83.33% (n = 36)	χ^2=0.39; DF=1;	81.25% (n = 32)	χ^2=0.38; DF=1;
	IFG+IGT	91.17% (n = 34)	p=0.534	85.29% (n = 34)	p=0.537

Significance of all abbreviations is explained in the text.

Table 20. Values of anthropometric and biochemical parameters in male and female subjects with isolated IFG and IFG+IGT

		IFG	**IFG+IGT**	
WC (cm)	Men	105.81 ± 11.76	106.14 ± 11.75	t=0.09; DF=48; p>0.05
	Women	95.33 ± 12.77	100.12 ± 12.94	t=1.58; DF=70; p>0.05
WHR	Men	1.0 ± 0.007	1.0 ± 0.07	t=0; DF=48; p>0.05
	Women	0.91 ± 0.07	0.95 ± 0.08	t=16.96; DF=70; **p<0.001**
Total cholesterol (mg/dL)	Men	206.4 ± 41.2	203.0 ± 27.0	t=0; DF=48; p>0.05
	Women	216.6 ± 58.2	207.2 ± 34.7	t=0.84; DF=70; p>0.05
HDL-cholesterol (mg/dL)	Men	46.59 ± 11.82	48.13 ± 14.69	t=0.40; DF=48; p>0.05
	Women	50.66 ± 14.65	50.96 ± 13.16	t=0.09; DF=70; p>0.05
LDL-cholesterol (mg/dL)	Men	126.50 ± 40.83	124.25 ± 35.52	t=0.21; DF=48; p>0.05
	Women	137.95 ± 50.12	127.53 ± 30.62	t=1.07; DF=70; p>0.05
Triglycerides (mg/dL)	Men	160.11 ± 83.77	166.78 ± 102.99	t=0.25; DF=48; p>0.05
	Women	150.45 ± 97.20	143.54 ± 63.81	t=0.47; DF=70; p>0.05
Uric acid (mg/dL)	Men	6.18 ± 1.04	6.37 ± 1.65	t=0.48; DF=48; p>0.05
	Women	4.76 ± 1.22	5.20 ± 1.54	t=1.34; DF=70; p>0.05

Significance of all abbreviations is explained in the text.

Prevalence of hypercholesterolemia was high and without differences neither between males and females in each of the two groups, nor between subjects of the same gender in each of the groups with isolated IFG and with IFG+IGT. The low values of HDL-cholesterol had significantly different prevalences between men and women only in subjects with isolated IFG, but not in those with IFG+IGT; no significant differences were noticed between subjects of the same gender in the groups with isolated IFG and with IFG+IGT (Table 21).

There were no significant differences in the prevalences of hyperuricemia between males and females in each of the two groups. Slight correlations between BMI and the values of total and LDL-cholesterol were found only in subjects with IFG+IGT (total cholesterol: r=+0.24, LDL-cholesterol: r=+0.19), while no correlation was observed in subjects with IFG (total cholesterol: r=+0.01, LDL-cholesterol: r=-0.08). WHR appeared to have only slight correlations with the values of total and LDL-cholesterol, negative in the case of subjects with isolated IFG (total cholesterol: r=-0.15, LDL-cholesterol: r=-0.16) and positive in subjects with IFG+IGT (total cholesterol: r=+0.19, LDL-cholesterol: r=+0.14). Triglycerides had slight positive correlations with BMI and WHR both in patients with isolated IFG (BMI: r=+0.34, WHR: r=+0.14) and IFG+IGT (BMI: r=+0.10, WHR: r=+0.19). Uric acid also had positive correlations with BMI and WHR both in patients with isolated IFG (BMI: r=+0.33, WHR: r=+0.47) and IFG+IGT (BMI: r=+0.28, WHR: r=+0.21).

Table 21. Prevalences of abnormal values of the lipid profile in subjects with isolated IFG and with IFG+IGT

		Hypercholesterolemia		**Low values of HDL-cholesterol**		**Hypertriglyceridemia**	
IFG	Men	66.66% (n = 27)	χ^2=0.29; DF=1; p=0.587	25.92% (n = 27)	χ^2=3.98; DF=1; **p=0.046**	40.74% (n = 27)	χ^2=0.04; DF=1; p=0.845
	Women	56.75% (n = 37)		54.05% (n = 37)		35.13% (n = 37)	
IFG+IGT	Men	56.52% (n = 23)	χ^2=0.11; DF=1; p=0.746	30.43% (n = 23)	χ^2=1.71; DF=1; p=0.191	43.47% (n = 23)	χ^2=0.01; DF=1; p=0.918
	Women	51.42% (n = 35)		51.42% (n = 35)		45.71% (n = 35)	
Men	IFG	66.66% (n = 27)	χ^2=0.20; DF=1; p=0.657	25.92% (n = 27)	χ^2=0.13; DF=1; p=0.723	40.74% (n = 27)	χ^2=0.01; DF=1; p=0.927
	IFG+IGT	56.52% (n = 23)		30.43% (n = 23)		43.47% (n = 23)	
Women	IFG	56.75% (n = 37)	χ^2=0.05; DF=1; p=0.828	54.05% (n = 37)	χ^2=0.05; DF=1; p=0.824	35.13% (n = 37)	χ^2=0.45; DF=1; p=0.500
	IFG+IGT	51.42% (n = 35)		51.42% (n = 35)		45.71% (n = 35)	

Significance of all abbreviations is explained in the text.

Insulin resistance indexes and adipokines had, in most cases, similar values between the two groups; the only statistical difference was observed in QUICKI values which was not accompanied, however, by similar differences in HOMA-IR values (Table 22). In patients with isolated IFG, HOMA-IR and QUICKI values correlated both with adiponectin and leptin, but not with LAR. In patients with IFG+IGT, these insulin resistance indexes showed correlations with adiponectin only for QUICKI, but not for HOMA-IR, while both of them correlated with leptin and LAR (Table 23).

Table 22. Insulin resistance indexes and adipokine levels in patients with isolated IFG and IFG+IGT

Parameter	IFG	IFG+IGT	Statistical significance
HOMA-IR	1.44 ± 1.55	1.94 ± 1.74	t=1.67; p>0.05
QUICKI	0.63 ± 0.07	0.59 ± 0.08	t=2.93; **p=0.01**
Adiponectin (ng/mL)	11450 ± 4678	11738 ± 4801	t=0.33; p >0.05
Leptin (ng/mL)	17.28 ± 14.74	22.47 ± 16.20	t=1.04; p >0.05
LAR (mg/g)	2.16 ± 3.56	2.15 ± 2.05	t=0.02; p>0.05

Significance of all abbreviations is explained in the text.

Table 23. Correlations of insulin resistance indexes with adipokine profile in patients with isolated IFG and IFG+IGT

		Isolated IFG		IFG+IGT	
		HOMA-IR	**QUICKI**	**HOMA-IR**	**QUICKI**
	r-value	**-0.374**	**0.442**	-0.167	**0.280**
Adiponectin	p-value	**0.002**	**0.0001**	0.210	**0.033**
	n	64	63	58	58
	r-value	**0.263**	**-0.362**	**0.415**	**-0.440**
Leptin	p-value	**0.038**	**0.004**	**0.001**	**0.001**
	n	63	63	57	57
	r-value	0.221	-0.248	**0.350**	**-0.381**
LAR	p-value	0.082	0.050	**0.008**	**0.003**
	n	63	63	57	57

Significance of all abbreviations is explained in the text.

2.6.3.2. Results of study 2: Analysis of the association between subclinical atherosclerosis and the metabolic profile in prediabetes

Neither carotid, nor femoral IMT were correlated to the anthropometric data or to the lipid profile (all p>0.05).

Age appeared related to carotid IMT (r=0.202, p=0.027), but not to femoral IMT (r=0.013, p=0.889). Neither adiponectin, leptin nor the LAR values were correlated with any of the anthropometric data, except for a positive relationship between leptin and the WHtR. There were no correlations between leptin or LAR and the lipid profile; adiponectin correlated only with triglycerides, HDL-cholesterol, cholesterol-to-HDL ratio and triglycerides-to-HDL ratio. Leptin and LAR correlated with HbA_{1c}, while adiponectin showed no correlation with HbA_{1c}. HOMA-IR was not related to the anthropometric parameters, except for an association with the WHR, but correlated with some components of the lipid profile (triglycerides, HDL-cholesterol, cholesterol-to-HDL ratio and triglycerides-to-HDL ratio) and HbA_{1c} (Table 24). The adipokines correlated with HOMA-IR, as shown in Table 25.

Table 24. Correlations of clinical and biochemical data with HOMA-IR and adipokine values

	HOMA-IR	**Adiponectin**	**Leptin**	**Leptin to Adiponectin Ratio (LAR)**
Age (years)	r=0.402 p=0.077	r=0.056 p=0.540	r=0.025 p=0.788	r=- 0.010 p=0.913
BMI (kg/m^2)	r=0.010 p=0.917	r=- 0.029 p=0.759	r=- 0.114 p=0.222	r=- 0.035 p=0.710
WC (cm)	r=0.103 p=0.269	r=0.069 p=0.463	r=- 0.013 p=0.891	r=0.103 p=0.270
WHR	r=0.106 p=0.283	r=- 0.036 p=0.714	r=0.055 p=0.577	r=- 0.091 p=0.359
WHtR	**r=0.258** **p=0.006**	r=- 0.153 p=0.108	**r=0.240** **p=0.011**	r=0.159 p=0.094
SBP (mmHg)	r=0.113 p=0.566	r=- 0.174 p=0.071	r=0.210 p=0.086	r=0.183 p=0.767
DBP (mmHg)	r=0.126 p=0.069	r=- 0.214 p=0.069	r=0.098 p=0.616	r=0.108 p=0.213
Total cholesterol (mmol/L)	r=0.054 p=0.554	r=0.049 p=0.594	r=- 0.016 p=0.859	r=- 0.037 p=0.689
Triglycerides (mmol/L)	**r=0.393** **p=0.000**	**r=- 0.316** **p=0.000**	r=0.050 p=0.586	r=0.078 p=0.396
HDL-cholesterol (mmol/L)	**r=- 0.326** **p=0.000**	**r=0.392** **p=0.000**	r=- 0.039 p=0.675	r=- 0.079 p=0.397
LDL-cholesterol (mmol/L)	r=0.055 p=0.562	r=0.049 p=0.607	r=- 0.009 p=0.927	r=- 0.032 p=0.733
Cholesterol / HDL	**r=0.354** **p=0.000**	**r=- 0.369** **p=0.000**	r=- 0.007 p=0.944	r=0.069 p=0.465
Non-HDL cholesterol (mmol/L)	r=0.151 p=0.105	r=- 0.063 p=0.505	r=- 0.007 p=0.937	r=- 0.022 p=0.815
Triglycerides / HDL	**r=0.399** **p=0.000**	**r=- 0.378** **p=0.000**	r=0.025 p=0.791	r=0.102 p=0.280
HbA_{1c} (%)	**r=0.227** **p=0.012**	r=0.004 p=0.963	**r=0.322** **p=0.000**	**r=0.259** **p=0.004**

Significance of all abbreviations is explained in the text.

Table 25. Correlations between HOMA-IR and the adipokine values

	HOMA-IR	
	r	p
Adiponectin	**- 0.329**	**0.0001**
Leptin	**0.298**	**0.001**
LAR	**0.252**	**0.006**

Significance of all abbreviations is explained in the text.

The initial relationships between IMT values and the metabolic profile (HOMA-IR, adipokines and HbA_{1c}) are shown in Table 26. In order to minimize type I statistical errors, Bonferroni's corrections were applied on the results of previously presented correlations,

without statistically changing the data. The results of multiple regression analysis are mentioned in table 27.

Table 26. Correlations of IMT values with the metabolic profile

	Carotid IMT		**Femoral IMT**	
	Pearson	p	Pearson	p
HOMA-IR	-0.016	0.859	0.015	0.873
Adiponectin	**0.190**	**0.036**	0.041	0.661
Leptin	-0.098	0.287	-0.080	0.390
LAR	**-0.212**	**0.021**	-0.174	0.058
HbA_{1c}	-0.023	0.803	0.131	0.154

Significance of all abbreviations is explained in the text.

Table 27. Multiple linear regression models: the relationships between IMT and the metabolic parameters adjusted for age and HOMA-IR

		Carotid IMT			**Femoral IMT**		
		Beta	Sign.	Std. Err.	Beta	Sign.	Std. Err.
Adjustments for age	HOMA-IR	-0.059	0.519	10.628	-0.069	0.451	10.780
	Adiponectin	0.096	0.297	10.587	0.109	0.240	10.707
	Leptin	0.055	0.548	10.620	0.044	0.634	10.761
	LAR	0.006	0.952	10.637	-0.018	0.850	10.770
	HbA_{1c}	**0.234**	**0.009**	10.337	**0.228**	**0.012**	10.518
Adjustments for HOMA-IR	Adiponectin	**-0.328**	**0.0001**	1.902	**-0.330**	**0.0001**	1.900
	Leptin	**0.300**	**0.001**	1.920	**0.304**	**0.001**	1.917
	LAR	**0.257**	**0.006**	1.948	**0.265**	**0.004**	1.942
	HbA_{1c}	**0.271**	**0.003**	1.932	**0.271**	**0.003**	1.933

Significance of all abbreviations is explained in the text.

Carotid IMT correlated only with adiponectin and LAR, while femoral IMT had no relationships whatsoever with the previously mentioned metabolic factors. Neither carotid, nor femoral IMT had any initial correlations with HbA_{1c} values. When adjusting for age, all initial correlations carotid IMT had with HOMA-IR and with adipokines disappeared and the regression coefficients for femoral IMT were left unchanged, while both IMT values showed moderate positive associations with HbA_{1c}. After using HOMA-IR as the adjustment factor, carotid IMT became negatively correlated with adiponectin and positively correlated with leptin and LAR; all these correlations had moderate intensity. Similar results were noticed when adjustments for HOMA-IR were applied on the relationships between femoral IMT and adipokine values. Both carotid and femoral IMT became positively correlated with HbA_{1c} after adjusting for HOMA-IR values. Adjustments for adipokines, lipid profile parameters and HbA_{1c} were then applied on the relationships between carotid/femoral IMT and HOMA-IR (Table 28).

Table 28. Multiple linear regression models: the relationships between IMT values and HOMA-IR adjusted for adipokines, lipid profile and HbA_{1c}

		Carotid IMT			Femoral IMT		
		Beta	Sign.	Std. Err.	Beta	Sign.	Std. Err.
	Adjustments for:						
HOMA-IR	Adiponectin	-0.326	0.0001	49.211	-0.330	0.0001	41.747
	Leptin	**0.297**	**0.001**	14.984	**0.303**	**0.001**	14.985
	LAR	**0.246**	**0.006**	2.803	**0.257**	**0.004**	2.816
	Total cholesterol	0.052	0.570	45.020	0.057	0.536	45.038
	HDL-cholesterol	**-0.325**	**0.0001**	12.850	**-0.326**	**0.0001**	12.859
	LDL-cholesterol	0.052	0.582	41.401	0.057	0.546	41.477
	Non-HDL-cholesterol	0.148	0.115	44.309	0.153	0.103	44.418
	Triglycerides	**0.391**	**0.0001**	80.469	**0.392**	**0.0001**	80.491
	Cholesterol-to-HDL ratio	**0.348**	**0.0001**	1.356	**0.355**	**0.0001**	1.363
	Triglycerides-to-HDL ratio	**0.396**	**0.0001**	2.974	**0.400**	**0.0001**	2.978
	HbA_{1c}	**0.271**	**0.003**	0.406	**0.270**	**0.003**	0.406

Significance of all abbreviations is explained in the text.

Both carotid and femoral IMT became correlated with HOMA-IR after adjustments for leptin and LAR; when adjustment for adiponectin was used, the relationships between IMT values and HOMA-IR were marked by a large standard error. As to lipid profile parameters, only adjustments for triglycerides, HDL-cholesterol, cholesterol-to-HDL ratio and triglycerides-to-HDL ratio induced new correlations between both carotid/femoral IMT and HOMA-IR. Significant correlations appeared when the relationships between carotid/femoral IMT and HOMA-IR were adjusted for HbA_{1c}.

An interim analysis of our study focused on the comparison of aspects related to subclinical atherosclerosis between the groups with isolated IFG and with IFG+IGT and between patients with lowest and highest values of the HOMA-IR index in 68 of our patients newly diagnosed with prediabetes. According to the IDF & WHO criteria, 31 of them had IFG and 37 had IFG+IGT. The gender distribution was 47% men vs. 53% women. Most patients were normal weight (38.2%) or overweight (30.9%); 19.1% were 1^{st} degree obese and 11.8% were 2^{nd} degree obese. 33.8% had low values of HDL-cholesterol and 42.6% had hypertriglyceridemia, with no significant differences between the IFG and the IGT patients (p_1=0.14, p_2=0.33).

When the patients were divided in quartiles based on the HOMA-IR index values, the highest quartile contained significantly more cases of metabolic syndrome than the lowest quartile ($p<0.05$), but in any of the four quartiles there were no statistically significant difference between the median values of BMI and WC, nor in the distribution of the IFG and the IGT patients ($p>0.10$ in all cases).

17.6% of the patients had abnormal carotid IMT; they were equally represented in the IFG and the IGT groups (Table 29), but they were all belonging to the two superior quartiles of HOMA-IR values, with a significant difference between the 3^{rd} and the 4^{th} quartile ($p<0.01$) (Figure 9 & Table 30); 83.3% of them met the criteria for metabolic syndrome.

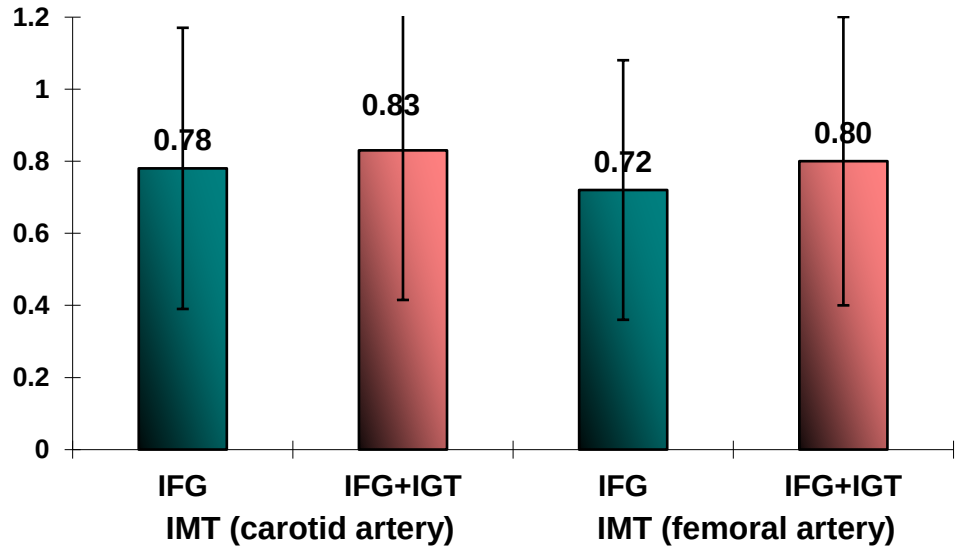

Figure 9. Mean IMT values in patients with isolated IFG and with IFG+IGT

Table 29. Values of carotid and femoral IMT in patients with isolated IFG and with IFG+IGT

	IMT (carotid artery)	**IMC (femoral artery)**	**Statistical significance**
IFG	0.78 ± 0.35	0.72 ± 0.32	t=1.01; p>0.05
IFG+IGT	0.83 ± 0.36	0.80 ± 0.35	t=2.93; p>0.05
Statistical significance	t=0.76 p>0.05	t=1.31 p>0.05	

Significance of all abbreviations is explained in the text.

Table 30. Values of carotid and femoral IMT in the 25th and 75th HOMA-IR quartiles

	Mean	Standard deviation	Quartiles			Q25 vs. Q75 differences
			25th	50th (Median)	75th	
HOMA-IR	2.5334	1.98885	1.3450	1.7150	3.1200	**p=0.001**
Carotid IMT	0.8177	0.33581	0.7425	0.8600	0.9600	**p=0.001**
Femoral IMT	0.7675	0.31740	0.6975	0.7950	0.9125	**p=0.0001**

Significance of all abbreviations is explained in the text.

2.6.4. Discussion

2.6.4.1. Relevance of study 1

The comparison between subjects with IFG and IGT was rarely performed by medical researchers in the last years. Hence, this analysis contributes to the detection of differences or similarities between incipient fasting and postprandial hyperglycemia.

Prediabetes is defined by either the presence of isolated IFG, isolated IGT, or that of both IFG and IGT. IFG, diagnosed as fasting plasma glucose levels slightly higher than normal, even after an overnight fast, occurs as a result of an impaired regulation of endogenous glucose production in the liver. IGT reveals the inability of an individual to

respond to the glucose loading induced by a meal, which corresponds to an elevated glycemia in postprandial conditions (*American Diabetes Association, 2020*). Both IFG and IGT are consequences of an increased insulin resistance, but the former results from the hepatic insulin resistance while the latter results from an increased insulin resistance in the skeletal muscle (*Abdul-Ghani et al, 2006*). The pancreatic β-cell dysfunction is common to both IFG and IGT. In the United States, mildly elevated levels of the glycated haemoglobin (HbA_{1c}) are also accepted for the diagnostic of prediabetes (*Ferrannini, 2014*).

Diagnostic of prediabetes is based on guidelines formulated by the World Health Organization (WHO) in 1999 and the American Diabetes Association (ADA) in 2003. Both guidelines provide the same cut point for IGT, but distinct cut points for IFG values (*Tabak et al, 2012*). Both the 1999 WHO guidelines and 2003 ADA guidelines recommend an IGT threshold of 7.8 to 11.0 mmol/L, where the plasma glucose measurement takes place 2 hours after the ingestion of 75 g glucose in an oral glucose tolerance test (OGTT). The threshold for IFG was set to 6.1 to 6.9 mmol/L by the 1999 WHO guidelines (*World Health Organization, 2006*), but later lowered to 5.6 to 6.9 mmol/L by the 2003 ADA guidelines. This distinct approach by the ADA resulted in reports of a higher global prevalence of prediabetes, and even if the test sensitivity increased, its specificity was definitely reduced (*Borch-Johnsen et al, 2004*; *Genuth, 2003*; *Schriger et al, 2004*; *Vaccaro et al, 2005*). In the years to come, the lower IFG threshold has been challenged by the WHO, which argued that the progression rate to type 2 diabetes is lower for those with liver insulin resistance (the IFG individuals) than for those with skeletal muscle insulin resistance (the IGT individuals) (*Leiva et al, 2014*). The Diabetes Epidemiology: Collaborative analysis of Diagnostic Criteria in Europe (DECODE) group, working under the auspices of the European Diabetes Epidemiology Group, which was subsequently established to assess the direct consequences of these revised diagnostic criteria, concluded later that IFG and IGT differ as predictors of all-cause mortality, of cardiovascular morbidity and mortality (*DECODE Study Group, 1999*).

The diagnostic criteria for prediabetes are still based on the same IFG and IGT thresholds and have not been changed in the most recent edition of ADA guidelines published in 2020 (*American Diabetes Association, 2020*). Clinical trials performed over the years have used one set of diagnostic criteria or the other to identify prediabetes, but most recent research predominantly used the 2003 ADA guidelines to report IFG. Some studies have set both IFG and HbA_{1c} values as thresholds to identify prediabetes (*Block et al, 2015*; *Parker et al, 2014*; *Perez et al, 2015*), while some other trials defined their inclusion criteria by both IFG and IGT values (*de Kreutzenberg et al*, 2015; *Liu et al, 2014*; *Perreault et al, 2014*).

Even though the prevalence of prediabetes may vary as a result of these differences in diagnostic criteria, the global prevalence of IGT was estimated by the International Diabetes Federation at 7.5% of the adult population in 2019, which is equivalent to 373.9 million individuals. About half (48.1%) of IGT subjects are aged under 50 years (*International Diabetes Federation, 2019*). By 2045 the prevalence is believed to increase to 8.6% of the worldwide adult population, which is equivalent to an estimated 548.4 million individuals, and constitutes a worrying trend (*International Diabetes Federation, 2019*; *Hostalek, 2019*).

The large proportion of patients (20.77%) that were identified in our study with diabetes mellitus, cardiovascular, renal or hepatic disease is an alarming finding in a population previously considered as having a good state of health. However, the design of this study had

limits that did not allow us to further analyze the etiology of these health issues, as to find if, for example, the hepatic disease might have a metabolic cause or the renal disease – an atherosclerotic one.

When analyzing the anthropometric data, we found the majority of patients (48.4%) to have first- or second-degree obesity, while fewer had a BMI over 40 kg/m^2. These findings might have multiple explanations. First, the large number of obese patients can be attributed to the fact that increased values of BMI associate with increased levels of insulin resistance and therefore with an increased risk of dysglycemia. Second, the lower number of cases with third degree obesity might be explained by speculating that such patients feature a greater risk of already having diabetes, which would have excluded them from our analysis. As to increased values of WC and WHR, we must emphasize their high prevalence both in men and women with isolated IFG and with IFG+IGT, most probably explained by the close association prediabetic dysglycemia has with abdominal obesity (*Petersen et al, 2005*), as both conditions are associated with elevated circulating concentrations of free fatty acids, oxidative stress, mitochondrial dysfunction, disordered nitric oxide release and endothelial dysfunction, all leading to insulin resistance (*Standl, 2012*).

Our data showed a high prevalence of hyperuricemia and lipid profile anomalies in both groups with isolated IFG and IFG+IGT. This is not a surprising result, given the known associations between prediabetes and these biochemical changes (*DeFronzo et al, 1991*). When the groups with isolated IFG and IFG+IGT were compared, most of the anthropometric and biochemical results were found to have similar mean values. As most studies up to this moment concentrated only on the comparison between normoglycemic, prediabetic and/or type 2 diabetic subjects (*Liu et al, 2009*), our paper adds knowledge to a chapter not yet sufficiently explored. Our findings add to data from other studies, which found no difference between subjects with isolated IGT and isolated IFG (*Lin et al, 2007*), but contradicts results other authors published recently, where BMI, WC, total, HDL and non-HDL-cholesterol, triglycerides and triglycerides-to-HDL ratio presented differences that argued for a more unfavorable cardiovascular risk profile in subjects with IFG+IGT than in those with isolated IFG (which they found to be close to normal controls) (*Lorenzo et al, 2013*). These differences, if confirmed by other future studies, might be explained by the distinct sites insulin resistance has in the two categories of incipient hyperglycemia – the liver in isolated IFG (with a normal insulin sensitivity in muscle) and both the liver and the muscle in IFG+IGT category.

About the uric acid, studies performed so far have concentrated mostly on its relationship with diabetes (*Ishizaka et al, 2005*; *Kodama et al, 2009*). There are only a few analyses to evaluate the relationships, if any, between uric acid and different stages of incipient hyperglycemia (*Whitehead et al, 1992*). In our female subjects, prevalence of hyperuricemia was different between the groups with IFG and IFG+IGT, but this difference disappeared in male subjects. This result supports data recently published by German researchers, who also found a stronger association of uric acid with isolated IFG and IFG+IGT in females than in males (*Meisinger et al, 2012*).

As to the relations between anthropometric data, lipid profile and uric acid, the strongest correlations to be identified, in both groups with isolated IFG and with IFG+IGT, were those between BMI and WHR as compared to triglycerides and uric acid. One plausible explanation

is that these two biochemical parameters – considered as components of the metabolic syndrome – might have an increasing trend in subjects with an excess fat mass (which also implies higher values of BMI and WHR), which tend to be more insulin resistant. Hyperuricemia, for instance, is strongly associated with insulin resistance, being thought to increase insulin resistance by stimulating inflammation and by leading to endothelial dysfunction and nitric oxide inhibition (*Nakagawa et al, 2005*). On the other hand, hyperinsulinism secondary to insulin resistance may reduce urate renal excretion and induce hyperuricemia. Other studies also reported an association between uric acid values and excess weight (especially if centrally distributed) in a population including normoglycemic obese subjects, prediabetics and type 2 diabetics (*Urbanavicius et al, 2008*).

It is estimated that 37% of the persons with prediabetes may develop diabetes in the next 4 years if their condition is not treated (*Yang et al, 2010*; *Tuso, 2014*; *Shen et al, 2016*; *Alanazi et al, 2017*; *Papaetis, 2014*). On the other hand, lifestyle optimization may lower the risk of prediabetes progressing to diabetes for approximately 10 years (*Tuso, 2014*). Prediabetes may therefore be considered a reversible turning point, where appropriate intervention may reduce the risk for diabetes development (*Tuso, 2014*; *Papaetis, 2014*). However, an expert panel which reviewed the literature data concluded that continuous, rather than dichotomous risk scores, predict better the risk of developing diabetes (*Bloomgarden, 2008*; *Perreault et al, 2019*). Diabetes risk scores based on accessible variables such as age, sex, ethnicity, fasting glucose, systolic blood pressure, HDL-cholesterol, BMI and history of diabetes in parents or siblings has been proven to predict the risk of progressing to diabetes better than the isolated use of IFG or IGT (*Stern et al, 2002*; *Richter et al, 2018*).

Comparison of insulin resistance indexes between subjects with isolated IFG and IFG+IGT led to similar results in our study groups when HOMA-IR was used, the only difference being noted for QUICKI. Data from the literature are conflicting, suggesting either that insulin resistance is more pronounced in IFG subjects, where it constitutes the main mechanism (while IGT is predominantly generated by defaults in insulin secretion), or that insulin resistance also plays an important role in subjects with IGT (*Hanefeld et al, 2003*).

The few studies that investigated levels of plasma adiponectin (*Bluher et al, 2007*) and leptin (*Tonjes et al, 2010*; *Wang et al, 2010*) in prediabetes gave contradictory results. In our subjects, no differences were identified when the mean values of adipokines were compared, which describes a similar profile of subjects with isolated IFG and IFG+IGT. Other authors consider that adiponectin is a marker for the progression of glucose intolerance in prediabetic stages, so we might have expected its mean values to be higher in patients with isolated IFG than in those with IFG+IGT. Nevertheless, other studies identified similar adiponectin levels in prediabetic and normoglycemic male subjects (*Luo et al, 2010*), suggesting that some categories of incipient dysglycemia are not necessarily accompanied by a decline in plasma adiponectin levels.

When the relationship between insulin resistance indexes and adipokines was studied in the two groups, different correlation patterns were identified. In subjects with isolated IFG (in other words, isolated fasting dysglycemia), adiponectin seemed to have the strongest connection with insulin resistance indexes and leptin – a more limited one, while in those with IFG+IGT (influenced not only by fasting, but also by postprandial hyperglycemia) HOMA-IR and QUICKI correlated predominantly to leptin. This might be explained by speculating that

postprandial metabolic anomalies are mostly linked to the variations in leptin secretion, known to modulate the appetite and energy balance, while fasting anomalies are predominantly linked to adiponectin, whose secretion is less correlated to food intake. If this is true, then the study of insulin resistance should be made by means of dosing different adipokines in subjects with isolated IFG and IFG+IGT. Moreover, our data support other findings which assert that IFG and IGT are associated with distinct adipokine patterns, reflecting the pathophysiological differences that underlie these two incipient hyperglycemic states. The connection between leptin and insulin resistance seems to be a bidirectional one, given the fact that leptin inhibits insulin synthesis and secretion, while insulin stimulates leptin secretion (*Faerch et al, 2009*).

One of the limitations of our study is that a temporal or a causal relationship between insulin resistance indexes and adipokine profile were impossible to demonstrate due to the crosssectional design of the study. Moreover, as this research is entirely observational, we cannot rule out the possibility that unmeasured or residual confounding factors might contribute to the observed or missing associations. Another limit of our data is that insulin resistance was not measured by clamp techniques, but using surrogate parameters (HOMA-IR and QUICKI). On the other hand, the study has several strengths, as it includes a relatively homogenous population and by focusing on prediabetes subjects instead of diabetic patients – it excludes some confounding factors such as drug treatment effect and decreased β-cell function within diabetic individuals, both having a direct effect on circulating insulin levels. A further step would be the initiation of a prospective study that would allow us to follow the evolution of our prediabetic subjects and to search for a cause-effect relationship in the case of the associations identified by the present study.

As an interim conclusion, our data on anthropometric data, biochemical profile and insulin resistance indexes support the existence of only minor differences between subjects with isolated IFG and with IFG+IGT, while no difference was noted in the mean values of adipokines. As to the relation between anthropometric data and biochemical profile, the strongest correlations that were identified in both groups were those between body mass index and waist-to-hip ratio on one hand and triglycerides and uric acid on the other hand. In subjects with isolated IFG, insulin resistance indexes seemed to have the strongest connection to adiponectin, while in those with IFG+IGT they appeared to be better correlated to leptin.

2.6.4.2. Relevance of study 2

Up to this moment, the association between low insulin sensitivity and IMT measured both at carotid and femoral levels was rarely analysed (*Vaudo et al, 2007*) and none of these previous studies concentrated on prediabetic patients as to analyse their adipokine profile. In the quoted interim analysis on 68 patients of our study, focused on the comparison of aspects related to subclinical atherosclerosis between the groups with isolated IFG and with IFG+IGT and between patients with lowest and highest values of the HOMA-IR index, we took into account that research comparing patients with IFG and IGT, or with lower and higher insulin resistance, in terms of their subclinical cardiovascular disease status is still scarce, and therefore an open matter for discussion. The conclusions of this interim analysis found no differences in the prevalence of incipient vascular abnormalities existed between our patients

with isolated IFG and with IFG+IGT, and patients with higher IMT expressing higher insulin resistance indexes, and metabolic syndrome in most cases.

Our data indicate that age is correlated to carotid IMT, but not to femoral IMT. This result is different from a previous research in a nondiabetic healthy geriatric population, where no relationship between carotid IMT and age was identified. However, as this correlation was not yet analysed in prediabetic patients, we might hypothesize that the metabolic anomalies that characterize this specific population might potentiate the effect of age on IMT values. As the association between age and femoral IMT was rarely analysed before and other data on prediabetic populations are unavailable, the lack of correlation we found between these two variables needs supplementary analyses on other prediabetic samples, but may indicate a lower or delayed sensibility of femoral IMT to the subclinical atherosclerotic modifications induced by age in the incipient hyperglycemic milieu. We did not observe any correlation between IMT values and the anthropometric data or the lipid profile of our subjects. On the other hand, some previous reports on healthy subjects mention a direct relationship between carotid IMT and some anthropometric measurements, especially those related to visceral adiposity; similarly, an association between the risk for ischemic heart disease and WHtR was reported on a large Lithuanian population sample (*Luksiene et al, 2011*). Correlations of carotid IMT with different components of the lipid profile in healthy individuals are inconstantly reported (*Acevedo et al, 2012*). In all previous studies regarding IMT in relation to anthropometric and lipid parameters, measurements of femoral IMT were not done and separate analyses on prediabetic subjects are lacking. Therefore, one possible explanation for our data might be that mild hyperglycemia induces specific metabolic changes that contribute to atherosclerosis through separate pathways than those secondary only to adipose excess or to classical lipid anomalies, thus reducing their relative influence upon IMT values. It is also possible that, by excluding subjects with clinical cardiovascular disease, we set aside some individuals in which the anthropometric or lipid anomalies might have influenced more the IMT and might have been better correlated to its values. Blood lipids also presented variable relationships with insulin resistance and adipokines levels in our subjects. Adiponectin was inversely correlated with markers of atherogenic dyslipidemia (triglycerides, cholesterol-to-HDL ratio and triglycerides-to-HDL ratio) and positively correlated to HDL-cholesterol. Inversely, HOMA-IR was positively associated to triglycerides, cholesterol-to-HDL ratio and triglycerides-to-HDL ratio and negatively associated to HDL-cholesterol. This is the first study to examine the relationships HOMA-IR and these adipokines have with lipid parameters in a prediabetic population, so results must be confirmed by subsequent research. Previous analyses found inconstant correlations between leptin and blood lipids in healthy subjects (*Tungtrongchitr et al, 2001*), while low adiponectin and high HOMA-IR levels were seldom reported as being associated to atherogenic dyslipidemia in non-diabetic subjects with different cardiovascular risk profiles (*Marso et al, 2008*). On the contrary, leptin and LAR, but not adiponectin, were correlated to HbA_{1c} levels in our study, suggesting a greater sensitivity of leptin than adiponectin to the small HbA_{1c} increases induced by prediabetes. Up to this moment, reports about the relationship between leptin and HbA_{1c} in diabetic populations provided contradictory results (*Taghdir et al, 2010*), while no previous study tested it in prediabetes subjects.

In our data, IMT values initially had inconstant correlations with the metabolic parameters. One possible explanation might be that the influence of insulin resistance and adipokines on this atherosclerosis marker was diminished by excluding from our study all individuals with a history of clinical cardiovascular disease. The lack of any initial correlations between IMT values and HOMA-IR can be interpreted in line with other studies, which found that in patients with incipient hyperglycemia, prediabetes is accompanied by an increased cardiovascular risk only if associated with metabolic syndrome, in other words if plain insulin resistance is present. It is therefore possible that a part of the subjects in our sample, having no sign of clinical atherosclerotic disease and maybe only minimally increased HOMA-IR values, to be the reason for the initial lack of correlations between IMT and HOMA-IR. Other studies also found that IMT does not correlate to insulin resistance measurements, but to postprandial hyperglycemia (*Marini et al, 2012*), which was not tested in our research; one possible explanation is that hyperglycemia can also induce atherosclerosis independently of insulin, through glycation of proteins and lipids and by increasing oxidative stress. Nevertheless, this hypothesis is not yet sufficiently tested in subjects with incipient hyperglycemia and needs to be tested in future research. On the other hand, adjustment of IMT for HOMA-IR and adipokine levels was necessary in our study to allow disentangling confounding effects by these variables, since both insulin resistance and secretion products of adipocytes are known to be associated with cardiovascular risk in subjects with advanced atherosclerosis (*Oliveira et al, 2011*). In our case, adjustments of IMT values based on leptin, LAR, triglycerides, HDL-cholesterol, cholesterol-to-HDL ratio, triglycerides-to-HDL ratio and HbA_{1c} induced the appearance of statistically significant correlations with HOMA-IR, with similar regression coefficients for carotid and femoral IMT. First, our results agree to observations from other studies, which found insulin resistance measured by HOMA-IR to be directly associated to carotid IMT values in non-diabetic subjects or in obese adolescents (*de Lima Sanches et al, 2011*). Second, our data do not suggest that femoral IMT is better correlated with insulin resistance than carotid IMT, as other studies did (*Vaudo et al, 2007*). Hence, we cannot share the idea that femoral IMT would make a more useful tool than carotid IMT to evaluate the connexions between the cardiovascular outcomes and the metabolic status of prediabetic patients. Third, appearance of new correlations between IMT and HOMA-IR after adjustments for some components of the lipid profile and HbA_{1c} suggests a residual relationship between low insulin sensitivity and atherogenesis, which is not mediated by the influence of insulin resistance-induced lipid and glucose metabolism changes upon IMT. When adjusting for HOMA-IR, moderate correlations of carotid and femoral IMT with adiponectin, leptin and LAR emerged. The inverse relationship between IMT and adiponectin, also identified in studies on healthy subjects, confirms that such negative correlations are also found in prediabetic patients, independently of the insulin resistance level. It might be possible that low adiponectin levels would induce atherogenesis by favouring inflammation and consequently abnormal changes in glucose and lipid metabolism. Chronic subclinical inflammation is known to be a predictor of both hyperglycemia and cardiovascular disease (*Perticone et al, 2008*). However, other observations in healthy individuals suggest that the inverse relationship between adiponectin and IMT is not an independent one, being mostly mediated by other cardiovascular risk factors associated with metabolic syndrome (*Nilsson et al, 2006*). Hence, we can only

speculate that prediabetic patients might manifest supplementary metabolic abnormalities that would induce lowering of adiponectin values parallel to atherogenesis. This hypothesis would explain results of other researches, where adiponectin was identified as a risk factor for atherosclerotic heart disease, in particular the myocardial infarction. Moreover, our study only analyzed the total adiponectin level, without measuring its isoforms, therefore no conclusion is possible on relationships between subclinical atherosclerosis and alterations in adiponectin oligomerization (*Dallinga-Thie et al, 2010*) that might exist in prediabetic patients. After adjustment by HOMA-IR, our data suggested a positive association between leptin and IMT values, showing a possible influence of body fat mass on the incipient atherosclerotic changes. These results are in agreement with other observations on normal subjects, which found plasma leptin to be correlated to carotid IMT. Our study seems to be the first to analyse the relationship that plasma leptin has with both carotid and femoral IMT. These are the first data about the comparative utility of adiponectin, leptin and LAR in prediabetic patients and seem similar to those from studies conducted on high cardiovascular risk individuals. On the contrary, in other types of patients, such as healthy middle-aged Italian men or Japanese type 2 diabetics, LAR seemed to be better correlated than adiponectin or leptin alone to subclinical atherosclerosis evaluated by IMT (*Norata et al, 2007*). As our findings do not support the use of LAR as a preferable estimate of atherosclerosis susceptibility compared to adiponectin or leptin alone, these apparent discrepancies need to be verified on larger samples, but might suggest a different pattern of associations between IMT values and adipokine profile in prediabetic patients. Moreover, as adiponectin did not seem to be substantially better correlated with IMT than leptin, we can speculate that both adipokines are related to this marker of subclinical atherosclerosis through a common underlying mechanism.

Prediabetes was also reported by several researchers to associate with an increased risk of chronic kidney disease and nephropathy (*Echouffo-Tcheugui et al, 2016*; *Plantinga et al, 2010*; *Xu et al, 2009*). This association may be induced by either the increased progression to diabetes or the coexistence of other factors inducing both hyperglycemia and nephropathy (*Thomas et al, 2011*; *Kim et al, 2019*; *Markus et al, 2018*). Other associations reported for prediabetes are those with the cardiac autonomic dysfunction, as seen in a reduced variability of heart rate (*Tesfaye et al, 2010*; *Schroeder et al, 2005*; *Wu et al, 2007*), the reduced parasympathetic modulation of cardiac activity (*Wu et al, 2007*) and the greater prevalence of male erectile dysfunction (*Grover et al, 2006*). IGT subjects associate greater neural anomalies such as impairment of up to four out of five cardiovascular reflex tests, increased prevalences of hyper- and hypoesthesia, increased heat sensitivity (*Putz et al, 2009*), higher proportions of idiopathic polyneuropathy, painful sensory neuropathy and small fiber neuropathy (*Hoffman-Snyder et al, 2006*; *Nebuchennykh et al, 2008*; *Stino et al, 2017*). Up to 8% of prediabetic subjects enrolled in the Diabetes Prevention Program were found to display early diabetic retinopathy (*Diabetes Prevention Program Research Group, 2007*). The association of prediabetes with a higher risk for atherosclerotic cardiovascular disease is widely reported, but it is still unclear whether it is due to prediabetes itself, to the high risk of developing diabetes or to the other risk factors common to both cardiovascular diseases and prediabetes (*Sarwar et al, 2010*; *Seshasai et al, 2011*; *Huang et al, 2017*; *Brannick et al, 2018*; *Kleinherenbrink et al, 2018*; *Huang et al, 2016*; *Barr et al, 2007*).

As prediabetes is a reversible condition, proper therapy has the chance to spare certain individuals from the long-term complications. Interfering of the two major pathways leading to prediabetes, insulin resistance and the relative deficiency of the β-cell function, may warrant the chance for a successful treatment of prediabetes as long as it is done early, before it aggravates to the point when the condition is no longer reversible (*Kanat et al, 2015*).

Most guidelines support lifestyle optimization, focused on the dietary interventions, weight loss and an increased physical activity, as a foundation stone of diabetes prevention. However, lifestyle optimization, even though safe and efficacious, is not reimbursed by most healthcare systems (*Bansal, 2015; Glechner et al, 2018*). Some consistent recent evidence also proves the efficacy of pharmacotherapy in persons with prediabetes. Organizations such as the ADA have recommended metformin as a potential pharmacological intervention in certain high-risk individuals (*American Diabetes Association, 2020*). Thiazolidinediones were also reported by some clinical trials to reduce the progression to diabetes in prediabetic subjects (*Knowler et al, 2005*; *Gerstein et al, 2006*). However, their use in prediabetes is not yet routinely advised due to issue related to cost and side effects. Ability to reduce the risk of progressing to diabetes has also been reported for α-glucosidase inhibitors (*Chiasson et al, 2004; Kawamori et al, 2009*), but they are not able to improve insulin sensitivity, so combination with other groups of antihyperglycemic drugs may be needed (*Shimabukuro et al, 2017*). No trials have yet been carried out to study the effect of either incretin-based drugs or SGLT-2 inhibitors on the progression of prediabetes to diabetes (*Khan et al, 2019*; *Hemmingsen et al, 2016*). Orlistat, an anti-obesity drug which reduces the intestinal absorption of fat, was found to lower the progression of prediabetes to diabetes if given in association with a low-calorie diet and weight loss (*Torgerson et al, 2004*). Bariatric surgery interventions, targeting obesity and therefore one of the major risk factors for diabetes development, were proven in several studies as a promising method of consistenly reducing the risk of progression from prediabetes to diabetes (*Sjöstrom et al, 2004*; *Pories et al, 1992*).

Among the limitations of our data, the first one is the small sample size we used; nevertheless, as our study is the first to simultaneously investigate the relationships between insulin resistance markers, adipokine levels and IMT values in subjects with incipient hyperglycemia and no clinical atherosclerosis, it must be credited for the novelty of its idea. Second, we are limited in interpretation given the cross-sectional design of our study, which precludes defining causal relationships. Therefore, we cannot state that abnormal adipokine profile or insulin sensitivity really precede the development of subclinical atherosclerosis measured by IMT values. Another limit in our research was represented by the use of a surrogate marker for insulin resistance (HOMA-IR); however, the value of this marker is already validated by numerous studies (*Gast et al, 2012*), while more complex measurements, as the hyperinsulinemic euglycemic clamp, have costs and technical difficulty levels that make them less accessible for usual clinical practice. Moreover, our choice of estimating subclinical atherosclerosis based on IMT values can be considered as generating accurate results, as IMT is seen as a direct measure for the arterial wall status and therefore directly reflects the extension of atheroma. Strengths of our research include the exclusion of subjects treated with glitazones, metformin or lipid-lowering drugs, which would have influenced the lipid and adipokine profiles, insulin sensitivity or HbA_{1c} levels. Besides, this study adds to the

limited information about the associations between metabolic profile (insulin resistance indexes, adipokines and HbA_{1c}) and subclinical atherosclerosis in prediabetic patients.

In conclusion, we should point out once more that this is the first study to investigate the associations between insulin resistance, adipokines and carotid and femoral IMT in prediabetic subjects without clinical signs of cardiovascular disease. Relationships between this atherosclerosis marker and the metabolic profile seem to exist even if manifest arterial obstructions are not yet present. The associations between IMT values and HbA_{1c} that emerge after adjusting for age prove that hyperglycemic changes, although minor, might have a significant effect on the arterial wall structure. Correlations of IMT with adipokines after HOMA-IR adjustments suggest that secretion anomalies in the adipose cells may modulate the evolution of atheromatous plaques independently of the insulin resistance level. Finally, the relationships identified between IMT and HOMA-IR after adjustments for leptin, LAR, HbA_{1c} and some lipid parameters indicate that correlations between insulin resistance and atherogenesis are not entirely mediated by adipokine, lipid and glycemic abnormalities.

CHAPTER 3. DIGESTIVE DISEASES AND DIABETES MELLITUS

3.1. State of the Art

Complications from diabetes mellitus involve a wide range of internal organs and systems. Some of the best researched forms of digestive disease in diabetes mellitus are due to the diabetic autonomic neuropathy, which concerns the vegetative nervous system. The vegetative (or autonomic) nervous system adds together the enteric, parasympathetic and sympathetic nerve systems. The autonomic neuropathy can bring upon the patients with diabetes mellitus some of the most burdensome symptoms, which severely lower the quality and sometimes the duration of life. On the other hand, autonomic neuropathy goes frequently unrecognized and under-diagnosed. The first forms of autonomic neuropathy to appear seem to concern the vagus nerve, which probably is one of the most vulnerable segments of the vegetative nervous system. Among patients with a duration of diabetes longer than 20 years, up to half of them may display gastrointestinal symptoms (*Sandireddy et al, 2014*).

The nervous link between the brain and the gut is a bidirectional network comprising some sensory inputs leaving from the gastrointestinal tube to the brain and some efferent output circuits leaving the brain and directing the secretion of digestive hormones, regulation of homeostasis and gut motility. Within this bipolar network, the autonomic nervous system, the enteric nervous ramifications and the sympathetic and parasympathetic efferences are closely connected in reciprocally regulating interactions.

Early stages of diabetic autonomic neuropathy are mostly silent and therefore difficult to detect. In some cases, the anomalies concerning the vagus nerve may induce some clinical manifestations, as its length and widespread functions (transmission of signals from the gut wall receptors, sensitivity to chemical and mechanical gut stimuli, multifactorial control of gut motility, gut secretions and eating behaviour) become most vulnerable to overt disfunctions (*Yuan et al, 2016*). It is therefore not a random fact that most research on the diabetic vegetative neuropathy focuses on the vagus function. Some neurons in the central nervous system display glucose-responsive properties which may drive, at least partially, the vagal efferent activity. These neurons may explain why changes in plasma glucose concentrations seem to alter the parasympathetic tone. Moreover, an enhanced vagus tone may be responsible for the activation of the cholinergic anti-inflammatory reflex and may modulate the immune system (*Koopman et al, 2016*). Such pathways, if they indeed had a practical significance, may explain why an enhanced vagal activity could be associated with a protective function against diabetes-induced neuroinflammation.

The diversity of pathways connecting together the enteric, the sympathetic and the parasympathetic nervous system justifies the wide variety of symptoms trigerred by the diabetic autonomic neuropathy (*Bonaz et al, 2016*). Reports of gastrointestinal symptoms are more frequent in patients with diabetes than in subjects without diabetes (*Bytzer et al, 2002*). Up to a fifth of diabetic patients complain about diarrhoea, and up to 60% about constipation (*Mjornheim et al, 2003*).

Non-alcoholic fatty liver disease (NAFLD) has become the today's epitome of metabolic chronic liver disease. Defined as the lipid overload in the hepatocytes that is caused by neither excessive alcohol intake (over 20 g/day in women and 30 g/day in men), nor hepatitis B or C viruses, autoimmune hepatitis, drug liver toxicity, or rare gene defects, NAFLD is estimated

to be present in up to one-third of the adult population of developed countries. The nomination of NAFLD covers a wide range of distinct histological and clinical entities, from the simple hepatic steatosis (fatty liver – NAFL), through steatohepatitis (NASH), which features hepatocyte ballooning, lobular inflammation and sometimes fibrosis, to the other extreme form of cirrhosis or, sometimes, hepatocellular carcinoma. At present, NAFLD is the second indication for liver transplant in the United States (*Wong et al, 2015*) and the most frequent cause of hepatocellular carcinoma (HCC) in both the United States (*Yu et al, 2013*) and the United Kingdom (*Dyson et al, 2014*).

Last but not least, the metabolic syndrome (MS) and chronic infection with hepatitis C virus (HCV) are both widely spread in the general population, and their superposition involves a wide range of patients with mixed metabolic and viral disease. The association of MS and HCV is not incidental, as there are common etiopathogenic links between the 2 conditions: insulin resistance, type 2 diabetes mellitus (T2DM), and liver steatosis. Nevertheless, the published data are still controversial (*Cheng YL et al, 2015*). HCV induces a specific form of metabolic syndrome called "hepatitis C-associated dysmetabolic syndrome" (HCADS), which includes hyperuricemia, reversible hypocholesterolemia, insulin resistance, hypertension, and visceral obesity.

In conclusion, another research direction I was preoccupied with refers to digestive disease in diabetes mellitus and other metabolically diseased individuals, involving both the digestive tube and the liver. My preoccupations in this research field have been directed towards the following three important axes:

- Digestive motility and diabetes mellitus
- Non-alcoholic fatty liver disease and diabetes mellitus
- Liver-targeted therapy in virus C compensated liver cirrhosis associated with metabolic syndrome

The results of my research have been materialized in the following papers:

Published papers in this field

- Digestive motility and diabetes mellitus

1. ***Mihai BM***, Mihai C, Cijevschi-Prelipcean C, Grigorescu ED, Dranga M, Drug V, Sporea I, Lăcătușu CM. Bidirectional relationship between gastric emptying and plasma glucose control in normoglycemic individuals and diabetic patients. *J Diabetes Res* 2018: 1736959. DOI 10.1155/2018/1736959.
2. Lăcătuşu Cristina Mihaela, ***Mihai BM***. Gastric motility in diabetic patients – Friend or foe? *Acta Diabetologica Română* 2015; 41: 36, 84-86.

- Non-alcoholic fatty liver disease and diabetes mellitus

1. Grigorescu E-D, Floria M, Lăcătușu CM, ***Mihai BM***, Crețu I, Popa AD, Onofriescu A, Jaba I, Șorodoc V, Ceasovschih A, Șorodoc L. The relationship between hepatic steatosis, inflammation and insulin resistance in type 2 diabetes with metabolic imbalance. *Internal Medicine* 2019; 16(4): 13-25.
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- Liver-targeted therapy in virus C compensated liver cirrhosis associated with metabolic syndrome

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3.2. Digestive motility disorders in diabetes mellitus

3.2.1. Rationale for the study of gastric emptying in relation with plasma glucose levels

Gastric emptying and glycemic control pathways are closely interrelated processes. Gastric chyme is transferred into the duodenum with velocities depending on its solid or liquid state, as well as on its caloric and nutritional composition. Once nutrients enter the intestine, the secretion of incretins (hormonal products of intestinal cells) is stimulated. Among incretins, glucagon-like peptide-1 (GLP-1) has multiple glycemic-regulatory effects that include delayed gastric emptying, thus triggering a feedback loop lowering postprandial serum glucose levels. Glycemic values also influence gastric emptying; hyperglycemia slows it down, and hypoglycemia accelerates it, both limiting glycemic fluctuations.

The relation between the stomach and diabetes mellitus was alleged ever since ancient times. In the first century AD, Aretaeus of Cappadocia, whose reputation is due to his work related to diabetes more than any other physician in antiquity, would say “We must, therefore, strengthen the stomach by all means, which is the fountain of thirst” when speaking of diabetes mellitus treatment (*Laios et al, 2012*). This hypothesis is obviously no longer valid in the 21st century, but the close bond between gastric emptying and glycemic control is

recognized today as a reality beyond any doubt. This relationship is bidirectional; on the one hand, gastric emptying is influenced by glycemic control (as hyperglycemic values slow it down and hypoglycemia accelerates it), while on the other hand, gastric emptying may influence glycemic values, particularly postprandial ones (*Plummer et al, 2015*).

In clinical medicine, gastroparesis is frequently encountered in association with diabetes, as about one-third of all cases of gastroparesis originate from complicated diabetes. Prevalence of gastroparesis is about 5% in type 1 diabetes patients and about 1% in type 2 diabetes. As gastroparesis is often associated with delayed gastric emptying, most research papers analyzed the velocity of gastric emptying, which is reduced in 30 to 50% of cases with long-lasting diabetes (*Camilleri et al, 2013*). In most cases, patients with overt gastroparesis have a long duration of insulin-dependent diabetes and a poor glycaemic control for at least several years. Usual gastroparetic symptoms may include nausea, vomiting, bloating, early satiety and epigastric pain. 80% of the patients presenting with gastroparesis are predisposed to the dysfunction of the small intestine, expressed as bacterial overgrowth in the small bowel or as pathogenic interactions between host and gut microbiota (*Camilleri et al, 2013*).

3.2.2. Materials and methods

We searched Medline and Scopus databases using the logical string "diabetes" OR "diabetes mellitus" AND "gastric emptying" to identify these key terms in the title or abstract of English-written articles published between January 2000 and January 2018. Clinical studies or trials, meta-analyses, and systematic reviews focusing on human subjects were selected. After eliminating duplicates, this initial search returned 794 results. We screened all titles and abstracts to select papers that could be considered relevant to the aim of our review. This operation led to a further reduction to only 52 titles. A second search using the same algorithm and replacing the key term of "gastric emptying" with "gastroparesis" OR "gastric motility disorders" and the key term of "diabetes" with "glucose disorders" OR "hyperglycemia" issued 14 supplementary relevant papers, which were also included in our analysis (*Mihai et al, 2018*).

3.2.3. Analysis of the mechanisms involved in the physiologic control of gastric emptying

When foods enter the stomach, the proximal gastric region initially relaxes in order to "accommodate" the ingested nourishments. They subsequently reach the distal areas of the stomach and, by means of antral contractions, are ground and mixed with the gastric hydrochloric acid secretion. Gastric chyme (Greek: *khymos* = juice), a semifluid mass, is thus formed. When the resulting particles are less than 1–2mm in diameter, they go through the pylorus into the duodenum. Their transit is backed by antral contractions and relaxation of the pyloric sphincter. In normal conditions, the rate of gastric emptying may vary between 1 to 4 kcal/min (*Marathe et al, 2013*), depending on the composition of gastric content (solids or liquids) and the macronutrient type. As a result of their high caloric content, lipids are evacuated more slowly from the stomach than carbohydrates or proteins (*Tappenden, 2017*). Digestion of solids begins after feeding with a lag time of 20 to 40 minutes, required for their grounding into 1–2mm diameter particles, and then, a quasilinear gastric emptying begins. Therefore, their evacuation from the stomach begins approximately 40 minutes after food intake and may last for a few hours. Evacuation of liquids is immediate (without any lag

phase) and usually mono-exponential. The more nutritionally dense the liquids are, the slower the gastric emptying becomes (*Phillips et al, 2015*). In fact, caloric content of foods may exert a greater influence on gastric emptying than previously thought. Some recent results suggest not only liquids of equal energetic densities are evacuated from the stomach with similar speeds, but meals with the same caloric content given with equal amounts of water have nearly identical gastric emptying curves, no matter if their initial form was solid or liquid (*Okabe et al, 2017*).

Nearly thirty years ago, Malbert and Ruckebusch described an intermittent transpyloric flux, with antral contractions and pyloric relaxation turning up at approximately 20-second intervals, while duodenal flux was continuous and uniform. At that moment, the mechanical function of the duodenal bulb was thought to be the only factor to transform this flux from intermittent to continuous. The mechanism is in fact far more complex. When nutrients reach the intestine, they become a signal stimulating the blood release of intestinal hormones known as the incretin system. K cells from the upper intestine (duodenum) secrete glucose-dependent insulinotropic peptide (GIP); in the distal segment of the intestine, L cells secrete glucagon-like peptide-1 (GLP-1). Both GLP-1 and GIP have glucose-dependent insulin secretion effects; GLP-1 inhibits glucagon secretion, and GIP exerts glucagonotropic actions (*Phillips et al, 2015*; *Wu et al, 2016*). While it was demonstrated that GIP has no effect on gastric emptying, GLP-1 induces an inhibitory feedback effect, delaying gastric emptying. Recent data also suggest a relation between glycemic values and stomach emptying (*Wu et al, 2016*). High velocity gastric emptying allows nutrients to reach the intestine more rapidly, thus increasing postprandial glycemia; on the other hand, hyperglycemia delays stomach emptying, so the nutrients are propelled more slowly for absorption at the intestinal level (*Plummer et al, 2015*). Hypoglycemia induces reverse effects, by accelerating gastric emptying and increasing the nutrient absorption speed, thus allowing for a prompter correction of glycemic levels (*Marathe et al, 2013*). The ability to increase gastric emptying was found to persist in healthy individuals even after repeated hypoglycemic episodes (*Wu et al, 2016*). This is in contrast to other hypoglycemia-induced reactions, such as the clinical signs induced by adrenergic response, which are subdued by impaired hormonal counter-regulation and tend to fade out during recurrent hypoglycemia.

As mentioned before, gastric emptying establishes a bidirectional relationship with glycemic levels: glycemia influences gastric emptying, while the latter may also influence the value of postprandial glycemia. Postprandial serum levels of glucose are essential in diabetic patients. Postprandial hyperglycemia is associated with increased oxidative stress and thus directly involved in the pathogenesis of chronic micro- and macrovascular complications of diabetes mellitus (*IDF Guideline Development Group, 2014*).

Several research teams found a direct relationship between the rate of gastric emptying and postprandial serum glucose levels. In patients with type 1 diabetes, altered rates of gastric emptying may impair efforts to adjust doses of prandial insulin according to the amounts of ingested nutrients. The most difficult problem in patients with type 1 diabetes is not the issue of too high or too low speeds of gastric emptying but its unpredictability. Most authors found that gastric emptying is increased even after recurrent episodes of hypoglycemia, not only in healthy individuals but also in diabetic patients (*Kar et al, 2016*). Evaluation of gastric emptying in insulin-treated diabetic persons with frequent hypoglycemia found it to be

delayed in most situations; in fact, such individuals exhibit discrepancies between the action of prandial insulin and the rates of gastric emptying. Hereupon, the evaluation of diabetic patients with frequent hypoglycemia should best include the assessment of gastric emptying; the "gastric hypoglycemia" (by delayed gastric emptying) proves to be an important concept in the management of diabetes mellitus (*Parthasarathy et al, 2016*).

3.2.4. Analysis of the effects of nutrients on gastric emptying and plasma glucose levels

Effects exerted by the main nutrients or other food components on both these aspects can hardly be separated from other perspectives, given that both gastric emptying and postprandial glycemic values imply the coexistence of meals and therefore nutrients. Adding sources rich in proteins to carbohydrate-based meals determines a 20% to 30% reduction in postprandial glucose levels. Besides the stimulation of insulin secretion (driven by direct stimulatory effects, but also indirectly, through an increased incretin response), underlying pathways include delayed gastric emptying under the influence of the same incretin hormones. Whey or soy proteins seem to give the best responses, but favorable effects on postprandial glycemic levels were also reported for rice, pea, and oat proteins (*Sun et al, 2017; Tan et al, 2017*). The structure of whey and soy proteins is rich in branched-chain amino acids, allowing faster digestion and absorption times, and therefore a quicker insulin release from pancreatic beta cells (*Giezenaar et al, 2017*).

In contrast with whole-structure proteins, intragastric administration of isolated amino acids such as lysine, leucine, or isoleucine does not seem to influence gastric emptying, even though it may reduce postprandial glycemic levels, most probably by direct stimulation of insulin secretion (*Mano et al, 2018*). Other three amino acids, histidine, glutamate, and aspartate, were reported to increase both postprandial glycemic levels, velocity of gastric emptying, and GLP-1 serum concentrations (*Mano et al, 2018*). L-Tryptophan isomeric form was found to significantly delay gastric emptying, even though the effect on GLP-1 secretion was minimal.

Classical nutrition information considers that meals with high lipid content reduce the velocity of gastric emptying. Recent data seem to confirm this theory by showing, for example, that high-fat meals may worsen symptoms of diabetic gastroparesis when compared to low-fat ones (*Homko et al, 2015*). More than the absolute lipid load, the degree of emulsification and the lipid droplet size seem to influence gastric emptying. Fine emulsions of olive oil in water slowed gastric emptying more than a coarse emulsion or a nonemulsified mixture of olive oil and water. Dietary fiber-rich foods are also able to reduce postprandial glycemia, with soluble fiber exerting the most pronounced effect. Most common explanations usually refer to an unmediated ability of soluble fiber to delay glucose absorption (*Homko et al, 2015*). However, the exact magnitude and conditionality of the effect fibers may exert on gastric emptying are still debatable.

3.2.5. Analysis of the effects of GLP-1 receptor agonists on gastric emptying and plasma glucose levels

GLP-1 receptor agonists are classified based on their half-life; the short-acting agents are designated as prandial agonists (exenatide BID, lixisenatide), while long-acting agents are considered nonprandial agonists (exenatide QW, liraglutide, dulaglutide). Their glycemic

effects differ, as prandial agonists mostly influence postprandial glycemia and nonprandial agonists exert a greater effect on fasting glycemia (*Meier et al, 2015*). As nonprandial GLP-1 receptor agonists have longer half-lives and prolonged action, the gastric emptying effect is reduced by tachyphylaxis and their influence on postprandial glycemia is thus diminished by comparison with prandial agonists (*Nakatani et al, 2017*). However, effects of short-acting and long-acting GLP-1 receptor agonists on postprandial glycemia are not always different, since semaglutide, a longer-duration GLP-1 receptor agonist seems able to lower postprandial glycemia and the velocity of gastric emptying in obese subjects.

Novel therapeutic guidelines for type 2 diabetes recommend the association of prandial GLP-1 receptor agonists to basal insulin; benefits of such pharmacologic combinations bring together the predominant effect of basal insulin on fasting glycemia and the effect of prandial GLP-1 receptor agonists on postprandial glycemia, based on their ability to inhibit gastric emptying (*Albèr et al, 2017*). This association between basal insulin and prandial GLP-1 receptor agonists is preferred today to the classical intensification of basal insulin therapy by adding prandial insulins, as it offers advantages of both a lower risk for hypoglycemia and a reduced weight gain (*Albèr et al, 2017*).

The effect of GLP-1 receptor agonists in diabetic patients with autonomic neuropathy and delayed gastric emptying, even though less studied, is usually feared to be deleterious in clinical practice by inducing or aggravating digestive intolerance, and therefore, their administration is intuitively avoided by most physicians in the case of patients with diabetic gastroparesis. Even if no detrimental effects would occur, choosing short-acting GLP-1 receptor agonist therapies in patients with diabetic gastroparesis seems an illogical and useless option, since their benefits are based on delay of gastric emptying. Patients with type 2 diabetes and slow gastric emptying at baseline may benefit more from treatment with long-acting GLP-1 receptor agonists, while in those with preserved gastric emptying, short-acting GLP-1 receptor agonists may be preferable.

In conclusion, gastric emptying and glycemic control exert an ongoing influence upon each other. Normal rates of gastric emptying, of 1 to 4 kcal/min, correspond to the best balance between intestinal propulsion and absorption of macronutrients (especially carbohydrates), incretin hormone secretion, and postprandial glycemic levels. Contrary to this equilibrium state, higher rates of gastric emptying may induce postprandial hyperglycemia but also represent a compensatory mechanism intervening when hypoglycemia occurs, while slower gastric emptying limits postprandial glycemic excursions or even acts as compensator under hyperglycemic conditions. In diabetic patients, fluctuations in gastric emptying are induced by complex pathophysiological pathways; these fluctuations may have a highly variable, unpredictable time pattern and limited correlations with the severity of clinical manifestations but strongly associate with variations in postprandial glycemic levels. Increased knowledge of this relation between gastric emptying and postprandial glycemic values allowed therapies targeting both factors to be updated, including diets with modified content or incretin-based medications. Future research and development will probably expand the range of both types of interventions, with diets based on reconsidered meal content or sequence and more GLP-1 receptor agonists approved for clinical use (*Mihai et al, 2018*).

3.3. Non-alcoholic fatty liver disease (NAFLD) and diabetes mellitus

3.3.1. Rationale of the study of NAFLD in association with diabetes mellitus

Medical literature acknowledges today a close relationship between chronic liver disease and diabetes mellitus. Alcoholic liver disease, portal hypertension of various aetiologies, but most of all chronic hepatitis C, NAFLD and NASH are frequently associated with a wide range of impaired glucose tolerance. As chronic hepatitis of any aetiology progresses towards the final stage of liver cirrhosis, hyperglycaemia becomes more and more frequent, and more and more severe. Chronic liver disease is associated with functional anomalies of all types of liver cells, thus leading to an increased insulin resistance, which seems to represent the major pathway underlying the liver disease-associated hyperglycaemia. The two best known examples for this associations are NAFLD (considered today as the liver determination within the metabolic syndrome) and chronic hepatitis C, which is progressively seen as a mixed disease, both viral and metabolic. On the other hand, recent research suggests that insulin resistance is associated with an increased risk of fibrosis progression, thus becoming a negative prognostic factor for the patient with chronic liver disease (*Watt et al, 2019*).

I was a member of a research team which developed two projects based on these theoretical considerations. In the first project, we evaluated the severity of liver fibrosis in patients with metabolic and/or viral liver disease and correlated this finding with several metabolic parameters of great interest during the last years. Our team used accurate inclusion criteria, which allowed the formation of homogenous study groups, and therefore valid statistical results. We focused our research on type 2 diabetes patients with NAFLD, and compared them with patients with chronic hepatitis B or C with either type 2 diabetes or normal glucose values. These choices were supported by the high prevalence of these liver diseases in the general population of Romania, our geographical region comprised. We searched for the variations of excess weight, lipid profile, insulin resistance, adiponectin and fibrosis associated to each of the metabolic or viral etiologies of liver disease (*Lăcătușu et al, 2011*; *Grigorescu et al, 2019*).

3.3.2. Materials and methods

3.3.2.1. Materials and methods of the first study

We formed five groups of patients with distinct etiologies of liver disease: type 2 diabetes mellitus with NAFLD, but no viral disease (DM), type 2 diabetes, steatosis and chronic hepatitis B (DM-CHB), type 2 diabetes, steatosis and chronic hepatitis C (DM-CHC), chronic hepatitis B, but no type 2 diabetes or steatosis (CHB), and chronic hepatitis C, but no type 2 diabetes or steatosis (CHC). All subjects gave a written informed consent to participate in the study prior to any study procedure. We collected data on age, gender, weight, height, waist circumference (WC), coexistence of hypertension, fasting plasma concentrations of glucose, lipids, insulin and adiponectin. The body mass index (BMI), waist-to-hip ratio (WHR), homeostasis model assessment for insulin resistance (HOMA-IR) index were calculated with the consecrated formulas found in the literature.

All previously mentioned data were centralized in a database using Microsoft Excel. Statistical analysis was performed using SPSS version 16.0. For descriptive statistics, discrete and continuous variables were expressed as frequencies and percentages, and means and standard deviations, respectively. Student t-test (for continuous variables) and $\chi 2$-test (for

categorical variables) were used to compare differences between subjects. Pearson and Spearman correlations were used to analyse associations between variables. When needed, multiple linear regression analysis was performed to disclose independent contributions between variables. A two-sided p-value below 0.05 was considered as statistically significant.

3.3.2.2. Materials and methods of the second study

The general methodology of the second study was already described in section 2.3.2. From the initial database, we selected 120 patients whose biochemical tests for liver disease were also available. In this group of patients with type 2 diabetes, we developed a secondary objective of analysing the severity of lipid overload in the liver. 84 patients formed the study group, who received therapy with a GLP-1 receptor agonist (15 patients) or a DPP-4 inhibitor (69 patients). Other 36 patients, receiving therapy with non-incretin drugs (sulfonylurea or acarbose), according to the diabetologist's clinical judgment, formed the control group (30%). In addition to the study procedures that were already described, we calculated the Fatty Liver Index (FLI), Hepatic Steatosis Index (HSI) and Non-Alcoholic Fatty Liver Disease-Liver Fat Score (NAFLD-LFS) using online and mobile interfaces provided by MDCalc, a well-known and widely used medical reference and e-tool. HOMA-IR C-peptide = (fasting glycemia (mg/dl)/18 × C-peptide (ng/ml) × 3.003) / 22.5 and C-peptide index = 20/[(C-peptide (ng/ml) × 3,003) × (fasting glicemia (mg/dl)/18)] were also calculated. Statistical procedures were the same as previously described.

3.3.3. Results

3.3.3.1. Results of the first study

Our study groups included 55 DM patients, 30 DM-CHB patients, 37 DM-CHC patients, 55 DM patients, 17 CHB patients and 19 CHC patients. Gender distribution was similar in the five groups; mean age was lower in the CHB and CHC groups than in the groups with diabetic patients (all $p<0.01$).

Patients in groups DM and DM-CHB had higher mean values of the body mass index (BMI) compared to group DM-CHC ($p=0.05$) and to groups CHB and CHC (both $p=0.001$) (Table 31). Obesity, defined as a BMI value of 30 kg/m^2 or higher, was significantly more frequent in the groups DM and DM-CHB than in group CHB ($p=0.013$) and significantly more frequent in group DM than in group CHC ($p=0.011$), with no significant differences between groups CHC and DM-CHC. Mean waist circumference (WC) values were higher in all groups including diabetic patients, compared to groups CHB and CHC (all $p<0.05$) (Table 32). Mean values of WHR were significantly lower for CHC, but not for CHB group, compared to all groups including diabetic patients ($p=0.001$ for DM and DM-CHB groups, $p=0.002$ for DM-CHC group) (Table 33).

High values of serum triglycerides (over 150 mg/dl) were significantly more frequent in the DM group than in DM-CHB and DM-CHC patients ($\chi^2=14.53$; GL=2; $p=0.0007$), but also in the DM-CHB group compared to DM-CHC patients ($\chi^2=7.41$; GL=1; $p=0.007$). Mean values of total cholesterol were significantly higher in DM and DM-CHB groups than in DM-CHC ($p=0.001$ and $p=0.01$, respectively) and CHC ($p=0.001$ and $p=0.05$, respectively) groups (Table 34). Mean values of LDL-cholesterol were significantly lower in all patients having

CHC, with or without diabetes, than in groups where liver disease was induced by diabetes and/or CHB (Table 35).

Table 31. Differences among groups in the mean values of BMI

Group	**DM**	**DM-CHB**	**DM-CHC**	**CHB**	**CHC**
DM (n=55)	**1**				
DM-CHB	t=0.93	**1**			
(n=30)	>0.05				
DM-CHC	t=2.09	t=2.32	**1**		
(n=37)	**0.05**	**0.05**			
CHB	t=3.48	t=3.52	t=2.12	**1**	
(n=17)	**0.001**	**0.001**	**0.05**		
CHC	t=3.75	t=3.61	t=2.16	t=0.31	**1**
(n=19)	**0.001**	**0.001**	**0.05**	>0.05	

Significance of all abbreviations is explained in the text.

Table 32. Differences among groups in the mean values of WC

Group	**DM**	**DM-CHB**	**DM-CHC**	**CHB**	**CHC**
DM (n=55)	**1**				
DM-CHB	χ^2=0.01	**1**			
(n=30)	0.925				
DM-CHC	χ^2=0.01	χ^2=0.09	**1**		
(n=37)	0.940	0.763			
CHB	χ^2=12.23	χ^2=9.22	χ^2=7.16	**1**	
(n=17)	**0.0005**	**0.002**	**0.007**		
CHC	χ^2=9.37	χ^2=7.27	χ^2=5.24	χ^2=0.11	**1**
(n=19)	**0.002**	**0.007**	**0.022**	0.740	

Significance of all abbreviations is explained in the text.

Table 33. Differences among groups in the mean values of WHR

Group	**DM**	**DM-CHB**	**DM-CHC**	**CHB**	**CHC**
DM (n=55)	**1**				
DM-CHB	t=0.88	**1**			
(n=30)	>0.05				
DM-CHC	t=1.50	t=0.68	**1**		
(n=37)	>0.05	>0.05			
CHB	t=1.99	t=1.54	t=1.12	**1**	
(n=17)	>0.05	>0.05	>0.05		
CHC	t=4.60	t=3.90	t=3.23	t=1.32	**1**
(n=19)	**0.001**	**0.001**	**0.002**	>0.05	

Significance of all abbreviations is explained in the text.

Table 34. Differences among groups in the mean values of total cholesterol

Group	DM	DM-CHB	DM-CHC	CHB	CHC
DM (n=55)	**1**				
DM-CHB	t=0.92	**1**			
(n=30)	>0.05				
DM-CHC	t=4.12	t=3.00	**1**		
(n=37)	**0.001**	**0.01**			
CHB	t=0.86	t=0.23	t=1.76	**1**	
(n=17)	>0.05	>0.05	>0.05		
CHC	t=3.65	t=2.52	t=0.60	t=1.42	**1**
(n=19)	**0.001**	**0.05**	>0.05	>0.05	

Significance of all abbreviations is explained in the text.

Mean values of serum adiponectin were significantly higher in DM-CHC group than in DM (p=0.001), DM-CHB (p=0.05) and CHB groups (0.001) (Figure 10 & Table 36). Adjustment of serum adiponectin by HOMA-IR values led to the intensification of these differences, while adjustment by BMI values partially mitigated these differences (Table 37 & Table 38).

Table 35. Differences among groups in the mean values of LDL-cholesterol

Group	DM	DM-CHB	DM-CHC	CHB	CHC
DM (n=55)	**1**				
DM-CHB	t=0.12	**1**			
(n=30)	>0.05				
DM-CHC	t=5.27	t=4.28	**1**		
(n=37)	**0.001**	**0.001**			
CHB	t=0.98	t=0.94	t=2.83	**1**	
(n=17)	>0.05	>0.05	**0.01**		
CHC	t=5.23	t=4.42	t=0.56	t=3.08	**1**
(n=19)	**0.001**	**0.001**	>0.05	**0.01**	

Significance of all abbreviations is explained in the text.

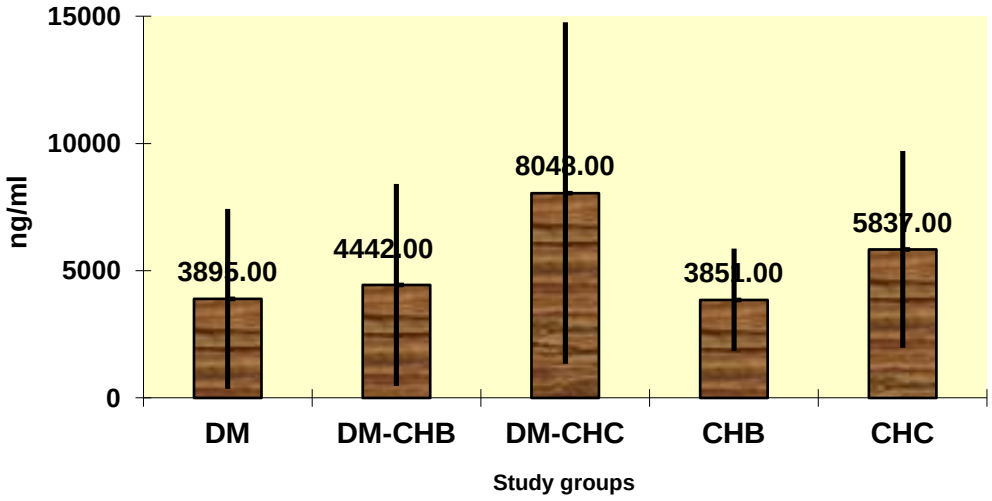

Figure 10. Mean values of adiponectin in the studied groups

Table 36. Differences among groups in the mean values of adiponectin

Group	DM	DM-CHB	DM-CHC	CHB	CHC
DM (n=55)	**1**				
DM-CHB (n=30)	t=0.63 >0.05	**1**			
DM-CHC (n=37)	t=3.45 **0.001**	t=2.73 **0.05**	**1**		
CHB (n=17)	t=0.06 >0.05	t=0.68 >0.05	t=3.54 **0.001**	**1**	
CHC (n=19)	t=1.93 >0.05	t=1.22 >0.05	t=1.58 >0.05	t=1.96 >0.05	**1**

Significance of all abbreviations is explained in the text.

Table 37. Differences among groups in the mean values of adiponectin adjusted by HOMA-IR

Group	DM	DM-CHB	DM-CHC	CHB	CHC
DM (n=55)	**1**				
DM-CHB (n=30)	t=2.23 **0.05**	**1**			
DM-CHC (n=37)	t=7.55 **0.0001**	t=3.90 **0.001**	**1**		
CHB (n=17)	t=2.45 **0.05**	t=5.86 **0.0001**	t=10.96 **0.0001**	**1**	
CHC (n=19)	t=2.40 **0.05**	t=3.87 **0.001**	t=11.02 **0.0001**	t=0.11 >0.05	**1**

Significance of all abbreviations is explained in the text.

Table 38. Differences among groups in the mean values of adiponectin adjusted by BMI

Group	DM	DM-CHB	DM-CHC	CHB	CHC
DM (n=55)	**1**				
DM-CHB (n=30)	t=0.34 >0.05	**1**			
DM-CHC (n=37)	t=2.48 **0.05**	t=2.11 **0.05**	**1**		
CHB (n=17)	t=1.13 >0.05	t=1.50 >0.05	t=3.84 **0.001**	**1**	
CHC (n=19)	t=0.48 >0.05	t=0.11 >0.05	t=2.14 **0.05**	t=1.79 >0.05	**1**

Significance of all abbreviations is explained in the text.

Mean HOMA-IR values were also higher in all groups including diabetic patients, compared to groups CHB and CHC (all $p<0.05$), but not different between groups DM-CHB and DM-CHC, or CHB and CHC (Table 39). Differences in HOMA-IR disappeared when adjusted based on BMI, but were maintained (to a reduced significance level though) when adjusted based on WC values (Table 40 & Table 41).

Table 39. Differences among groups in the mean values of HOMA-IR

Group	**DM**	**DM-CHB**	**DM-CHC**	**CHB**	**CHC**
DM (n=55)	**1**				
DM-CHB	t=0.44	**1**			
(n=30)	>0.05				
DM-CHC	t=0.52	t=0.80	**1**		
(n=37)	>0.05	>0.05			
CHB	t=4.86	t=3.55	t=3.29	**1**	
(n=17)	**0.0001**	**0.0001**	**0.002**		
CHC	t=6.05	t=4.13	t=4.08	t=1.29	**1**
(n=19)	**0.0001**	**0.0001**	**0.0001**	>0.05	

Significance of all abbreviations is explained in the text.

Table 40. Differences among groups in the mean values of HOMA-IR adjusted by BMI

Group	**DM**	**DM-CHB**	**DM-CHC**	**CHB**	**CHC**
DM (n=55)	**1**				
DM-CHB	t=0.72	**1**			
(n=30)	>0.05				
DM-CHC	t=0.51	t=0.26	**1**		
(n=37)	>0.05	>0.05			
CHB	t=0.46	t=0.32	t=0.06	**1**	
(n=17)	>0.05	>0.05	>0.05		
CHC	t=0.54	t=1.34	t=1.19	t=1.16	**1**
(n=19)	>0.05	>0.05	>0.05	>0.05	

Significance of all abbreviations is explained in the text.

Table 41. Differences among groups in the mean values of HOMA-IR adjusted by WC

Group	**DM**	**DM-CHB**	**DM-CHC**	**CHB**	**CHC**
DM (n=55)	**1**				
DM-CHB	t=0.99	**1**			
(n=30)	>0.05				
DM-CHC	t=0.64	t=0.39	**1**		
(n=37)	>0.05	>0.05			
CHB	t=2.45	t=3.44	t=3.25	**1**	
(n=17)	**0.05**	**0.002**	**0.002**		
CHC	t=2.99	t=3.95	t=3.82	t=1.0	**1**
(n=19)	**0.01**	**0.001**	**0.001**	>0.05	

Significance of all abbreviations is explained in the text.

3.3.3.2. Results of the second study

66.7% of the patients were obese and 29.2% overweight, with mean BMI values of 32.75±5.65 kg/m^2 and mean abdominal circumference values of 109.48±12.62 cm. These features are consistent with known patterns of obesity among T2DM patients worldwide. Most patients had dyslipidemia and/or hypertension among data in their medical history. Non-alcoholic hepatic steatosis was also diagnosed by ultrasonographic measurements in 15% of the cases, while only 15 patients had no proof of other disease than type 2 diabetes mellitus. 70% of the patients had antihyperglycemic monotherapy, with only 3 subjects on sulfonylurea, and the rest taking metformin. The other 30% were prescribed a combination of oral metformin and sulfonylurea (23.3%) or metformin and acarbose.

Statistically significant correlations were noted between HOMA-IR index and the inflammation markers IL-6 (r=0.22, p=0.012) and hsCRP (r=0.29, p=0.001). At the same time HOMA C-peptide correlated weakly only with hsCRP levels (r=0.22, p=0.01) (Table 42).

Table 42. Association between inflammation markers and insulin resistance indexes

Spearman correlations		**insulin**	**C-peptide**	**IL-6**	**hsCRP**	**HOMA-IR**	**HOMA-C-peptide**
insulin	r	1.00	**0.74**	**0.21**	**0.25**	**0.93**	**0.67**
	p	.	0.00	0.02	0.005	0.00	0.00
C-peptide	r	**0.74**	1.00	0.13	**0.21**	**0.72**	**0.86**
	p	0.00	.	0.13	0.02	0.00	0.00
IL-6	r	**0.21**	0.13	1.00	**0.41**	**0.22**	0.15
	p	0.02	0.13	.	0.00	0.012	0.08
hsCRP	r	**0.25**	**0.21**	**0.41**	1.00	**0.29**	**0.22**
	p	0.005	0.02	0.00	.	0.001	0.01
HOMA-IR	r	**0.93**	**0.72**	**0.22**	**0.29**	1.00	**0.80**
	p	0.00	0.00	0.012	0.001	.	0.00
HOMA-C-peptide	r	**0.67**	**0.86**	0.15	**0.22**	**0.80**	1.00
	p	0.00	0.00	0.08	0.01	0.00	.

Significance of all abbreviations is explained in the text.

Assessment of the statistical relationship between the inflammation markers, insulin resistance and hepatic steatosis indexes provided the results summarized in Table 43.

The diagnostic performance of the liver steatosis indexes was tested by the area under the receiver operating characteristic curve (AUROC). The AUROC for FLI was 0.78 [95% CI (0.70, 0.86)], for HSI – 0.70 [95% CI (0.60, 0.79)], and for NAFLD-LFS – 0.87 [95% CI (0.81, 0.94] (Figure 11).

Table 43. Correlations between inflammation markers, hepatic steatosis and insulin resistance markers indexes

Spearman correlations		HbA1c	IL-6	hsCRP	HOMA-IR	HOMA-C-peptide	C-peptide index
FLI	r	-0.04	**0.19**	**0.3**	**0.53**	**0.45**	**-0.45**
	p	0.68	0.03	0.001	0.00	0.00	0.00
HSI	r	-0.08	**0.18**	**0.32**	**0.35**	**0.25**	**-0.257**
	p	0.38	0.03	0.000	0.000	0.005	0.005
NAFLD-LFS	r	-0.25	**0.19**	**0.29**	**0.77**	**0.55**	-0.55
	p	0.18	0.03	0.002	0.00	0.000	0.000

Significance of all abbreviations is explained in the text.

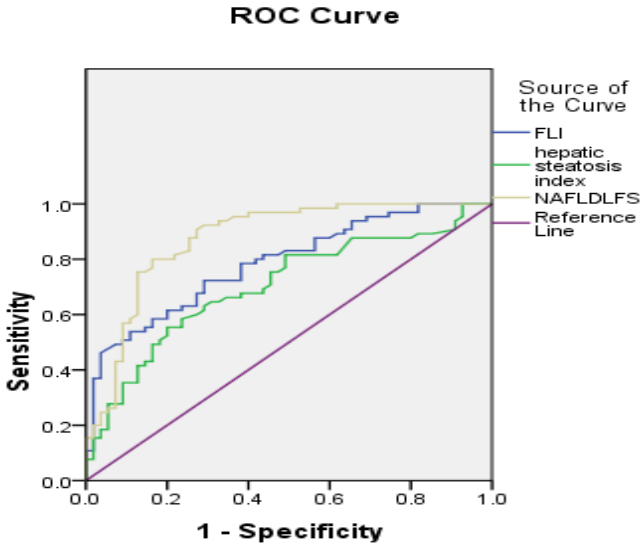

Figure 11. AUROCs for the predictive values of severe insulin resistance by hepatic steatosis indexes

Table 44. Variation of hepatic steatosis indexes depending on insulin resistance levels and quality of glycemic control

Distribution by insulin resistance levels / glycemic control	Mean rank (Kruskall Wallis Test)					
	FLI	p	HSI	p	NAFLD-LFS	p
HOMA-IR<2	27.94	<0.001	36.38	<0.001	26.27	<0.001
HOMA-IR=2-5	48.12		52.18		37.26	
HOMA-IR>5	76.22		71.67		76.15	
HbA1c<7.5	62.62	0.75	65.25	0.59	65.56	0.25
HbA1c=7.5-8	61.96		58.73		54.16	
HbA1c>8	57.30		57.91		54.93	

Significance of all abbreviations is explained in the text.

The Kruskall Wallis test revealed statistically significant differences (p<0.001) between the average ranking for each of the three hepatic steatosis indexes in groups of patients with

HOMA-IR values of below 2, 2 to 5, and above 5. No significant differences were seen when values of hepatic steatosis indexes were analyzed in patients with HbA_{1c} values below 7.5%, of 7.5% to 8%, and above 8%) (Table 44).

3.3.4. Discussion

3.3.4.1. Relevance of the first study

Based on the data presented above, we could hypothesize that levels of insulin resistance seem to be associated rather to the global degree of excess weight measured by BMI, than to the presence of T2DM or CHC; by comparison, abdominal excess weight seems to have a more limited influence on the insulin resistance indexes, which remains more significant in cases where CHC adds on to the rage of metabolic risk factors. Addition of CHC to type 2 diabetes seems to substantially modify the metabolic profile, even though excess weight is more limited in these patients and less associated to lipid profile anomalies. On the contrary, addition of CHB to type 2 diabetes does not significantly impair the metabolic profile of these patients; the rather discrete differences that can be observed are most probably due to the predominance of the metabolic diseases by themselves (obesity and type 2 diabetes).

Type 2 diabetes mellitus, insulin resistance, obesity, metabolic syndrome and NAFLD are multiple clinical facets of the same phenotype. Obesity determines multiple dysfunctionalities in the adipose tissue; at its turn, lipotoxicity promotes insulin resistance and pancreatic β-cell dysfunction. As it may be associated with abdominal obesity, hypertriglyceridemia and high-normal values of ALT, NAFLD may progress independently of the progression of diabetes (*Leite et al, 2009*). The presence of NAFLD is predictive for an increased risk of type 2 diabetes, independently from age or obesity, even though it is less clear if NAFLD identification is a pragmatic approach for establishing the diabetes risk in clinical practice. Prevalence of NAFLD accounts for 65 to 87% of patients with type 2 diabetes (*Leite et al, 2009, Doycheva et al, 2016*).

The insulin resistance syndrome (metabolic syndrome – MS) is also closely related to both type 2 diabetes and NAFLD. In the case of MS, overt hyperglycemia develops only in patients whose beta-cells are not able to sustain an adequately high hyperinsulinemia to fight the insulin resistance (*Yki-Jarvinen, 2014*). However, no matter if diabetes is also present or not, the liver of MS patients is insulin resistant and produces high amounts of glucose and triglycerides packed as very low-density lipoprotein (VLDL) particles. The highest the levels of VLDL, the lower the concentration of high-density lipoprotein (HDL) cholesterol become. Such anomalies are frequent in obese subjects, but may also exist independently of the excess weight (*Yki-Jarvinen, 2014*). Therefore, NAFLD closely interrelates to the MS pathogenesis, thus raising the possibility that NAFLD becomes a good predictor for the development of type 2 diabetes.

As obesity and diabetes, two of the main components of the metabolic syndrome, are strongly related to a higher risk for hepatocellular carcinoma (HCC), there is enough motivation for the increased risk of HCC seen in NAFLD patients. In some developed countries, NAFLD surpassed other major etiologies for HCC. Solid epidemiologic data prove a parallel rise in the prevalences of obesity, type 2 diabetes, NAFLD, and HCC. As obesity and its related diseases became pandemic in the worldwide populations, the incidence of HCC

is expected to furthermore increase in the future. Development of NAFLD-related HCC seems to be due to the same pathogenic mechanisms that underlie the appearance of NAFLD and its subsequent development into NASH and cirrhosis: insulin resistance and subsequent hyperinsulinism, increased oxidative stress, activation of the hepatic stellate cells, cytokine and adipocytokine signaling pathways, genetic and environmental factors (*El-Serag, 2011*).

3.3.4.2. Relevance of the second study

When assessing the patients' poor glycemic control in correlation with the inflammation markers, weak positive associations of HbA_{1c} with hsCRP were found ($r=0.18$, $p=0.042$), but of less statistical power than those found in other studies (*Elimam et al, 2019*). HbA_{1c} also correlated with IL-6 ($r=0.41$, $p<0.001$). These findings suggest that the link between the quality of glucose control and subclinical inflammation is not necessarily a strong one. However, this hypothesis requires additional analyses to address potentially confounding factors which may diminish the strength of the relationship (e.g. duration of diabetes, other comorbidities, age, gender, treatment). Also, note should be made that our cohort comprised only diabetic subjects, whereas other studies included non-diabetic as well as diabetic patients, which might explain the difference. Moreover, NAFLD is not necessarily a consequence, but rather one of the factors causing the metabolic syndrome or even type 2 diabetes mellitus, and worsening their progression. For instance, patients suffering from both T2DM and NAFLD struggle harder to achieve glucose control compared to diabetic subjects without NAFLD (*Leoni et al, 2018*).

At the distribution of the values of the inflammation markers based on an insulin resistance grading scale, using HOMA-IR values of below 2, 2 to 5, and above 5, both IL-6 and hsCRP levels accurately confirmed the presence of a higher degree of insulin resistance defined as HOMA-IR >5. When the diagnostic performance of the liver steatosis indexes was tested by AUROC, all three hepatic steatosis indexes also acted as predictors of severe insulin resistance expressed as HOMA-IR>5. On the other hand, the predictive markers for hepatic steatosis were not influenced by the severity of uncontrolled diabetes and of insulin resistance.

Evidence gathered by now suggests that lifestyle optimization (including hypocaloric diet, physical exercise and weight loss) is, in most cases, the most effective method for treating NAFLD. Even though simple to enunciate, the nonpharmacologic therapeutic approach is not always successful, leaving place for the implementation of potentially useful pharmacological therapies. Drug therapy should be considered predominantly in NASH patients, and particularly in those with significant fibrosis (F2 stage or higher). Another borderline indication is represented by patients with less severe fibrosis, but displaying a high progression risk (e.g., diabetes, MS, persistently increased values of ALT, high levels of necroinflammation).

Current levels of proof do not allow firm recommendations to be made. However, pioglitazone seems to have the highest amount of efficacy data, even though its prescription is off-label outside the frame of type 2 diabetes. Vitamin E has better safety and tolerability proof for the short-term, but evidence linked to efficacy is rather limited. Some authors suggest that their combination may be useful for NASH. The best duration for such therapies is also unknown; in patients with increased baseline values of ALT, stopping the therapy

seems a reasonable approach if no reduction in aminotransferases is seen after 6 months; however, in patients with normal baseline values of ALT at baseline, such recommendations are no longer useful. Statins keep their specific benefits on the LDL-cholesterol reduction and on the prevention of cardiovascular risk, while inducing neither supplementary benefits, nor harm on the metabolic liver disease. Similar benefits on plasma and liver lipids are seen for ω-3 polyunsaturated fatty acids, but current guidelines do not advise their use in NASH patients *(EASL-EASD-EASO, 2016).* In patients having failed after combined lifestyle optimization and pharmacotherapy, implementation of bariatric (metabolic) surgery is a resource able to substantially reduce weight and metabolic complications, including the metabolic liver disease. The advantage of bariatric surgery consists in the stability of its long-term results. Study of surrogate markers suggest bariatric surgery to be effective on NAFLD-induced liver injury, and possibly on necroinflammation and fibrosis (*Schauer et al, 2014*). Its favourable effects on obesity and diabetes are probably the reasons behind the success of bariatric surgery in reducing NASH progression. For now, there is not enough evidence to sort out the comparative effects of various bariatric procedures on the liver fat load.

When NAFLD-associated cirrhosis occurs, it represents a clear indication, at present among the top three positions, for liver transplantation. There are no differences between the 3-year and 5-year survival in NAFLD and non-NAFLD patients. However, NAFLD patients display a higher risk for cardiovascular death and sepsis, while the risk for graft failure is smaller (*Schauer et al, 2014*). The overall mortality in NAFLD-transplanted recipients is proportional with the BMI levels and the coexistence of diabetes; in the first year after transplantation, death is recorded in half of cases with preoperative BMI values higher than 35 kg/m^2 (*Schauer et al, 2014*). Transplant failure occurs in 10% of patients at 10 years and in 45% of patients at 20 years, but obese patients seldom display recurrent NASH cirrhosis (only about 2% of cases) (*Wang et al, 2014*). Despite the higher risk for cardiovascular mortality, NASH patients with end-stage liver disease (terminal liver failure and/or HCC) have a strong indication for liver transplantation, and overall survival is comparable to other liver transplant indications (*Mikolasevic et al, 2018*).

Diabetologists were proven to grossly underestimate the prevalence and severity of advanced fibrotic NAFLD in their diabetic patients. Furthermore, only a few reports exist that use or intend to use non-invasive staging algorithms (*Marjot et al, 2017*). Such issues related to the clinician′s perceptions and practices should be explored in further research, as well as analysis of the pathways by which NAFLD contributes to chronic inflammation and insulin resistance, in order to define optimal preventative and therapeutic targets in diabetic patients with NAFLD.

In conclusion, our data highlight significant correlations between hepatic steatosis, insulin resistance and subclinical inflammation in type 2 diabetes patients with poor glycemic control. Screening for NAFLD in all type 2 diabetic becomes therefore a major necessity, as recommended in current guidelines (*EASL-EASD-EASO guidelines, 2016*).

3.4. Liver-targeted therapy in virus C compensated liver cirrhosis associated with metabolic syndrome

3.4.1. Rationale for the study of drug efficacy in virus C liver cirrhosis associated with metabolic syndrome

There are many pathogenic mechanisms involved in the development of hepatic insulin-resistance in patients with HCV, both through a direct viral effect and through inflammatory cytokines: the inhibition of insulin receptor substrate (IRS), and the activation of suppressor of cytokine signalign (SOCS) with inhibition of phosphatidylinositol 3 kinase-transducer, which has a key role in insulin metabolism. As far as the lipid metabolism is concerned, HCV relies on the host lipid metabolism to enter the hepatocytes and replicate, consequently impacting on the lipid metabolism itself. The hepatic accumulation of lipids is caused both by virus (viral steatosis in genotype 3 virus) and other associated metabolic factors in other genotypes (metabolic steatosis). They have multiple mechanisms: increased availability of lipogenic substrate, increased de novo lipogenesis, decreased oxidation of fatty substrates, and decreased export of fatty substrates from the hepatocyte into the bloodstream. Insulin-resistance and hepatic steatosis are the negative risk factors in HCV infection, leading to accelerated progression of hepatic fibrosis, risk of hepatocarcinoma, and in mortality through cardiovascular complications (*Kralj et al, 2016*).

In the recent years, HCV therapy has been revolutionized through the development of directly acting antiviral therapy (DAA), an efficient, quick, and well tolerated therapy with the potential to cure HCV infection. Whilst metabolic parameters were the negative prognostic risk factors in peginterferon and ribavirin therapy, they do not seem to affect the sustained viral response (SVR) in DAA. Genotype 3 virus (associated with hepatitis virus-induced steatosis) is an exception, as it is now the most difficult type to treat. There is no consensus in this regard, with some data suggesting that certain metabolic parameters (for examples, the low levels of LDL-cholesterol) prior to treatment have a negative impact on SVR even in DAA therapy (*Lim, 2014*).

I was part of a research team who developed a project evaluating the association of MS in patients with HCV liver cirrhosis (compensated genotype 1b) and changes in MS parameters after SVR, following a 12-week therapy with paritaprevir, ritonavir, ombitasvir, dasabuvir, and ribavirin (PrOD+R) (*Mihai et al, 2017*).

3.4.2. Materials and methods

A multicenter retrospective study included 809 patients diagnosed with compensated HCV cirrhosis (Child class A), all 1b genotype treated for 12 weeks with DAA (PrOD+R) regimen (according to the protocol practiced in Romania), and achieved SVR. Clinical characteristics and laboratory data were collected from medical records. The diagnosis of HCV liver cirrhosis was based on clinical, biological, and imaging parameters, as well as non-invasive methods (FibroMax) of assessing liver fibrosis. Treatment continued for 12 weeks from 01 December, 2015 to 20 June, 2016, with an undetectable viral load at 12 weeks from completion of therapy (SVR). MS was defined according to the definition of the International Diabetes Federation, 2009 (*Alberti et al, 2009*). All patients were assessed prior to starting DAA therapy and at the time of SVR (24 weeks after the commencement). The results were

collected in a central database and analyzed with SPSS 18.0. Statistical analysis used both descriptive and analytical methods with a significance level of 95% (CI 95%).

3.4.3. Results

The studied cohort included 438 females (54.1%) and 371 males (45.9%), with an age range of 34 to 79 years and the mean age of 59.21±8.72 years.

The descriptive MS parameters according to the gender are presented in Table 45.

Table 45. Descriptive indicators of metabolic syndrome parameters according to the gender

Parameter	Gender	N	Mean ± Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
AC, cm	Male	371	90.02 ± 9.43	0.49	89.06	90.98	67	132
	Female	438	81.52 ± 10.62	0.51	80.52	82.52	58	163
TG, mg/dL	Male	371	131.51 ± 58.64	3.04	125.52	137.49	38	610
	Female	438	125.89 ± 45.35	2.17	121.63	130.15	40	410
HDL, mg/dL	Male	371	54.65 ± 5.67	0.29	54.07	55.23	31	68
	Female	438	54.88 ± 5.51	0.26	54.37	55.40	33	68
SBP, mmHg	Male	371	125.08 ± 12.30	0.64	123.82	126.33	91	159
	Female	438	124.60 ± 12.43	0.59	123.43	125.77	90	170
DBP, mmHg	Male	371	75.22 ± 9.49	0.49	74.25	76.19	60	95
	Female	438	76.24 ± 9.11	0.44	75.38	77.09	60	110
FPG, mg/dL	Male	371	108.53 ± 30.44	1.58	105.43	111.64	61	291
	Female	438	108.67 ± 33.18	1.59	105.55	111.78	63	310

Abbreviations: AC, abdominal circumference; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL, high-density lipoproteins; SBP, systolic blood pressure; TG, triglycerides.

We identified the following results based on the diagnostic criteria for MS:

- Five diagnostic criteria were present in 3 patients (0.4%);

- Four diagnostic criteria were present in 3.5% of the patients as follows: 16 patients with abdominal obesity, hypertriglyceridemia, low HDL-cholesterol values, and high fasting plasma glucose levels, 7 patients with abdominal obesity, hypertriglyceridemia, high blood pressure, and high fasting plasma glucose levels, 5 patients with abdominal obesity, low HDL-cholesterol values, high blood pressure, and high fasting plasma glucose levels, and 1 patient with hypertriglyceridemia, low HDL-cholesterol values, high blood pressure, and high fasting plasma glucose levels;

- Three diagnostic criteria were present in 9.1% of the patients: 37 patients with abdominal obesity, hypertriglyceridemia and high fasting plasma glucose levels, 6 patients with abdominal obesity, low HDL-cholesterol values and high fasting plasma glucose levels; 13 patients with abdominal obesity, high blood pressure and high fasting plasma glucose levels, 3 patients with hypertriglyceridemia, high blood pressure, and high fasting plasma glucose levels, 4 patients with abdominal obesity, hypertriglyceridemia and high blood

pressure, and 8 patients with abdominal obesity, hypertriglyceridemia and high blood pressure.

The most frequent association in MS was abdominal obesity, hypertriglyceridemia, and high fasting plasma glucose levels (37 patients; 35.2%).

The re-assessment of MS parameters after SVR showed favorable changes which were statistically significant: a significantly lower serum triglyceride level (182.32 vs. 153.50 mg/dL, p=0.001), lower systolic blood pressure (130.57 vs. 124.85 mmHg; p=0.001), lower diastolic blood pressure (80.26 vs. 78.42 mmHg; p=0.001), and lower glycemic levels (130.06 vs. 120.71 mg/dL; p=0.001), as well as a significant rise in HDL-cholesterol levels (48.61 vs. 50.50 mg/dL; p=0.003). Abdominal circumference was the only parameter, which did not change after SVR (Table 46).

Table 46. Changes in metabolic syndrome markers after the achievement of sustained viral response

Marker	**Initial**		**SVR**		**p value**
	Mean ± SD	Standard Error	Mean ± SD	Standard Error	
Triglycerides, mg/dL	182.32 ± 76.34	7.45	153.50 ± 63.38	6.19	0.001
HDL, mg/dL	48.61 ± 8.86	0.86	50.50 ± 7.80	0.76	0.003
SBP, mm Hg	130.57 ± 13.58	1.33	124.85 ± 12.97	1.27	0.001
DBP, mm Hg	80.26 ± 8.83	0.86	78.42 ± 8.77	0.86	0.001
Glycaemia, mg/dL	130.06 ± 42.44	4.14	120.71 ± 34.70	3.39	0.001

Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure; SVR, sustained viral response.

Following the changes sustained after SVR, 26.7% of the patients no longer fulfilled the minimum 3 criteria for MS (Table 47). The most significant improvements from a frequency perspective were noted in the reduction of blood pressure and triglyceride levels.

In the studied cohort, there were 23 severe adverse events (2.84%): 8 decompensations of liver disease, 12 hepatocarcinomas, and 3 deaths. No correlation was found between the presence of MS and the risk of severe adverse events (Table 48). Instead, it was noted that 37.5% of the patients who decompensated, 66.7% of the ones who developed hepatocarcinoma, and 100% of those that died had abnormal glycemic levels (*Mihai et al, 2017*).

Table 47. Proportion of changes in metabolic syndrome parameters after achieving a sustained viral response[a]

MeS Criteria	**Initial**	**SVR**	**p Value**
Abdominal obesity	100 (95.2)	100 (95.2)	1.000
↑ Blood pressure	42 (40.0)	23 (21.9)	0.001
↑ Glycaemia	91 (96.7)	84 (80.0)	0.034
↓ HDL-cholesterol	37 (35.2)	26 (24.8)	0.024
↑ Triglycerides	79 (75.2)	55 (52.4)	0.001
MeS	105 (100.0)	77 (73.3)	

Abbreviation: MeS, metabolic syndrome; high (↑) or low (↓) values according to MeS definition.
[a]Values are expressed as No. (%).

Table 48. Metabolic syndrome and severe adverse events[a]

Adverse Events	No MeS (N = 704)	MeS (N = 105)	MeS Criteria					MeS	p value
			↑AC	↑ TG	↓HDL	↑BP	↑FPG		
LCD (N = 8) (1.0%)	7 (1.0)	1 (1.0)	1 (12.5)	1 (12.5)	1 (12.5)	1 (12.5)	3 (37.5)	1 (12.5)	0.968
HCC (N = 12) (1.5%)	9 (1.3)	3 (2.9)	7 (58.3)	4 (33.3)	1 (8.3)	1 (8.3)	8 (66.7)	3 (33.3)	0.212
Death (N = 3) (0.4%)	3 (0.4)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	1 (33.3)	3 (100)	0 (0.0)	0.503

Abbreviations: AC, abdominal circumference; BP, blood pressure; FPG, fasting plasma glucose; HCC, hepatocarcinoma; HDL, high-density lipoproteins; LCD, liver cirrhosis decompensation; MeS, metabolic syndrome; TG, triglycerides; high (↑) or low (↓) values according to MeS definition.
[a]Values are expressed as No. (%).

3.4.4. Discussion

Multiple definitions of MS exist, but all include obesity, insulin resistance/hyperglycemia, dyslipidemia, and arterial hypertension as the inclusion criteria. MS leads to a rise in mortality, especially as a result of cardiovascular causes. The hepatic manifestation of MS is steatosis, in the context of non-alcoholic fatty liver disease (NAFLD). Similarly, HCV infection induces hepatic steatosis through direct mechanisms (valid especially in genotype 3) and is associated with multiple metabolic abnormalities: "hepatitis C-associated dysmetabolic syndrome". In our cohort, the prevalence of MS was 13%, with a female-to-male ratio of 1.4:1, lower than estimated in the general population of Romania 38.5%. In the published literature, the presence of MS in patients with HCV varied, 12.4% in Europe and 35% in the United States (*Banks et al, 2017*). These differences are explained by the different definitions of MS used, extremely different cohort studies on patients with HCV infection and variations in the prevalence of MS in the populations investigated.

Many authors consider that, despite the fact that insulin resistance is the common etiological link, the prevalence of MS in HCV infection is similar to that of the general population. Visceral obesity is one of the features of hepatitis C-associated dysmetabolic syndrome, observed in 38.6% of the studied patients. It is demonstrated that insulin-resistance has a hepatic origin, as well as a peripheral one, with a hypothesis stating that HCV could infect fatty tissue. The association of obesity-hyperglycemia-hypertriglyceridemia was the most frequent occurrence in MS. Currently, it is known that HCV infection is associated with insulin-resistance, T2DM, and liver steatosis. In a recent meta-analysis, DM is considered as the second most common extra-hepatic manifestation of HCV (after depression), found in 15% of the patients. The high proportion of patients with raised glycemic values could be explained by the advanced stage of liver fibrosis in the cohort studies, knowing that in HCV infection, T2DM correlates with the degree of hepatic fibrosis (*Younossi et al, 2016*).

The interaction of HCV with lipid metabolism is a complex one, which is incompletely understood. Hepatitis C-associated dysmetabolic syndrome, in particular, features the associations of hepatic steatosis with those of hypocholesterolemia and reduced triglyceride levels. The virus enters the hepatocyte by binding to LDL receptors. The low levels of LDL

are caused by geranylgeranyl-diphosphate in viral replication, which is a substrate for the synthesis of cholesterol. The high levels of LDL compete with LDL receptors and reduce viral replication, whilst the high levels of HDL support the entry of the virus into the hepatocyte. In the hepatocyte, HCV interferes with the lipid metabolism of the host to replicate and assemble leading to hepatic steatosis. The main mechanisms through which HCV leads to accumulation of triglycerides in the hepatocyte are the activation of lipogenesis, its impact on mitochondrial lipid oxidation, the lowering of microsomal triglyceride transfer protein activity, and the reduced activity of peroxisome proliferator activating receptor (PPAR). The release of the virus from hepatocytes occurs by means of the very low-density lipoprotein (VLDL). In the studied group of patients, hypertriglyceridemia was found in 26.08% of patients, whilst low HDL was encountered in only 5% of the patients. It was explained by the particular features of lipid metabolism associated with HCV (*Zhu et al, 2014*).

Whilst there are a number of controversies, some evidence may suggest an involvement of HCV in the development of cardiovascular complication, insulin resistance, hepatic steatosis, and T2DM as a common link (*Lonardo et al, 2016*). In our study, hypertension was observed in 12.1% of the patients.

Our data showed that all MS parameters, with the exception of abdominal circumference. had a significant improvement after SVR. The most significant improvements were noted in the reduction of blood pressure and triglyceride levels. Consequently, almost one-third of the patients no longer featured the diagnostic criteria for MS. Moreover, our data confirmed that the significant drop in glycemic levels (both in terms of mean value and the percentage of patients with hyperglycemia) after SVR is attained using DAA therapy. The reversibility of hypocholesterolemia, hypotriglyceridemia, and hepatic steatosis is demonstrated after the attainment of SVR in therapy regimens based on interferon. In our study, a rise in HDL-cholesterol was noted and a drop in triglyceride levels after attainment of SVR in patients treated with PrOD+R. The changes in lipid metabolism, which quickly appear after viral clearance, highlight once again the direct effect of HCV in lipid homeostasis (*Meissner et al, 2015*).

The effects of viral eradication on cardiovascular risk are controversial. In the current study, there was a significant reduction in mean blood pressure post SVR, as well as a lower proportion of patients with hypertension. Some suggest that the attainment of SVR leads to a rise in blood lipids and consequently an increase in cardiovascular risk. In the current study, the improvement of arterial blood pressure after SVR emphasized the direct link between HCV and cardiovascular complications.

The role of HCV in the complexity of metabolic abnormalities remains a highly interesting topic for future research. Patients with HCV liver cirrhosis (compensated genotype 1b) frequently display features of MeS (high glycaemia, visceral obesity), but not MeS itself. Glycemic abnormalities are associated with a higher risk of hepatic decompensation, hepatocarcinoma, and death. The attainment of SVR through PrOD+R led to the short-term improvements in MeS parameters and the disappearance of this diagnosis in almost one-third of the treated patients.

CHAPTER 4. CLINICAL NUTRITION

4.1. State of the Art

A necessary flash-back to take when speaking about nutrition research focuses on its very beginnings and its subsequent development. The early era of nutritional research focused on the study of populational feeding and the proportion of food consumption from each main food group. This approach then developed into complex views that take into account not only the minimal and optimal intakes from food groups, but also primary prevention of nutrition-related diseases, health maintenance, optimal support for the quality of life and professional activities. The modern world came only recently to realize the real dimensions nutrition-related diseases may present with, when some of them, such as obesity, type 2 diabetes, atherosclerotic disease, or some types of cancer, took pandemic proportions. Another facet of the diet-health correlation has thus become evident, and hence a subject for research (*Isaak et al, 2013*).

An even more modern approach of nutrition research is the study of the diet-genome interaction, which encompasses the two opposite directions of nutrigenomics (study of the influence diet exerts over the genome, by measuring the cellular or gene response induced by nutritional stimuli) and nutrigenetics (study of the genotype's influence over the cellular or gene response to nutritional stimuli). However, genetic predisposition is not enough in most cases, as environment, often including a predominant nutritional component, may induce the difference between health and disease (*Isaak et al, 2013*).

An important facet for those practicing clinical nutrition is to respect its ethical coordinates. The same as in other fields of medicine, there are no medical progresses without newly appeared ethical dilemmas. Nutrition is subject to the same rule, and there is perhaps no other more controversial subject than ethical issues existing in cases where artificial nutrition therapy is implemented. The oral feeding is one of the fundamental dimensions of a free and independent life of any human being. However, modern medicine may use artificial nutrition in most cases where patients, from a variety of reasons, are not able to eat or drink enough amounts as to keep themselves alive and in good health. Artificial nutrition is defined as the whole range of medical therapies used to supplement or completely replace oral feeding. The artificial nutrition techniques refer to any of the technical procedures by which a person receives foods and fluids which cannot be provided by oral intake. According to most authors, the total amount of time hospitalized patients spend on artificial nutrition should best be limited to a maximum of 7 days. Nutritional support by artificial feeding should also be initiated when a person has lost about 10 to 15% of the initial body weight (*MacFie, 2005*).

A new and interesting concept in clinical nutrition is that of dietary pattern. It seems more and more plausible that the effects of diet upon health is generated not only by the individual dietary content in one or other of the key nutrients, but also by the synergistic interactions between them and by the overall effect of the mixed composition of that diet. In the last few decades, some dietary patterns have proven health benefits in the primary and even secondary prevention of major chronic diseases. There is perhaps no other dietary pattern that gathered favorable clinical evidence than the Mediterranean diet.

The Mediterranean diet originates in the food cultures of ancient civilizations which developed around the Mediterranean Basin and is based on the regular consumption of olive oil (as the main source of added fat), plant foods (cereals, fruits, vegetables, legumes, tree nuts, and seeds), the moderate consumption of fish, seafood, and dairy, and low-to-moderate alcohol (mostly red wine) intake, balanced by a comparatively limited use of red meat and other meat products. A few decades ago, the Mediterranean diet drew the attention of medical professionals by proving extended health benefits. The first reports ascertained cardiovascular protection, as multiple large-scale clinical studies, starting with Ancel Keys' Seven Countries Study, showed a marked reduction of atherosclerotic clinical events in populations with a Mediterranean dietary pattern. Ensuing trials confirmed favorable influences on the risk for metabolic syndrome, obesity, type 2 diabetes mellitus, cancer, and neurodegenerative diseases. While its health benefits are universally recognized today by medical professionals, the present state of the Mediterranean diet is challenged by major difficulties in implementing this protective dietary pattern in other geographical and cultural areas and keeping it alive in traditional Mediterranean territories, also tainted by the unhealthy eating habits brought by worldwide acculturation.

Last but not least, my preoccupations in the field of metabolic anomalies associated with digestive diseases brought me to the study of nutritional issues associated with various digestive health problems. One of these nutrition-related diseases is the inflammatory bowel disease (IBD), exerting an increased incidence in the last years, mostly in developed countries and predominantly motivated by the unhealthy Western dietary pattern.

My preoccupations in this research field have been directed towards the following three important axes:

- Ethical issues in clinical nutrition
- Dietary patterns of proven benefit in the field of clinical nutrition
- Clinical nutrition and inflammatory bowel diseases

The results of my research have been materialized in the following papers:

Published papers in this field

1. Lăcătușu C, Cijevschi-Prelipcean C, Mihai C, ***Mihai B***. Ethics of artificial nutrition. *Rev Rom Bioet* 2014; 12(1): 44-55.
2. Lăcătușu CM, Grigorescu ED, Floria M, Onofriescu A, ***Mihai BM***. The Mediterranean Diet: from an environment-driven food culture to an emerging medical prescription. *Int J Environ Res Public Health* 2019; 16(6): 942. DOI 10.3390/ijerph16060942.
3. Mihai C, Cijevschi Prelipcean C, Pintilie I, Nedelciuc O, Jigaranu A-O, Dranga M, ***Mihai B***. Nutrition in inflammatory bowel diseases. *Rev Med Chir Soc Med Nat* 2013; 117(3): 662-669.

4.2. Ethical issues in clinical nutrition

4.2.1. Rationale for the analysis of ethical issues surrounding artificial nutrition

As nutritional approach is not enough, in most cases, to cure a disease by itself, artificial nutrition cannot be considered a curative therapy. Artificial nutrition should rather be seen as an adjunctive treatment, offering the patient supplementary nutritional resources during the course of background curative or palliative therapies. Administration of nutrients using gastric or jejunal feeding tubes, or intravenous catheters, may increase the chances of survival in patients who are not able of oral feeding (*Fuhrman, 2008*).

However, these benefits are complemented by a wide range of side effects, which transform the matter of choosing between one or the other of artificial nutrition's specific procedures into a medical, and often an ethical, dilemma. Enteral nutrition may be associated with nausea, vomiting, diarrhoea, esophageal perforations or pulmonary aspiration of the feeding mixtures. Parenteral nutrition induces an increased risk of phlebitis, catheter site infections, dyselectrolytemia, peripheral edema or acute pulmonary edema, if renal failure develops (*Slomka, 2003*). Both enteral and parenteral nutrition are associated with higher risks for bleeding. They also trigger a wide range of medical and ethical consequences when restraint of a non-compliant patient is brought into discussion in order to avoid his potentially detrimental removal of tubes and catheters needed by artificial nutrition procedures.

4.2.2. Materials and methods

We searched Medline and Scopus databases using the logical string “artificial nutrition” OR “enteral nutrition” OR “parenteral nutrition” AND “ethics” to identify these key terms in the title or abstract of English-written articles published between January 2000 and January 2018. Clinical studies or trials, meta-analyses, and systematic reviews focusing on human subjects were selected. After eliminating duplicates, this initial search returned 609 results. We screened all titles and abstracts to select papers that could be considered relevant to the aim of our review. This operation led to a further reduction to only 47 titles. A second search using the same algorithm and replacing the key term of “artificial nutrition“ with “feeding tube“ OR “nutrition support” and the key term of “ethics” with “ethical issues” OR “patient consent” issued 8 supplementary relevant papers, which were also included in our analysis (*Lăcătuşu et al, 2014*).

4.2.3. Tools and procedures used in artificial nutrition

Artificial nutrition includes several procedures (*Lăcătuşu et al, 2014*):

- Intravenous fluid administration provides the daily needed fluids for the patient’s proper hydration, but it does not deliver, in most cases, an adequate supply of nutriments;

- Nasogastric tubes inserted through the patient’s nasal passages and upper digestive tract into the stomach provide a proper supply of liquids and nutriments. They are mostly used in patients with acute illnesses, since their placement cannot be permanent;

- Hypodermoclysis represents the subcutaneous infusion of less than a liter of fluid per day;

- Percutaneous endoscopic gastrostomy is the procedure of inserting a tube into the patient’s stomach through the abdominal wall; this technique is used when it is anticipated that the patient will not be able to feed orally for several weeks. Rarely, the tube may be

passed through the abdominal wall and placed directly into the jejunum (jejunostomy). Both techniques are capable to ensure a proper supply of nutriments and liquids.

For a long time, controversies emerged whether artificial nutrition is part of the basic medical care or a medical treatment. The cultural and religious concepts left their marks upon the perspectives from which nutrition is regarded in the daily life and as a part of the medical care. The Catholic Church sees life as a benefit, no matter how severe a person's disabilities may be. Therefore, feeding the patient is considered to be basic care under any circumstances (*Monod et al, 2011*). Pediatricians claim that in newborns enteral nutrition is part of the basic care. However, in adults, modern ethics sees artificial nutrition as a medical procedure, so the decision to initiate or withdraw needs to comply with the same ethical rules like transfusion or dialysis (*Monod et al, 2011*; *Körner et al, 2006*).

Since artificial nutrition is considered a medical treatment, competent adults may accept or not this procedure, like any other medical treatment. The patients' consent is crucial to initiate or continue artificial nutrition, even if refusing the respective techniques may cause their death. The issue of initiating or withdrawing artificial nutrition becomes even more delicate with incompetent patients.

If the patient is not able to understand or express his wish, the physician may have several options. Obviously, if the patient has previously mentioned his wish in writing regarding the therapy he would accept under these circumstances, these wishes need to be obeyed. In some countries, family has legal rights to make decisions or – if the patient orally stated his wish in front of family members, friends or even the physician – these forms of orally expressing his decision may be taken into consideration when making a decision. Some countries consider that the patient may be legally represented by a person (a family member, a friend or a lawyer) previously appointed to represent his interests when he becomes unable to decide. In Romania there are no clear legal provisions concerning either the advance directive or the other persons who might decide on behalf of a patient who became incompetent to make decisions on the possibility of artificial nutrition. Therefore, legal norms and medical practice guidelines in this field are strongly required in our country.

The patient's expressed wish is essential for establishing the total time for which artificial nutrition is to be provided (*O'Sullivan Maillet, 2008*). Still, many times the patient's wishes are not known. It might be advisable that such discussions with the patients would be carried out during the early stages of the disease and their wishes on the measures to be taken in the final stages of their life would be documented. Nonetheless, only 20-30% of the patients leave such directives.

There are no legal differences between withholding and withdrawing artificial nutrition. If the burden of artificial nutrition is greater than its benefit or if it no longer meets its intended goals, then this treatment can be withdrawn with consent of the patient or – in some countries – of the legal representative. If the patient or his legal representative withdraws his initial consent, artificial nutrition can be interrupted. Withdrawing support therapy will be made in the following order: dialysis, vasopressors, blood tests and afterwards the nutritional support and mechanical ventilation. If a patient's diagnosis or prognosis is uncertain, a test period for that nutritional intervention could be useful: a set of therapeutic goals and a period of time in which they should be reached are to be established and the treatment may start. At

the end of the initially established time, the efficiency of the artificial nutrition is assessed; if it has not proved to be beneficial, it will be discontinued (*Monturo, 2009*).

The efficiency of artificial nutrition depends on the patient's overall condition and the reason it is recommended. Artificial nutrition has good results in patients with temporary swallowing or superior gastrointestinal tract diseases and in those with certain time-limited disabling conditions. We can mention here patients with non-neoplastic upper gastrointestinal tract obstructions, patients who receive treatment which prevents them from eating for more than two weeks, patients with persistent or recurrent intestinal obstructions, patients with post-medication disorders or intestinal resections (*Körner et al, 2006*). Artificial nutrition might even prolong life, therefore allowing time for a more precise assessment of a patient's recovery chances, if his initial prognosis is uncertain. Therefore, ethically speaking, artificial nutrition is highly recommended in these cases. If a patient is dependent on artificial nutrition for an adequate nutriment supply and enjoys his life, artificial nutrition is clearly useful, not only physiologically, but also in terms of quality of life.

In elderly persons with acute diseases, artificial nutrition can reduce complications and mortality. In persons receiving home care assistance, artificial nutrition determines only a minor weight improvement, without any change in mortality.

4.2.4. Ethical issues in neurologic and neurodegenerative diseases

Patients with strokes

Around 40% of the patients having suffered a stroke have swallowing disorders and cannot eat. In these situations, the nutritional status progressively fails and leads to an unfavorable prognosis. This is the reason why some authors support the early provision of artificial nutrition (insertion of a nasogastric tube or percutaneous endoscopic gastrostomy), although there are no studies to prove the benefits of this therapy. However, even if dysphagia is present in more than a third of the patients when being hospitalized, this percentage decreases in a week to 16%; therefore, enteral nutrition is generally recommended to be postponed for a week. This period of time allows a more accurate assessment of the patients, as well as their recovery from aphasia or dysarthria, so that they can express their own options. It is usually preferred to initially use a nasogastric tube, since it involves low mortality and can be easily removed if deglutition recovers. If the patient does not recover from deglutition disorders and needs long-term enteral nutrition, resorting to percutaneous endoscopic gastrostomy may be necessary. Most authors recommend the invasive methods of enteral nutrition only two to four weeks after the stroke (*MacFie, 2005*).

Patients in a persistent vegetative state

The persistent vegetative state is a form of permanent alteration of consciousness where patients are in a state of partial wakefulness and have physiological sleep-wake cycles, but are completely unconscious about themselves or the surrounding world. It is induced by any pathological situation in which the functioning of the cerebral cortex is totally damaged, but the nervous activity of the brainstem is preserved (*Fine, 2006*). Providing nutrition support to these patients to provide comfort and reduce suffering has not a scientific basis (*Fuhrman, 2008*). However, artificial nutrition can definitely prolong life for some patients under a persistent vegetative state, so it might be prudent to provide artificial nutrition when the diagnosis is uncertain.

Patients with dementia

Population ageing results, among other consequences, in the dramatic increase of Alzheimer's disease and cerebrovascular disease with cognitive impairment incidences. In mild or moderate forms of dementia patients do not remember having eaten or not. It is then necessary to monitor their meals and even place some snacks within the patients' reach, in order to help themselves and properly maintain their nutritional status (*Körner et al, 2006*). Eating disorders usually appear in advanced forms of dementia; patients may present deglutition alterations, pulmonary aspiration of feeding material, inability to self-feed, loss of interest in food or even resistance to feeding (*MacFie, 2005*). All this frequently leads to malnutrition and food supplements may be useful (*Körner et al, 2006*). Some physicians appeal to enteral nutrition (usually through percutaneous endoscopic gastrostomy), aiming to improve the patient's nutritional status, prevent or heal pressure sores and prevent aspiration through the trachea and secondary pneumonia. Sometimes, healthcare staff may use enteral nutrition simply due to the fact that it meets their needs (even if not those of the patient), since feeding a dementia patient by mouth takes more skills and time than tube feeding. However, studies have shown that, in patients with dementia, enteral nutrition does not reduce the risk of pressure sores or pneumonia, does not induce an improvement of the cognitive abilities or daily performances and does not increase the patients' comfort, weight or functional status or their survival (*Fuhrman, 2008*). Overall, enteral nutrition in patients with dementia has not proved any real long-term benefit. Moreover, the immediate risk of death resulting from the insertion of a feeding tube can be quite high, varying between 4 and 54% (*Fuhrman, 2008*). The use of a nasogastric tube may induce diarrhea, aspiration syndrome, tube obstruction or its removal by the patient. Percutaneous endoscopic gastrostomy may be associated with discomfort, aspiration syndrome, infections, oral hypersecretion, feeding tube dysfunctions (*MacFie, 2005*; *Monod et al, 2011*). There are cases when patients with dementia are physically restrained to prevent them from removing the feeding tubes. In order to increase the quality of life, it is recommended that patients with advanced dementia be provided oral feeding. In their case effort should be made to remove dietary restrictions. In the latest studies on dementia, artificial nutrition is considered to have more risks than benefits and not to be initiated; providing comfort and dignity to the patient is more important than the nutritional treatment (*MacFie, 2005*). Exception should be made for the patients with vascular dementia, who may improve their cognitive functions. This emphasizes the importance of accurate neurological assessment to confirm the diagnosis.

4.2.5. Ethical issues in terminal illnesses

Patients with neoplasms

In some forms of cancer, patients actually "starve to death" and in these cases artificial nutrition is beneficial. We mention here cancers localized in the cephalic and cervical regions, esophageal neoplasm with secondary local obstruction and ovarian cancer with intermittent small-bowel obstruction. Weight loss is often an inevitable result of antineoplastic therapy, so in such cases nutritional interventions may prevent nutritional and physical impairment (*Fuhrman, 2008*). If there are doubts about the patient's prognosis, artificial nutrition may be provided for a limited period of time and the decision to continue or withdraw will be made depending on the clinical results. In patients with terminal cancer, malnutrition is responsible

for 25% of deaths. Patients with terminal cancer are rarely hungry and, if hunger occurs, it is tempered by small amounts of food. It is recommended to provide appetizing meals, according to the patient's preferences, at an adequate temperature, in a quiet environment, accompanied by comforting music. Patients must not be forced to eat against their will (*Fuhrman, 2008*). Providing comfort and improving symptoms are, on the other hand, more important than an aggressive nutritional support.

Patients with end-of-life stage of chronic illnesses

Patients with terminal illnesses, nearing death, lose their appetite and become incapable to self-feed. Until recently, death from malnutrition and/or dehydration was thought to induce supplementary pain to the patient, so that artificial nutrition was recommended by routine. Nowadays it is known that the decrease of liquid and nutrients intake determines an improvement of the symptoms. Most terminal patients often do not feel hungry or thirsty. The starvation ketosis leads to the body's release of endogenous opioids, which are thought to block pain and discomfort (*Slomka, 2003*). The role of hydration in terminal patients is controversial. More studies have shown that liquids play just a small role in these patients' comfort as long as they are provided rigorous oral hygiene. The sensation of dry mouth may be treated with pieces of ice chips, moistened swabs or lip balms. There is no evidence that medically assisted hydration at the end of life prolongs survival. Water deprivation increases the intern production of endogenous opiates that lead to a euphoric state and seem to be associated with pain reduction, inducing the patient a state of somnolence before death. Moreover, intravenous hydration can exert a negative impact upon the quality of life because of risks of patient's physical restrain, increasing pulmonary secretions and urinary output, bleeding, nausea, vomiting, fecal incontinence and edema. Since in terminal patients the goal is to keep their dignity and not to prolong their sufferance, there are voices who claim that – ethically speaking – it is human to let such patients die after withdrawing artificial nutrition (*Pasman et al, 2005*).

In conclusion, artificial nutritional support is a therapy raising numerous ethical issues and which needs to be implemented according to the patient's wishes, diagnosis, prognosis and therapeutic goals. The communication between the patient, his family and the healthcare team is crucial. If the discussion is open and sincere, it will help making decisions to the best interest of the patient. Patient's autonomy always needs to lie at the foundation of these decisions. Moreover, once artificial nutrition has been initiated, it is necessary to periodically monitor its efficiency and possible side effects, so that the initial decisions may be adjusted consistent with the upcoming reality. In Romania adequate legislation and specific protocols need to be set in order to facilitate the progress of making decisions related to artificial nutrition from the perspective of the risk/benefit balance.

4.3. Dietary patterns of proven benefit in the field of clinical nutrition

4.3.1. Rationale of the analysis of the current place occupied by the Mediterranean diet

Traditional eating habits seen in geographical territories surrounding the Mediterranean Sea, although differentiated by some food choices and cooking practices specific to each country and culture, share a common set of basic features. The specific dietary dimension of the Mediterranean lifestyle consists of a plant-based cuisine using vegetables, fruits, cereals, nuts, and legumes, most of them cooked by adding substantial amounts of olive oil, with

moderate usage of fish, seafood or dairy, and limited intake of meat and alcohol (mostly red wine) (*Davis et al, 2015*). This unique dietary pattern, the result of a complex and multi-millennial interaction between the natural food resources available in the Mediterranean environment and the human element inhabiting the Mediterranean basin throughout history, came to acquire new valences in the last century and to become a precious medical tool in the contemporaneous world.

In the moment when recognition of the health benefits associated with the Mediterranean diet has become universal, its paradoxical fate is that it is at risk of being extinguished in its homeland territories. Globalization, importation of Western habits, changes in lifestyle and the environment specific to modern civilization have brought a heavy toll on the traditional Mediterranean diet (*Davis et al, 2015*). At the same time, when international guidelines include it among the recommended healthy dietary patterns, the United Nations Educational, Scientific and Cultural Organization (UNESCO) considers the Mediterranean diet an "Intangible Cultural Heritage of Urgent Safeguarding" (*Lăcătușu et al, 2019*).

4.3.2. Materials and methods

We searched Medline and Scopus databases using the logical string "healthy eating" OR "healthy diet" OR "chronic disease" AND "Mediterranean diet" to identify these key terms in the title or abstract of English-written articles published between January 2010 and January 2019. Clinical studies or trials, meta-analyses, and systematic reviews focusing on human subjects were selected. After eliminating duplicates, this initial search returned 1214 results. We screened all titles and abstracts to select papers that could be considered relevant to the aim of our review. This operation led to a further reduction to only 78 titles. A second search using the same algorithm and replacing the key term of "chronic disease" with "cardiovascular disease" OR "cancer" OR "neurologic disease" OR "metabolic disease" issued 21 supplementary relevant papers, which were also included in our analysis (*Lăcătușu et al, 2019*).

4.3.3. The Mediterranean diet acknowledged as an environment-driven food culture

The term "Mediterranean diet" is used today to describe the traditional dietary habits of countries neighboring the Mediterranean Sea, mostly Greece and Southern Italy. Nevertheless, it should be understood as more than a strict reference to the preferences these populations exhibit in their daily food selection, since the original meaning of the word *diaita* in Greek does not refer to just food or eating choices, but to a certain "way of living" which corresponds better to the modern concept of "lifestyle" (*Dernini, 2011*). Throughout history, the Mediterranean diet incorporated some of the habits brought by conquerors, while keeping most of the previous local traditions alive and functional. Roots of the Mediterranean diet may be seen in ancient societies belonging to the Fertile Crescent – the Near East geographical area located between the eastern extremity of the Mediterranean Sea and the Persian Gulf, which included Mesopotamia, Canaan and, according to some, Northern Egypt. As a plant-based diet, the Mediterranean diet received sequential influences, as successive vegetal species were imported from other geographical regions of the world and acclimatized in the Mediterranean Basin. The food patterns on the shores of the Mediterranean Sea were

largely influenced by the three main monotheistic faiths succeeding in this area: Judaism, Christianity, and Islam (*Dernini, 2011*). As a result of geographical variations in food selection, diverse combinations of food groups are considered by current guidelines to form a Mediterranean diet pattern. Diet pyramids (graphic representations of the main principles within a diet, where foods allowed in larger amounts are represented in the inferior floors of the pyramid and restricted foods are represented towards its top) have today three main variants to describe a Mediterranean diet: the Oldway's Preservation and Exchange Trust pyramid, the traditional Mediterranean diet of the Greek nutrition guidelines, and the Mediterranean Diet Foundation pyramid. Some of these models kept the features of the traditional food habits, while the others were modified in time in order to better suit nowadays the availability of food supplies, nutritional needs, and eating habits (*Davis et al, 2015*).

The man responsible for noticing the health protective effects of the Mediterranean lifestyle and for coining the term "Mediterranean diet" is Ancel Keys. A specialist in biology and animal physiology, Keys concentrated at the end of World War II on the effects of starvation on the human body, searching for nutritional techniques able to restore health after starvation. He was surprised to notice the major drop in acute coronary attacks in countries where famine led populations to limit their typical high-fat, high-calorie diets, and also the inverse trend when the same countries recovered after the war and the population feeding changed again. At the same time, Keys was well aware of the high incidence of heart attacks in affluent middle-aged businessmen in the United States, and so he came to suspect that diet may influence health in general and especially the risk for cardiovascular disease (*Aboul-Enein et al, 2017*). While working in Oxford during a one-year sabbatical in 1951, he came to hear about the very low incidence of heart disease in Southern Italy. When Ancel Keys first presented his ideas of diet causing heart disease at an international meeting of the World Health Organization in 1955, he was met with skepticism and was even challenged by Sir George Pickering, a world-famous cardiologist, to present additional evidence. Unable to do so for the moment, he took this as motivation to design and implement a research project that was to become the so-called Seven Countries Study (*Aboul-Enein et al, 2017*). He chose to evaluate tobacco use, diet, physical activity, weight status, blood pressure, heart rate, lung capacity, blood cholesterol levels, and electrocardiographic readings in seven cohorts formed from all men aged 40 to 59 inhabiting some well-selected rural communities in the former Yugoslavia, Italy, Greece, Finland, the Netherlands, the United States, and Japan. In total, 12,763 subjects were screened. In 5 and respectively 10 years, the study team returned to all of the populations that were initially screened and collected data about the participants who in the meantime experienced a coronary attack (*Aboul-Enein et al, 2017*).

When the medical data were submitted to statistical analysis, the results showed significant differences between geographical areas. The lowest rates in heart attack incidences were found in Crete, Japan, and Corfu, in this order. At the other end of the spectrum, the highest rates were identified in Finland, with the United States coming second. Ancel Keys then realized that the dietary habits inherited in traditional Mediterranean populations, especially in Greece and Southern Italy, were associated with a reduced risk of developing cardiovascular disease. He coined these eating habits under the phrase of "Mediterranean diet" and co-authored two books on the subject: "*Eat well and stay well"* and "*How to eat well and stay well the Mediterranean way"* (*Keys et al, 1975*). He took his own advice on

adopting the Mediterranean dietary pattern and died in 2004, at 100 years old, his efforts and research having gained worldwide recognition and respect in the meantime.

4.3.4. The Mediterranean diet acknowledged as a healthy eating pattern

The Seven Countries Study had an observational design and limited power to demonstrate a cause-effect relationship. Keys and his team dwelt on the relationship between total serum cholesterol levels and the dietary factors influencing them, more than on the possibility that the Mediterranean dietary pattern as a whole had beneficial effects on cardiovascular health.

The Lyon Diet Heart Study was a secondary prevention randomized controlled trial to assess the effects of a modern, French-adapted version of the Mediterranean diet in patients having already suffered from an acute myocardial infarction. In order to best mimic the features of the Greek diet, naturally rich in omega-3 alpha-linolenic acid but poor in omega-6 linoleic acid, the authors decided to use rapeseed oil in association with olive oil. Coming as something of a surprise, the results of this research showed not just a 50% reduction of new acute coronary episodes, but also a reduction in the number of new cancer cases and in all-causes mortality (*de Lorgeril et al, 1999*).

In the following years, confirmation of the cardiovascular benefits of the Mediterranean diet became more robust. In the large cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC)–Elderly Prospective Cohort Study, including 74,607 healthy participants from nine European countries, aged 60 or over at the time of recruitment, a variant of the Mediterranean Diet Score was used to estimate the adherence to the Mediterranean diet. The score was obtained by adding nine partial scores of 0 or 1, which represented the intake of nine specific dietary components, and thus varied between a total of 0 (lowest adherence) and 9 (highest adherence). After a 4-year-follow-up, a 2-points increase in the values of this Mediterranean Diet Score was found to be associated with a significant 33% reduction in cardiovascular death (*Trichopoulou et al, 2003*).

Two other Spanish cohort studies, as well as the multinational Healthy Ageing: a Longitudinal study in Europe (HALE) project, confirmed the association between a higher adherence to the Mediterranean diet and a reduced number of cardiovascular events, also in primary prevention settings. A reduction in the rate of cardiovascular events was also seen in several secondary prevention studies (*Trichopoulou et al, 2007*).

One of the recent large trials to provide strong evidence in favor of the Mediterranean diet was the Spanish Prevención con Dieta Mediterránea (PREDIMED) study. Designed as a primary prevention randomized controlled trial, it enrolled 7447 subjects with no clinical signs of cardiovascular disease into either a control group advised to follow a low-fat diet and two active experimental groups set to follow a Mediterranean diet supplemented with extra-virgin olive oil or mixed nuts. Even though all three groups showed a rather small number of acute cardiovascular events, since all of the three diets were healthy cardioprotective eating patterns, the groups randomized to the Mediterranean diet still displayed a 30% reduction in the risk of cardiovascular complications, with an impressive 40% reduction in the risk of stroke. Adherence to the Mediterranean diet was measured in PREDIMED with a dedicated, validated, 14-item screening tool (the Mediterranean Diet Adherence Screener, or MeDiet

score) and was found to be inversely associated with the rate of cardiovascular events (*Schröder et al, 2014*).

Attempts to adapt to the Mediterranean eating style and search for related cardiovascular benefits exist today far beyond the borders of the Mediterranean region. Indian patients with pre-existing coronary heart disease or with high cardiovascular risk were included in another randomized trial using a so-called "Indo-Mediterranean diet" rich in whole grains, fruits, vegetables, walnuts, almonds, mustard or soybean oil, all bringing a high content of alpha-linolenic acid, and compared to a control group randomized to a step I National Cholesterol Education Program (NCEP) diet. Patients following the "Indo-Mediterranean diet" had an approximately 60% reduction in the rate of cardiovascular death and an approximately 50% reduction in the risk for non-fatal myocardial infarction (*Martínez-González, 2016*).

Adherence to the Mediterranean diet was associated with a significantly lower rate of cardiovascular events in a large cohort study following 23,902 UK participants for an average time of 12.2 years; the magnitude of beneficial effects in this study, statistically significant yet inferior to that in PREDIMED, might be attributed to an imperfect, limited transferability of the dietary habits comprised within a typical Mediterranean diet to a British population (*Martínez-González, 2016*).

Two cohort studies in the United States confirmed that significant reductions in the rate of cardiovascular events were also seen in the American population at higher rates of adherence to the Mediterranean diet (*Martínez-González, 2016*).

A meta-analysis of randomized controlled trials searching for the effects of the Mediterranean-like food patterns in the primary prevention of cardiovascular disease suggested benefits on total and LDL-cholesterol levels. Separate studies confirmed that adherence to the Mediterranean diet was associated with a favorable evolution of abdominal obesity, favorable weight changes, and a reduced incidence of overweight and obesity (*Rees et al, 2013*).

After studies showing protective effects of the Mediterranean diet against cardiovascular and metabolic diseases, analyses concentrating on possible benefits in other chronic diseases followed. A first indication of a possibly favorable effect of the Mediterranean diet on cancer morbidity and mortality was seen in a secondary analysis of the Lyon Diet Heart Study. Reduced rates of death by cancer were then seen in several studies in Swedish and United States populations (*Lagiou et al, 2006*).

Some data seem to show a protective effect of the Mediterranean diet against non-alcoholic fatty liver disease, with a higher adherence being associated with a lower severity of hepatic steatosis and reduced levels of alanine–aminotransferase both in cross-sectional and in some low-number, short-term prospective studies (*Suárez et al, 2017*).

Last but not least, the Mediterranean diet might offer protection against the development of neurodegenerative diseases. In European and United States populations, a better adherence to the Mediterranean diet were found to be associated with a lower risk for cognitive decline and development of Alzheimer's disease. A large prospective study on 131,368 participants in the American Health Professionals and Nurses' Health Study showed that higher adherence scores to the Mediterranean diet were associated with a 25% reduction in the risk of developing Parkinson's disease (*Feart et al, 2009*; *Gao et al, 2007*).

Research attempting to decipher the mechanisms involved in the positive effects of the Mediterranean diet on the risk of cardiometabolic, cognitive or neoplastic diseases covers an increasing number of publications in recent years. Maybe the best way to explain the benefits of the Mediterranean diet is to see it as one of the best illustrations of the concept of "food synergy", which is a fundamental principle in modern nutrition. Various nutrients and foods present multiple interactions and reciprocally enhance their positive effects, in such a measure that no separate food principle can be taken apart from the context of the whole dietary pattern or be used as an isolated explanation for the benefits brought by the Mediterranean diet altogether. In short, pathways leading to a favorable effect of the Mediterranean diet on various diseases can be systematized as belonging to one or more of the following: lipid lowering and modulating effects; anti-inflammatory, anti-oxidative, and anti-aggregating effects; modulation of cancer-prone mediators such as hormones or growth factors; decreased stimulation of hormonal or other extra- and intracellular transmitting pathways involved in the development of metabolic diseases and cancer, due to the changes in the amino acid content of the diet, compared to other eating styles; changes in gut microbiota, driving a modified production of bacterial metabolites. A sub-analysis in the PREDIMED trial found a higher polyphenol intake to be associated with reduced all-cause mortality; statistically significant differences were seen for stilbenes and lignans, with no significant relationship between flavonoids or phenolic acids and overall mortality. Other data also originating from the PREDIMED trial pointed to the benefits induced by consumption of higher amounts of olive oil in the diet. Moreover, the total antioxidant potential of the Mediterranean diet is completed by the phytochemicals found in whole grains and antioxidant vitamins found in vegetables and fruits. Besides olive oil, the healthy balance of fatty acids in the Mediterranean diet is completed by the polyunsaturated fatty acids brought by the sustained consumption of nuts, seeds, and whole grains and by the moderate or high fish intake. The high content of vegetal fiber brought by the rich consumption of whole grains, legumes, and fruits reduces insulin resistance, inhibits cholesterol absorption in the intestine and cholesterol synthesis in the liver, thus contributing to the overall cardiovascular protection. Phytosterols comprised in nuts, whole grains, seeds, vegetables, and fruits also contribute to the control of the intestinal absorption of cholesterol (*Jacobs et al, 2009*; *Tosti et al, 2018*).

4.3.5. The Mediterranean diet between worldwide recognition and cultural erosion

Like all the other territories of the world, Mediterranean countries were not able to get rid of the current trend of globalization interfering with all cultures, including the one relating to food. Worldwide acculturation is setting a marked stamp on food choices, and exchanges of agricultural products, recipes, and traditions have become a daily rule. As Western food culture, technologies, and advertising are driven by a powerful economic force, they tend to exert a marked influence on traditional eating habits and to substitute them even in their traditional homelands. All of this has resulted in a continuously growing prevalence of excess weight and other eating-related chronic diseases between the last generations of Mediterranean-neighboring populations. Lifestyle standardization, retail sales development, the lesser awareness and appreciation modern generations have for traditional food cultures, which tend to be abandoned in favor of new, socioeconomic-driven changes, women's integration into the labor market, resulting in limited time for culinary activities, also seem to

have a role in the erosion of Mediterranean food cultures. Several surveys of dietary habits performed in Mediterranean regions previously participating in the Seven Countries Study, and featuring low rates of cardiovascular events, showed a decreasing adherence to the Mediterranean dietary traditions manifested by increased intakes of saturated fatty acids, animal foods, cakes, pies, cookies, and sweet beverages, and decreased intakes of monounsaturated fatty acids. Most worryingly, low rates of adherence to the Mediterranean diet were seen in multiple studies among children and adolescents in Cyprus and Greece (*Dernini et al, 2015*).

While fighting for sustainability and economic survival in its homelands, the Mediterranean diet must also overcome barriers in other territories of the world, where its health benefits are recognized by the medical community, but adoption by communities is still limited due to the dominance of less healthy Western behaviors. Countries in Northern Europe have started to adopt a Mediterranean-like eating pattern due to the increased availability of Mediterranean fruits and vegetables in local stores and to well-driven public health policies. Acquisition in the United States is still restricted, even though modern nutrition guidelines have already included the Mediterranean eating pattern into their advisable healthy dietary patterns. A higher education level is certainly more able to drive people towards learning more about healthy diets, taking into consideration the dietary health advice coming from local and international authorities and finally giving their food choices a higher variability and diversity.

However, when speaking strictly about money expenditure, the costs of the Mediterranean diet are close to those of a Westernized diet, because supplementary expenses on fruits and vegetables are counterbalanced by less money spent on red meat, desserts, sweets, and fast foods. A realistic approach to implementing Mediterranean-like eating habits in populations living elsewhere than the Mediterranean Sea coasts could be to search first for local dietary habits by taking an adapted nutritional survey, and then to compare these newly identified eating patterns to the original Mediterranean diet, to identify the major differences and to adapt the local habits to the healthier Mediterranean ones in some key points, without giving up completely on the specific character of local food cultures (*Bonaccio et al, 2016*).

As a compelling and provocative conclusion, the Mediterranean diet now lies at a crossroads. A product of three millennia of culture and traditions, the Mediterranean lifestyle entered the medical consciousness approximately half a century ago and progressively gained recognition as one of the healthiest patterns of living. Besides cardiovascular, metabolic, cognitive, and possibly anti-neoplastic benefits, the Mediterranean diet seems to be associated with good adherence scores in some extra-Mediterranean populations and with an improved quality of life. Henceforth, it is advised today by a large majority of medical professionals all over the world. At the same time, the erosion of traditions and cultures in the Mediterranean-neighboring populations makes its survival back home an ever more difficult matter. Efforts in these apparently disjunctive directions of both Mediterranean and non-Mediterranean populations are required, in order to make the entire human race benefit from this complex network of food-associated habits that began in times of old as a mixture of lifestyle, religion, and lay culture and which ended up as an emerging medical prescription for health.

4.4. Clinical nutrition and inflammatory bowel diseases

4.4.1. Rationale for the study of nutrition involvement in the pathogenesis of inflammatory bowel disease

Beside my preoccupation in the field of metabolic diseases, which has been detailed in the previous sections of this thesis and permanently takes into account their nutritional facet, my involvement in clinical nutrition extended to the field of digestive diseases.

The incidence of inflammatory bowel disease (IBD) has increased over the last decades, particularly in developed countries, countries that have adopted habits specific to other geographical areas (Japan, India), or in people that migrated to developed countries and took over new alimentary habits. This epidemiological trend is primarily accounted for by dietary change. Increased consumption of sugars, fats, low-fiber or high raw diets may cause resistance to degradation, or by incorporation in Peyer plaques macrophages, exacerbation of inflammation in Crohn disease in genetically predisposed individuals. Excessive consumption of meat and alcohol favors relapse in ulcerative hemorrhagic rectocolitis (the sulfur compounds in these foods intensifies the inflammatory process) (*Latella, 2012*).

Fats with higher ω-6/ω-3 polyunsaturated fatty acids (PUFA) ratio increase the incidence of Crohn disease. European studies have shown that higher linoleic acid levels are associated with increased risk for ulcerative hemorrhagic rectocolitis. If ω-3 PUFA have anti-inflammatory properties (fish oil rich in ω-3 PUFA decreases the production of inflammatory mediators interferon γ, PGE 2), ω-6 PUFA (arachidonic acid, linoleic acid) have proinflammatory properties (*Viladomiu et al, 2013*).

These considerations led me to being involved in a research group aiming to review the main correlations between nutrition and the pathogenesis of inflammatory bowel disease (*Mihai et al, 2013*).

4.4.2. Materials and methods

We searched Medline and Scopus databases using the logical string “diet” OR “nutrition” AND “inflammatory bowel disease” to identify these key terms in the title or abstract of English-written articles published between January 2000 and January 2013. Clinical studies or trials, meta-analyses, and systematic reviews focusing on human subjects were selected. After eliminating duplicates, this initial search returned 230 results. We screened all titles and abstracts to select papers that could be considered relevant to the aim of our review. This operation led to a further reduction to only 31 titles. A second search using the same algorithm and replacing the key term of “inflammatory bowel disease” with “Crohn disease” OR “ulcerative hemorrhagic rectocolitis” OR issued 11. supplementary relevant papers, which were also included in our analysis (*Mihai et al, 2013*).

4.4.3. Analysis of the main correlations between nutrition and IBD

Malnutrition is represented by the discrepancy between body needs and food intake and its components, from macronutrients - carbohydrates, lipids, proteins - to micronutrients - vitamins, minerals.

Malnutrition is present in 85% of IBD patients, and weight loss in 80% of Crohn disease and 18-62% of ulcerative hemorrhagic rectocolitis cases. Growth deficiency, especially in Crohn disease, is not only due to inadequate calorie intake but rather to the inhibition of the

growth factor due to elevated leves of proinflammatory cytokines. Deficiency in vitamins A, D, K, E promotes inflammation which is found in 40 to 90% of IBD patients, commonly without clinical signs and without any decrease in the nutritional intake (*Mijac et al, 2010*).

A single indicator is not enough to determine the nutritional status, its assessment requiring nutritional history, physical examination and laboratory data (*Mijac et al, 2010*). Subjective global assessment, originally used in oncology, could be a tool for the screening of IBD patients (using the following parameters: history of weight loss, food intake, gastrointestinal symptoms, functional capacity). Serum levels of albumin may be used as a marker of nutritional status. Hypoalbuminemia occurs in 25-80% of Crohn disease patients and 25-50% of those with ulcerative hemorrhagic rectocolitis and reflects the metabolic response to stress, so that in IBD its level can be normalized by proper nutrition and by the correction of inflammatory process (*Mihai et al, 2013*).

There are multiple causes and mechanisms that explain the occurrence of malnutrition in IBD. Decreased food intake is due to anorexia, nausea, vomiting, abdominal pain and diarrhea, as well as to restrictive diets. In explaining anorexia in IBD, an important role is played by pro-inflammatory cytokines: TNF-alpha, IL-1β, IL-6, leptin, produced by the activation of monocytes, macrophages, lymphocytes to various stimuli that cause disturbances in the use and absorption of proteins, carbohydrates, lipids (*Alpers et al, 2008*).

Clinical nutrition is a supportive component of the IBD therapy, which corrects specific nutritional deficits and determines the induction and maintenance of the remission.

Individual features of oral nutrition must be established following a dietitian advice for a healthy, individualized diet with an elementary, fractioned intake with liquid oral supplements. Compliance with elemental or semi-elemental diets in Crohn disease and acute ulcerative hemorrhagic rectocolitis episode is poor due to their cost, taste, smell, texture (*Prince et al, 2011*).

Resting energy requirements are not increased in Crohn disease patients, and the body mass index (BMI) can be used to assess the caloric needs. The energy requirements of IBD patients are strictly dependent of the BMI (Table 49).

Table 49. Energy requirements of IBD patients according to BMI values (*Klein S, 2002*)

BMI (kg/m^2)	**Energy requirement (kcal/kg body weight)**
<15	36-45
15-19	31-35
20-29	26-30
>30	15-25

BMI, body mass index

Patients with IBD have increased protein needs due to intestinal losses and increased catabolism (infection/abscess/required surgery). Protein needs are assessed by body weight and disease status. The recommended daily protein intake for IBD patients without kidney disease is 1-1.5 g/kg, being reduced to 0.8 g/kg body weight for the patients with renal failure not receiving dialysis (*Prince et al, 2011*).

Vitamin and mineral requirements in IBD patients, as well as supportive measures in case of nutritional deficiencies, are found in Table 50.

Table 50. Vitamin and mineral requirements and treatment of deficiencies in IBD patients

Nutrient	Recommended daily requirements	Signs and symptoms of deficiency	Recommended replacement oral dose
Zinc	15 mg	Dry, flaky skin, palm peeling, diarrhea, mental status changes	50 mg elemental/day
Iron	10-15 mg	Microcytic anemia, fatigue	300 mg 1-3/day
B_{12}	3 mcg	Megaloblastic anemia, ataxia, diarrhea, mental status changes	1000 mcg/day
Folate	400 mcg	Sore mouth, glossitis, diarrhea, inattentiveness, megaloblastic anemia	1 mg/day
Calcium	800-1500 mg	Osteopenia, osteoporosis, tetany	1500-2000 mg/day
Magnesium	400 mg	Nausea, muscle weakness, arrhythmia, confusion, convulsions	150 mg elemental 4x/day
Vitamin D	400 IU	Rickets, osteomalacia, bone pains, muscle weakness, tetany	Variable

Vitamin B_{12} deficiency is found in distal ileal Crohn disease or in case of gastric resection (intrinsic factor deficiency) or terminal ileum (absorption deficit). Due to these aspects serum methylmalonic acid (S-MMA) concentration is elevated, vitamin B_{12} concentration is decreased, and homocysteine is elevated. The treatment consists in the administration of vitamin B_{12}.

Folic acid deficiency occurs either through increased requirements or by decreased intake (the main sources of folic acid – fruits, vegetables – are difficult to tolerate for IBD patients). Supplementation with folic acid 1 mg/day corrects the deficiency and protects against the risk for colorectal cancer. A special mention must be made on increased homocysteine levels that occur in vitamin B_{12} and folate deficiency, with increased risk for thrombosis, atherosclerosis, cardiovascular diseases and stroke (*Eiden, 2003*; *Massironi et al, 2013*).

Bone metabolism disorders, usually due to calcium and vitamin D deficiency, increase the risk of fractures in IBD by 40%. DEXA scan (dual energy X-ray absorptiometry) evaluates osteoporosis diagnosis and the therapeutical programme. The prevalence of osteoporosis is evaluated at 3-58% in ulcerative hemorrhagic rectocolitis and 4-50% in Crohn disease, due to deficiencies in proteins, vitamin D, and Ca intake, presence of inflammatory cytokines and corticosteroids. A major role is represented by old age, weight loss, disease status, and treatment with corticosteroids. Supplementation with calcium + vitamin D preparations (1000-15000 mg calcium + vitamin D 400-600 IU/day) is recommended and taking into consideration the fact that Prednisone can induce vitamin D resistance by increasing parathyroid hormone level and calcium losses. In patients with severe malabsorption 2,000-4,000 IU of vitamin D/day need to be administered parenterally, and in osteoporosis, bisphosphonates are preferred (*Massironi et al, 2013*).

Zinc deficiency occurs in fistulas and short bowel syndrome. The recommended dose for substitution is 15 μg/kg/day. Iron deficiency secondary to hemorrhage occurs in 80% of ulcerative hemorrhagic rectocolitis patients and in 40% of those with colonic Crohn disease.

In iron deficiency anemia injectable iron (100-200 mg ferrous sulfate) is preferred because iron administered p.o. is accompanied by gastrointestinal intolerance in 21% of cases, and intestinal inflammation and increased risk for colorectal cancer. Magnesium deficiency occurs in case of fistulas and colectomies, and short bowel syndrome and parenteral administration is preferred: 5% magnesium gluconate which has a high solubility (*Eiden, 2003*).

4.4.4. Alternatives to oral nutrition in IBD

Enteral nutrition (EN), used since the 70's and 80's in IBD, may be used as an autonomous therapy or as an adjunctive to drug therapy. Studies on EN showed several characteristic aspects for patients with Crohn disease (*Gupta et al, 2013*): corticosteroid therapy is more effective in obtaining remission compared to EN in adults; EN in children, particularly ileum located, is superior to corticosteroid therapy, being the choice of first-line treatment for reducing the negative effects of corticosteroids on growth and bone metabolism. The studies on EN in ulcerative hemorrhagic rectocolitis have shown inconsistent results, most of them not recommending it either in inducing or maintaining remission. Combined therapy is indicated perioperatively in malnourished patients with intestinal stenosis (weight loss > 10%, BMI <18.5 kg/m^2, and albumin below 30 g/l). The response to enteral nutrition is assessed after 3 to 6 weeks (*Levine et al, 2013*). EN diminishes inflammation and induces remission without the side effects of 5-amino-salicilyc derivates (5 ASA), steroids, or Azathioprine (AZA). It has also a role in maintaining remission, especially in patients with EN-induced remission. It can cause "mucosal healing" through its local immunstimulatory action (*Levine et al, 2013*). Enteral nutrition is administered orally, by nasogastric or nasoenteric tubes. If EN should be administered for more than 4 weeks, a percutaneous gastrostomy or jejunostomy is required (in CD patients this procedure increases the risk for fistulas). The main complications are: aspiration, hemorrhage, perforation, nose bleeding, pneumothorax, tube obstruction, intestinal ischemia and in stoma there is a high risk of infections, fissures or ulcerations (*Gupta et al, 2013*).

Parenteral nutrition (PN) is indicated in the following situations associated with IBD: obstruction, fistula, toxic megacolon, short bowel syndrome, severe malabsorption, loss of fluids and electrolytes, other conditions that make EN impossible/ineffective. Crohn's disease is one of the main indications for PN at home. The most common complications of PN can be: liver, biliary, catheter sepsis, thrombosis, decreased quality of life (*Alastair et al, 2011*).

In conclusion, nutrition in IBD is a complex problem that requires a good nutritionist-gastroenterologist collaboration and patients with IBD will benefit by the assessment and correction of existing deficits. Enteral nutrition is an adjuvant therapy in inducing and maintaining clinical remission and in mucosal healing. Parenteral nutrition is reserved for severe cases with complications.

SECTION II
FORTHCOMING PROJECTS AND DEVELOPMENT IN THE SCIENTIFIC FIELD

My career is marked by the extensive approach allowed by my double specialization in Internal Medicine and in Diabetes, Nutrition and Metabolic Diseases. Another pillar of my professional development is represented by the perpetual scientific update needed by my academic endeavours. The constant necessity of keeping the pace with the ongoing developments, breakthroughs and changes in the scientific approach of medical diagnostic and therapy is, in fact, an intensive stimulus for my medical work, which is therefore continuously adapting to the newest scientific findings, to the undoubted benefit of my patients, residents, scientific collaborators, and – in the future – of my PhD students.

As any activity field, my medical career always leaves place for improvement. As I am now accessing the new phase of forming PhD students, I see now the need for the following concepts to be implemented among the forthcoming updating directions of my academic career:

- A valoric selection of my PhD students among the residents with best performances and a broad vision upon their professional development, who will be therefore willing, able and most fitted to endorse a multidirectional career, as the one in the medical research field definitely is.
- Widening the interactivity dimension of my research team, where the young PhD students must be offered the opportunity to increase their professional autonomy, whilst continuously remaining under the surveillance of more experienced members of the team, within a concept of “gradual learning”.
- An increased involvement of my PhD students in projects of my professional team which will provide them the opportunity to draft original papers, and thus a visibility of their own in various journals, at national and international scientific conferences.
- A continuous improvement of the quality of research provided by the team I am part of, by stimulating the current members both to acquire a wider range of novel scientific knowledge and to publish in more and more performant journals, as well as by introducing PhD students as new members to the same development phases, in order to enlarge this research team.
- Widening the interdisciplinary dimension of the research team I am part of by stimulating the active involvement of PhD students from other medical or surgical specialities. The high level of academic interaction I have been developing in the last years is certainly an advantage in the practical development of this endeavour.
- Adaptation of my research team to the overriding technology facet that made its entrance in our medical specialty in the last years, which will make us able to implement modern technologies such as continuous glucose monitoring, unconventional insulin delivery systems (insulin pumps, sensor-augmented devices, closed-loop systems, or even the artificial pancreas) into our research themes.
- Development of an interactive formation network (workshops, journal clubs, clinical lectures with interdisciplinary content), involving colleagues in other research areas of

Internal Medicine and clinical care involving the diabetic patient, to support the educational growth of my PhD students.

- Identification of time, work and teaming resources needed to elaborate a wide range of research themes in the fields of diabetes mellitus, dyslipidemias and clinical nutrition, which will be provided to my PhD students.

In line with my medical and academic profile, my view upon my upcoming scientific activity focuses on clinical research centered upon metabolic diseases and clinical nutrition.

In my view, there are several specific tools and methods to develop these research areas. First of all, I am currently planning to develop a multidisciplinary research team, based on the principle of cooperation with other research teams from other disciplines and departments of the "Grigore T. Popa" University of Medicine and Pharmacy Iași, but also from other specialized research institutions, both in the country and abroad. I consider the access of funding sources to finance the projects to be of vital importance for my scientific development plans. I include here, as potential examples, internal grants of the "Grigore T. Popa" University of Medicine and Pharmacy Iași, grants of the Romanian Society of Diabetes, Nutrition and Metabolic Diseases, national grants from UEFISCDI, international grants from several funding agencies, including the professional organizations in the field of diabetes such as the European Association for the Study of Diabetes (EASD), but also private funding from the pharmaceutical industry.

At this moment, I have devised the plans of expanding my clinical research preoccupations into two major ***research directions***:

- Clinical research into the field of glucose variability
- Clinical research into the field of blood pressure variability in the diabetic patient.

1. Considerations supporting clinical research in the field of glucose variability

1.1. General considerations supporting the research opportunities within this area

Glucose control in patients with diabetes mellitus is an issue that should include strategies aiming to interfere with the three main components of uncontrolled diabetes: chronic hyperglycaemia, hypoglycaemia, and plasma glucose variability. These three items contribute to the development and progression of chronic diabetic complications (*Forbes et al, 2017*). Long-term interventional trials which compared intensive and standard glucose control as part of diabetes management have clearly shown the association between the long-term lack of glucose control, the development of microvascular complications and, to a smaller extent, that of macrovascular complications (*Roussel et al, 2018*; *Zoungas et al, 2017*). However, further series of observations have been published during the last decade, when the negative effects of both short-term plasma glucose variability (within-day glucose fluctuations; peaks to nadirs), as well as long-term variations (changes in fasting plasma glucose and HbA_{1c}), have been brought to the attention of physicians.

Nevertheless, strong evidence in the field of glucose variability, expressed by hard clinical outcomes and a widened consensus, is still scarce for now, thus leaving enough place for further original research (*Monnier et al, 2017*; *Lachin et al, 2017*). It should be pointed out that availability of glucose monitoring, including the high standard of continuous glucose monitoring (CGM), already became a valuable tool for informed management decisions, complementing the isolated use of HbA_{1c}, which can be otherwise misleading. Short-term

glucose variability is an increased concern of diabetes specialists, as it should reveal its potential risk of precipitating hyperglycaemic or hypoglycaemic episodes and of impairing the quality of life in our patients and trigger appropriate measures of preventing excessive glycaemic fluctuations. Moreover, the combination of short-term and longer-term plasma glucose variability seems to be associated with a higher number of severe hypoglycaemic episodes, related at their turn with adverse cardiovascular outcomes and all-cause mortality (*Zinman et al, 2018*). However, the same as above, there is a scarcity of categorical evidence supporting the role of glucose variability in the development and progression of negative clinical outcomes and complications in people with diabetes, especially when compared with the strong evidence supporting the negative effects of chronic exposure to hyperglycaemia, as assessed by HbA_{1c} (*Forbes et al, 2017*). This significant gap of evidence offers enough room for newly constituted research teams to develop, to put into practice clinical research projects and to obtain subsequent original results.

One cannot approach the topic of glucose variability research without tackling its limitations. The main limit to clinical investigations based on the use of CGM systems is the relatively high cost of such devices. Long-term intervention studies using CGM systems would definitely be associated with increased costs of the research. However, along with the broadening interest from the medical public and the subsequent development of the glucose-dedicated technology, prices are partially falling due to an increasing competition between producers. Moreover, the high standard represented by the CGM system itself makes research on this topic to become very up-to-date and to elicit the interest of more and more potential financial organizations, sponsors and fundraisers. Some categories of diabetic patients, such as individuals with type 1 diabetes, have witnessed the implementation of governmental policies of financial support which make them able to access these technologies for free. Last but not least, flash glucose monitoring, a CGM-related technology involving a lower financial burden, has proven some satisfactory quality results in the last years, thus making it a potentially valuable, but cheaper, surrogate of the classical CGM technologies. Under these circumstances, further development in the field of clinical investigation using CGM systems, and of subsequent indexes reflecting glucose variability, may provide an improved understanding of the real place control of glucose variability should indeed occupy in diabetes care (*Ceriello et al, 2018*).

Potential tools for reducing glycaemic variability in clinical practice include the use of pharmacological and non-pharmacological methods. In the following paragraphs I will examine the currently existing scientific background in each of these fields.

1.2. Considerations supporting the research opportunities provided by the interaction between non-pharmacological therapies and glucose variability

Findings of the HypoCOMPaSS trial suggest that training adult patients with type 1 diabetes, frequent severe hypoglycaemias and some degree of hypoglycaemia unawareness in the direction of avoiding low glycaemic levels might decrease glycaemic variability (*Tan et al, 2016*). Moreover, moderate physical exercise proved able to lower plasma glucose variability and reduce oxidative stress in people with type 2 diabetes or impaired glucose tolerance (*Farabi et al, 2015*). The combination of CGM and appropriate education seems to offer promising results of improved glucose control and reduced glycaemic variability.

1.3. Considerations supporting the research opportunities provided by the interaction between pharmacological therapies and glucose variability

Use of antihyperglycaemic drugs to achieve a normal or near-normal HbA_{1c} without increasing the risk for hypoglycaemic episodes seems nowadays crucial for an optimal diabetes management, especially during the early stages of type 2 diabetes, when lack of glucose control is often limited to an accentuated dawn phenomenon or to abnormally high postprandial glycaemic excursions (*Monnier et al, 2015*). For instance, management of type 2 diabetes using dipeptidylpeptidase-4 (DPP-4) inhibitors at an early stage, or glucagon-like peptide-1 (GLP-1) receptor agonists at a later stage of the disease, can reduce both chronic hyperglycaemia and glycaemic variability and can optimally avoid the risk of hypoglycaemia. Adding of ultra-long-acting insulins from the second generation of basal insulin analogues (i.e., insulin degludec, glargine 300) is also a strategy able to reduce glucose variability (*Haahr et al, 2014*). However, the scrutiny on the ability of newer classes of non-insulinic antihyperglycemic drugs to influence glucose variability is yet limited, while modern insulin analogues are typically the subject of a more extended research in the field of drug-influenced glycemic variability. Development of a solid research project tackling the topic of glucose variability should always take into account the fact that most antihyperglycemic drugs work by concomitantly reducing both overall hyperglycaemia (which reflects into a subsequent HbA_{1c} reduction) and glycaemic variability. In order to avoid the HbA_{1c} reduction becoming a confounding factor in the assessment of glucose variability effects, an ideal intervention trial testing these specific effects of the latter on cardiometabolic health indicators (and potentially on overt cardiovascular outcomes) should avoid the use of insulin therapy as an active comparator and should aim to achieve similar glycemic targets, and therefore a similar degree of overall hyperglycaemia, in the subjects tested in the active and control groups (*Ceriello et al, 2018*).

1.3.1. Considerations supporting the research opportunities provided by the interaction between incretin-based therapies and glucose variability

The OPTIMA study, a multicenter, randomized, prospective trial on 30 type 2 diabetes patients previously treated with metformin and then randomized to either vildagliptin or sitagliptin for 8 weeks, used CGM techniques at baseline and at the end of the study to evaluate the post-prandial glucose exposure (AUCpp) and the dawn phenomenon (∂ dawn) (*Ceriello et al, 2019*). In patients with an initially frank dawn phenomenon (∂ dawn $\geq$ 20 mg/dl), the introduction of any of the two DPP-4 inhibitors led to a significant ∂ dawn reduction (32 mg/dl to 15 mg/dl) and to a 64% lower frequency of the dawn phenomenon. A *post-hoc* analysis of the data in the OPTIMA study suggested that treatment with these two DPP-4 inhibitors reduced AUCpp, total glucose exposure (AUCtotal) and the mean amplitude of glucose excursion over 24 h (MAGE), a marker of glucose variability. Changes in AUCpp and MAGE were strongly positively correlated, supporting the idea that glucose variability in type 2 diabetes patients may be strongly influenced by the magnitude of post-prandial glucose excursions (*Monnier et al, 2015*).

The FLAT-SUGAR study, (*FLAT-SUGAR Trial Investigators, 2016*) tested whether therapy with exenatide twice daily added to an ongoing basal insulin therapy is able to reduce

short-term glycaemic variability and to improve cardiometabolic risk markers in type 2 diabetes patients requiring insulin therapy and displaying a high cardiovascular risk profile. The results of the FLAT-SUGAR study showed that the coefficient of variation (CV), the surrogate marker for glucose variability chosen for this study, was only moderately differing between the two groups, which was obviously not enough to support a difference in the cardiometabolic risk markers. However, the study spanned on only 26 weeks, and both groups were having underlying basal insulin therapy. As insulin inhibits inflammation, thrombosis, and oxidative stress, the potentially beneficial effect of twice-daily exenatide on the reduction of postprandial glucose excursion might have been confounded by the predominant actions of insulin on the cardiometabolic risk markers (*Monnier et al, 2010*).

Other than these two isolated studies, the ability of incretin-based therapies to modify glucose variability is relatively untackled by current research. This fact is all the more surprising, as the benefits offered by these drugs in the glycemic control of type 2 diabetes patients are well-acknowledged today. As incretin-based therapies are strong-acting antihyperglycemic drugs while showing only a minor risk of hypoglycemia, exert their pharmacological actions through a glucose-dependent β-cell stimulation and a glucagonostatic activity, they may prove able to reduce glucose variability in type 2 diabetes patients, by fighting both the post-prandial hyperglycemia and the dawn phenomenon, possibly due to the glucagon overproduction in the latest part of the night (*Monnier et al, 2015*).

1.3.2. Considerations supporting the research opportunities provided by the interaction between SGLT-2 inhibitors and glucose variability

CGM systems were used to investigate the administration of dapagliflozin in type 1 diabetic patients in the 24-week, double-blind, randomized, phase 3 studies DEPICT-1 and DEPICT-2. Pooled data from these two studies, including 1,591 patients, showed better results of dapagliflozin than placebo on time in range (TIR), MAGE, post-prandial and mean 24-h glucose (MG) values, without higher risks for hypoglycemia (*Mathieu et al, 2019*). However, these results were obtained in type 1 diabetic individuals, and – the same as above – the confounding effect of insulin therapy cannot be excluded. Therefore, the previous conclusions cannot be extrapolated for type 2 patients, in the case of which insulin is not always an underlying therapy for dapagliflozin administration.

In a 4-week, double-blind, placebo-controlled, multicenter, parallel-design study on 100 patients with type 2 diabetes, CGM was used to assess glucose variability at baseline and at the end of the study (*Henry et al, 2018*). TIR (70-180 mg/dL) was significantly greater in patients receiving dapagliflozin, compared to placebo; post-prandial glucose levels and overall glucose variability were also significantly improved, and only a small increase in the time below range (TBR) was seen in the dapagliflozin group, most probably due to the interfering actions of underlying insulin therapy in some patients. However, use of insulin in some patients, the short duration, small number of patients, lack of cardiometabolic risk markers assessment are all serious limits of this study. Dapagliflozin was also investigated as opposed to an active comparator represented by DPP-4 inhibitors in a small study on 29 type 2 diabetic patients using basal insulin and a CGM system (*Nomoto et al, 2017*), without generating any significant difference in MAGE or any other glucose variability parameter. The design of this study is obviously flawed by the same limits mentioned above.

Canagliflozin, a dual SGLT-1 and SGLT-2 inhibitor not available in our country, was tested for glucose variability versus placebo in an 18-week, double-blind study on 351 type 1 diabetes patients, of whom a subset of 89 participants underwent CGM (*Rodbard et al, 2017*). The standard deviation (SD), MG and TIR improved with canagliflozin therapy, while a significant reduction in the time above range (TAR) was also seen in these patients. In a 4-week randomized study on 75 type 1 diabetes patients in whom empagliflozin was added to insulin therapy, 7-day CGM was used to assess glucose variability (*Famulla et al, 2017*). Higher doses of empagliflozin (25 mg/day) reduced MG and increased TIR at week 1 and week 4. However, the same remarks made above for DEPICT-1 and DEPICT-2 studies with dapagliflozin are valid for these studies using canagliflozin or empagliflozin in type 1 diabetic patients.

As seen above, most studies on the topic of glucose variability with SGLT-2 inhibitors were performed off-label, in type 1 diabetic patients obviously displaying a higher glucose variability and the confounding effect of baseline insulin therapy.

2. Considerations supporting clinical research in the field of blood pressure variability in diabetic patients

2.1. General considerations supporting the research opportunities within this area

Disordered homeostasis, which may occur at the level of multiple metabolisms and feedback loops, constitutes a common model for risk factors inducing several chronic diseases, and also triggering events in the cardio-reno-metabolic continuum. Beside plasma glucose variability, which was discussed in the previous section of this chapter, variation of serum cholesterol concentrations also appears to be associated with upcoming cardiovascular events (*Xu et al, 2016*). Likewise, blood pressure (BP) variability was proven to represent an independent cardiovascular risk factor, acting separately from the background blood pressure profile. Hemodynamic homeostasis does allow continuous fluctuations within hour-to-hour, day-to-day, or month-to-month periods of time. However, these fluctuations tend to reproduce a specific pattern. On the contrary, an increased BP variability would lead to a higher hemodynamic stress upon the arterial wall, thus inducing endothelial dysfunction and consequently target organ damage (*Choi et al, 2017; Ohara et al, 2019*).

Even though differing in the specific underlying mechanisms, the fluctuations of physiological parameters such as plasma glucose and BP levels share the same property of contributing, or at least predicting, adverse disease outcomes. Among the most exposed to health risks, patients with type 2 diabetes seem to experience negative outcomes not only due to chronic hyperglycemia or high BP levels, but also due to glucose variability, as shown before, and to BP variability. The last two phenomenons induce an increased risk for macrovascular and microvascular complications in type 2 diabetes patients (*Hirakawa et al, 2014; Hata et al, 2013*). This detrimental effect adds to the already increased sumative risk of diabetes and hypertension coexisting together, leading to an increased risk for death, atherosclerotic cardiovascular events, and progression of diabetes-specific microvascular complications (*Ohara et al, 2019*). Increasing evidence in the last years suggests that, besides long-term unsatisfactory glucose control (high values of HbA_{1c}), an increased BP variability may be an independent risk factor for albuminuria development and for the loss of kidney

function, as reflected in a decreased estimated glomerular filtration rate (eGFR), in type 2 diabetes patients (*Gorst et al, 2015; Hata et al, 2013*).

2.2. Considerations supporting the research opportunities provided by the interaction between glucose variability, blood pressure variability and cardiovascular outcomes

Both short-term and longer-term variabilities have been reported for glucose and BP levels. As medical technology advances, detection of short-term glycemic and BP variability can be easily made by using relatively common devices such as continuous glucose monitoring (CGM) systems and 24-hour ambulatory blood pressure monitoring (ABPM).

To make matters even more complex, increased variabilities of BP and plasma glucose concentrations may originate in a variable degree of adherence to a healthy diet or in self-induced changes upon one's dietary habits or body weight (*Kim et al, 2018*).

Up until now, the triumvirate relationship between glucose variability, BP variability and cardiovascular outcomes has been hardly explored in type 2 diabetic patients. A small, inpatient study on 60 subjects with type 2 diabetes and hypertension investigated performed CGM and ABPM simultaneously with the assessment of oxidative stress using the diacron-reactive oxygen metabolites (d-ROMs) test. Glucose variability was assessed by MG level, glucose CV, MAGE, and AUCpp (see previous section for abbreviations). BP variability was estimated by the assessment of average BP, standard deviation (SD) of systolic BP (SBP) and diastolic BP (DBP), and coefficient of variation (CV) of SBP and DBP during daytime and nighttime ABPM. MAGE, nighttime SDs of SBP and DBP, and nighttime CV of SBP were significantly correlated with d-ROMs. At stepwise multiple regression analysis, MAGE, nighttime SD and CV of DBP were identified as independent contributors to d-ROMs, and therefore to the oxidative stress (*Ohara et al, 2019*).

A larger study on 11,791 patients, with a 5-year follow-up, evaluated the effect of long-term glucose variability, measured by HbA_{1c} variability, and of long-term SBP and DBP variability, on the risk of worsening renal outcomes (*Ceriello et al, 2017*). BP variability had the stronger correlation with the eGFR decline, while HbA_{1c} variability had a stronger connection with albuminuria development. On the other hand, the large population sample used in this study made it impossible to be screened by more precise methods of estimating glucose and BP variability, such as periodical CGM and ABPM procedures. Moreover, assessment of cardiovascular outcomes was not performed.

A cohort of 6,748,773 healthy subjects from South Korea was submitted to a statistical analysis of the evolution of standard clinical (SBP and body mass index – BMI) and biological (fasting plasma glucose and total serum cholesterol) data over a median follow-up of 5.5 years (*Kim et al, 2018*). Coefficients of variation (CVs), standard deviations (SDs), means variability and average real variability were calculated for all parameters. Higher variabilities for each parameter was associated with higher risks for all-cause mortality, myocardial infarction, and stroke. Risk for these cardiovascular outcomes further increased along with the number of high-variability metabolic parameters. However, the same as above, methods for estimating glucose and BP variability in this population were only basic and precision of results should be questioned, no matter how large the population sample.

Objectives of my future research

I can therefore argue that the opportunity of further investigation in the two research directions I previously mentioned is obvious, as there are multiple areas within this field where the gap of evidence is still consistent. My potential ***research objectives*** in these areas are:

- Development of a clinical research project using CGM techniques to evaluate the evolution of glucose variability in type 1 diabetes patients undergoing intensive therapeutic education programmes in the field of physical exercise training. In the case of type 1 diabetes patients, the limit represented by the financial barrier (relatively high costs of CGM systems) can be approached by using the legal opportunity generated by the last extensions in the National Health Programme for diabetes care in Romania, which offers free CGM devices to a continuously increasing number of type 1 diabetes patients.
- A similar study of changes in glucose variability induced by the implementation of systematic physical training in type 2 diabetes patients. The scientific value of such an objective is supported by the fact that the fight against a previously sedentary lifestyle that contributed to the genesis of type 2 diabetes is often a valuable tool used to regain glucose control.
- A research project exploring glucose variability in CGM-controlled type 2 diabetes patients where implementation of incretin-based therapies (DPP-4 inhibitors or GLP-1 receptor agonists) is needed due to insufficient glucose control.
- Development of a clinical research project investigating glucose variability under therapy with SGLT-2 inhibitors in type 2 diabetic patients with suboptimal glycemic control.
- A research project exploring short-term glucose and blood pressure variability in type 2 diabetes patients, using the simultaneous implementation of CGM and ABPM procedures.
- Development of a more complex, long-term clinical research project aiming to investigate a composite cardiovascular outcome in type 2 diabetes patients in whom CGM and ABPM procedures should be periodically implemented, along with repeated HbA_{1c} determinations and active involvement of study subjects in the self-monitoring of blood glucose and blood pressure.

SECTION III
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