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TEZĂ DE ABILITARE
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TABLE OF CONTENT

Table of figures

ABBREVIATIONS

AAD - antiarrhythmic drugs

ACEI - angiotensine-converting enzyme inhibitor

ACT - activated clotting time

AF - atrial fibrillation

APO-A1 - apolipoprotein A1

ALA-α - linolenic acid

AMI - acute myocardial infarction

ANS - autonomic nervous system

AUC - area under curve

BP - blood pressure

BMI - body mass index

BMS - bare metal stents

CAD - coronary artery disease

CAFE - complex atrial fractionated electrograms

CAVB - complete atrio-ventricular block

CB - cryoballoon

CK - creatin-kinase

CK-MB - creatin-kinase-MB

CVD - cardiovascular disease

DES - drug-eluting stents

DM - diabetes mellitus

DHA - docosahexaenoic acid

DNA - deoxyribonucleic acid

EAR - estimated area reference

EC - endothelial cell

ECG - electrocardiogram

ED - erectile dysfunction

EDRF - endothelial-derived relaxing factors

EDCF - endothelial-derived constricting factors

ESC - European Society of Cardiology

EPA - eicosapentaenoic acid

EPC - endothelial progenitor cell

FG - fasting glucose

HDLc - high density lipoprotein cholesterol

HOMA-R - model assessment-estimated insulin resistance

HR - heart rate

HS - haemorrhagic stroke

HsCRP - high sensitivity C reactive protein

HTN - arterial hypertension

IL - interleukine

IL1β - interleukine 1β

IMT - intima-media thickness

IR - ischemia-reperfusion

ISR - in-stent restenosis

α-HBDH - α-hidroxi-buthyrate-dehidrogenase

LA - left atrial

LVEF - left ventricular ejection fraction

MAAC - Multi-Array Ablation Catheter

MAPK - nitrogen-activated protein kinase

MASC - Multi-Array Septal Catheter

MESH - Mesh Ablator Catheter

MES - microembolic signal

MI - myocardial infarction

MS - metabolic syndrome

MSC - mesenchimal stem cells

NO - nitric oxide

Ox-LDLc - oxidized low density cholesterol

PAD - peripheral arterial disease

PBMC - peripheral blood mononuclear cells

PCI - percutaneous coronary intervention

PTCA - percutaneous transluminal coronary angioplasty

PNS - parasympathic nervous system

PUFA - Polyunsaturated fatty acids

PV - pulmonary vein

PVAC - multipolar circular duty-cycled catheter for pulmonary vein ablation

PVI - pulmonary veins isolation

RBC - red blood cells

RF - radiofrequency

RIPC - remote ischemia preconditioning

ROS - reactive oxygen species

RTD - research technology department

SNS - symphatic nervous system

STEMI - ST-segment elevation myocardial infarction

TCD - transcranial Doppler

TIA - transient ischemic attack

TNFα - tumor necrosis factor alpha

TG - triglycerides

UF - unfractionated heparin

WBC - white blood cells

WJ - Wharton's Jelly

Section 1. ABSTRACT

Summary

The professional, academic and scientific activity, in the period that followed the PhD thesis until present, belongs mainly to the field of cardiology and it was centered on two main directions: ischemic heart disease (from risk factors to treatment of clinical manifestations and prognosis) and atrial fibrillation with emphasis on the study of new interventional treatment techniques (ablation therapy).

The personal research in the field of acute myocardial infarction brought new elements regarding the study of risk factors and the prognosis estimation in such patients.

Even if, currently, primary coronary angioplasty with stent implantation represents the first-line therapy in acute myocardial infarction, there are still many patients, particularly in Romania, which do not have access to this method of treatment. In this context, fibrinolytic therapy still plays an important role and, especially in this field, I conducted important research that brought new data concerning the assessment of the long-term prognosis in patients with acute myocardial infarction treated with thrombolytic therapy. These publications have appeared in the context of a relative abundance of data concerning the short-term estimation of the risk of death and unfavorable outcome, but studies regarding the long-term monitoring of the evolution of these patients have been extremely limited.

In direct correlation with the extension of myocardial infarction and its impact on prognosis, my research allowed the validation of a new and cost-efficient assessment method for the estimation of the area of necrotic myocardium. Previous research regarding the estimation of necrotic area was carried out using mainly the determination of the area under the curve of a certain enzymes of myocardial necrosis: creatin-kinase (CK), creatin-kinase-MB (CK-MB). We utilized and validated a much simpler method that

only involved two measurements of plasma level of α-hidroxi-buthyrate-dehidrogenase (α-HBDH) determined at 36 and 72 hours after infarction.

The identification of some clinical and hemodynamic parameters that are easy to quantify at the patient bedside, in conjunction with a biochemical method that allows the cheap and simple estimation of the amount of myocardial necrosis, led to development of an original algorithm useful to assess the risk of death at two years post myocardial infarction. All these aspects are important elements of originality in my research activity. This model for long term prognosis estimation was developed in a period of time when the factors influencing long-term death were relatively less investigated.

My interest to identify risk factors for myocardial infarction and to establish their impact on prognosis of such patients and the type of care they should receive, reached a higher level by the involvement in a large national project aimed to create the first Romanian Registry for ST-segment Elevation Myocardial Infarction, RO-STEMI. It has become one of the most comprehensive registries in Europe, collecting until now more than 50,000 patients. The data in the registry have provided an important amount of information about the demographic characteristics and about the treatment of patients with acute myocardial infarction in Romania. I was actively involved in both the recruitment of patients in the registry, as well as in the data analysis (especially data related to demographic and risk factors and their influence on the vital prognosis and mortality of patients with acute myocardial infarction). The publications authored by me on this subject represented the natural continuation of the research in the field of my PhD thesis.

After the PhD thesis, I have completed my professional training in the area of myocardial ischemia by performing a 6-month fellowship in Israel, with focus on interventional treatment of coronary artery disease. Expanding preoccupation in this area has been also materialized in publishing new articles that covered various aspects of the interventional treatment of acute myocardial infarction.

At the same time, the research area was widened, aiming also the characterization of the early stages of ischemic heart disease with a focus on early detection of vascular atherosclerosis with emphasis on the mechanisms underlying the progression of atherosclerosis and its complications. On this topic, it is noteworthy my collaboration with a group of researchers from the Nutrition Department, University of North Carolina in the United States. Together with the American researchers, we developed a research project aimed to position the role of therapy with α-linolenic acid in the evolution of the metabolic syndrome. The original aspects of the research consisted mainly in the characterization of the evolution of some parameters related to metabolic syndrome, chronic inflammatory syndrome and insulin resistance in patients treated with α-linolenic acid (ALA), but also in an attempt to characterize changes in the deoxyribonucleic acid (DNA) methylation profile and gene expression correlated with the level of DNA methylation, in human lymphocytes.

Winning a two-year European Fellowship with emphasis on electrophysiology and ablation therapy, allowed me to develop the knowledge and further research in a new area focused on the electrophysiological study of rhythm disorders and their interventional treatment.

Throughout the period of this fellowship, I was actively involved in research activities of Department of Cardiology of Debrecen University, Hungary, most of them dedicated to the study of the newest ablation therapies of atrial fibrillation. The collaboration with the Department of Cardiology of Debrecen University continued after the conclusion of the two-year fellowship until present, I still participate in research projects developed in that center.

After the PhD thesis, I published 37 scientific papers, most of them in the field of cardiology. Fourteen papers have been published in journals indexed in ISI Thomson Reuters data base. Of all articles, 21 have been published as first author. My publications have accumulated a total of 66 citations and an H index of 5 in Google Scholar database and an H index of 4 in ISI Thomson Reuters database.

Since the time of the PhD thesis preparation until now, I was director of a scientific grant won through national competition, I was project responsible and scientific coordinator of an international research grant and I was a team member in another research project also obtained through a national competition. On top of these are the participations as principal investigator in four international and two national research studies, and as a team member in other five international studies.

My activities in the field of cardiology provide significant scientific and academic contribution through both original works and books published as a single author, co-author and editor. The involvement in the activity of the continuous training of doctors is noteworthy, leading to numerous post-graduate continuing education medical courses.

Future research are mainly centered on the cardiac ischemia, one research direction aiming the metabolic changes of the myocytes and endothelial cells during and after an ischemic episode and the protective mechanisms against ischemia such as the ischemic preconditioning.

The second research direction is done in a multinational consortium and aims to develop a new type of coronary endovascular prosthesis.

Concerning teaching activities, the internal medicine courses will be improved by the permanent addition of new information and internal medicine courses in English. The teaching activity will be complemented by collaboration with the Technical University and the creation of a new discipline within the Faculty of Materials Engineering, targeting implantable devices in cardiovascular pathology.

Given the involvement of teaching and academic activity, some of my further actions still regard the publication of new books in the field of cardiology and proposing new courses for master and doctoral school in order to ensure better training of medical students and young doctors.

Rezumat

Activitatea științifică, profesională și academică, în perioada ce a urmat după susținerea tezei de doctorat și până în prezent, s-a desfășurat cu precădere în domeniul cardiologiei și a vizat două mari direcții: boala cardiacă ischemică (de la factori de risc la manifestări clinice, tratament și prognostic) și fibrilația atrială, cu accent pe studiul noilor tehnici de tratament intervențional.

Cercetările personale în domeniul infarctului miocardic acut, au adus elemente noi în ceea ce privește studiul factorilor de risc și estimarea prognosticului pacienților cu infarct miocardic acut. Deși, în prezent, angioplastia primară cu implantare de stent constituie terapia de primă linie în infarctul miocardic acut, există încă numeroși pacienți, în special în România, care nu au acces la această metodă de tratament. În acest context, terapia fibrinolitică ocupă încă un loc important în arsenalul terapeutic al acestei patologii, iar studiile mele au contribuit la consolidarea datelor privitoare la estimarea prognosticului, pe termen lung, al pacienților cu infarct miocardic acut tratat cu terapie trombolitică. Aceste publicații au apărut în contextul unei relative abundențe de date care estimau, pe termen scurt, riscul de deces și evoluție nefavorabilă, în timp ce studiile dedicate urmăririi, pe termen lung, a evoluției a acestor pacienți au fost extrem de limitate.

În directă corelație cu extinderea infarctului și impactul acesteia asupra prognosticului, am studiat o metodă nouă și cost-eficientă de estimare a ariei miocardului necrozat. Dacă cercetările deja existente în această direcție, au avut la bază metode consacrate, (cum ar fi determinarea ariei de sub curbă a enzimelor de necroză miocardică CK și/sau CK-MB), studiile personale au vizat evaluarea unei metode mai simple, bazată pe doar două măsurători succesive ale nivelului plasmatic al unei alte enzime (α-HBDH). Validarea acestei metode biochimice de evaluare a ariei de necroză miocardică, relativ ieftină și ușor de efectuat, identificarea unor parametri hemodinamici ce pot fi cuantificați direct la patul bolnavului și combinarea acestor date într-un algoritm nou de evaluare a riscului de deces la doi ani post infarct miocardic, reprezintă elementele de originalitate a contribuției personale din cadrul cercetărilor pe care le-am efectuat.

Principala contribuție în domeniul prognosticul pacienților cu infarct miocardic tratați cu terapie fibrinolitică a constituit-o elaborarea unui model de estimare a riscului de deces la doi ani, într-o perioadă în care, cercetarea acorda o atenție mai mică factorilor ce influențează decesul la un interval lung de timp după la producerea evenimentului

Preocuparea de a identifica factorii de risc ai infarctului miocardic și de a stabili impactului acestora asupra prognosticului pacienților și asupra tipului de terapie pe care aceștia urmează să o primească, a cunoscut o nouă dezvoltare prin implicarea activă într-un important proiect național, de creare a primului Registru Român pentru infarct miocardic acut cu supradenivelare de segment ST - registrul RO-STEMI. Acesta a devenit unul dintre cele mai cuprinzătoare registre din Europa, reușind să înroleze peste 50.000 de pacienți. Datele acestui registru au furnizat informații importante despre particularitățile demografice și terapeutice ale pacienților cu infarct miocardic acut din România. Personal am fost implicată atât în includerea pacienților în registru, cât și în analiza datelor, în special a acelora privitoare la factorii demografici, factorii de risc și la influența acestora asupra prognosticului vital al pacienților cu infarct miocardic acut. Publicațiile proprii pe această temă constituie o continuare firească a cercetării care a făcut subiectul tezei de doctorat.

După susținerea tezei de doctorat, mi-am completat pregătirea profesională în domeniul ischemiei miocardice prin efectuarea unui stagiu de șase luni în Israel, stagiu ce a vizat tratamentul intervențional al bolii cardiace ischemice. Extinderea preocupărilor privind aceasta terapie s-a concretizat prin publicarea unor articole ce au vizat studiul diferitelor aspecte ale tratamentului intervențional în infarctul miocardic acut.

Într-un plan paralel, mi-am lărgit aria de cercetare aplecându-mă asupra caracterizării etapelor incipiente ale bolii cardiace ischemice, cu accent pe detectarea precoce a modificărilor aterosclerotice și cu implicare în descifrarea mecanismelor ce stau la baza evoluției aterosclerozei și a complicațiilor ei.

În acest context se înscrie colaborarea cu un grup de cercetători din Departamentul de Nutriție al Universității Carolina de Nord - Statele Unite ale Americii, în vederea derulării unui proiect

de cercetare menit să poziționeze rolul terapiei cu acid α-linolenic în evoluția sindromului metabolic. Aspectul original al cercetării a constat pe de o parte în caracterizarea a numeroși parametri ce țin de sindromul metabolic, de sindromul inflamator cronic și de rezistența la insulină la pacienții tratați cu acid α-linolenic și, pe de altă parte, în încercarea de a caracteriza modificările ce survin în profilul metilării ADN-ului și ale expresiei genice, corelate cu metilarea ADN, la nivelul limfocitelor umane.

Câștigarea unui grant european de doi ani, care a vizat instruirea în domeniul electrofiziologiei, mi-a permis dezvoltarea unei noi arii de cunoaștere și de cercetare ce a avut ca obiectiv studiul electrofiziologic al tulburărilor de ritm și al tratamentului intervențional al acestora. În toată această perioadă, am fost activ implicată în activitățile de cercetare ale Departamentului de Cardiologie al Universității din Debrecen, dedicate studiului celor mai noi terapii ablative folosite în special pentru fibrilația atrială. Colaborarea cu centrul din Debrecen a continuat și după încheierea celor doi ani de activitate din cadrul grantului și se menține și în prezent, cu participarea mea la unele proiectele curente de cercetare.

După susținerea tezei de doctorat, am publicat 25 lucrări științifice în special în domeniul cardiologiei, 11 dintre acestea fiind publicate în reviste indexate ISI Thomson Reuters. Din totalul articolelor, 15 au fost publicate în calitate de prim autor. Publicațiile mele au cumulat un număr de 66 de citații și un indice H de 5 în baza de date Google Scholar și un indice H de 4 în baza de date ISI Thomson Reuters.

În perioada de timp cuprinsă între elaborarea tezei de doctorat și până în prezent, am fost director al unui proiect de cercetare obținut prin competiție națională, responsabil de proiect și coordonator științific al unui grant internațional și membru în echipa de cercetare a unui proiect de cercetare național. La acestea se adaugă participarea ca membru în echipă în alte două proiecte educaționale, participarea în calitate de investigator principal la patru studii de cercetare internaționale și două naționale, și participarea ca membru în echipă în alte cinci studii internaționale.

Activitatea pe care am desfășurat-o în domeniul cardiologiei aduce o importantă contribuție științifică și academică, atât prin lucrările originale, cât și prin cărțile publicate atât în calitate de autor principal, cât și de co-autor și editor. La acestea se adaugă implicarea intensă în activitatea de pregătire continuă a medicilor, în principal ca director/responsabil a numeroase cursuri de formare continuă post-universitară.

Cercetările viitoare sunt centrate în principal tot pe domeniul ischemiei cardiace. Una dintre direcțiile de cercetare vizează studiul modificărilor metabolice la nivel miocitar și endotelial în timpul ischemiei și în perioada post-ischemică și studiul mecanismelor protective de precondiționare ischemică. A doua direcție de cercetare se realizează în cadrul unui consorțiu multinațional, fiind centrată pe dezvoltarea unui nou tip de proteză coronariană endovasculară.

Referitor la activitatea didactică, cursurile la disciplina de medicină internă vor fi perfecționate prin adăugarea permanentă de informație nouă precum și prin redactarea și susținerea lor în limba engleză pentru studenții străini. Activitatea didactică va fi completată prin colaborarea cu cadre didactice din domeniul tehnic și crearea unei discipline noi în cadrul Facultății de Știința și Ingineria Materialelor, disciplină ce vizează dispozitivele implantabile în patologia cardiovasculară.

Ținând cont de implicarea didactică și academică, am în continuare în vedere publicarea de noi cărți de specialitate în domeniul cardiologiei și propunerea de noi cursuri de master și școală doctorală, în scopul asigurării unei mai bune formări profesionale a studenților la medicină și a tinerilor medici.

Section 2. SCIENTIFIC, PROFESSIONAL AND ACADEMIC ACHIEVEMENTS

2.1 SCIENTIFIC, PROFESSIONAL AND ACADEMIC ACHIEVEMENTS IN THE FIELD OF ACUTE MYOCARDIAL INFARCTION

2.1.1 Introduction

Acute myocardial infarction represents a major health problem even in developed countries and remains a leading cause of morbidity and mortality worldwide.

Improvements in the treatment of acute myocardial infarction (AMI), especially use of reperfusion therapy, had an important impact on decreasing mortality.

For patients with acute ST segment elevation myocardial infarction (STEMI) with the clinical presentation within 6-12 hours after the onset of symptom, early mechanical percutaneous coronary intervention (PCI) or pharmacological reperfusion should be performed. [1]

Fibrinolysis is an important reperfusion strategy, particularly in those settings where primary PCI cannot be offered to STEMI patients within the recommended timelines. [2]

The benefit of fibrinolytic therapy in patients with STEMI is well established with 30 deaths prevented per 1000 patients treated. [3-4].

In patients who would have survived despite reperfusion therapy, use of this treatment should lead to greater myocardial salvage and a reduced extent of ventricular injury in many. All randomized trials demonstrate a significant decrease in mortality in patients receiving fibrinolytic therapy versus conventional treated patients. [5]

Compared with thrombolysis, treatment of STEMI by mechanical reperfusion has been shown to further improve prognosis in terms of survival, reinfarction, and hemorrhagic complications. [6]

In recent years, the use of new devices, such as stents, and the development of new antiplatelet drugs have led to improvements in the results obtained with the technique. [7] The in-hospital mortality of unselected STEMI patients in the national registries of the European Society of Cardiology (ESC) countries varies between 6% and 14%. [8]

In Romania, according to first STEMI report (1997-2009), the global in-hospital mortality was 15.31% in patients treated with no reperfusion therapy. Reperfusion therapy added an important improvement on mortality, with a 9.16% deaths in patients treated with thrombolysis and 6.62% deaths in patients treated with primary percutaneous coronary angioplasty. [9]

Despite the fact that primary angioplasty represents the gold standard in treatment of the STEMI, there are still many patients who cannot take benefit for this procedure. In these situations, pharmacological reperfusion should be administered (unless contraindicated). These patients however, will remain at risk of further cardiovascular complications and death. The risk of death remains important both in hospital and after discharge.

The mortality of STEMI is influenced by many factors, among them: age, Killip class, time delay to treatment, mode of treatment, history of prior myocardial infarction, diabetes mellitus, renal failure, number of diseased coronary arteries, ejection fraction, and treatment. There are plenty of data regarding the estimation of the risk of death at 30 days until 6 months after the onset of AMI, but relatively few regarding long term predictors of mortality. [10]

Data referring on prognosis in survivors of acute STEMI myocardial infarction particularly on long-term survival and the risk of recurrence are very limited. Thus, a significant proportion of patients with STEMI may suffer from long term sequelae of myocardial damage. About 5% of patients treated for ST-elevation myocardial infarct will develop heart failure after 1 year and in 7.5% the rhythm disturbances may severely affect the prognosis. [11-12]

An important number of patients already have or will develop heart failure during admission, and the percentage range between 12-29%. [13-15]

Registries provide further confirmation that this complication of AMI is common. The National Registry of Myocardial Infarction, consisting of 606 500 patients with AMI, identified heart failure in 20.4% of individuals at admission with 8.6% developing heart failure subsequently. [16]

There are limited population-based national data on prognosis in survivors of AMI, particularly on long-term survival and the risk of recurrence.

As short-term survival from AMI improves, the study of long-term prognosis becomes ever more important. This information is of interest to clinicians, public health professionals and decision-makers because it can be used to support clinical and funding decisions.

2.1.2. Long-term prognosis in patients with acute myocardial infarction treated with fibrinolytic therapy.

Despite the therapeutic advances in STEMI, large-scale, randomized clinical trials have reported 69% early 30-35 days mortality rates, even for patients receiving thrombolytic therapy within 6 h after the symptoms onset. [17-19]

Careful identification of pivotal factors that increase the risk of early mortality might elucidate the role of further interventions or adjunctive pharmacotherapies that would further lower the mortality rate associated with acute myocardial infarction. Though many studies have attempted to define the prognosis of patients with myocardial infarction or provide risk algorithms or both, studies have been limited by small sample sizes, diverse medical care systems, restricted spectrum of clinical data, and mostly targeting short–term follow-up. The long-term evaluation of the post myocardial infarction period is lacking. [20-23]

The principal aim of my doctoral thesis was to develop a clinical risk prediction tool for estimating the cumulative long term (two years) risk of death in patients with STEMI who received pharmacological reperfusion therapy.

In this respect, my research consisted in a clinical, prospective, open study of patients with STEMI treated with fibrinolytic therapy.

The patients were initially evaluated for the traditional cardiovascular risk factors (age, hypertension, dyslipidemia, diabetes, and smoking status). In addition, we assessed the inflammatory status (fibrinogen, C-reactive protein) and we estimated the magnitude of myocardial damage. The magnitude of myocardial damage was reflected by CK and CK-MB plasma activity, assessed by calculating the enzyme area under curve (AUC). In order to calculate these AUCs, blood samples for CK and CK-MB were taken every 4 hours after thrombolysis. We also took blood samples for another enzyme capable to reflect myocardial damage, thus α-hydroxibutirat dehidrogenase (α-HBDH) activity was estimated by measuring the plasma level of α-HBDH at 36 and 72 hours following the onset of the myocardial infarction. [24-26]

The infarct diagnosis was considered using classical criteria: ST segment elevation in at least two additional leads, in association with increased CK, CK-MB plasma level. [27]

For pharmacologic reperfusion, Streptokinase 1500000 UI in one hour or Actylise 100 mg in 90 minutes was used, in addition with anticoagulation and antiplatelet therapy.

A 2D and Doppler ultrasound examination was performed in all patients in first day of AMI (Agillent Sonos 4500) and the left ventricular ejection fraction (LVEF) was determined using the Simpson method.

All patients were followed-up in regular outclinic visits every 3 months after the enrollment until they have reached 2 year follow-up interval.

Statistical analysis was made using Pearson $\chi 2$ and survival analysis for groups, in order to assess individual relation between predictors. For significance testing, we used Pearson $\chi 2$ and Gehan-Wilcoxon methods respectively. Both methods were used for each individual predictor due to follow-up characteristic of observations (patients was censored at late time points, 2 years). We also used linear regression to find out if α-HBDH activity at 72 hours and LVEF were correlated.

Results: The study group included 72 patients, mean age 58.7 year (31-79), 59 males, 13 females admitted in Cardiology Department of the Brasov County Hospital.

We assessed overall death and we sought to identify the main risk predictors for mortality.

For the study group, the overall mortality rate at two years was 18%. The factors which significantly modified survival were:

- blood pressure and heart rate at admission;
- age over 65 years;
- male gender;
- the presence of peripheral obstructive disease and
- reduced LVEF.

Long term survival after myocardial infarction appeared to be in relation with classical cardiovascular risk factors (diabetes, dyslipidemia, smoking status, age) and the extension of the atherosclerotic disease evaluated through the presence of stroke and/or peripheral arterial disease (PAD). [28-32]

In our study the older patients (>65 years) have had a higher proportion of deaths (36.3% >65 years vs. 12% <65 years), with the segregation of the survival curves after 90 days of follow up. (Fig. 1)

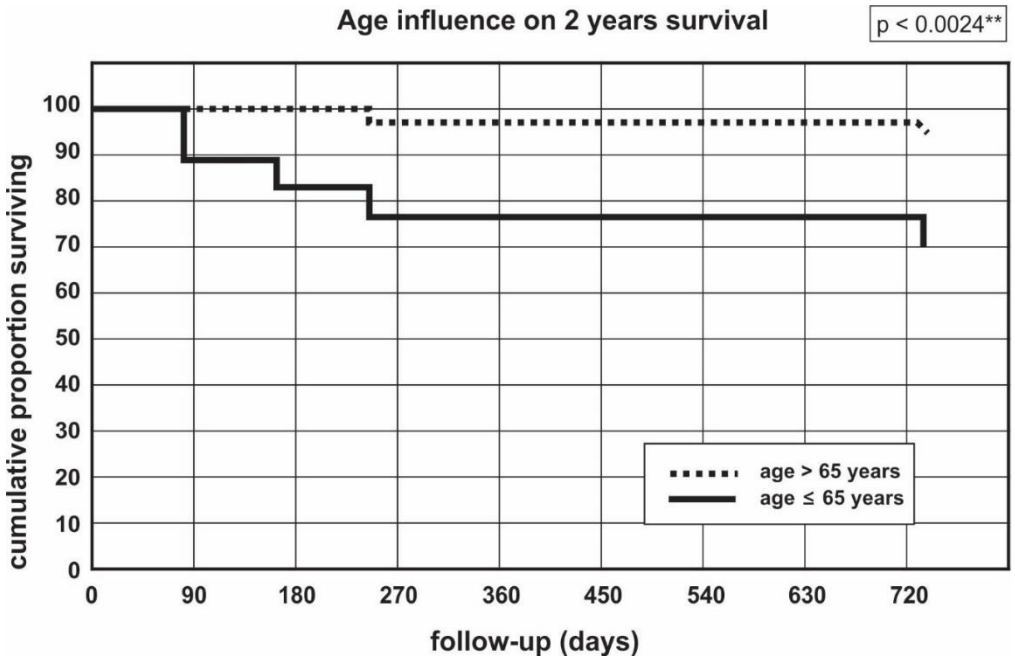

Fig. 1 - The age influence on 2 years survival [D. Țînț, M. Rădoi. Medicina Internă 2004; I(2):39-43]

The admission heart rate was also strongly correlated with 2 years mortality (50% for HR>90 beats/min vs 14% HR<90 beats/min), but the most powerful predictor of death was the value of the systolic blood pressure at admission. (Fig. 2)

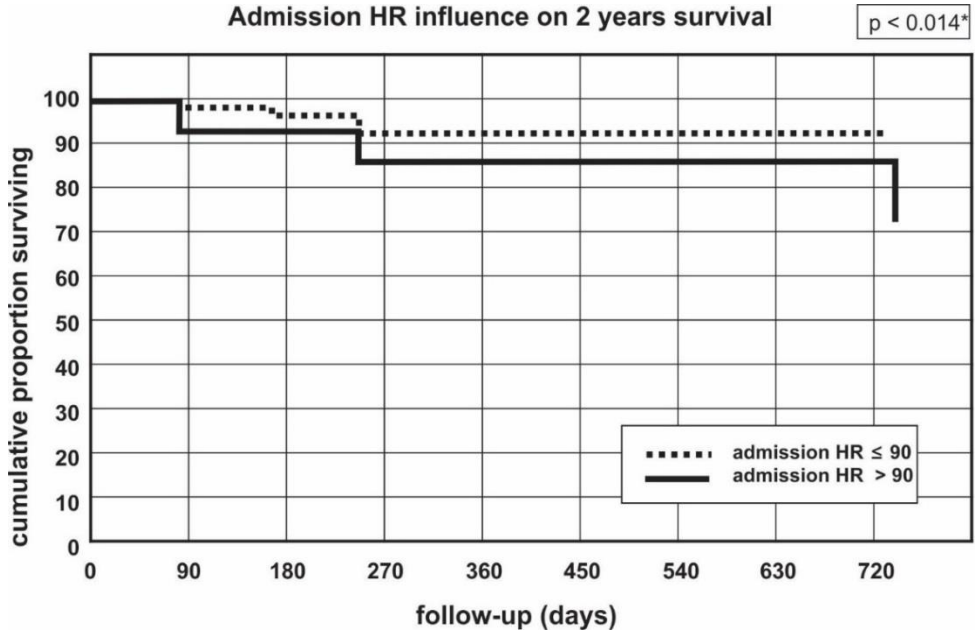

Fig. 2 - The admission heart rate influence on two years survival. [D. Țînț, M. Rădoi. Medicina Internă 2004; I(2):39-43]

The patients with admission systolic blood pressure below 100 mmHg have had a significantly higher mortality rate at 2 years in comparison with those in whom the systolic blood pressure was above 100 mmHg (83.3% vs. 12,1%). (Fig. 3)

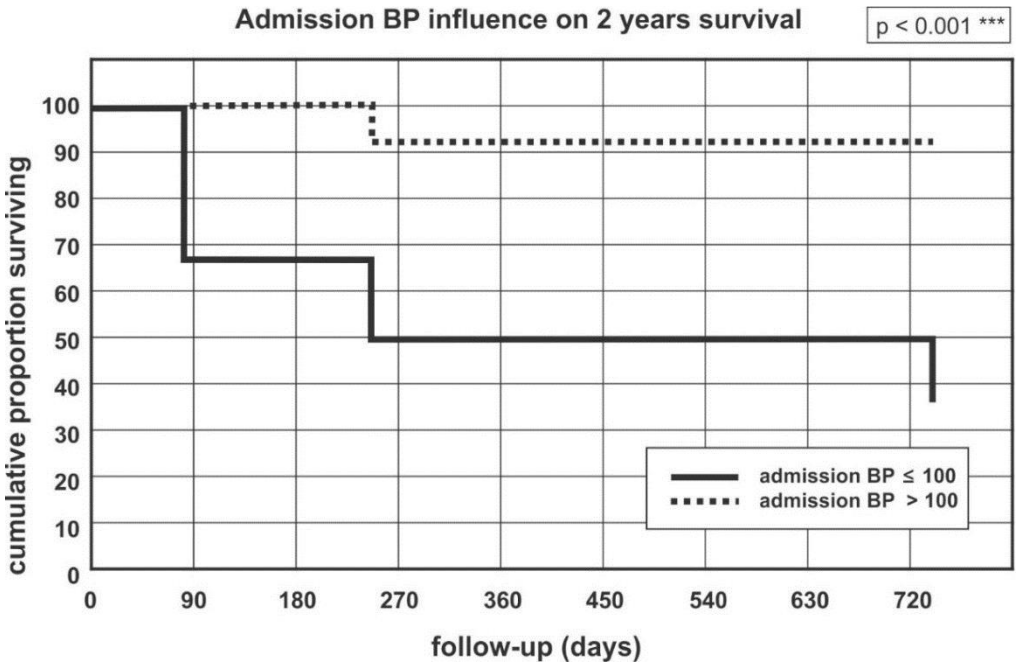

Fig. 3 - The admission blood pressure influence on two years survival. [D. Țînț, M. Rădoi. Medicina Internă 2004; I(2):39-43]

The risk of death was significantly greater in females than in males (38% vs. 13.5%) and in patients with LVEF below 40% (37.5% for LVEF<40%, vs. 5.7% for LVEF >40%). (Fig.4 and 5)

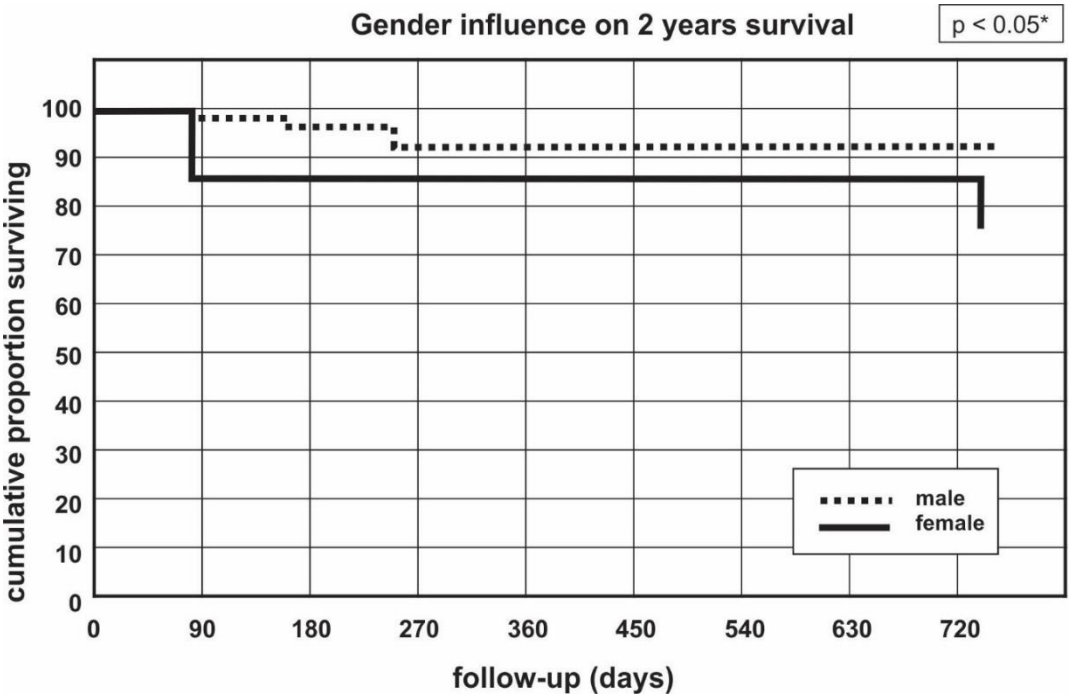

Fig. 4 - Gender influence on two years survival. [D. Țînț, M. Rădoi. Medicina Internă 2004; I(2):39-43]

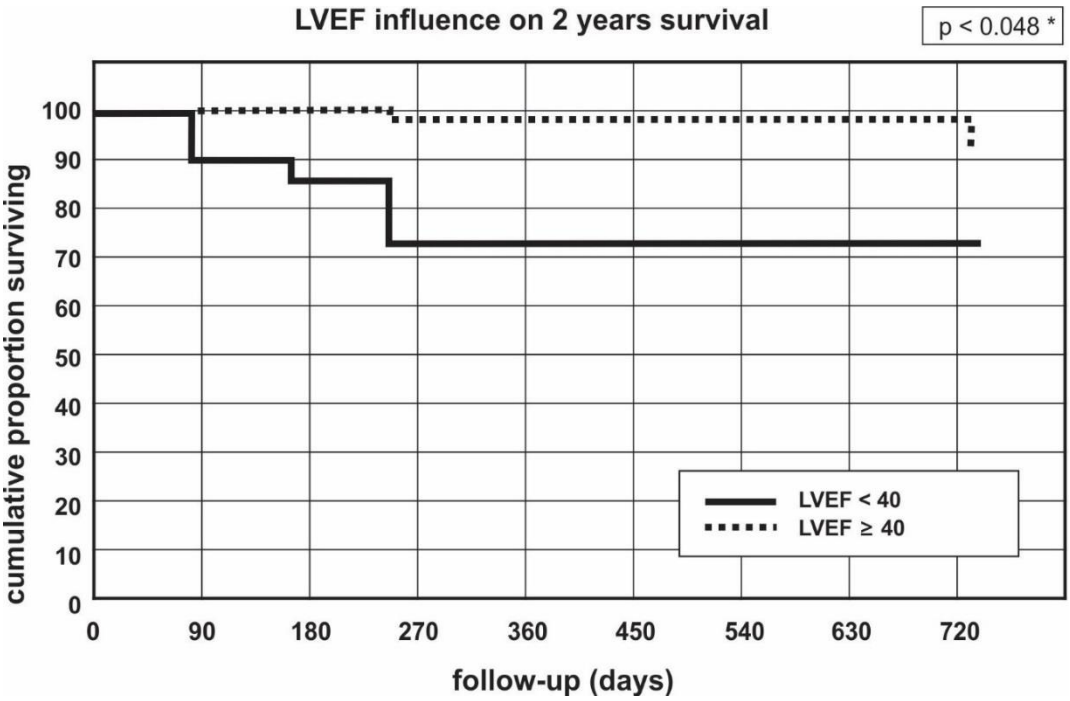

Fig. 5 - Left ventricular ejection fraction influence on two years survival. [D. Țînț, M. Rădoi. Medicina Internă 2004; I (2):39-43]

2.1.3. Estimation of the myocardial necrosis damage after myocardial infarction

Whereas in 2000 in Romania, thrombolysis (pharmacological fibrinolysis) represented the main method of reperfusion in patients with acute ST segment elevation myocardial infarction (STEMI), assessing the effectiveness of this method in order to re-open the culprit artery and preserve myocardial mass have had particular importance. One of the methods used for this purpose was to determine the area of residual myocardial necrosis after thrombolysis.

The importance of developing methods to assess the extent of myocardic irreversible injury arises from the hypothesis that the size of the necrotic area is a major determinant of prognosis in patients with AMI. [33]

One of the most promising techniques used to estimate the infarct size is quantification of the release of CK by frequent serum samples. Studies of AMI in man have demonstrated reasonable agreement between infarct size estimated by serum CK models and left ventricular hemodynamics, prognosis and post-mortem macroscopic infarct size measurements. [34-36]

Size estimation of the necrotic area using enzymatic tests includes assessment of the area under curve for CK and CKMB. This method is relatively difficult to assess and require multiple serologic determinations. [34-38]

Moreover, in patients with AMI treated with fibrinolytic therapy, the accuracy of estimation of necrotic size area is controversial due to an excess of seric enzymes produced by the reperfusion injury. [39, 40]

I studied a new and cost-effective method for determining of necrotic myocardium mass using a new biomarker called α hydroxi-buthyrate-dehidrogenase based on data already existing in literature [41] and I validated it by simultaneous calculation of AUC using CK and CK-MB.

The results of my research were communicated at 6th International Congress on Coronary Artery Disease in 2005 and published in extenso in the Proceedings book of the Congress.

Estimation of necrotic size area after myocardial infarction and the influence of fibrinolytic therapy on myocardial enzymes trends in patients with acute myocardial infarction - *Țînț D., Rădoi M, Pamfil G. - Progress in Coronary Artery Disease, 2005, Medimond S.r.l, Monduzzi Editore, 151-156. ISBN 88-7587-201-5.*

The aim of this research was to investigate the value of seric level of α-HBDH measured 36 and 72 hours after the onset of myocardial infarction (α-HBDH 36, α-HBDH 72) in patients with AMI treated with fibrinolytic therapy as an indicator of necrotic size area, and to study the enzymatic curves trends in the presence and in the absence of clinic and paraclinic syndrome of reperfusion.

Material and method: we prospectively included 72 patients with AMI with ST segment elevation admitted in hospital in the first 6 hours after the onset of symptoms. We have administered Streptokinase 1.500.000 UI in one hour in 47 patients and Actilyse 100 mg in 90 minutes in 15 patients. We performed repeated determinations of the seric level of CK and CK-MB at 6 hours interval (minimum 72 hours) and we have calculated the area under curve (AUC) for both enzymes.

We also assessed α-HBDH 36 and α-HBDH 62 using α-HBDH - optimized Monotest (Boehringer Manheim GmbH, Mannheim, Germany). Uper limit of a-HBDH normal level was 140 UI/l.

We have correlated the seric levels of a α-HBDH 36 and a α-HBDH 72 with values of area under curve for CK (AUC-CK) and CK-MB (AUC-CK-MB) and with value of LVEF. We comparatively studied the levels of myocardial enzymes in patients with and without reperfusion.

All statistical analyses were performed using Statistica 7/StatSoft Inc.

Results: There were 51 males and 11 females included, mean age 58.7 years (31-79 years). The AMI was anterior in 43 patients and inferior in 19 patients. Based on clinical, electrocardiographic and biochemical criteria, we appreciate that the reperfusion [42] occurred in 33 patients and failure of the reperfusion was present in 29 patients.

We have found a significant correlation between α-HBDH 72 and AUC-CK (R=0,5578***) - Fig. 6, α-HBDH 72 and AUC-CK-MB (R=0,633***) - Fig. 7, and α-HBDH 72 and LVEF (R=0,52***) - Fig. 8. The seric level of α-HBDH 36 does not correlates with any of the above factors.

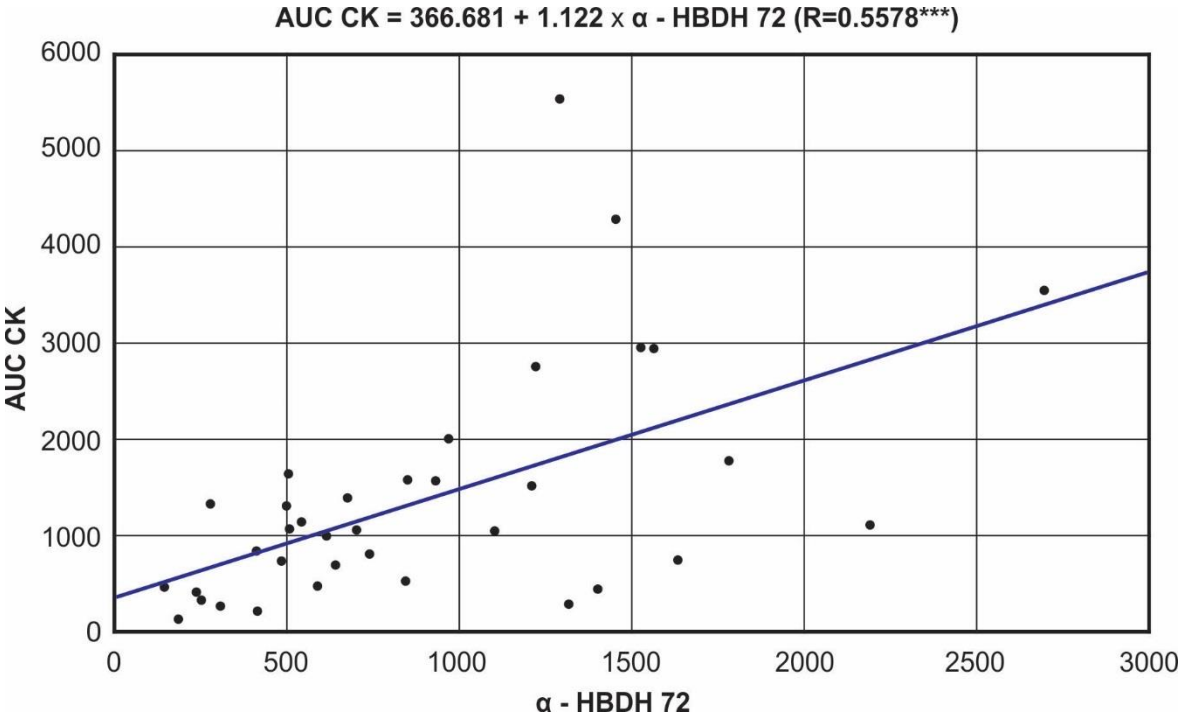

Fig. 6 - Relation between Creatine - Kinase area under curve and plasma level of α-Hydroxi-buthyrate-dehydrogenase at 72 hours after the onset of myocardial infarction

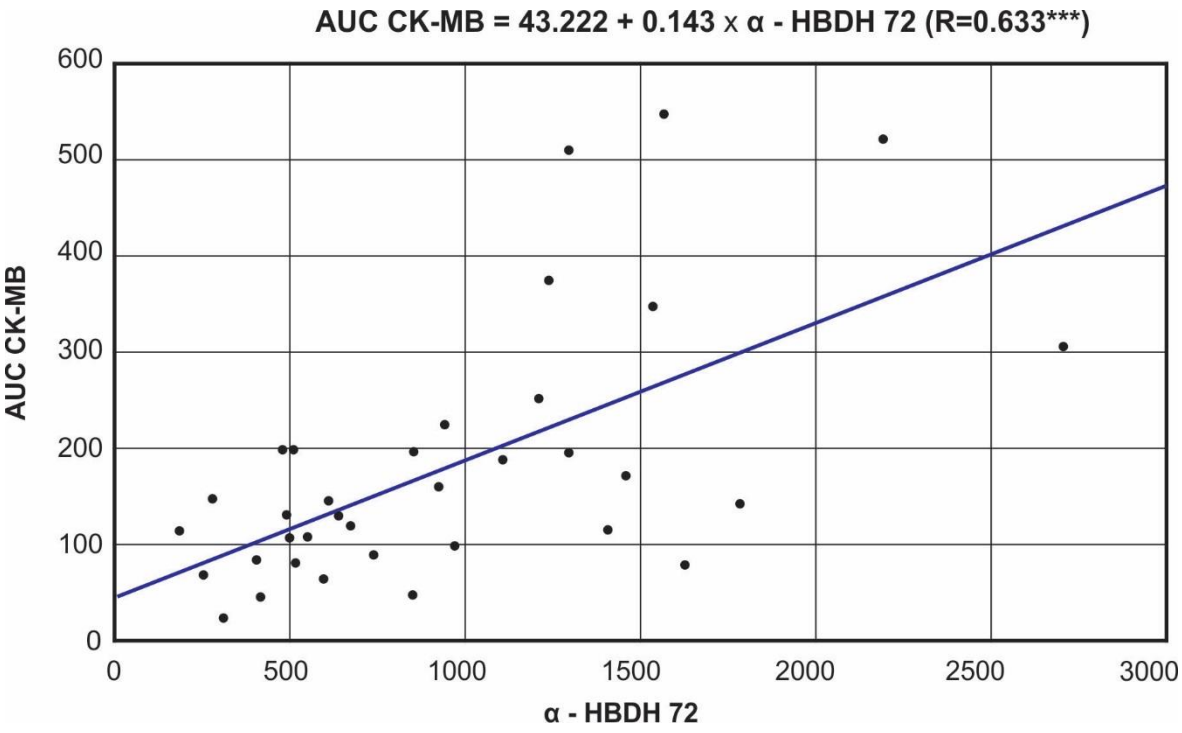

Fig. 7 - Relation between Creatine – Kinase-MB area under curve and plasma level of α-Hydroxi-buthyrate-dehydrogenase at 72 hours after the onset of myocardial infarction

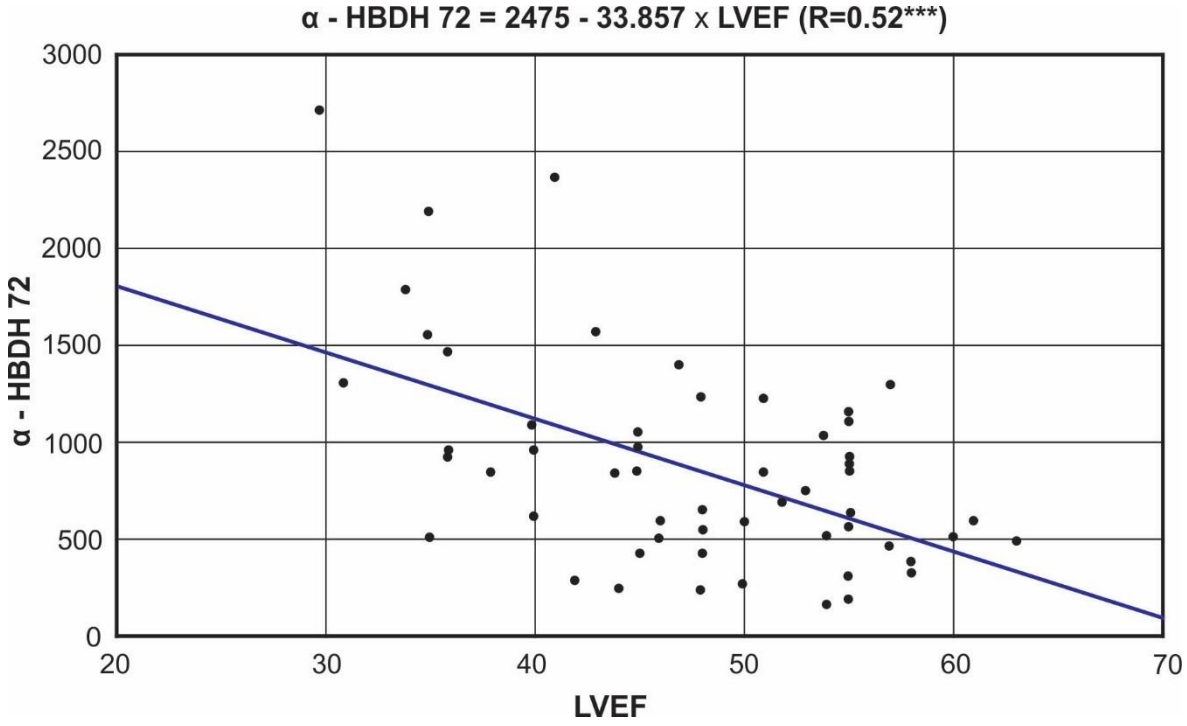

Fig. 8 - Relation plasma level of α-Hydroxi-buthyrate- dehydrogenase at 72 hours after the onset of myocardial infarction and left ventricular ejection fraction

We also found a significant correlation between AUC-CK and LVEF (R=0,64***) - Fig. 9 and between AUC-CK-MB and LVEF (R=0,51***) - Fig. 10.

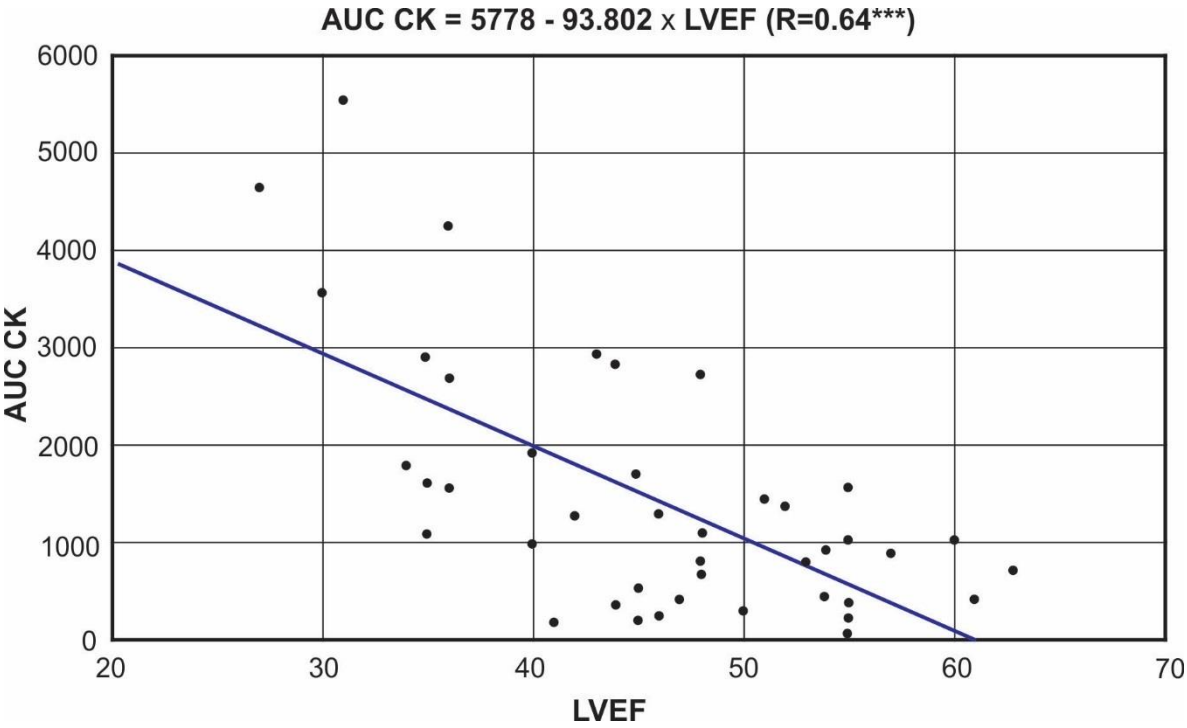

Fig. 9 - Relation between Creatine - Kinase area under curve and left ventricular ejection fraction

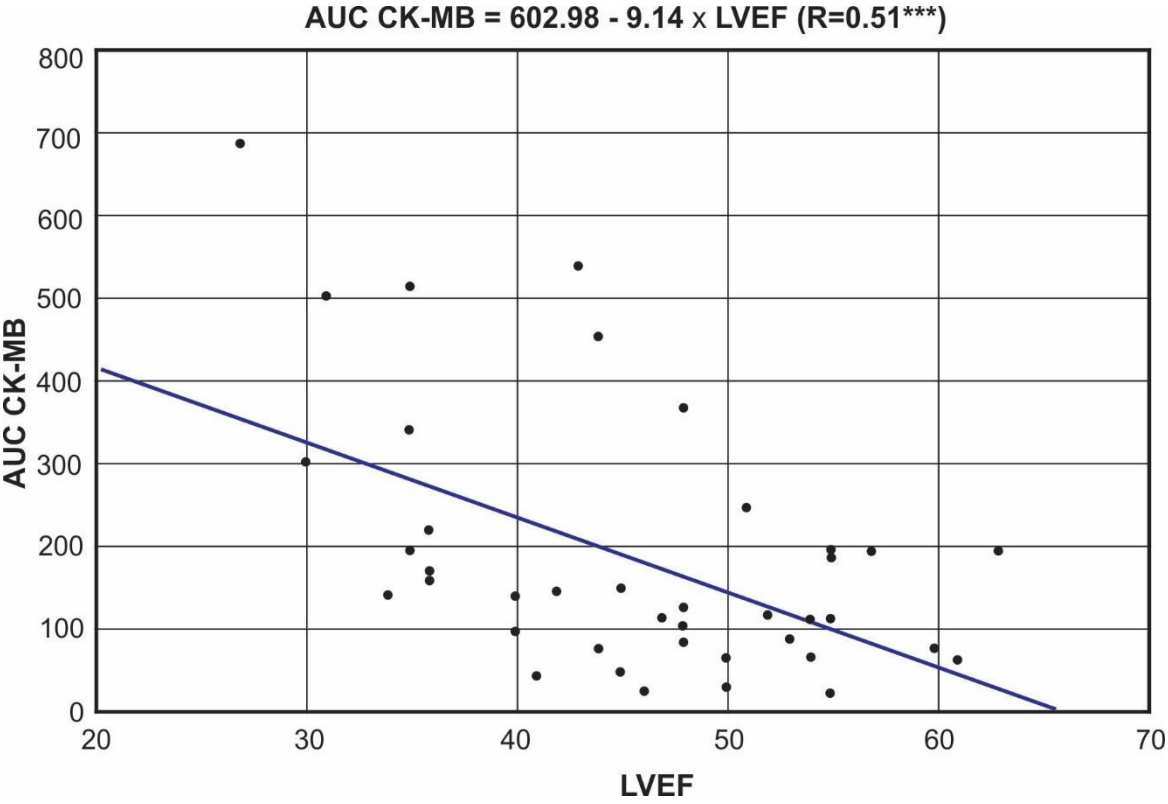

Fig. 10 - Relation between Creatine – Kinase- MB area under curve and left ventricular ejection fraction

The mean seric level α-HBDH 72 in patients with reperfusion syndrome was significantly lower comparing with mean seric level of α-HBDH 72 in patients without reperfusion syndrome. The trend for AUC-CK and AUC-CKMB was similar but did not reach statistical significance. (Table 1).

Table 1 - Myocardial enzymes trend in patients with and without reperfusion

	With reperfusion	**Without reperfusion**	**p**
αHBDH 36 (UI/l)	965.1	997.8	0.91 NS
αHBDH 72 (UI/l)	748.33	1050.58	*p= 0.023
CK – AUC (UI/l)	1088	1899	p = 0.26 NS
CK-MB – AUC (UI/l)	253.1	261.9	p = 0.91 NS

Comparing the mean serum level of α- HBDH assessed at 72 hours in patients without heart failure with mean serum level at 72 hours α- HBDH patients that have had heart failure revealed a significant difference, distinct for all ranges of tracking. (Table 2)

Table 2 - The correlation between mean serum levels of α-HBDH and heart failure

Follow-up interval	Mean level of αHBDH 72 in patients without heart failure	Mean level of αHBDH 72 in patients with heart failure	P
One month	761,1 UI/L	1249,4 UI/L	P = 0.002**
Three months	717,0 UI/L	1305,8 UI/L	P = 0,00003***
Six months	721,3 UI/L	1199,7 UI/L	P = 0,0005***
One year	731,11 UI/L	1202,8 UI/L	P = 0,0009***
Two years	730,53 UI/L	1152,4 UI/L	P = 0,0054**

The lack of correlation between plasma level of α-HBDH measured at 36 hours after the onset of AMI and the LVEF in our study, suggests an inadequate representation of the necrotic size by assessing the seric level of α-HBDH at this moment. We found in turn, a highly significant correlation between the seric level of α-HBDH measured 72 hours after the onset of AMI and the LVEF suggesting the fact that this is the appropriate time for the assessment of seric level of α-HBDH in order to reflect the size of infarction.

Moreover, in our study we find a very strong correlation between AUC-CK and AUC-CK-MB, supporting the idea that measuring the seric level of α-HBDH 72 hours after the onset of AMI, we can obtain a reliable estimation of the necrotic size area.

Conclusions:

Seric level of α-HBDH assessed by a unique determination 72 hours after onset of myocardial infarction may represent a cost/efficient method for assessing size of the necrotic area. Due to

slower dynamics of this enzyme, it probably permits a more accurate determination of the size of post myocardial necrosis in patients with AMI treated with fibrinolytic therapy.

2.1.4. Risk of death stratification after acute myocardial infarction

Elaboration of a risk stratification model designed to give a quantitative estimation of the risk of death in a long term perspective (2 years) was the main objective of my PhD thesis. We initially elaborated an original risk model of prediction which was eventually completed and re-designed and presented at The International Applied Computer Conference in 2009. This scientific contribution was published in extenso in the Proceedings Book of the Conference.

At that time, the preoccupation of developing prediction risk score leaded to the development of a numerous type of risk scales, mostly assessing the short-term risk score. [43-45]

The development of long term risk scale assessment was mainly taking in consideration the newer mechanical reperfusion therapies. [44, 46]

The topic is still actual and reflected by a recent publication. [47]

Two years risk of death prediction after acute myocardial infarction treated with fibrinolytics. A statistical model. *- Țînț D., Pamfil Gh., Rădoi M, Leașu F. Proceedings of the applied computer conference 2009 (ACC 2009), ISBN 978-960-474-124-3; ISSN 1790-2769, 547-550.*

The risk model was elaborated including predictors that in my research have had a significance level $p \leq 0.05$ for both methods. [48]

The risk model provided a clinical risk prediction tool for estimating the cumulative two years risk of death in patients with AMI who received pharmacological reperfusion therapy.

The significance for 2 years mortality predictors Predictor *p* level Pearson and Gehan-Wilcoxon were depicted in the table below (Table 3)

Table 3 - The significance for 2 years mortality predictors

Predictor	*p* level	
	Pearson	Gehan-Wilcoxson
PAD	0.04*	0.0015**
admission HR	0.0128*	0.014*
LVEF	0.0114*	0.048*
Gender	0.0499*	0.05*
Age	0.0024**	0.0024**
admission BP	0.0005***	0.001***

We entered selected individual predictors into the multiple logistic regressions, in order to obtain a preliminary clinical death risk prediction model. Then, we subsequently added in our model α-HBDH activity at 72 hours as a surrogate of myocardial damage, obtaining the final working prediction model. For both models (preliminary and working), the predictive accuracy was good (Table 4).

Table 4 - Models accuracy

Goodness of fit estimator	Model	
	Preliminary	working
Nagelkerke R^2	0.686	0.720
Hosmer-Lemeshow	0.99	0.98
C statistic	0.962	0.971
C statistic 95% CI	0.885 to 0.993	0.892 to 0.996
Overall model fit χ^2 (associated *p*)	31.6171 (0.0001)	32.2274 (0.0001)

We developed a clinical risk prediction model for estimating the cumulative two years risk of death in patients with AMI who received pharmacological reperfusion therapy.

Developed models, preliminary and working, comprise predictors of death (Table 5). Two predictor factors consisted in categorical variables (the presence of peripheral obstructive disease and gender).

Table 5 - Factors associated with fatality, included in the model

Predictor	Model	
	preliminary	working
PAD	27.91 (1.70 – 45.9)	25.98 (1.46 - 46.2)
admission HR	1.075 (0.998 – 1.157)	1.076 (0.995 – 1.164)
LVEF	0.776 (0.645 – 0.933)	0.755 (0.588 – 0.968)
Gender	5.221 (0.259 – 105.10)	3.874 (0.181 – 82.57)
Age	1.135 (0.967 – 1.332)	1.137 (0.969 – 1.333)
admission BP	0.999 (0.954 – 1.046)	0.996 (0.950 – 1.044)
α – HBDH 72	-	0.999 (0.997 – 1.002)

Like similar models already published in literature, [49, 50] this model also intended to be a very simple tool which uses variables that are very easy to assess and are not expensive (Ex: α-HBDH). The model was codified for simplicity in Excel and can be executed on any computer that has MS Office installed. Based on this model, the patients are classified into 4 risk groups

- low < 10% mortality risk;
- medium 10-24 % mortality risk;
- high 25-59% mortality risk and
- very high > 60% mortality risk

Examples of such a model results are depicted in the figures below:

Diana TINT	Two years risk of death prediction after acute myocardial infarction treated with fibrinolytics		
Risk factor	**Data**		
Gender	**m**	[M / F]	✔
Age [years]	**62**	[31 - 79]	✔
α-HBDH 72	**560**	[140 - 2000]	✔
PAD	**y**	[Y / N]	✔
Admission BP	**135**	[70 - 200]	✔
Admission HR	**74**	[36 - 170]	✔
LVEF	**33**	[18 - 63]	✔
Calcule	**Results**		
Complete data?	**Y**	[Y / N]	
Percent risk	**80.016**	[0 - 100]	
Estimated risk	**very high**		

Fig. 11 - Example of the model - a very high-risk result

Diana TINT	Two years risk of death prediction after acute myocardial infarction treated with fibrinolytics		
Risk factor	**Data**		
Gender	**m**	[M / F]	✔
Age [years]	**65**	[31 - 79]	✔
α-HBDH 72	**400**	[140 - 2000]	✔
PAD	**n**	[Y / N]	✔
Admission BP	**115**	[70 - 200]	✔
Admission HR	**77**	[36 - 170]	✔
LVEF	**32**	[18 - 63]	✔
Calcule	**Results**		
Complete data?	**Y**	[Y / N]	
Percent risk	**29.395**	[0 - 100]	
Estimated risk	**medium**		

Fig. 12 - Example of the model - a medium risk of death result

The difficulty of generating such model came from the fact that including more and more factors, we did not have the possibility to compare our model with similar already existing models in literature.

Meanwhile, by multiplying the factors that we have taken in consideration into the model, little variations of these factors have generated greater variations of the risk scores. This led to the need of use “ponderate factors”.

This ponderation can be performed either by statistical calculation, or based on own observation in correlation with similar data already existing in literature. For our model, we used the second option, and we intended to further refine the risk factors ranking after we have developed a more consistent database.

The validation of our conclusions is supported by minimum two distinct statistical methods of calculation, permitting the supposition that further enlargement of the database will not change the significance of the factors, but rather the factors variability.

The mathematic formula of model function through which one can estimate the risk of death is a multidimensional logistic one. Every risk factor included into the model has very strict variation limits based on our study, and the applicability of this model is valid between these limits.

However, the application of this model beyond the experimental space edges can lead to results which are non-concordant with the studied phenomenon.

2.1.5 Large cohort analysis: national RO-STEMI Registry reports

As a continuation on my research area in the field of acute myocardial infarction, I was actively involved in creation, development and analysis of the National Romanian Registry for Acute Myocardial Infarction.

The occurrence of coronary reperfusion procedures (thrombolytic therapy and primary coronary angioplasty) have spectacularly improved the prognosis of the patients with acute myocardial infarction with ST-segment elevation. Therefore, randomized trials published in the

last two decades have reported levels of mortality of 6-8%, unimaginable values before coronary reperfusion era. [51-59]

However, randomized clinical trials have enrolled patients selected according to strict criteria and which have usually a lower risk of death. For example, mortality at 30 days reported in the GUSTO -I was 6.9% and 16.8 % of eligible patients to those considered as non-eligible. [56, 60]

Registries may be contributing to an image closer to the truth about the main characteristics, evolution and prognosis of patients with STEMI. The registries provide data from a large number of patients, regarding morbidity, demographic and clinical particularities, mortality. Meanwhile, these records reflect the degree of adherence of practicing physicians to the diagnostic and treatment guidelines generated by clinical trials.

Registries can demonstrate differences in clinical practice between different geographical regions or even within the same geographical region and, if carried out over the long term, they can demonstrate trends of change for the different parameters to hand, such as risk factor dynamics, or changes in the therapeutic attitude. Thus, registries can have an important contribution in changing strategies of general or specific Health Care policies regarding a specific disease. [61]

The **RO**manian Registry for **ST**-segment **E**levation Acute **M**yocardial **I**nfarction (RO-STEMI Registry) started in 1997 as a national initiative to include all the patients with STEMI. The registry has had two main purposes:

1. Comparative tracking the evolution and prognosis of patients treated with different thrombolytic regimens and

2. Tracking the effectiveness and tolerability of enoxaparin as compared with unfractionated heparin in patients with STEMI treated with thrombolytics.

Starting in 2000, the number of the centers enrolling patients in this Registry has progressively increased. Simultaneously, the investigators have started, out of their own initiative, to enter

into the common database both thrombolysed and conventionally treated patients. The year 2001 has marked the inclusion of the first RO-STEMI patient treated with primary angioplasty.

In 2003, the Registry was formally adopted, under its current name, by the Acute Cardiac Care Working Group of the Romanian Society of Cardiology. As a result of the effort of this Task Force, RO-STEMI has marked, since 2004, an even more rapid increase in enrollment.

I was actively involved in this National Registry starting with year 2000 and my activity consisted in patient's enrollment and statistical analysis and interim follow-up reports.

The first RO-STEMI report was published in 2009 and this report was followed by certain other sub-analysis published in national and international journals and communicated in professional conferences.

2.1.5.1. Clinical presentation, outcome and therapy in patients with acute myocardial infarction - correlation with demographic factors

Clinical presentation, outcome and therapy in patients with acute myocardial infarction. Correlation with demographic factors - a RO-STEMI analysis report. *- Tînț D., Rădoi M., Pamfil Gh., Ionescu D.D., Militaru C., Dan G.A., Daha I., Tatu-Chiţoiu G., Nechita E. and RO-STEMI investigators. New Horizons in Coronary Artery Disease. Proceedings of the 7th International Congress on Coronary Artery Disease, 2007, Medimond S. r. l, Monduzzi Editore ISBN 978-88-7587-402-5, 281-284.*

The study represents an analysis of national RO-STEMI Registry data base included STEMI patients, hospitalized in different Romanian Cardiology Departments between 01.01.1990-31.12.2006.

We sought to evaluate the influence of demographic factors on clinical and therapeutic aspects in patients with STEMI.

This research took in consideration data of 10037 STEMI patients. In order to perform this analysis, we divided the follow-up period in three intervals: interval I 1990-1995, interval II 1996-2000, and interval III 2001-2006.

We assessed the correlations between:

a) demographic factors (age, gender);

b) associated cardiovascular risk factors (diabetes mellitus -DM, arterial hypertension-HTN, smoking status, obesity);

c) previous vascular events;

and

1) time to hospital presentation;
2) clinical status at presentation (Killip class) and type of administrated therapy, and
3) incidence of complications (bleedings, stroke, death).

Statistical analysis was performed using Statistica 7.0.

Results

- Administration of thrombolytic therapy has constantly increased from 1990 to 2006, while primary percutaneous coronary angioplasty (pPTCA) appeared as a therapeutic method since 2001.
- The percent of patients receiving thrombolytic therapy was significantly increased from interval I to interval III [224 patients, (8.3%) vs. 1318 patients (50.8%) and vs. 3880 patients (77.3%) respectively; p<0,0001 for all comparing intervals].
- Type of administered therapy was strongly influenced by age: patients treated with pPTCA were younger than those receiving thrombolysis and the oldest patients were receiving mainly conventional therapy (mean age 47.16 years for PTCA, 58.7 years for thrombolysis and 67.05 years for conventional therapy; p<0.001).

- Thrombolysis was administered in significantly fewer patients with DM [934 patients (46.6%) vs. 4370 patients (56%); p<0.001] and with associated HTN [2544 patients (48%) vs. 2459 patients (54.4%), p<0,0001].
- The history of an old myocardial infarction (MI) also influenced the type of therapy, the percent of patients with previous MI receiving thrombolytic agents was significantly lower than the percent of patients without previous MI [486 patients (49.6%) vs. 4819 patients (54.5%); p=0.004].
- The promptitude of hospital presentation significantly correlated with age (p<0.001): mean age was 60.53 year for those with early presentation (< 6 hours) vs. 65.72 year for the patients with late presentation (> 12 hours).
- Clinical status at admission (Killip class ≥3 vs. Killip class ≤ 2) was significantly influenced by: mean age (66.7 year vs. 61.2 year), gender (20% women vs. 11% man), the presence of DM (22 % with DM vs. 13 % without DM); p<0,001 for each factor.
- Age was the main factor that significantly influenced the occurrence of hemorrhagic complications. The mean age of patients presenting major bleeding was greater than that of patients without bleeding (64.99 year vs. 61.99 year; p=0.03).

Analyzing the occurrence of minor bleedings, we found no significant differences regarding mean age between the two groups (62.05 year vs. 61.58 year respectively; p=0.67). Thrombolytic treatment was associated with a significant higher incidence of both major and minor bleedings [major bleedings 62 patients (1.1%) in patients with thrombolysis vs. 15 patients (0.3 %) in those without thrombolysis, p<0.0001] and minor bleedings 109 patients (2.07%) in patients with thrombolysis vs. 16 patients (0.3 %) in those without thrombolysis, p<0.0001).

There was a trend toward an increased risk of major hemorrhagic complications in patients associating DM, but the difference did not reach statistical significance: [18 patients (0.9%) with DM vs. 62 patients (0.7%) without DM, p=0.06].

The incidence of stroke was also higher in elderly patients (mean age 65.36 year for patients with stoke vs. 61,99 years for patients without stroke; p=0.007) and in patients receiving thrombolytic therapy than in those treated with conventional therapy (1.3% vs. 0.7%; p=0.0029). We found no correlation between stroke incidence and gender, smoking status, association of HTN, DM, and dyslipidemia.

The total number of deaths was 1315 (13.1%). The patients who died were older (mean age 68.29 years vs. 61.09 years, p<0.0001) and mostly women [591 women (19.3%) vs. 725 man (10.46%), p<0.001].

The other factors associated with an increased mortality were:

- DM [334 patients (16.36%) deaths in diabetic patients vs. 979 patients (12.25%) in nondiabetic patients, p<0.0001],
- Killip class at presentation [657 deaths (44%) for Killip class III and IV vs. 656 deaths (7.6%) for Killip I and II class, p<0.0001] and
- The presence of an old MI [219 deaths (21.9%) in patients associating an old MI vs. 1096 deaths (12.1%) in patients who did not experience an old MI, p<0.001].

The administration of thrombolytic treatment was associated with a significant decrease of death rate as compared to the conventional therapy [545 deaths (10.2%) vs. 751 (16.6%) respectively, p<0.0001] as well as interventional strategies [19 deaths (9.2%) in PTCA vs. 751 (16.6%) deaths for conventional therapy, p<0.0001].

At the time of analysis, there were no differences regarding mortality between patients who received thrombolysis and those who received interventional treatment. This aspect however could be explained by the reduced number of primary PTCA performed at that time and by the performance of the STEMI team at that time in the full learning curve.

Conclusions:

1. In Romania treatment strategy in patients with STEMI was significantly influenced by age, invasive reperfusion being reserved for younger patients.

2. Administration of thrombolytic regimens was limited by age and co-morbidities (association of hypertension, diabetes mellitus and old myocardial infarction).
3. Clinical status at admission was worse in older patients, in women and in diabetic patients.
4. Complications rate (bleedings and stroke) was significantly higher in older patients and in those who received thrombolytic therapy.
5. Mortality rate was significantly correlated with Killip class at admission, age, diabetes mellitus, old myocardial infarction and conventional therapy.

2.1.5.2. Anticoagulation strategy in patients with ST-segment elevation myocardial infarction

Enoxaparin, unfractionated heparin or both in patients with ST-segment elevation myocardial infarction? Data from the Romanian registry for ST-elevation myocardial infarction (RO-STEMI). *Tatu-Chitoiu G., Dorobanțu M., Vinereanu D., Stănescu C., Petris A., Pop C., Olariu C., Vlădoianu M., Craiu E., Minescu B., Țînț D., Stănciulescu P., Babes K. - Cardiology International Journal of Cardiovascular Medicine, Surgery, Pathology and Pharmacology, 2009;113 (supl1):63. ISSN 008-6312.*

Regarding the tracking the effectiveness and tolerability of enoxaparin as compared with heparin in patients with STEMI treated with thrombolytic therapy, the following analysis was presented at the XXII Nordic-Baltic Congress of Cardiology held in Reykjavik, Iceland, in June 2009 and published as an abstract in International Journal of Cardiovascular Medicine, Surgery, Pathology and Pharmacology.

Background: A better outcome in patients with fibrinolythic therapy for ST-segment elevation myocardial infarction who received enoxaparin for 8 days, compared with unfractionated heparin (UF) for 48 hours was reported. A “rebound” effect following UH cessation was suspected as the reason of this difference.

Objective: To compare the rates of the in-hospital mortality and hemorrhagic stroke (HS) in patients receiving fibrinolytic and either UH, or Enoxaparin or their combination (UH & enoxaparin).

Methods: Between 1.01.2004 and 31.12.2008 a group of 2691 consecutive patients enrolled in the Romanian registry for STEMI (RO-STEMI) received thrombolytic therapy (Streptokinase, Alteplase, Reteplase or Tenecteplase) followed by either UH [1000 IU/hour for 72–96 hours, (n=1600)] or Enoxaparin [(1 mg/kg bodyweight for 8 days, n=470)] or combination of UH and enoxaparin: UH 1000 IU/hour, 48 hours followed by enoxaparin 1 mg/kg until day 8, (n=377) within the first 6 hours after STEMI onset.

All patients received aspirin, clopidogrel, beta-blockers, angiotensin-conversion enzyme inhibitors (ACEI)s, and statins.

Results: From 2691 patients included in this analysis, 1667 (61.9%) received UH, 517 (19.2%) patients received enoxaparin and 507 (18.8%) were administered the combination of UH and enoxaparin.

A lower in-hospital mortality was seen with UH & enoxaparin compared with UH (3.44% vs 9.00%, $p<0.0001$).

A lower mortality at the limit of significance was also seen with Enoxaparin (6.17%) compared with UH ($p=0.064$). Non-significant difference in mortality was found between Enoxaparin and UH and Enoxaparin ($p=0,098$).

After adjustment for age and aspirin/clopidogrel, patients treated with UH&enoxaparin had a 1.61-time lower risk for death compared with those treated with UH ($p=0.003$).

The rates of HS with UH (0.56%), enoxaparin (0.42%) and UH & Enoxaparin (0.26%) were similar.

Conclusion: The unfractionated Heparin and Enoxaparin combination was the most efficacious anticoagulant strategy in the RO-STEMI patients treated with fibinolytic therapy.

2.1.5.3. The age, cardiovascular risk factors, therapy and in-hospital mortality in patients with STEMI.

I was also the lead author of another important subanalysis of data from RO-STEMI Registry, published in the Romanian Journal of Cardiology in 2011.

[Rom] The age, cardiovascular risk factors, therapy and in-hospital mortality in patients with STEMI - *Țînț D., Rădoi M., Petriş A., Datcu D. M., Sinescu C., Craiu E., Vinereanu D., Dorobanţu M., Ionescu D.D., Macarie C., Ginghină C., Georgescu C., Şerban L., Stănciulescu P., Petrescu I., Petrescu L., Tase A., Nechita E., Topolniţchi L, Benedek I., Dobreanu D., Gârbea S., Gheorghe A.D., Vida-Simiti L., Olinic D., Pop C., Tatu-Chiţoiu G., on behalf of RO-STEMI investigators. A STEMI Romanian Registry (RO-STEMI) report. Romanian Journal of Cardiology Vol. 26, No. 1, 2011:4-13. ISSN 1583-2996.*

The aim of this study was to compare the main demographic characteristics, outcome and in hospital prognosis in patients enrolled in RO-STEMI. The analysis was made on four groups of patients divided according the age.

Method:

RO-STEMI Registry included between 01.01.1997 - 31.12.2009 - a number of 19510 STEMI patients from 43 centers in Romania. Of these, 15484 patients were entered directly in the central database by the investigators. Data from the other 4026 patients were taken either form a registry coordinated by regional myocardial County Hospital Târgu Mureș, either from other local database systematized by their own criteria.

The 15484 patients included in the analysis were divided into four age groups: Group A (patients under the age of 45 years); Group B (45-59 years); Group C (60-74 years); Group D (over 75 years). For each age group we have calculated the incidence of the cardiovascular risk factors, the time intervals between the onset of myocardial infarction and admission and the interval between admission and the onset of treatment. We have alos assessed the incidence of complications and in-hospital mortality rate.

Data stored in the RO-STEMI database were introduced and processed in the program SPSS 15.0 for Windows (LEAD Technologies, Inc.). Analysis and statistical processing of data, was made independently and compared, by two investigators.

The assessment of a RO-STEMI frequency variable was carried out strictly in patients for whom existing data have been validated. The results are presented as the average proportions, with standard deviation and median. For the comparison of mean values, we used "t-test" and the „chi square" test was used for comparing the proportions. Statistical significance was represented by p values < 0.05.

Results:

General characteristics

The overall mean age of RO- STEMI patients was 63.63 +12.8 year.

The distribution by age group of these patients was as follows: Group A, 1329 patients (8.5%); Group B, 5152 (33.2%) patients; Group C 6054 patients (39%) and Group D 2949 patients (19%).

In the entire cohort women with STEMI accounted for 31% (n = 4795). The percentage of women with STEMI has increased proportionately and significantly (p < 0.0001 for tendency). Male/female ratio was maintained above 1 for the first three age groups, but was below 1 in the age group D.

Myocardial infarction localization of was reported in 15338 patients (99.05%). Of these, 7630 (49.7%) patients had anterior STEMI and 7708 patients (51.3%) had nonanterior STEMI. There were no significant differences between the four study groups with respect of STEMI localization.

Distribution of main cardiovascular risk factors in four age groups:

The percentage of hypertensive STEMI patients significantly increased with age (A = 28.2% vs. B = 48.8% vs. C = 57.7% vs. D = 59.7%) with far-reaching differences for the entire trend until the age of 75 years (p<0.0001).

For dyslipidemia, smoking and obesity instead, the trend has been reversed. Dyslipidemia was significantly less frequent after 60 years (A = 41.7% and B = 45.0% vs. C = 37.16% and D = 28.17%; p<0.0001), the number of smokers has decreased progressively with age (A = 76%, B = 65.2%, C = 37.85% and D = 20.7%, p<0.0001). The obese patients were present in a smaller proportion after 75 years (D = 14.13% vs. A = 24.7%, B = 25.48% and C = 22.4%; p<0.001). Diabetes incidence was highest in the age group of 60-74 years. The lowest incidence of diabetes was recorded in patients under 45 years of age (A = 9.7% vs. B = 20.82% vs. C = 25.5%, vs. D =21.08%, respectively; p<0.0001).

As was expected, the number of patients with history of myocardial infarction, proportionally increased with age, reaching statistical significance between the youngest patients and other groups (A = 5.7% vs. B =8.8%, vs. C = 10.56%, vs. D = 11.4%, p<0.0001). The difference between group C and D was not statistically significant.

There was a linear relationship in STEMI patients, between age group and severity of hemodynamic profile at admission estimated by Killip class. Thus, 82 of the patients from Group A (6.16%), 470 of patients belonging to group B (12%), 910 of patients in Group C (15.02%) and 665 of the patients in Group D (22.55%), had Killip class III or IV at admission.

Time from onset of symptoms and admission was recorded in 11872 patients. The median time was 240 minutes. The majority of patients with STEMI (n = 7407, 62.3%) were presented to the hospital within the first 6 hours after the onset of pain, in the window of time accepted for initiation of reperfusion therapy.

The proportion of patients in this time frame was significantly higher among patients below 60 years of age [A and B = 3522 patients, (80.9%) vs. C and D = 3537 patients, (60.6%) p<0.0001]. More than a third of the patients enrolled in the register [(n = 4578 patients, (37%)], were presented to the hospital within the first three hours of the onset of STEMI. The proportion of patients in this category fell proportionately with increasing age (A=39.3%; B=36.87%; C=27.6%; D=21.6%; p<0.0001 for the tendency). There were no

differences among the four age groups in terms of the proportion of patients presented at the hospital in the range of 180-359 minutes from the onset of symptoms.

At the opposite pole were patients with late hospital presentation, over 24 hours after the onset of STEMI (n = 1654 patients; 13.25%). This group was dominated by elderly patients (D = 13.37% vs. C = 13.37%, vs. B = 10% vs. A= 9.66%; p<0.0001).

The time of the onset of thrombolytic therapy, of primary angioplasty, or administration of the conventional treatment (usually time for the administration of the first dose of anticoagulant) was regarded as the moment of initiation of treatment. Overall, the median elapsed time from admission to the initiation of treatment was 30 minutes. Initiation was performed in an optimal range (≤ 30 min) at 6535 (54.2%) patients. Treatment was initiated as earliest as patients were younger. Thus, 59% of patients belonging to the Group A were treated in the first 30 minutes of admission compared with only 50% of patients belonging to Group D (p < 0.0001).

Treatment

A number of 7739 STEMI patients (50.2%) have benefited from coronary reperfusion therapy, (thrombolysis or primary percutaneous coronary angioplasty). The main method of pharmacological reperfusion consisted in pharmacological reperfusion (69254 STEMI patients treated with fibrinolytic therapy, 44,72%), primary percutaneous coronary angioplasty being accessible for a smaller group of patients 815 (14.8%).

It is important to note, however, that in this analysis, we have not been included a substantial number of patients treated in two interventional centers (Cluj-Napoca and Târgu Mureș) due to the impossibility of separating them by age group and time intervals between the onset of symptoms and admission, respective the onset of therapy.

The chance of a patient to be treated with thrombolytics has abruptly decreased with his affiliation to an older age group. Thus, if 54,77% (728 patients) belonging to the Group A of and 54,28% (2797 of patients) in Group B have received thrombolytic therapy, this percentage has

been reduced from 44.26% (2680 patients) in Group C and only 24.41% (720 patients) in Group D (p for trend < 0.0001).

We analyzed the management of the three types of therapy depending on the age of patients in patients hospitalized in optimal therapeutic range (0-360 minutes after the onset of STEMI). Fibrinolytic therapy was administered in 5363 patients (72.6%) hospitalized in this time frame. Primary coronary angioplasty has been performed to 286 patients (3.8%) and 1732 patients (23.46%) were treated conventionally with anticoagulants agents (p<0.0001 for thrombolysis as compared with the other two groups). Interventional therapy was applied preferentially to the young patients (A = 9.2% vs. B = 6.39%, C = 4.26%, D = 3.38%, p<0.0001). The proportion of patients who received fibrinolytic treatment was significantly reduced after the age of 60 years (A = 55.11% vs. B =34% vs. C = 44.4% and D = 24.52%, p<0.0001). The percentage of patients admitted in the optimum time frame, but not administrated reperfusion therapy, increased parallel with the age, the differences being statistically significant after the age of 60 years (A = 14.88%, B = 15.33% vs. C = 22.72% vs. D =50.48%; p<0.0001).

Overall, more than 90% of STEMI patients were treated with anticoagulants (n=14215 patients, 94.2%). The proportion of patients that were not administered with anticoagulants was significantly higher in the groups of age over 60 years [C and D = 596 patients (6.7%) vs. A and B = 294 patients (4.6%), p = 0.001].

The most commonly used anticoagulant was unfractionated heparin. The percentage of patients treated with this anticoagulant was significantly higher as compared to the percentage of patients treated with enoxaparin for all age groups (A = 49% vs. 28%; B = 49% vs. 27%; C = 48% vs. 28%; D = 41% vs. 35%; p ≤ 0.001).

Antiplatelet therapy was administered in 13172 (86%) of RO-STEMI patients. However, the percentage of patients who took no benefit from this treatment, was significantly higher among the elderly population (over 75 years) (D = 14.14% vs. C = 12%; p = 0.092; D = 14.14% vs. B = 10.24%; p<0.0001 and D = 14.14% vs. A = 10.18%; p=0.004). Overall, aspirin was administered to 7036 (47.1%) patients, Aspirin plus Clopidogrel was used in 5420 (36.32%)

patients and the administration of Clopidogrel was only registered in 325 patients (2.1%). Other antiplatelet agents or antiplatelet drug combinations were used at 2163 patients (14.55%). The rate of the administration of Aspirin as single therapy, significantly increased with age (A= 40.96% vs. B = 48.91 C =58.67% vs. D = 64.84% p<0.0001 for tendency).

Beta-blocker therapy has been administered to approximately 80% of patients younger than 60 years, the proportion of treated patients decrease subsequently with age and thus make significant measure of referral (A = 80.85 % vs. B = 78.7% vs. C = 67.8 % vs. D = 56,27%; p<0.0001 for all comparisons except A vs. B in whom the difference was not significant).

The administration of angiotensin-converting enzyme inhibitors was relatively uniform except in patients over 75 years of age who benefit from this therapy significantly less as compared to other age groups (D = 65.1% vs. C = 71.12% vs. B = 72.68% and vs. A = 71.5% p<0.0001 for patients over 75 years compared with the other three age groups).

The rate of statins administration has also recorded a progressive decline after the age of 60 years (A = 75.5%. B = 73.7% vs. C = 67.14% vs. D = 59.4%; p<0.0001).

Stroke and major bleedings

The overall incidence of stroke in RO-STEMI patients was 0.98%. Patients aged over 75 years have had more frequent strokes compared to other age groups (D = 1.69% vs. C = 0.95%, vs. B = 0,067% vs. A = 0.76%; p <0.05 for trend).

Ischemic stroke was more common among patients aged over 60 years (3.6% vs 1.2%, p<0.0048), the same data trend being recorded for hemorrhagic stroke, without reaching statistical signification. There were no differences between age groups in terms of percentage of major bleedings.

In-hospital mortality

The overall mortality among patients enrolled in the register of RO-STEMI was 11.9% (1849 patients). Mortality progressively and significantly grew with age (A = 4.4% vs. B = 6,29% vs. C = 13,32% vs. D = 22,39%; p<0,001 for tendency).

Conclusions:

Elderly patients represent a subgroup with high risk of death due to the existence of comorbidities, the history of coronary events and the more severe hemodynamic profile at admission. These patients arrive significantly later to the hospital as compared to the young ones. We identified an inverse relationship between patient age and "intensity" treatment administered.

First, the current classes of drug recommended for STEMI are significantly less frequently used in elderly patients. The existence of a higher number of comorbidities and contraindications for various therapeutic measures can only partially explain the attitude more "passive" to the elderly. On the other hand, reperfusion procedures are significantly rarely applied in elderly patients. These characteristics may contribute to the worsening prognosis with the age. The contrast between data relative to interventional therapy recorded from randomized trials and from registries (including RO-STEMI), require a more active attitude toward elderly patients.

2.1.6. Mechanical reperfusion in patients with STEMI - Percutaneous transluminal coronary angioplasty (PTCA)

As long as mechanical reperfusion therapy (PTCA) became the main treatment in patients with STEMI, my research area was extended in this direction.

The treatment of acute myocardial infarction was a continuous evolving field. One important complication associated with acute myocardial infarction is complete atrio-ventricular block (CAVB) which is still associated with worse prognosis, despite the implementation of novel therapies. [62,63]

The patients in whom myocardial infarction is complicated with CAVB, have poorer outcomes and higher mortality as compared to those without complete AVB. Complete atrio-ventricular block develops in more than 5% of patients with myocardial infarction and the incidence of CAVB complicating myocardial infarction remained cvasi unchanged from the pre-thrombolytic era until today. [64]

Revascularization therapies were expected to improve the prognostic and to decrease mortality of these patients. Indeed, data coming from thrombolytic era, demonstrated a slightly improvement prognosis in patients with myocardial infarction and CAVB, especially in those patients with inferior MI. Percutaneous transluminal coronary angioplasty is a procedure performed in order to open clogged heart arteries caused by coronary artery disease and to restore arterial blood flow to the heart tissue without open-heart surgery. A stent is a tiny, expandable metal coil that is inserted into the newly-opened area of the artery to help keep the artery from narrowing or closing again. Newer stents (drug-eluting stents or DES) are coated with medication to prevent the formation of scar tissue inside the stent. These drug-eluting stents release medication within the blood vessel itself.

Coronary stents improved procedural safety and efficacy and eliminated the need for surgical standby. However, stent-mediated arterial injury elicited neointimal hyperplasia, leading to restenosis and the need for repeat revascularization in up to one third of patients.

Drug-eluting stents with controlled local release of antiproliferative agents have consistently reduced the risk of repeat revascularization, as compared with bare-metal stents [65].

The introduction of the DES proved to be an important step forward in reducing rates of restenosis and target lesion revascularization after PTCA. However, a new problem appeared: DES in stent restenosis which occurs in 3–20% of patients, depending on patient and lesion characteristics and DES type [66].

In this setting, dual antiplatelet therapy remains the cornerstone of medical therapy after PCI.

However, despite these advanced revascularization technologies, patients with anterior MI, remained at high risk of mortality and some required pacemaker implantation. With the increasingly widespread availability of mechanical reperfusion for MI, the prognosis of these high-risk patients would be expected to further improve, although limited data have been available to date [67-69].

These aspects were addressed in the following publication:

Advanced Metallic Stents and Their Efficiency in Complicated Myocardial Infarction Treatment

Țînț D., Sima S., Rău I., Orțan F.O., Moga M.A. Mol. Cryst. Liq. Cryst., 2014; 603: 99–104.

The aim of this study was to assess the in-hospital outcome of patients with acute ST segment elevation myocardial infarction (STEMI) complicated with CAVB and treated with primary percutaneous intervention (pPTCA).

The method used in this study consisted in a retrospective analysis of 868 files of patients hospitalized with acute ST segment elevation myocardial infarction (STEMI). The patients were admitted in ICCO Clinics between February 5th 2009 and December 31th 2013 and were treated with pPTCA according to guidelines.

STEMI was defined as ST segment elevation >1 mm in two or more contiguous limb leads or ST-segment elevation >2 mm in two or more contiguous precordial leads, in association with chest pain lasting >30 minute. All STEMI were confirmed by demonstrating an elevated level of creatinine-kinase, creatinine- kinase MB and/or troponin.

After the ECG recordings analysis, we divided the patients in two groups: Group A–patients with STEMI complicated with CAVB and Group B–patients with STEMI without CAVB. We intended to find out whether prompt mechanical revascularization has had an influence on resolution of CAVB and on the outcome of these patients.

From statistics point of view, all demographic and clinical characteristics were compared for patients with and without CAVB using chi square test or Fisher's exact test. A multivariate logistic regression model was fitted to evaluate the association between baseline demographic and clinical characteristics and CAVB. Two-sided P values of <0.05 were considered statistically significant. All analysis were performed using SPSS software (version 13.0, SPSS Inc., Chicago, IL, USA).

Results:

The Incidence of the CAVB

From the whole studied group, we have identified 57 patients (6.56%) in whom myocardial infarction was complicated with CAVB. These patients were included in group A. The baseline characteristics of patients from the two groups A and B were depicted in Table 6.

The patients with STEMI complicated with CAVB were significantly older (mean age 65.72 ± 11.97 vs. 56.52 ± 13.18 years; p < 0.0000001), were women in higher proportion (31.57% women vs. 17% men; p = 0.0043) and have had diabetes mellitus in greater proportion that patients with STEMI without CAVB (28.07% vs. 15.67%; p = 0.014). There were no other significant differences between the two groups in respect of other cardiovascular risk factors (smoking, hypertension, dyslipidemia).

As expected, CAVB occurred in higher proportion in patients with inferior myocardial infarction than in patients with anterior infarction (87.71% vs. 48.14%; p < 0.0000001).

Baseline Characteristics

Clinical characteristics of patients with and without CAVB were depicted in Table 6.

Table 6 - Clinical characteristics of patients with CAVB (group A) and without CAVB (group B)

Characteristic	Group A	Group B	p
Mean age (years)	65.72 + 11.97	56.52 + 13.18	<0.0000001
Female	18 (31.57%)	135 (17%)	0.0043
History of hypertension	24 (42.1%)	400 (40.98%)	0.36
Smoking	21 (36.8%)	332 (40.98%)	0.53
Diabetes mellitus	16 (28.07%)	127 (15.67%)	0.014
Mean LVEF (%)			
Mean Na	138 + 5.42	137.41 + 3.96	0.285
Mean K	4.08 + 0.71	4.42 + 5.31	0.64
Glucose	148.67 + 64.99	190.44 + 94.64	0.06
Inferior MI	50 (87.71%)	390 (48.14%)	<0.0000001
Killip class III and IV	2 (3.5%)	18 (2.2%)	0.07
Ventricular fibrillation	5 (8.7%)	50 (6.1%)	0.43

Analyzing the characteristics of patients with CAVB according to the localization of myocardial infarction, we found out that the patients with anterior myocardial infarction were slightly older (although the difference was not statistically significant) and had more severe damage of

myocardium, reflected in significantly lower LVEF (35% vs. 50.65%, p = 0.0026). The difference persisted despite the prompt mechanical revascularization by pPTCA.

Table 7 - Characteristics of patients according to myocardial infarction localization

Characteristic	Anterior STEMI	Non-anterior STEMI	p
Mean age (years)	68 + 16.63	65.4 + 11.36	0.59
Female	3 (42.8%)	15 (30%)	0.49
LVEF (%)	35	50.65	0.002612
Na (mmol/l)	140.83 + 3.4	137.77 + 5.55	0.24
K mmol/l)	4.14 + 0.58	4.08 + 0.72	0.864

All patients received at least one stent, bare metal stents (BMS) (240 patients, 27.2%) or drug eluting stents (627 patients, 72.8%). There were no differences in respect of type and number of stents delivered in patients with and without CAVB.

The outcome was unfavorable in patients with CAVB as compared with patients without CAVB. In-hospital death was significantly higher in CAVB group than in non-CAVB group (14% vs. 2.4%, p < 0.00001). Of note, mortality was very high in patients with CAVB and anterior infarction (42.85%) and significantly higher as compared with patients with CAVB and inferior infarction (10%, p = 0.0189). The resolution of CAVB after revascularization appeared in 76% of patients with inferior myocardial infarction and in only 28% of patients with anterior myocardial infarction (p = 0.0072).

2.2 ATEROSCLEROSIS, METABOLIC SYNDROME AND CORONARY DISEASE

2.2.1 Atherosclerosis and erectile dysfunction

Looking beyond the atherosclerosis complications, I was interested in thoroughly study the mechanisms of atherosclerosis.

It is always difficult to evaluate early signs of atherosclerosis, thus in one of my researches I have try to establish a link between the erectile dysfunction (ED) and coronary events in men. It was already known that all the risk factors of atherosclerosis account as risk factors for ED. [70]

Increasing evidence suggested a link between ED and vascular disorders, leading to the hypothesis that ED may be representative of vascular disease and, furthermore, it may be an early sign of vascular impairment. [71, 72]

Data regarding the link between the ED and coronary atherosclerosis [73,74] were already published, but at that time, there were no studies centered on correlations between ED and the evolution with cardiovascular events.

Thus, I conducted a prospective research on 535 males associating erectile dysfunction (ED) in order to assess the prevalence of risk factors for atherosclerosis and the incidence of cardiovascular events in a 3 years follow-up period.

The paper was presented at the 7th International Congress on Coronary Artery Disease in Venice 2007 and published in the Proceeding book of the conference.

Erectile dysfunction – Early sign or Risk Factor for Vascular Disease? New Horizons in Coronary Artery Disease - *Țînț D., Stănescu C., Pamfil Gh., Rădoi M. Proceedings of the 7th International Congress on Coronary Artery Disease, 2007, Medimond S. r. l, Monduzzi Editore ISBN 978-88-7587-402-5, 277-280.*

The patient's assessment consisted in evaluation of the medical history, cardiovascular medication demographic parameters, clinical examination and blood samples collection for

plasma level of glucose, cholesterol, triglyceride, urea and creatinine at inclusion and each year during the follow-up period.

We have also assessed the Doppler flow velocities in the penile artery in each subject.

The evaluation of the ED was made using a specific questionnaire (the International Index of Erectile Dysfunction) [75]. This is a 15-item self-administered questionnaire with answers scored 0 to 5. Erectile dysfunction is defined as a score of less than 26.

Based on result of this questionnaire, the patients were divided in: group 1 (389 patients with ED) and group 2 (169 patients without ED). The patients were followed-up 36 months for cardiovascular events (angina, myocardial infarction, stroke, cardiovascular death and hospitalization).

Results

We found significant differences between group 1 and 2 regarding the incidence of common risk factors for atherosclerosis.

The patients associating ED were older (mean age 52.8 ± 5.3 years in Group I vs. 47.13±7.2 years in Group II; $p<0.001$), they had greater BMI (mean BMI 28.6 ± 5.8 kg/m^2 Group I vs. 26.9 ± 6.5 kg/m^2 Group II; $p=0.0003$). The incidence of hypertension was significantly higher in patients associating ED and we also found greater mean value of systolic blood pressure in group I: (158.52 ± 3.5 mmHg vs. 143.33 ± 4.2 mmHg) and greater mean value of diastolic blood pressure in Group I as compared with patients included in Group II. (93.48 ± 1.8 mmHg vs. 86.05 ± 0.92 mmHg, $p<0.001$). The mean pulse pressure was also higher in patients with ED (65.8 ± 2.5 mmHg) than in patients with no ED (57.28 ± 1.8 mmHg).

The values of Doppler flow velocities recorded in the penile artery were significantly lower in patients presenting ED than in patients with no ED (mean velocity 4,1 ± 1.5 cm/sec vs. 11,32 ± 2.3 cm/sec; $p<0,0001$).

Dyslipidemia was more common in group 1 patients in whom we found significantly higher levels of plasma cholesterol (mean level 229.45 ± 39.3 mg/dl Group in 1 vs. mean level 176.13

± 29.76 mg/dl in Group 2) and plasma triglycerides (mean level 251.87 ± 38.2 mg/dl Group I vs. mean level 154.33 ± 35.4 mg/dl in Group II).

Diabetes mellitus incidence was significantly higher in patients associating ED (135 patients, 33%) than in patients without ED (5 patients 3%), ($p<0.0001$).

Regarding the diabetes complications, in Group 1, we found diabetic nephropathy in 29 patients (7.2 %), diabetic retinopathy in 27 patients (6.7%) and diabetic neuropathy in 31 patients (7.7%). The patients included in Group 2 have had no complications, ($p<0.001$).

There were also differences in medical history between two groups:

- the percentage of patients having old myocardial infarction was higher in Group 1 than in Group 2 [17 patients, (4.2%) vs. 1 patient, 0.7%; $p=0.059$];
- the percentage of cardiac revascularization procedures PTCA and/or CABG [25 patients, (6.4%) vs. 0 patients; $p=0.0031$);
- the associated peripheric vascular disease [38 patients, (9.4%) vs. 0 patients; $p=0.00029$];
- the rate of peripheric revascularization and stroke was higher in Group 1 than in group 2, but did not reach statistical significance [8 patients, (2.1%) vs.1 patient (0.7%); $p=0.31$ and 14 patients (3.4%), vs. 1 patient, (0.7%); $p=0.1$ respectively].

Regarding the occurrence of the cardiovascular events during the 3 years follow-up period, there was a trend of higher incidence of acute myocardial infarction in patients associating ED but the difference has not reach statistical significance [8 patients, (1.9%) in Group 1 vs. 0 patients in Group 2; $p=0.1$].

However, for angina, stroke and hospitalization for a vascular event at 3 years, we found a significantly higher incidence in Group 1 than in Group 2 [84 patients, (20.9%) vs. 11 patients, (8.53%); $p=0.00185$ for angina; 17 patients, (4.23%) patients vs. 0 patients; $p=0.017$ for stroke and 92 patients, (22.89%) vs. 10 patients (7.75%); $p=0.0002$ for hospitalization]. There were 3 cardiovascular deaths occurring in Group 1.

Several prospective studies published later, reinforced the idea that ED acts as an independent risk factor for future cardiovascular events with an estimated increase of the relative risk of 2.0 over 5–10 years and the link between ED and cardiovascular risk was proved in a very interesting autopsy study. [76-78]

2.2.2 Metabolic syndrome

Since the mid-seventies, the prevalence of overweight and obesity within the U.S. population has increased sharply for both adults and children. Overweight and obesity constitute well defined risk factors for the onset of metabolic syndrome, which in 2000 has reached an age-adjusted prevalence of 27% within the same population. The metabolic syndrome (MS) is a well-recognized precursor of type-2 diabetes, hypertension, and atherosclerosis.

Overweight and obesity constitute well-defined risk factors for the onset of metabolic syndrome. [79-81]

Metabolic syndrome is a frequent precursor to type 2 diabetes, and is characterized by the presence of at least three of the following clinical and biochemical signs: central abdominal obesity, hypertension, insulin resistance, elevated fasting glucose, levated triglycerides, and reduced high density lipoprotein cholesterol (HDL-c). [82]

2.2.2.1 Dietary supplementation with omega-3 Polyunsaturated fatty acids (PUFA), and metabolic syndrome

Polyunsaturated fatty acids (PUFA), and especially dietary supplementation with omega-3 (ω-3) PUFAs (α-linolenic acid, ALA; eicosapentaenoic acid, EPA; and docosahexaenoic acid, DHA), has been reported to improve some of the clinical and biochemical features in individuals with metabolic syndrome. However, because of the variety of study models, as well as inconsistent findings, considerable debate still exists as to whether ω-3 PUFA supplementation may be successfully used as a dietary intervention against the metabolic syndrome. [83]

Beneficial effect of flaxeed oil supplementation in subjects with metabolic syndrome *- Țînț D., Anghel M., Fischer L., Niculescu M. Atherosclerosis Supplements 11, no. 2 (2010) 109–222.*

Background: Flaxeed oil is a rich source of α-linolenic acid (ALA), and may have some beneficial effects on cardiovascular risk factors but the effect on metabolic syndrome (MS) is unknown.

Objective: To assess the effect of Flaxeed oil in subjects with MS.

Method: Prospective, double-blind, placebo-controlled, randomized trial in male subjects with MS divided in two groups: 10 subjects (ALA group) received 2400 mg flaxseed oil/day and 10 subjects (CT group) received 2576 mg corn oil (placebo)/day for 3 months.

Clinical parameters and biochemical investigations [glucose, insulin, model assessment-estimated insulin resistance (HOMA-R) index, lipoproteins, oxidized low density cholesterol (ox-LDL) and high sensitivity C reactive protein (hsCRP)] were assessed at baseline and at day 90. Data analysis was performed using bivariate analysis (linear fit model between day 0 and day 90) and logistic regression.

Results: The BMI increased at day 90 vs. baseline in CT group while it decreased in ALA group, with significant difference between groups (1.1 vs -0.3; p=0.0215). The level of total cholesterol (mg/dl) at day 90 was higher than day 0 in CT with significant correlation (209 vs. 203.6; p=0.01). The insulin level (mU/ml) and the HOMA-R index decreased in ALA group with significant correlation on day 90 vs day 0 (14.55 vs. 12.92 p=0.0358 and 5.29 vs. 4.23 p=0.0242), while no differences in glucose level were found. There were no significant differences in other biochemical parameters trends.

Conclusion: In subjects with metabolic syndrome, 3 months administration of Flaxseed oil had no effect on cardiovascular risk factors, but significantly improved insulin sensitivity and decreased BMI.

The effect of Flaxseed Oil Supplementation upon Inflammatory Markers and Metabolic Syndrome Progression. *Țînț D., Anghel M, Lupu DS, Fischer LM and Niculescu MD.- presented in 80th EAS Congress 2012*

Aim: This study aimed to assess the effect of flaxseed oil supplementation upon the evolution of metabolic syndrome (MS) and inflammatory markers in men.

Method: Double blind, randomized study in men with MS, mean age 55.95 ± 5.23 year, assigned to receive flaxseed oil (Group A, 10 patients), or corn oil (Group B, 10 patients) for 90 days. Clinical and biochemical investigations were assessed on days 0 and 90. Clinical and biochemical investigations were assessed on days 0 (D0, one day before the beginning of supplementation) and 90 (D90, end of study) for each subject. Safety monitoring visits were performed monthly during this period and consisted of clinical examination, blood pressure measurements, distribution of capsules for the next month, and evaluation of compliance.

Results: Body mass index (BMI) remained unchanged in group A as compared to an increased BMI registered in group B (D90/D0 0.99 ± 0.01 vs. 1.12 ± 0.63, $p < 0.05$). Bivariate analysis fit for plasma insulin and derived HOMA index indicated that flaxseed oil maintained the individual correlation of each parameter between the start and end of study, while corn oil supplementation was associated with an increase in insulin resistance with no significant correlation between start and end of treatment (D90/D0 1.12 ± 0.17, $p<0.05$ vs. 2.11 ± 0.79, $p > 0.05$). Flaxseed oil induced an increase in interleukine 1β (IL1β), while opposite changes were induced by corn oil (D90/D0 2.90 ± 0.80 group A vs. 1.13 ± 0.17 group B, $p < 0.05$). In group A, tumor necrosis factor alpha (TNFα) individual values were correlated between D0 and D90 ($p<0.018$).

Conclusion: Low daily doses of flaxseed oil may improve clinical and metabolic parameters and may increase the D90/D0 IL1β ratio in middle-aged men without adequate treatment for metabolic syndrome.

Low dose Flaxseed Oil Supplementation Alters the Fatty Acids Profile and the Progression of Metabolic Syndrome in Men without Adequate Medical Treatment. *Țînț D., Anghel M., Lupu D.S., Fischer L.M., Niculescu M.D. (2011) J Nutr Disorders Ther 2011; S7:1-8.*

The aim of this study was to determine the consequences that low dose supplementation with flaxseed oil may have upon clinical and biochemical outcomes in subjects with MS who did not receive proper medical treatment.

Materials and Methods: The study was performed according to the Declaration of Helsinki and approved by the Institutional Review Board at the University of North Carolina at Chapel Hill, and by the Ethics Committee at Transilvania University. Written consent was obtained from all participants before study initiation.

Study population

The study was a double-blind, controlled, randomized intervention trial in 20 male volunteer subjects, between 50-65 years of age, with diagnosed MS, recruited from Romania (Brasov area) between August 2008 and September 2009. MS was assessed according to the National Cholesterol Education Program's Adult Treatment Panel III report criteria (ATP III), for the presence of three or more of the following criteria:

1) Waist circumference ≥ 40 inches (102 cm);

2) Triglycerides (TG) ≥ 150 mg/dl;

3) HDL-cholesterol ≤40 mg/dl;

4) Blood pressure (BP, systolic/diastolic ≥ 130/≥85 mmHg) or antihypertensive treatment;

5) Fasting glucose (FG) ≥110 mg/dl or antidiabetic medication (insulin or oral agents). [84]

Exclusion criteria consisted of:

1) Any coagulation syndrome and/or diagnosed blood disorder related to the number, morphological features, or physiology of red blood cells (RBC), white blood cells (WBC), or platelets;

2) Family history of hereditary blood disorder, even in the absence of a diagnosed blood disorder, any carrier of a diagnosed genotype known to primarily cause a blood disorder;

3) History or presence of an embolic event (any organ), stroke, transient ischemic attack (TIA), ischemic heart disease, myocardial infarction, unstable angina, or varicose veins;

4) Current use of a treatment or dietary supplements (for the past three months) containing any omega-3 fatty acid or any lipid modifying agent (e.g. statins, fibrates, ezetrol);

5) Any treatment including the administration of aspirin, ticlopidine/clopidogrel, non-steroidal anti-inflammatory drugs, and other hypocoagulant medication before and during the study.

Study design was described in details in project proposal.

Data analysis

Data was analyzed using the JMP 8 analysis software (SAS Institute, Cary, NC). Time-treatment interaction was assessed by two factor Anova. Student's t-test was used to assess significance between the two groups, for each variable and time point (D0 or D90). Paired t-tests were used for assessing significance of change in each group between start and end of study (D0 vs. D90). Because variances were, in most cases, not equal between groups, additional testing was performed.

Bivariate analysis was performed for each parameter and group, to determine the dependency relationship between D0 and D90 values.

Logistic fit testing was performed on D90/D0 ratios for each parameter and between groups, in order to assess the predictive value of changes for subject classification in either of the treatment groups. For all tests, $p < 0.05$ was considered to declare significance of change.

Results: In this study 64 parameters were analyzed for potential changes between the group receiving corn oil (C) and the group receiving flaxseed oil (F), for two time points (D0 and D90).

Results

The characteristics of subjects assigned to each group are presented in Table 8.

Table 8 - Baseline measurements

PARAMETER	C group	F group	P value
Age (years)	54.80 ± 1.98	57.10 ± 1.26	NS
BMI (kg/m^2)	30.92 ± 1.95	29.64 ± 0.84	NS
Waist (cm)	117.00 ± 4.49	111.45 ± 1.48	NS
SBP (mm Hg)	145.50 ± 5.13	149.00 ± 6.22	NS
DBP (mm Hg)	90.00 ± 3.07	90.50 ± 2.83	NS
Glucose (mg/dl)	126.00 ± 11.00	127.00 ± 19.00	NS
HDL – cholesterol (mg/dl)	56.78 ± 2.49	44.78 ± 3.37	<0.05
Triglycerides (mg/dl)	166.50 ± 25.56	220.40 ± 20.57	NS

Identical containers containing capsules with either 1.2 g of either corn oil or flaxseed oil were numbered by the Chapel Hill team according to each subject's assigned number, and shipped to Brasov. The lipid content of corn oil and flaxseed oil capsules is indicated in Table 9.

Table 9 - Fatty acid composition of supplements

FA species		FA concentration µmol/mL	
		Corn oil	Flaxseed oil
14:0	**myristic**	ND	1.26
16:0	**palmitic**	368.51	200.44
16:1n7	**palmitoleic**	3.78	3.26
18:0	**stearic**	55.47	119.01
18:1n9	**oleic**	844.36	528.58
158:2n6	**linoleic**	1653.74	554.79
18:3n3	**linolenic**	13.51	1638.40
20:0	**eicosanoic**	25.24	ND
20:1n9	**11 – eicosenoic**	9.31	5.79
20:2n6	**11, 14 – eicosadienoic**	ND	1.27
20:3n3	**11, 14, 17 – eicosatrienoic**	ND	1.53
22:0	**behenic**	3.62	3.93
22:1n9	**erucic**	ND	1.28
22:5n3	**7, 10, 13, 16, 19 – docosapentaenoic**	1.66	1.06
24:0	**lignoceric**	5.42	3.69

ND = not detectable

MS-related parameters

Analysis using paired-t tests revealed no changes between groups and time points. Analysis of two other related parameters, BMI and insulin concentrations, revealed significant changes when either logistic fit or bivariate analysis was applied.

While BMI averages between groups and time points did not indicate significant changes (Figure 13 A) the relative BMI ratios (D90/D0) were smaller in the F group (0.99 ± 0.01 SE) than in the C group (1.04 ± 0.03 SE), with significant predictive value (Fig. 13 B) for group assignment. BMI was assessed at start (day 0, DO) and end of study (day 90, D90) for both groups (flaxseed oil supplementation, F and corn oil supplementation, C). In Fig. 13 A, averaged BMi per group is indicated for both time-points, with no significant changes for either group as assessed by

paired t-tests. Error bars represent standard error. Fig. 13 B indicated significance for the D90/D0 BMI ratio as a predictor for group classification.

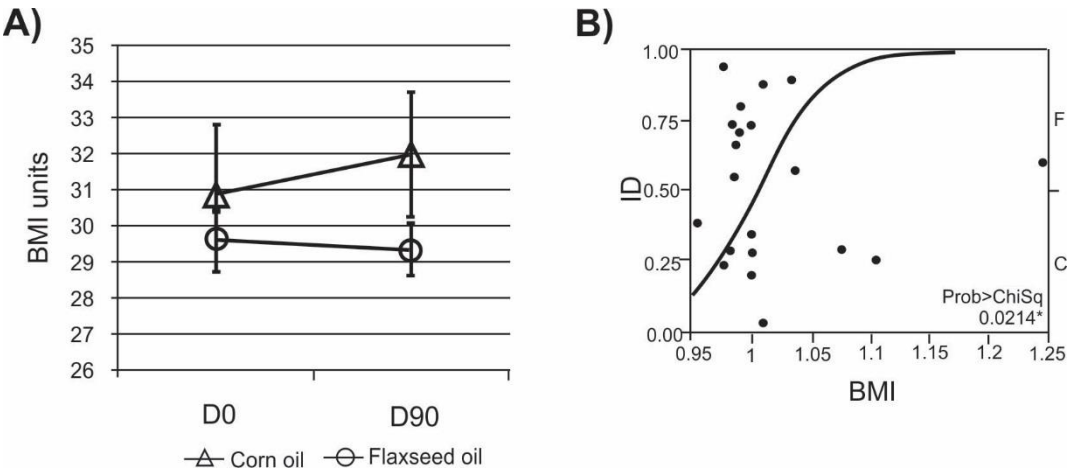

Fig. 13 - BMI assessment between the start (day 0) and the end of the study (day 90)

Markers of inflammation

Among the markers of inflammation, flaxseed oil supplementation induced an increase in IL1β, while opposite changes were induced by corn oil supplementation (Fig. 14 A). The D90/D0 ratios (2.90 ± 0.80 SE in F group versus 1.13 ± 0.17 SE in C group) also had predictive value for group assignment (Figure 13B). Within the F group, TNFα individual values were correlated between D0 and D90 ($p < 0.018$) while for the C group the correlation was not present between the same time-points.

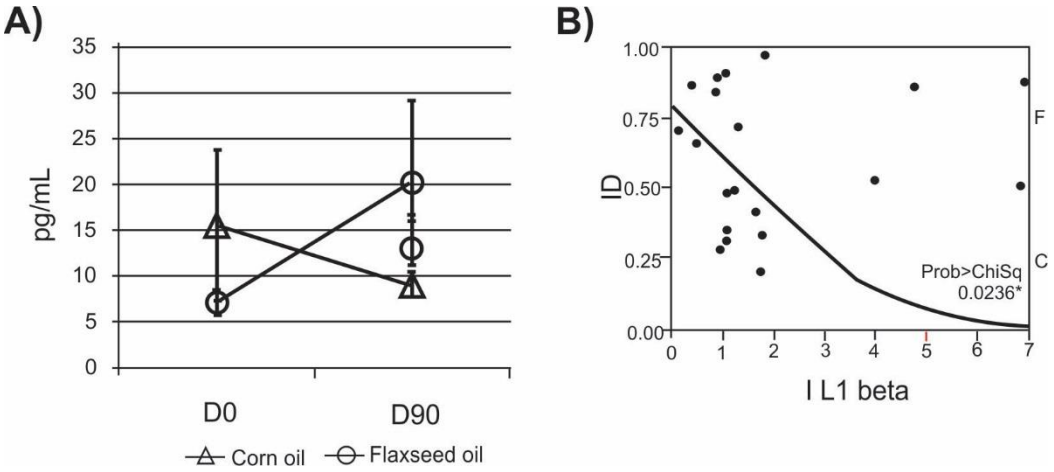

Fig. 14 - Serum ILβ1 assessment between start and end of the study

Lower apolipoprotein A1 (ApoA1) D90/D0 ratios were also predictive for the F group, as indicated in Fig. 15.

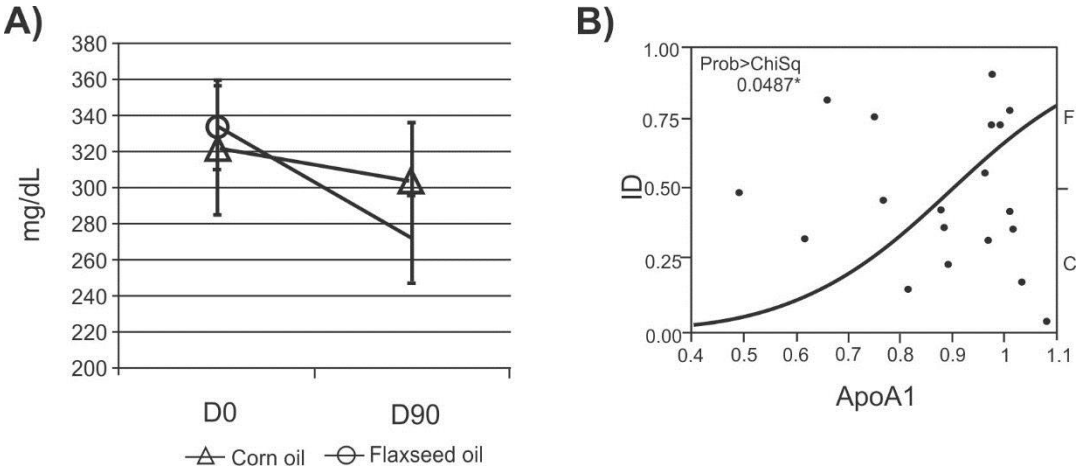

Fig. 15 - Serum apo-A1 assessment between start and end of the study

Plasma fatty acids composition

Among the fatty acid species in plasma, α-linolenic acid (ALA,18:3n3) had lower D90/D0 ratios assigned to the F group as compared to the C group values (0.63 ± 0.11 SE vs. 1.25 ± 0.32 SE, respectively, Fig. 15). Plasma profiles of α-linolenic acid (18:3, ALA) were measured at start (day 0, D0) and end of the study (day 90, D90) for both groups (flaxseed oil supplementation, F and corn oil supplementation, C). Fig. 16 A - ALA group averages are indicated for both time-points, with no significant changes for either group as determined by paired t-tests. Error bars represent standard error. Fig. 16 B- Logistic fit indicated significance for the D90/D0 ALA ratio as a predictor for group classification.

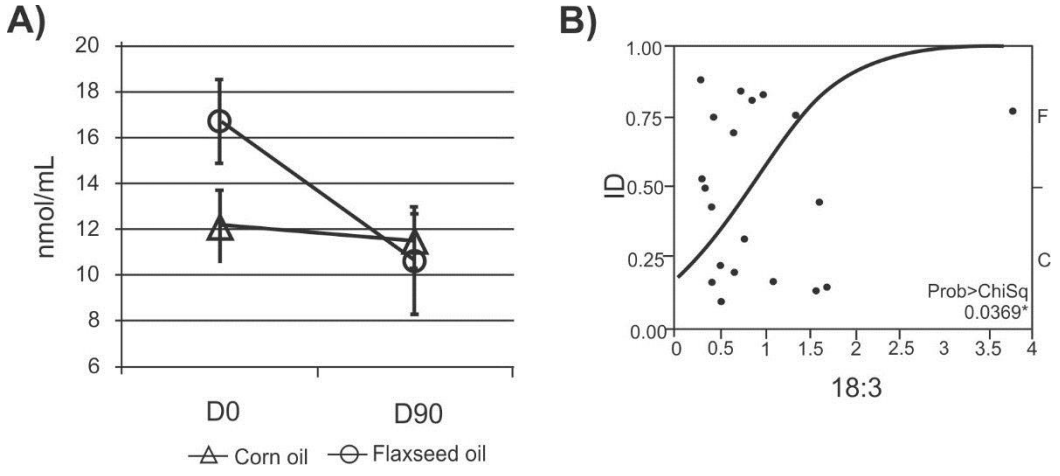

Fig. 16 - Plasma ALA levels between start and end of the study

Time-treatment interaction analysis indicated significance for 11-eicosenoic plasma levels (Fig. 17 A), as determined by the ANOVA-two factor test ($p < 0.05$). These changes were also significant for group assignment, as indicated by logistical fit analysis of the D90/D0 ratios, with higher ratios assigned to the C group (1.63 ± 0.20 SE) when compared to the F group (1.00 ± 0.11 SE) (Fig. 17). Plasma profiles of eicosenoic acid (20:1) were assayed at start (day 0, D0) and end of study (day 90, D90) for both groups (flaxseed oil supplementation, F; corn oil supplementation, C). Fig.17 A Eicosenoic acid group averages are indicated for both time-points, with no significant changes for either group as determined by paired t-tests. Significant time-treatment interaction was determined using ANOVA-two factor analysis ($p<0.05$). Error bars represent standard error. Fig.17 B Logistic fit indicated significance for the D90/D0 eicosenoic acid ratio as a predictor for group classification.

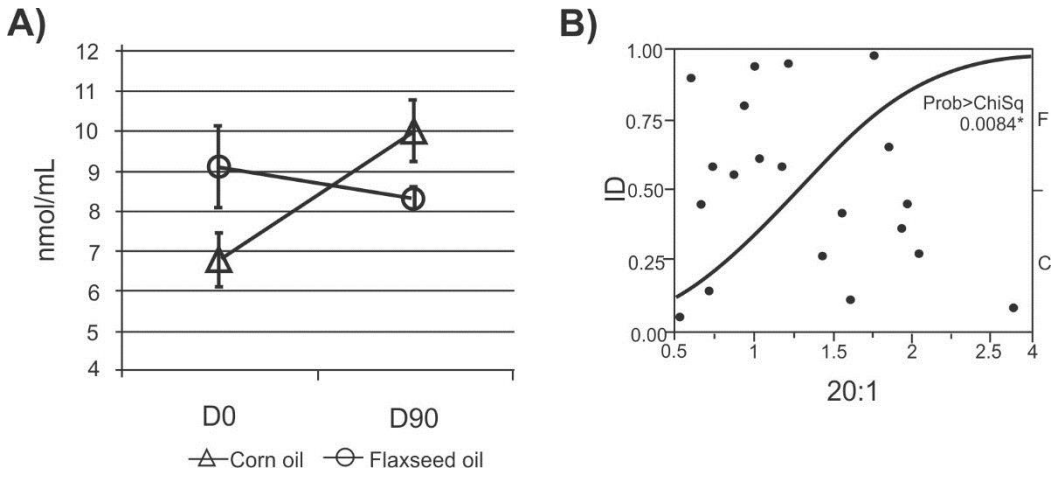

Fig. 17 - Plasma eicosenoic acid levels between start and end of study

Six other fatty acid species were differently correlated between the two groups, indicating that the low dose of either flaxseed or corn oil could induce specific changes in their plasma profiles as is depicted in Table 10.

Table 10 - Fatty acid species and their correlation between time points

Variable	Corn oil		Flaxseed oil	
	D90/D0 ratio	D90/D0 bivariate model (Prob > F)	D90/D0 ratio	D90/D0 bivariate model (Prob > F)
Fatty acid species				
14:0 (myristic)	0.94 ± 0.11	NS	**0.99 ± 0.09**	**0.0102**
18:0 (stearic)	1.02 ± 0.05	NS	**0.93 ± 0.04**	**0.0489**
18:2n6 (linoleic)	1.05 ± 0.07	NS	**0.99 ± 0.04**	**0.0032**
20:0 (arachidic)	**1.21 ± 0.21**	**0.0185**	1.88 ± 0.43	NS
20:2n6 (eicosadienoic)	1.23 ± 0.09	NS	**1.12 ± 0.06**	**0.0034**
20:3n6 (dihomo-gamma-linoleic)	1.14 ± 0.14	NS	**1.01 ± 0.08**	**0.0258**

Discussions: In our study, several parameters were modified by flaxseed oil supplementation, as compared with the group receiving corn oil. BMI did not change over the 90 days treatment period in the F group, while the C group registered an increase in BMI. Logistic fit of the BMI D90/D0 ratios indicated that such changes have predictive value for group assignment. Similar predictive values were obtained for changes in IL1beta, ApoA1, ALA, and eicosenoic acid. Bivariate analysis indicated that the flaxseed oil supplementation reduced insulin resistance as assessed by insulin levels and the derived HOMA index.

In our study, with the exception of a correlated increase of D90/D0 IL1β ratios within the F group, we found no changes of inflammation markers (averaged values). However, regression analysis revealed that TNF-α was correlated between start and end of study only in the F group. Similarly, insulin sensitivity was correlated in the F group. Our results are in agreement with published studies that have indicated that TNF-α plays an important role in mediating insulin resistance. [85]

Increased intakes of dietary ALA demonstrated anti-inflammatory effects by inhibiting IL-6, IL-1, and TNF-α production in cultured peripheral blood mononuclear cells (PBMCs). [86]

Our study aimed to determine whether a low-dose flaxseed oil supplementation regimen could be effective in improving physiological and biochemical parameters associated with metabolic syndrome and we found that low daily doses of flaxseed oil may improve clinical and metabolic parameters in middle-aged men without adequate treatment for metabolic syndrome.

The present study had however, important limitations. The most important factor was the high and unequal variance in data distribution, due to a small sample size in both groups, and to high physiological variations between individuals. Group randomization failed in regard to HDL-cholesterol distribution. Therefore, parametric assumptions for t-testing were frequently not met among the measured variables. While logistic fit or bivariate analyses rendered significance for the discussed variables, it is not clear whether the reported changes have biological significance. We surmise that flaxseed oil supplementation, in opposition to corn oil, was associated primarily with no increase in BMI and insulin resistance, while other reported changes are difficult to interpret in the context of our study design.

2.3. SCIENTIFIC DEVELOPEMENTS IN THE FIELD OF ATRIAL FIBRILLATION ABLATION THERAPY

Introduction:

This part of my research activity was performed in Department of Cardiology, University of Debrecen, Hungary. I was part of the research team between 2009-2011, while I worked in this Institute as a Fellow of the European Heart Rhythm Association. During this period of time, I was involved in most of the research activities of the department.

In Debrecen University Centre, there was a continuous preoccupation for simple and efficient ways to ablate atrial fibrillation. The research activity was centered on ablation of atrial fibrillation using a different ablation technique.

The role of catheter ablation in patients with atrial fibrillation (AF) was widely increased due to the high incidence of AF and the limitations of the medical therapy. Transcatheter isolation of the pulmonary veins (PVI) is considered the cornerstone of ablation for AF. Due to an already proved benefit of ablation techniques, ESC guidelines for the management of AF have indicated catheter ablation for paroxysmal and persistent AF in symptomatic patients who have previously failed or were resistant to antiarrhythmic medication (AAR) as a class II b treatment option. [87]

Although considered “gold standard procedure” pulmonary vein isolation using point-by- point radiofrequency (RF) ablation remains a complex and challenging procedure. Novel catheter designs and energy forms have been then introduced as alternatives to point-by-point RF ablation, aiming at a safer and simpler procedure with a faster learning process and comparable success rates even in less experienced hands.

Until now, four devices have been evaluated on a broader scale. Ablation with Bard HD Mesh Ablator Catheter (MESH), showed a high recurrence of AF within the first year despite a high acute success rate. [88]

The utilization of ultrasound energy based devices (high intensity focused ultrasound balloon catheters) was followed by a long-term success rate similar to RF-catheter based procedures,

however the good effectiveness was offset by severe post-procedural device-related complications. [89]

The utilization of cryoballoon technology has being proven to be a safe procedure, followed by good clinical results, very similar with classical point-by-point ablation, especially in paroxysmal AF. [90-97]

2.3.1 Pulmonary vein isolation using cryoballoon (Arctic Front, CryoCath Technologies Inc., Kirkland, Quebec, Canada).

The first single-shot technique for pulmonary vein isolation used in Debrecen was cryoballoon ablation. The center was the first one in Hungary using this technique and the first Hungarian experience using this technology was published in 2010.

Cryoballon isolation of the pulmonary veins in atrial fibrillation: Mid-term results after the first 55 patients. *Tóth Zs., Nagy-Baló E., Kertész A., Clemens M., Herczku Cs., Ţînţ D., Kun Cs., Edes I., Csanádi Z. Orv. Hetil. 2010 Jan 31;151(5):163-171.*

This paper summarizes the initial experience with cryoballon ablation for pulmonary vein (PV) isolation in patients with atrial fibrillation after the first 55 patients.

Method: Symptomatic patients refractory to antiarrhythmic medication mostly with paroxysmal atrial fibrillation without significant structural heart disease were enrolled. Cannulation and isolation of all pulmonary veins were attempted using a 28 mm double-wall cryoballon inflated at the ostium of the vein and abolishing electrical activity of atrial tissue around its perimeter by freezing to -70^0 C. Intravenous heparin during and oral anticoagulant after the procedure was administered.

Conventional electrocardiograms (ECGs), Holter ECGs and transtelephonic ECG recordings were used through 6 months follow-up for rhythm monitoring.

Results: In 55 patients enrolled (18 female; age: 56±33.64 years) 165 out to 192 (86%) pulmonary veins were successfully isolated. All pulmonary veins were isolated in 37 patients

(67%). Procedure time was 155.67± 100.66 min, while fluoroscopy time was 34.04± 31.89 min. In 34 patients with 6 months follow-up 24 (70%) either remained free of arrhythmia (17 patients) or had a significant decrease in arrhythmia burden (7 patients).

Conclusion: Based on our initial experience, cryoballon isolation of pulmonary veins appears to be a simpler procedure with similar efficacy to radiofrequency ablation in the treatment of atrial fibrillation.

2.3.2 Pulmonary vein isolation using multipolar circular duty-cycled catheter (PVAC).

The "one-shot" technology which seems to fulfil expectations regarding safety and efficacy is represented by multipolar circular duty-cycled catheter (PVAC). [98-100]

Atrio-esophageal fistula is a very rare complication of AF catheter ablation. Described for the first time in two very experienced centers in 2004, this complication is the most dreadful and lethal among all the others related to AF catheter ablation. [101,102]

Extensive RF delivery during AF procedures may lead to esophageal damage due to its anatomical course in close proximity of the left atrium. Although the esophageal fistulas rarely occur, endoscopic studies have shown that ulcerative lesions are relatively common even after cryoballoon ablation, a technique considered to be safer than those using RF energy.

Effect of left atrial radiofrequency ablation on the esophagus using a novel three-dimensional ablation catheter family. *Țînț D., Toth Zs., Nagy-Balo E., Beke I., Clemens M., Edes I., Csanadi Z. J. Kardiol 2010;16 (Suppl A) : 7.*

The aim of the study consisted in a prospective evaluation of the acute effect of the left atrial RF ablation on the esophagus, using a novel ablation system (Medtronic, Ablation Frontiers, Carlsbad, CA, USA.)

Method: Patients with symptomatic AF underwent left atrial (LA) ablation using multielectrode RF ablation catheters designed for pulmonary veins isolation (PVAC), ablation of the LA septum

(MASC) and along the posterior atrial wall (MAAC). RF energy was delivered in different ratio of bipolar and unipolar mode, using a target temperature of 60^0C and a maximum power of 10 W.

Procedure end-point was electrical isolation of all pulmonary veins in all patients. Sites showing complex atrial fractionated electrograms (CAFE) were also targeted in those patients with permanent AF.

Esophago-gastroscopy was performed within 24 hours post-ablation in all patients.

Results: A total of 25 patients (14 males), mean age 54.4±11.06 year (29-67), underwent LA ablation. Twelve patients had had previous PVI procedures (10 of them cryoablation). Ostial vein isolation was performed in all patients, and additional ablation using MASC and MAAC was performed in 9 patients. A total of 81 PVI were targeted. Acute successful isolation was achieved in 73 (90%) of PVs. The mean procedure time was 138 ± 56.6 minutes (65-120) and mean fluoroscopy time 38.7 ± 15.2 minutes (23.6-79.6). The mean PVAC time was 6.2 ± 4.5 minutes (2-19) and the mean number of application for PV ablation was 7.1 ± 5.24 (1-22). More applications were performed in the superior than in the inferior veins: (8.2 ± 5.6 vs. 5.9 ± 4.6) and in the left sided veins than in the right sided veins (8.3 ± 5.59 vs. 5.67 ± 4.45). The number of applications using MASC and MAAC was 6.25±4.25 minutes and 4.57 ± 2.42 minutes, respectively. Esophago-gastroscopy showed no lesion attributable to the ablation procedure in any patient.

Conclusion: Based on our initial experience, extensive left atrial ablation with 3D multielectrode catheters using different ratio of unipolar and bipolar RF delivery causes no significant thermal injury of the esophagus.

AF ablation using this single-shot device was further developed in Cardiology Center of Debrecen and we started to analyze different aspects derived of this technique such as: the learning curve, the short term efficacy and the long term efficacy.

I was an active part of the research team until the end of my fellowship and all the above mentioned aspects were summarized in the following paper.

Learning curve in circular multipolar phased radiofrequency ablation of atrial fibrillation *- Martirosyan M., Kiss A., Nagy-Baló E., Sándorfi G., Țînț D., Edes I., Csanádi Z. Cardiol J. 2014 22(3):260-266.*

We investigated the time-dependent changes in procedural parameters, complication rates, and in the 1-year clinical outcome during our initial experience with circular multipolar phased radiofrequency ablation.

Methods:

Study population

The present study included consecutive patients who underwent PVI with phased RF ablation for paroxysmal or persistent AF at Cardiology Department of Debrecen University, Hungary, between November 1st 2009 and April 30th 2012 and who had regular follow-up during the first 12 months post-ablation.

End points

The acute endpoint of the procedure was the electrical isolation of all pulmonary veins, as confirmed by an entrance block. Long-term efficacy was defined as freedom from any atrial arrhythmia without a Class I or Class III AAD after one procedure at 12-month follow-up with a blanking period in the first 3 months. Significant periprocedural complications were defined as any injury which resulted in death or had long-term sequels, required an immediate intervention or prolonged hospital stay.

Statistical analysis

The study period was divided into tierces to include the same number of patients who underwent AF ablation within each tierce. Clinical characteristics, procedural and follow-up data for subjects in each tierce were presented using numbers and frequencies (%) for categorical variables and means with standard deviations (SD) for continuous variables.

Statistical calculations were performed with IBM SPSS Statistics 20 Software. The distribution was examined with Kolmogorov-Smirnov test. Discrete variables were analyzed using Chi-

square test. ANOVA (Analysis of variances) and Kruskal-Wallis were used for comparisons of groups. Cox regression as univariate test was used to estimate the hazard ratio. P value less than 0.05 was considered significant.

Results:

A total of 132 patients were enrolled. There were no differences in preablation clinical characteristics of the first, second and third 44 patients who underwent PVI with phased RF ablation with the exception of the age and left atrial size.

A total of 177 PVs were successfully isolated out of the 177 targeted in Tierce 1, while 173/176 and 169/171 in Tierces 2 and 3, respectively ($p > 0.05$).

All PVs were successfully isolated in 44 (100%), 41 (93.8 %) and 42 (95.5 %) patients in Tierce 1, 2 and 3, respectively, ($p = 0.233$).

However, the number of RF applications (per PV) needed for isolation demonstrated a significant decrease with experience (6.22 SD: 2.43; 4.65 SD:.1.32 and 4.12 SD: 1.2 in Tierce 1, 2 and 3, respectively; $p < 0.001$).

Procedure times demonstrated a trend for lower values in Tierces 2 and 3 but the difference did not reach the level of statistical significance. In contrast, a significant decrease in fluoroscopy times was demonstrated. (Fig.18)

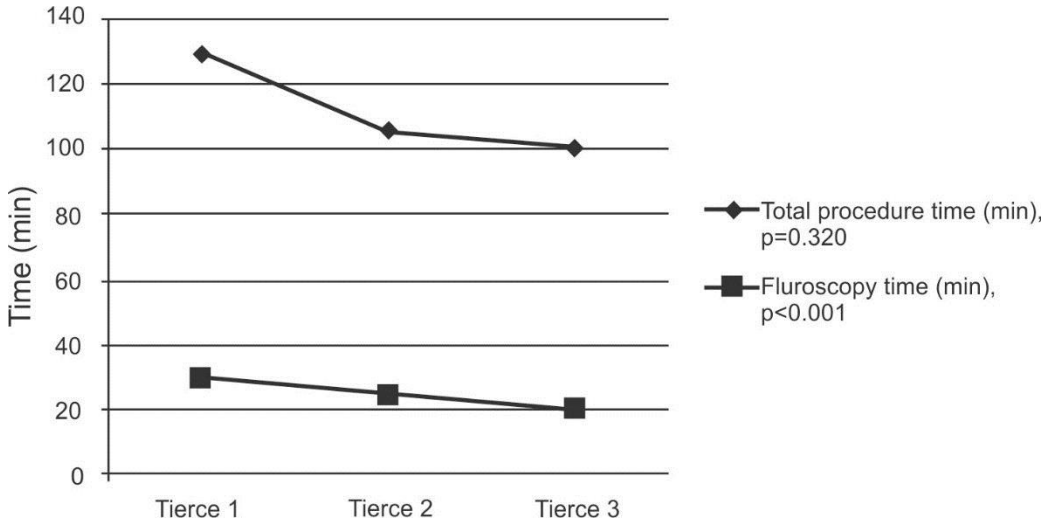

Fig. 18 - Procedure and fluoroscopy times in each Tierce

Pericardial tamponade requiring percutaneous subxiphoid drainage occurred in the 104th consecutive patient (Tierce 3) as the only significant procedural complication.

Atrial arrhythmia-free survival rates without AAD at 12 months postablation were 68%, 75%, and 70.75% in Tierce 1, Tierce 2 and Tierce 3, respectively (p=0.772). On Cox proportional hazard analysis which included clinical and procedural variables no significant predictor of arrhythmia recurrence was demonstrated.

Conclusions: A learning curve effect was demonstrated in fluoroscopy times and in the number of RF applications but not in the acute success and in the long-term arrhythmia-free survival with circular multipolar RF ablations.

Concerns have been raised about the safety of this system after detection of increased incidence of silent cerebral embolism using this phased RF ablation device in comparison with irrigated tip RF ablation or cryoablation has been reported. [103,104]

These aspects were analyzed and published in the paper below.

Transcranial measurement of cerebral microembolic signals during pulmonary vein isolation: a comparison of two ablation techniques. *Nagy-Baló E., Țînț D., Clemens M., Beke I., Kovács K.R., Csiba L., Édes I., Csanádi Z. Circ Arrhythm Electrophysiol. 2013; 3:473-480.*

Manifest stroke and transient ischemic attacks are among the most fearful adverse events during transcatheter ablation for atrial fibrillation, with an occurrence rate of around 1%. [105]

Although the clinical relevance of these lesions is unknown, they raise new concerns regarding the safety of AF ablation. Importantly, a significant correlation has been demonstrated between the incidence of these cerebral lesions and the ablation technology used: circular multipolar phased RF ablation has consistently been associated with the highest incidence of new lesion formation, in up to 37% of the procedures, whereas cryoenergy seems to be the safest technique. [106]

The aims of the present study were, therefore, to investigate cerebral microembolization during different stages of LA ablation with a cryoballoon (CB) versus multipolar phased RF technology and the pulmonary vein ablation catheter (PVAC), and also the ratio of solid/gaseous emboli and the influence of different anticoagulation strategies on the microembolic signal (MES) burden.

Methods: Thirty-four consecutive patients undergoing PVI for symptomatic paroxysmal or persistent AF not adequately controlled by more than one antiarrhythmic drug were eligible for inclusion in the study. After inclusion, patients were randomized into 3 different treatment groups:

- PVI with a CB catheter and the intraoperative administration of heparin to reach a minimum activated clotting time (ACT) level of 250 seconds according to the Venice Chart Consensus Protocol on Atrial Fibrillation Ablation (CRYO group);
- PVI with the PVAC and also the conventional intraoperative anticoagulation protocol (ACT>250 seconds; PVAC group); and
- PVI performed with the PVAC using an anticoagulation protocol with an ACT target of >320 seconds (PVAC high-ACT group).

Results:

Patient Characteristics

A total of 34 patients who participated in 35 procedures were enrolled in this study. There were no significant differences in the baseline characteristics of the patients in the 3 treatment groups

Procedural Data

The total procedure time, the total fluoro time, and the total energy delivery time were significantly longer in the CRYO group than in the PVAC groups. The mean ACT values also differed significantly in the 3 treatment groups according to the predefined protocol. No procedure-related complication was encountered in any patient.

Microembolic signals count

Because bilateral insonation of the middle cerebral arteries could not be achieved in all patients for technical reasons, MES counts were based on transcranial Doppler (TCD) recording of 16, 22, and 23 arteries in the CRYO, PVAC, and PVAC high-ACT treatment groups, respectively. Cerebral MES count was significantly lower in the CRYO group as compared with the 2 PVAC groups ($p = 0.0005$). No statistical difference was found between the 2 PVAC groups at this sample size ($p = 0.1419$).

With respect of the ratios of gaseous versus solid MESs detected in the 3 treatment groups, less than 20% of all microemboli were categorized as solid in all 3 groups. This ratio was constant across the different stages of the procedure.

Two ablation parameters potentially influencing the MES counts during the PVAC procedures were also analyzed:

- RF delivery with different bipolar/unipolar ratios, and
- the use versus the avoidance of simultaneous energy delivery on PVAC pairs 1 and 5.

The latter parameter was a significant predictor for a higher MES count in the normal-ACT PVAC group ($p = 0.036$), but the bipolar/unipolar ratio did not influence MES formation.

Correlation Between ICE and MES Count

A significant correlation was found between bubble formation on ICE and the generation of MESs in all 3 groups during the procedure ($P < 0.001$). The average number of MESs/minute corresponding to the different grades of bubble formation on the semiquantitative ICE scale. Bubble formation on ICE required a critical amount of MESs and was not sensitive to detect infrequent microembolization.

Conclusions: Cerebral microembolization was assessed by using TCD to compare AF ablation with a CB catheter versus PVAC and phased RF. In line with the results of previous MRI studies on the incidence of silent cerebral lesions, phased RF ablation was associated with significantly more MESs than CB ablation. Use of higher ACT target for intraoperative heparinization during

PVAC ablation resulted in a trend to a lower MES count that did not reach statistical significance at the size of our patient cohort. Although the occurrence of MESs exhibited an even distribution during CB ablation, it was concentrated during RF delivery with phased RF technology. The majority of MESs were gaseous, regardless of the ablation technique and the phase of the procedure.

2.4. PROFESSIONAL AND ACADEMIC ACHIEVEMENTS

2.4.1. Career overview

My entire professional and academic development is presented in the Curriculum Vitae included in Section IV. However, I considered useful to insert here a very brief synthesis of my career evolution in order to facilitate the interpretation of the scientific achievements presented in detail in Section I and their correlation with future research directions mentioned below. Thus, the most representative landmarks of my activity include:

- Fully trained (senior MD) in two medical specialties – Cardiology and Internal Medicine;
- Professional training in prestigious international centers: 2006 – Beer-Sheva, Israel Training in interventional cardiology and 2009-2011 Debrecen, Hungary – Training in electrophysiology
- Progressive development of the academic career up to the level of Associated Professor in the Department of Medical and Surgical Specialties of Faculty of Medicine, "Transilvania" University in Brasov
- PhD thesis in the field of Cardiology – prognosis of acute myocardial infarction;
- Fifty-seven papers since PhD thesis presentation, 37 of them in extenso, 14 of them published in ISI journals;
- Three books written in the field of Internal Medicine and Cardiology between 2005 and 2016, 8 book chapters of them as first/ single author and co-editor in two books;
- Sixty-six citations (h-index of 5);
- Three research grants, one of them won by international competition (scientific coordinator) and 2 won by national competition;
- One professional two-year formative grant won by international competition of European Society of Cardiology

- Principal investigator in 4 international and 2 national research trials and research team member in 5 international trials
- As a recognition of the academic activity, appointed member of the Council Staff between 2008-2011 and member in different commissions of the "Transilvania" University and national Universities as a referent for doctoral Thesis or referent for the files of candidates for certain professional positions contests in universities
- Member of the Romanian Society of Cardiology Board since 2014
- The President of the Acute Cardiac Care Working Group of the Romanian Society of Cardiology since 2014
- Fellow of the European Society of Cardiology – awarded in 2010 as a professional and academic activity recognition

2.4.2. PhD thesis and projects

2.4.2.1 PhD thesis

Title: „Long term prognosis in patients with acute myocardial infarction treated with fibrinolytic therapy"

Supervisor: Prof.dr. Cotoi Simion, University of Medicine and Pharmacy Târgu Mureș, Romania

Presentation: 16.12.2004

Confirmation 25.04.2005

Based on my previous research in the field of acute coronary syndromes, materialized by 8 papers published in cardiology journals and 7 oral presentations as first author or coauthor, communicated at National Conferences I choose to continue my research activity by studying the prognosis of myocardial infarction.

The aims of my doctoral thesis were:

1. Identification of risk factors for cardiovascular events post-infarction (death, post-myocardial angina and re-infarctization and heart failure)

2. The correlation of the identified factors with the occurrence of these cardiovascular events tracked for a period of two years follow-up in patients with acute myocardial infarction treated with fibrinolytic therapy.

3. Creating an experimental predictive model based on identified risk factors and evaluating the risk of death at to two years

Group definition and research methodology:

I realized a prospective study in 119 patients with STEMI myocardial infarction admitted in the Cardiology Department of Emergency Clinical County Hospital Brasov in between 01 January 2000 and 31 December 2001.

The patients were monitored for the evolution of post myocardial infarction cardiovascular events: angina post- infarction, re-infarction, arrhythmias, heart failure and death for a period of 2 years.

The clinical and non-clinical assessment at admission was done by a consultant in cardiology and the follow-up period was done mostly by me.

I studied the probability of certain post myocardial infarction cardiovascular events occurrence in relation with some demographic factors, mostly known in the literature, but I also tried to identify new factors pertaining to the associated pathology (peripheric artery disease) laboratory determinations (serum level of alpha hydroxy-butyrate dehydrogenase). These new factors have not been studied at that time in relation with the outcome of patients with acute myocardial infarction treated with fibrinolytics.

I considered useful to assess the probability of evolution with the heart failure in order to select a group of patients at high risk of death requiring sustained treatment from the very early, asymptomatic stages. In the same manner, the appreciation of the likelihood of reinfarctization

and post-infarction angina occurrence, selects another group of patients who could benefit from alternative therapy revascularization (surgical or interventional).

The conclusions of the PhD thesis were:

1. The main predictors of risk of death over a period of two years follow-up are: female gender, age over 65 years, plasmatic level of cholesterol over 250 mg/dl, inflammatory status (seric detection of C Reactive Protein), low blood pressure at admission (below 100 mmHg), increased heart rate at admission (over 100 beats/minute) and left ventricle systolic dysfunction (LVEF < 40%).

2. In our study, the most important predictor of death for a period of two years post- infarction was decreased systolic blood pressure at admission.

3. The administration of thrombolytic treatment was associated with significant decrease of 30 days cardiovascular mortality, without influence on post infarction angina and heart failure.

4. The presence of the symptomatic peripheral arterial disease in patients with acute myocardial infarction treated with thrombolytic therapy is associated with an increased risk of death and outcome with post-infarction heart failure.

5. The plasma level of alpha- hydroxybutyrate dehydrogenase measured at 72 hours of the onset of acute myocardial infarction allows the assessment of probability of developing heart failure in patients with acute myocardial infarction treated with thrombolythic therapy and can be used to estimate the size of myocardial necrosis area.

6. The occurrence of post-acute myocardial infarction angina is in relation to the presence of C reactive protein and increased levels of the fibrinogen and cholesterol, but the power of these predictive factors are limited to appearance of angina in the first three months post myocardial infarction.

7. In patients with acute myocardial infarction treated with fibrinolytic therapy, there is a strong correlation between the occurrence of post-infarction angina and the evolution with heart failure.

2.4.2.2 Projects/grants

1) Competition 2000

***Title*:** Chlamydia pneumoniae infection as a risk factor in acute coronary syndromes, stroke and peripheric atherotrombotic occlusions

Contract number: Grant CNCSIS nr. 256, contract nr. 3993/14.06.2000

***Period*:** 2000-2003

Project Director: Prof. Dr. Mariana Rădoi

Value: 250000 Lei

Concomitant with my PhD thesis I worked as a team member in a research project designed to study the outcome of patients with unstable angina, with emphasis on the inflammatory phenomenon and the highlighting of the role of the Chlamydia Pneumoniae infection at the level of the atheromatous plaque.

As a research team member I made research aiming the benefit of treatment with rowamicyne in patients with acute coronary syndromes and even in research focused on cellular activation: CD14 receptor assessed by immunohistochemistry and the analysis of serum antibodies level and the antioxidant status in acute coronary syndromes.

The research results were communicated in international conferences – National Congress of Pharmacotherapy in Amsterdam and in national cardiology conferences.

2) Competition 2007 Romanian Federation „Sport for everybody"

***Title:* "Cardiovascular pathology evaluation and supervision in young athletes. Risk of sudden death prevention Protocol"**

***Contract number*:** 236/15.07.2008

Value 15000 Lei

Project Director: Dr. Diana Țînț

Data from literature highlight the existence of an increased risk of sudden death in athletes, by configuring the main pathologies that lie at the origin of this dramatic event.

This project has proposed to drawn up a protocol for assessing the health of young people involved in various sporting activities such that to be done in a simple way, as complete as possible and kept within the optimal cost- efficiency.

The project consisted in two parts:

- The first part – a retrospective analysis of the health status and the incidence of the cardiovascular disease in young athletes in Brasov County.
- The second part - involves a prospective analysis.

The results were communicated in international and national conferences.

3) International research project – North Carolina Institute competition

„The role of α- linolenic acid supplementation in the modulation of epigenetic profile in subjects with metabolic syndrome"

***Contract number*: 3203/16.03.2009**

***Period*: 2010-2012**

***Project Director*:** Mihai D. Niculescu, MD, PhD, Research Assistant Professor UNC Nutrition Research Institute, Nutrition Department

***Study coordinator and Responsible for the "Transilvania"University Romanian center*:** Associate Prof. Diana Țînț, MD, PhD

Total project value 85000$, **Value for UTBv** 18000 $

The aim of this application was to **establish a new proof of concept** regarding the role that α-linolenic acid (an ω-3 fatty acid of plant origin) may have in the epigenetic regulation of gene expression in human lymphocytes.

Our hypothesis was that α-linolenic acid supplementation alters the epigenetic profile and, consequently, the gene expression profile in lymphocytes from subjects with metabolic syndrome.

Since the mid-seventies, the prevalence of overweight and obesity within the U.S. population has increased sharply for both adults and children. Overweight and obesity constitute well-defined risk factors for the onset of metabolic syndrome, which in 2000 reached an age-adjusted prevalence of 27% within the same population. The metabolic syndrome is a well-recognized precursor of type-2 diabetes, hypertension, and atherosclerosis. Several studies indicated that genes involved in insulin resistance, atherosclerosis, and the accompanying inflammatory syndrome are epigenetically regulated through changes in their DNA methylation, establishing a new paradigm regarding the role that dietary inputs may have upon the long-term regulation of metabolic syndrome.

The aim of this project was to determine the consequences that supplementation with α-linolenic acid (an essential ω-3 fatty acid of plant origin) may have upon the epigenetic regulation of gene expression in lymphocytes from human subjects with metabolic syndrome.

This hypothesis is based on published data indicating that α-linolenic acid alters the activation of mitogen-activated protein kinases (MAPK) pathways which, in turn, regulate the gene expression of DNA methyltransferases involved in the epigenetic regulation of gene expression.

The proposed study was a double-blind, placebo-controlled, randomized intervention trial, designed to determine if supplementation with alpha-linolenic acid for 3 months alters the epigenetic profile in subjects with metabolic syndrome, with the following specific aims:

1. To determine comprehensive changes in the DNA methylation profile of human lymphocytes;

2. To determine comprehensive changes in the gene expression profile of human lymphocytes which are correlated to changes in gene-specific DNA methylation;

3. To determine secondary clinical and biochemical outcomes related to changes in the phenotypic presentation of the metabolic syndrome during the intervention.

These aims were accomplished using CpG island arrays and expression microarrays for DNA methylation and gene expression profiling, respectively, and by clinical and biochemical measurements (including plasma fatty acid composition, blood cell counts, fasting plasma lipid profile, plasma inflammation markers, and coagulation and platelet aggregation markers).

This approach was very novel and allowed the first comprehensive characterization of the epigenetic and gene expression changes induced by α-linolenic acid in subjects with metabolic syndrome, along with potential changes in the accompanying clinical and metabolic variables.

Scientific significance

Although an adequate Intake level has been established for ALA, no Estimated Average Requirement on which to base a Recommended Dietary Allowance had been established. [107]

At the present time, it is still not known whether dietary ALA deficiency exists within the human population. However, beginning with the last decade, low linolenic oils were developed to increase the stability of canola oil, with the aim of reducing or eliminating the requirement for hydrogenation. Low linolenic canola oil has improved stability under storage conditions. [108]

Because ALA is found in negligible amounts in fish oil, it is conceivable that the only significant source of ALA in human nutrition is of plant origin. The impact of the drastic reduction of ALA in vegetable oils, upon the overall health, has not been yet evaluated.

In this light, the importance of ALA supplementation has to be established, along with the specific impact that ALA may have in various physiological and pathological conditions.

This proposal aimed to establish a new proof of concept on the relationship between ALA supplementation and the epigenetic regulation of the metabolic syndrome. The study was designed to characterize the epigenetic changes induced in lymphocytes from subjects with metabolic syndrome, correlated with concomitant gene expression changes, along with the evolution of clinical and biochemical markers. If successful, the study will allow us to determine the exact role that ALA plays in improving the quality of life for individuals with metabolic syndrome. It will also provide a broad conceptual frame (combined epigenomics and genomics analysis) that could be applied to investigating other nutrients or combination of nutrients.

Study sites

The proposal involves collaboration between the University of North Carolina at Chapel Hill (UNC-CH), and the School of Medicine within the "Transilvania" University, Brasov, Romania (UTB). The study was coordinated at UNC-CH, with UTB acting as subcontractor.

UTB responsibilities were: Subject enrollment, clinical intervention, and the clinical and biochemical measurements (except for plasma fatty acids determinations) will all take place at UTB (on an outpatient basis, at the UTB affiliated Cardiology Clinic within the Emergency Hospital, Brasov, Romania). Additionally, lymphocytes and plasma will be collected, processed, and shipped to UNC-CH for further determinations.

UNC-CH responsibilities: The study involving the epigenetic modifications and gene expression profiling will be performed at UNC, along with the statistical interpretation of all data. Additionally, plasma fatty acid composition will be performed at this site.

Study results were communicated in two international conferences and published as indexed abstracts in two prestigious journals. The publications gathered 7 citations.

4) Other projects

My research experience was enriched by participating in many national and international research projects as a research team member as well as a principal investigator. The participation in such research projects is depicted below:

- *Principal investigator in international research trials:*

  POET-COPD: Effect of inhalation of thiotropium once daily 18 mcg versus salmeterol twice daily 50 mg on time to first exacerbation in COPD patients (a randomized, double blind, double dummy, parallel group, one-year study) Coordinator: C. Volgemeier; Study number 205309/NCT00563381; period: 2008-2010.

  JUPITER: Rosuvastatin to prevent Vascular Events in Men and Women with Elevated C-Reactive Protein. Coordinator: P.M. Ridker; Study number D3560L0030; period: 2006-2007.

  DELPHI study: A twelve week, multicenter, double blind, randomized, parallel group, clinical study to assess the antihypertensive efficacy of Delapril 15 mg bid vs 30 mg bid versus Lisinopril and placebo. Coordinator A. Ficci; Study number CMA-0601-PR-0004; ENDURACT Nr. 2006-001823 period: 2007-2008;

  Efficacy and safety of the combination zofenopril + hidrochlorotiazide vs. irbesartan + hidrochlorotiazide in patients with arterial hypertension uncontrolled by monotherapy. Coordinator E. Agabiti-Rossei; Study number Men/09/Zof-IPE/001; period:2009-2012.

- *Principal investigator in national research trials:*

  PREFER – Prestarium new formulation, antihypertensive efficacy. Coordinator D.D. Ionescu, Study number EduraCT: 200-003140-30; period: 2007

TRUE – Trimetazidine MR, Unique Efficacy in stable Angina, Coordinator I. Brucker; Study number ICE-6790-42-ROM; period: 2006-2007.

- *Investigator in international research trials:*

EVEREST – Multicenter randomized, double-blind, placebo-controlled study to evaluate the long term of efficacy and safety of oral Tolvaptan tablets in subjects hospitalized with worsening congestive heart failure; Coordinator M. Konstarm, M. Gheorghiade; Study number 156-03-236; period: 2003-2006.

ROCKET AF – A prospective, randomized, double blind, double dummy, parallel group, multicenter, event-driven, non-inferiority study comparing the efficacy and safety of once-daily oral rivaroxaban (BAY 59-739) with adjusted dose oral warfarin for the prevention of stroke and non-central nervous system systemic embolism in subjects with non-valvular atrial fibrillation. Coordinator: M.R. Patel; Study number NCT00403767; period: 2006-2010.

PLATO – Ticagrelor versus Clopidogrel in Patients with Acute Coronary Syndromes; Coordinator L. Valentin; Study number D5130C05262; period: 2007-2009.

RLX. CHF.003 - (Pre-RELAX AHF/RELAX AHF); Serelaxin, recombinant human relaxin-2, for treatment of acute heart failure. Coordinator J.R. Teerlink; Study number BI 502.550; period: 2009-2013.

SMILE IV – Comparison between Zofenopril and Ramipril in combination with ASA on the extent of cardiovascular risk patients with systolic left ventricular dysfunction after acute myocardial infarction. Coordinator C. Borghi; Study number: MEN/03 ZOF-CHF/001; ENDURACT N. 2004-00115088; period 2006-2009.

2.4.3. Academic activity

I will refer below to the most important achievements in the field of teaching activities, dissertations coordinated, books published, as well as my attendance in different committees and commissions of the Faculty of Medicine, "Transilvania" University, Brasov and in other National Universities.

Between 2005 and 2015 I delivered lectures and practical lessons in the field of internal medicine, mostly cardiology to the medical students of 4th and 5th year of the Faculty of Medicine as well as to the students attending the 1st and 2nd year at the General Nursing specialization and Physical-Kinetic-Therapy. I was also lecturer in the master program of Palliative Care Strategies.

I was also involved in the professional continuous formation of the general practitioners in Brasov and Covasna County, and I was and lecturer in 5 Courses organized by the Faculty of Medicine and Course Director in 4 courses.

Starting with the year 2009, I delivered lectures in National Professional Courses organized under the aegis of the Romanian Society of Cardiology. I was the Course Director of 3 such national courses organized between 2014-2016.

Based on the theoretical and practical experience accumulated, between 2000 and 2015, I have supervised 15 dissertations for the medicine students, all of them in the field of Internal Medicine, most of them in the field of Cardiology. Along each thesis I tried to teach students how to read and interpret medical literature, how to perform a literature review (how to select good articles, how to extract useful information and then how to build a proper review) how to design a clinical study (which information should be extracted from every file, how to organize them, how to do simple statistical interpretations, how to correlate the results and how to formulate conclusions), how to compare their own results with the results of other research published in the literature. I tried to teach the students how to do a references list and insert the references in their work and how to design and present their work (Power Point presentation). All the theses passed the final evaluation with maximum mark. We have 3 studies presented in international conferences and one study published in an ISI indexed journal.

My academic activity also includes writing books. I was author/co-author in 4 books, co-editor in 2 books and I wrote 8 chapters as a single author/co-author in books published in prestigious Romanian publishers.

Of particular interest are the two books that I have also contributed to editing. The first one called "*Practical Guide for cardiovascular emergencies*" represents an up-date of the emergency protocols to be applied in the field of cardiology and also summarize our medical experience as clinicians. The book was written into a very practical format and is very easy to use by any doctor working in a field of emergency cardiology.

The second one "*Pulmonary embolism – a contemporary approach*" summarizes the new tools of diagnostic and the newest strategies of treatment, but add the personal clinical experience of the authors, refers to certain particular patient's categories and further perspectives concerning the diagnostic, treatment and patient education.

Concerning my attendance in different commissions of the University, I have to mention that I attended the commission for the entrance exam for graduate students every year. I have also been a member of many promotion and doctoral commissions in our own faculty and in other universities, as well as member of commissions for specialist or senior degree contest in Internal Medicine. Moreover, I have taught postgraduate lectures and supervised the practical training of residents in the field of internal medicine and cardiology.

Starting 2013 I was appointed as a Coordinator of Family Medicine Residents in our center. I have been appointed member of the board of professors in our university between 2008-2011. For all these jobs I have accomplished my mission as good as I could, trying to get the maximum benefit for all the parts involved and to encourage people to develop their potential.

2.4.4. International recognition

- In 2010 I was awarded as **Felow of the European Society of Cardiology**. This title is based on professional scientific merit and testifies one's commitment and achievement within the world of Cardiology.
- As the President of the Working Group of the Romanian Society of Cardiology, I represented Romania at the ACCA Committee on National Cardiac Societies relation. 30

august in London during the European Society of Cardiology Congress and in ACCA Summit held in 17 October 2015 in Vienna during the Acute Cardiovascular Care Congress

- My publications gathered 66 citations and a h-index of 5 in Google Scholar database and a h-index of 4 in ISI Thomson Reuters database – as depicted below (Fig. 19)

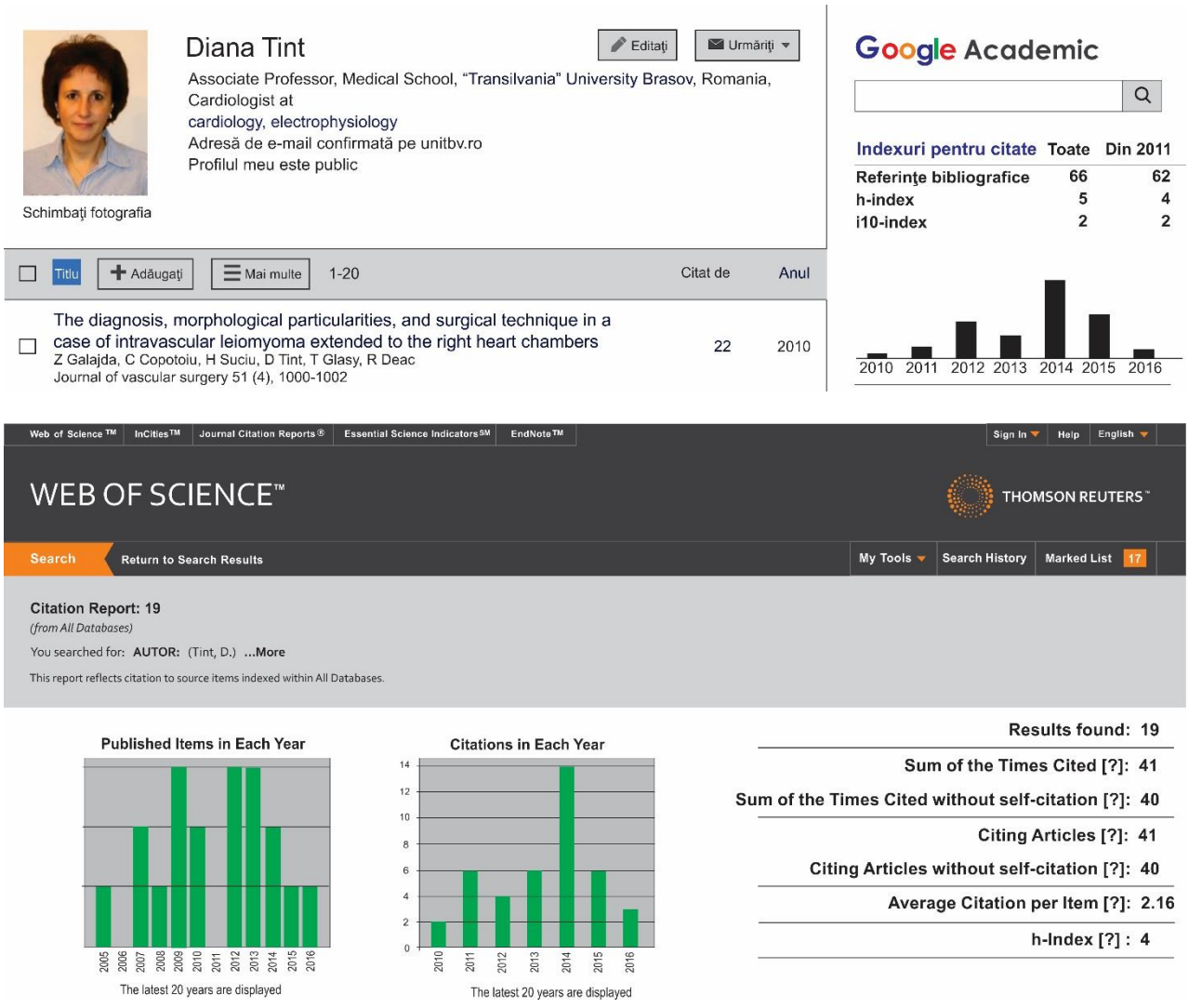

Fig. 19 – Citations and H-index top: Google Scholar database, bottom: ISI Thomson Reuters database

2.5. CONCLUSIONS

Along my entire academic career, the scientific research activity has been centered on two important directions.

The first one was directed to study myocardial ischemia, mainly the study of myocardial infarction, but also aimed to study the mechanisms and risk factors of atherosclerosis. The research in this area began before the doctoral thesis, continued with doctoral thesis and after it, with the broadning of the research area with the invasive treatment of ischemic myocardial disease and an active participation in the one of the largest myocardial infarction registries in Europe – mainly the ROmanian Registry of ST segment elevation MYocardial Infarction (RO-STEMI).

My research in the field of myocardial ischemia have resulted in many papers published in prestigious Romanian and international scientific journals and many participations in national and international conferences with the aim to disseminate the results of my research.

The assessment of the death risk after two years post an acute myocardial infarction was the subject of my doctoral thesis.

I have also been involved in one national research grant, one international research grant and few international clinical trials concerning the atherosclerosis and acute coronary syndromes.

The second research direction is dedicated to the interventional therapy of atrial fibrillation. This area of research was directly linked with my European Heart Rhythm Award of “Two-year basic electrophysiology with emphasis on ablation therapy” (international competition).

During this two-year period I was actively involved together with the research team in Cardiology Department, Debrecen University in Hungary.

The research focus was mainly on the ablative therapy of the atrial fibrillation. On this topic we have a number of papers published in prestigious international journals.

I have also participated as team member in two international trials focused on atrial fibrillation.

3. SCIENTIFIC, PROFESSIONAL AND ACADEMIC FURTHER DEVELOPEMENT PLANS

3.1. SCIENTIFIC FURTHER DEVELOPEMENT PLANS

3.1.1 Autonomic changes and endothelial function in patients with ischemic heart disease with and without revascularization. the role of preconditioning in patients undergoing cardiac surgery.

Research background

Endothelial dysfunction is a new pathway in cardiovascular disease (CVD) development and represents a new measure that is broadening our understanding of this field. Endothelial cells play an important role in maintaining the structural and functional integrity of the vasculature. In addition to regulating vessel tone, endothelial cells help to prevent the build-up of lipids and platelets that initiate the atherosclerotic process.

Endothelial dysfunction is believed to be one of the earliest stages of atherosclerosis and can be observed even in healthy people with risk factors for heart disease since it can be evidenced in people with risk factors for CVD even in the absence of overt CVD. [109]

Endothelial dysfunction is a pathological condition characterized by an imbalance between endothelial-derived relaxing factors (EDRFs) and endothelial-derived constricting factors (EDCFs). This imbalance is associated with changes in the synthesis, bioavailability and/or action of endothelial factors and results in reduction of endothelium-dependent vasodilatation and/or in an increased response to vasoconstrictor agonists. Additionally, endothelial dysfunction may contribute to ischemic or arrhythmic events by preventing needy cells, such as those in the heart, from receiving the blood supply and nutrients required for proper functioning. [110]

Endothelial function can be measured in the brachial artery of healthy samples using ultrasonography. Brachial artery endothelial function correlates well with the presence of

coronary artery disease (CAD). Impaired brachial vasodilatation in response to increased blood flow is evident in patients with coronary heart disease and patients with greater carotid artery intima-media thickness (IMT). Prospective studies of patients with CAD show that *impaired endothelial function predicts subsequent cardiac events.* [111]

The autonomic nervous system (ANS) may directly influence the endothelium. Elevated sympathetic nervous system (SNS) activity and suppressed parasympathetic nervous system (PNS) activity impair the ability of the ANS to regulate the cardiovascular system. Impaired ANS regulation may be linked with CVD development through impairments in endothelial function. Sympathetic hyperactivity is a common feature of cardiovascular diseases and is a relevant predictor of mortality rate in patients with heart failure. [112-114]

Prolonged ischemia and reperfusion (IR) are responsible for a cascade of reactions leading to endothelial injury characterized by a decrease in nitric oxide (NO) production. IR impairs endothelium-dependent, but not -independent, coronary vasodilation, indicating selective endothelial dysfunction.

Endothelial dysfunction may play a critical role in the pathogenesis of myocardial IR injury by setting the stage for adherence of neutrophils to the vascular endothelium, via expression of adhesion molecules by endothelial cells and the subsequent development of an inflammatory component of IR injury. Given the important vasodilator property of NO, such impairment may lead to an increased coronary vasoconstriction and an increased risk of vasospasm. Moreover, endothelial dysfunction after reperfusion may favor platelet aggregation and thus increase the risk of thrombosis. *Taken together, those observations indicate that the prevention of endothelial dysfunction/injury is an important therapeutic goal. However, little is known about the endothelial effects of most of the known anti-ischemic interventions.*

One of the most potent anti-ischemic interventions known to date is the concept of endogenic protection of ischemic myocardium described by Murry et al. and termed *preconditioning*. [115]

According to these experiments, submitting the heart to short episodes of ischemia separated by intermittent reperfusion renders the heart more resistant to prolonged ischemia and markedly limits infarct size. Preconditioning also confers protection against the severe ventricular rhythm disturbances that occur during subsequent ischemia and reperfusion. [116]

A Chinese group of researchers performed a systematic review and meta-analysis on randomized controlled clinical trials of remote ischemia preconditioning (RIPC) for the prevention of myocardial injury during cardiovascular surgery, showing a statistically significant benefit of RIPC over control treatment for reduction in biomarkers of myocardial injury in patients undergoing cardiovascular surgery. Most interesting is that remote ischemia preconditioning (RIPC) can prevent ischemic reperfusion injury of both the heart and extra-cardiac organs at the same time when patients are subjected to the open heart surgery. [117]

Most of the experimental studies of endothelial dysfunction after ischemia/reperfusion involve in vivo coronary occlusion in large species (especially dogs and pigs) followed by studies of endothelium-dependent vasorelaxation in isolated coronary arteries. [118]

These results suggest that the impaired response to acetylcholine is mostly the consequence of the absence of structurally intact endothelial cells and not that of a selective defect in NO synthetase activity, or a specific impairment of the transduction pathway linking muscarinic receptors to NO synthetase.

The observation that the reintroduction of molecular oxygen at reperfusion is required to produce post ischemic endothelial dysfunction is consistent with the view that ischemia/reperfusion injury to the endothelium may result from the generation of reactive oxygen species. This has now been clearly demonstrated by the observation that endothelial dysfunction can be attenuated or prevented by scavengers of these species.

TNF-α plays a major role in myocardial ischemic injury after IR. Heusch and Schulz observed that, in a model of coronary embolization, expression of TNF-α produced

progressive coronary constriction that exacerbated the initial magnitude of ischemia. Current evidence suggests that TNF-α participates in myocardial IR injury and cardiac allograft rejection antibody neutralization of TNF-α prevented coronary endothelial dysfunction during myocardial I/R injury. Neutralization of TNF-α reduced O2 - generation and xanthine oxidase activity during IR, and blockade of xanthine oxidase mimicked the actions of anti- TNF-α on O2 - production and endothelial function. [119, 120]

Endothelial cells (ECs) are more sensitive to IR than to ischemia alone. IR injury is associated not only with immediate vasoconstriction and decreased response to vasodilators at reperfusion but also prolonged vascular dysfunction. Remote conditioning reduces the incidence of myocardial infarction. The release of biomarkers of myocardial injury was reduced after remote conditioning. In contrast, however, there is no reduction in mortality, or length of stay in hospital or in the intensive care unit. [121]

Upon our best knowledge however, *there are no data regarding the influence of the preconditioning phenomena on endothelial function and no data regarding the correlation between autonomic dysfunction and related changes on endothelial function and this is at least in part the purpose of this research.*

On the other hand, cardiac surgery and cardiopulmonary by-pass initiate a systemic inflammatory response largely determined by blood contact with foreign surfaces and activation of complement. Persistence of the systemic inflammation may be considered potentially harmful for the cardiac surgical patient. One of the most important mechanisms that promotes inflammation in cardiac surgery also involves ischemia-reperfusion injury as a result of cross clamping. Restoration of perfusion on release of the aortic cross clamp is associated with activation of key indices of the inflammatory response. Generation of reactive oxygen species (ROS), such as hydrogen peroxide and the superoxide and hydroxyl radicals, occurs upon reperfusion following bypass, and these may be important contributors to tissue injury. Leukocytes activated during bypass may also release substantial amounts of cytotoxic ROS. [122]

The concrete objectives of this research are:

1. To assess the influence of the autonomic nervous system on endothelial function and clinical prognosis in patients with documented ischemic heart disease.
2. To assess the effect of preconditioning in patients with unstable angina and post conditioning in patients with acute myocardial infarction on endothelial aggression and inflammatory responses.
3. To assess the influence of the preconditioning in reduction of the endothelial dysfunction in patients undergoing coronary artery by-pass.
4. To evaluate the correlation between urinary neurotransmitter levels and endothelial dysfunction in patients with acute and chronic coronary syndrome.
5. To determine the correlation between **neurotransmitters**, inflammatory markers (**cytokines**), oxidative stress markers in patients with acute and chronic coronary syndrome prior and after intervention.

The impact of the research

In terms of research direction, we will be able to describe more specific the pathogenic mechanisms involved in ischemia/reperfusion injury and to open new direction to modulate the therapy targeting more specific elements from the pathogenic chain of ischemia-reperfusion injury. The relation between sympathetic activation and inflammatory response after IRI is not well characterized, and creating a specific model of I/R through remote preconditioning in patients undergoing coronary artery by-pass grafting is a new model that allow us a better understanding of this relationship.

If this hypothesis is true, by applying a simple technique we will be able to protect the myocardium during and after surgery, with better patient's outcome, reducing the health care costs (including hospitalization and post-hospitalization period) and provide a better survival rate.

3.1.2. Nanotopographic control of mesenchymal stem cell adhesion and proliferation on stent metal surfaces: towards a novel bioactive coating - STEMSTENT

Background

The percutaneous transluminal coronary angioplasty represents a well-established procedure to open up and revascularise coronary arteries with stenosis. This procedure is generally followed by the stents implantation, which is a common practice for the treatment of arterial lesions stenosis. [123, 124]

The two major causes of stent failure after implantation, stent thrombosis and in-stent restenosis represent the main reason to develop new materials used for implantable devices. [123, 125].

Bare metal stents (BMSs) were the first devices used for coronary stenting due to their favorable mechanical properties because of good expandability ratio; sufficient radial hoop strength and flexibility; negligible recoil and visibility on X-ray imaging. [126-130]

Interestingly, although these devices reduced rates of restenosis compared with balloon angioplasty, narrowing within the stented arterial segment continued to develop in 20-30% of lesions. Drug-eluting stents have been developed as an alternative to BMSs; however, issues with delayed arterial healing and late stage thrombosis have dampened their appeal. Also, the polymers used in DESs have been linked to irritation and chronic inflammation of the vessel wall at the site of the stent as well as to allergic reactions in some patients. Recent innovations to stent technology include biodegradable coated DES and bio absorbable nonmetallic stents. [131, 132]

However, both these systems present several drawbacks such as inconsistent resorption rate, early recoil post-implantation and radiopacity issues during device apposition. It is now generally believed that cell coating of the stent surface may be helpful for accelerating endothelialisation, promoting the healing of injured blood vessels, and effectively preventing in stent restenosis while retaining the structural/mechanical

advantages of metal stents. Several studies have demonstrated the reduction of restenosis after endothelial progenitor cell (EPC) delivery; however, there are drawbacks to this approach due to the inconsistencies in these studies resulting from difficulties in characterization the particular cell type. [133]

More recently, cell based therapeutic strategies have explored the use of Mesenchimal Stem Cells (MSCs) for the treatment of inflammatory, cardiovascular, and autoimmune diseases. A limited number of studies have assessed the effect of MSCs on endothelialisation and restenosis; [134] whereas at present no study has used MSCs originating from Wharton's jelly (WJ) for the treatment of in-stent restenosis (ISR). WJ-derived MSCs are non-hematopoietic progenitor cells capable of differentiating into functional cell types able to repair the diseased or injured arterial tissue. Compared to other tissue sources, WJ-derived MSCs may have a greater impact on future regenerative medicine applications because of low immunogenicity, easy donor accessibility, high proliferative capacity and greater sample homogeneity. [135]

Objectives of the project and expected results

This project aims to develop on the overall properties and biocompatibility of metal stents used in interventional cardiology by covering them with MSCs using an effective strategy for precise control and patterning of the local nanoroughness on stent surfaces, in order to mitigate ISR. A second goal is to control the mechanisms and pathways that regulate stem cell growth and differentiation on the machined surfaces and to improve the immune tolerance of the developed coating.

MSCs have significant therapeutic potential when coated on the stent surface, as they possess immunomodulatory features that make them attractive candidates for the treatment of the inflammatory response after percutaneous coronary intervention. Developing such bioactive surfaces requires the design and fabrication of materials that mimic selected properties of native extracellular matrices.

The response of MSCs to nanotopography of stent surfaces will be studied and the resulting coating will be used as a novel therapeutic approach to mitigate in-stent restenosis. Metal substrates will be subjected to controlled modification of the surface finishing using plasma treatment and chemical etching. All modified surfaces will also be separately evaluated after the deposition of a biocompatible film. The various samples will be pre and post-etching characterized in terms of mechanical-tribological and structural characteristics. MSC morphology, attachment and proliferation properties on the modified metal surfaces will be investigated with specific in vitro assays and molecular/imaging techniques. The biocompatibility of the developed biomaterial will be studied in a series of cytotoxicity, hemocompatibility and animal implantation tests. The proposed study will lead to the design of a novel biofunctional and biomimetic stent coating.

The therapeutic potential of the MSC technology will be evaluated in a murine atherosclerotic stent implantation model. The immune response at the implantation site will be dynamically monitored *in vivo*, using innovative molecular imaging techniques, and *ex vivo* through immunohistochemistry to elucidate the potential role of MSCs in mitigating the immune response that leads to ISR.

The ever growing need for more effective therapeutic approaches will be addressed through the successful transfer of knowledge and the commercial exploitation of the developed technology. This will eventually lead to the design of an innovative biofunctional and bioactive stent platform that will be utilized by clinicians to help the millions of cardiac patients who suffer from ISR complications in the EU and worldwide. Thus, the project holds the potential to generate significant revenue to benefit the local economies of the participating countries and expand the competitive edge of Europe.

The collaboration aims at supporting the development and commercial exploitation of innovative bioactive surfaces and to provide opportunities to advance biomaterial-based technologies closer to the market. Finally, the consortium will explore intellectual property

opportunities in order to benefit the local economies and expand the competitive edge of Europe, while strengthening the ties between the six partners and forming the basis for future collaboration in competitive research programs.

The project will undertake early stage pre-clinical trials in a small animal model that will allow the technology to progress in larger animals and eventually in clinical trials.

The proposed project will develop for the first time an effective nanofabrication strategy, to prime stent metal surfaces for MSC seeding, adhesion and proliferation. Existing approaches include ultrasonic atomisation spraying of the stent surface with a protein or antibody coating. Topographical sensing affects multiple cellular properties including morphology, migratory capacity, gene expression profile and eventually cell differentiation and fate.

The study marks the first time that WJ-derived MSCs will be used as therapeutic agents for ISR, and more importantly such a novel and complex process will be utilized for developing cell coated stent platforms. Recent *in vitro* and *in vivo* evidences support the usage of WJ-MSCs in tissue repair and regeneration primarily through secretion of trophic and immune regulatory factors, which aid repair, and resolution of injury. The clinical use of MSCs is preferable to induced pluripotent and embryonic stem cell therapies, as MSCs are devoid of the ethical, teratomas-formation and histocompatibility issues of the latter cell types. To date, bone marrow MSCs have been considered the gold standard in cell therapy, however, serious limitations still exist including invasive isolation procedures and higher risk of bacterial and viral contamination. The WJ-MSCs offer a more attractive alternative as they are isolated from already discarded tissue (following birth) and since they are less exposed than other adult tissues, have lower incidence of contamination with viral and bacterial antigens. The study will also take advantage of recent advances in large scale production of WJ-MSCs for clinical applications. WJ-MSCs show no signs of transformation like loss of anchorage dependence, contact inhibition and serum dependence over several passages and large quantities of the cells can be banked until use.

All these attributes make WJ-MSCs a reliable source for clinical grade expansion and seeding on the modified stent metal surfaces.

Expected impact of research

Research collaboration of academic institutions with biotech companies and healthcare providers is one of the most effective ways of transferring innovation and expertise and advancing new therapeutic technologies in the clinic. The expected impact for the consortium and each partner individually will be:

1) The development of improved biomaterial properties and stent design characteristics for novel biofunctional and bioactive implants that will deliver enhanced performance and improved cost/benefit ratio,

2) *Increased competitiveness of the involved SME (Trojantec) and medical centers (AMC, ICCO Clinics) in the European and international market.*

3) *Improved competitiveness of the European Health industry through a clear increase of the Technology Readiness Level for the proposed technologies,*

4) Improved market access through increased awareness on the part of the Research Technological Development (RTD) performers and medical partners of the regulatory protocols that must be followed before the developed implants reach the patients and 5) *Generation of a vigorous exchange between RTD performers and industrial and clinical stakeholders in the health sector.*

3.2. PROFESSIONAL AND ACADEMIC FURTHER DEVELOPEMENT PLANS

As a teacher, I will further try to remain involved as much as I can in active training of the medical students. I was involved and I will be involved in medical training of foreign students who visited our university through the Erasmus program.

Being awarded with sTANDEM certificate in English for Medical Purpose level C1 I will actively support the creation of a new Department with English teaching for foreign students.

Together with my colleagues from Department of Materials Science and Engineering we are in the process of implementing a new course concerning the implantable devices in cardiovascular pathology and I will be the course coordinator. The course will begin by the year 2017. The purpose of this course is to make the students of Department of Materials Science and Engineering familiar with the newest medical technique and to stimulate them to cooperate in order to try to develop newer or better medical devices.

I will continue to keep my all teaching materials up to date in order to connect the students to the newest information in the field of cardiology and internal medicine. I will encourage my students to participate in our Congresses and Conferences, as well as in our research projects in order to contribute to a full and comprehensive professional build-up.

I will be very involved in coordination of doctoral thesis and I will continue to support the young doctors to perform their own researches and to communicate the results of their research in the academic environment.

I will continue to focus my activity in continue postgraduate training of doctors, both by organizing and participating in continuing medical education courses and by writing books that contribute to raising the level of medical education and thus, the quality of patient care.

I also intended to remain actively involved in the structure of the Romanian Society of Cardiology and in the activity of the European Society of Cardiology, especially in the Acute Cardiac Care Working Group of the ESC. In this respect, I will try to contribute to the further development of the relationship between our University with other foreign universities in order to increase the quality of both didactic and scientific activity of our Department.

4. REFERENCES

1. Schomig A, Mehilli J, Antoniucci D, et al. *Beyond 12 h Reperfusion Alternative Evaluation (BRAVE-2) Trial Investigators. Mechanical reperfusion in patients with acute myocardial infarction presenting more than 12 hours from symptom onset: a randomized controlled trial. JAMA 2005, 293:2865–2872.*

2. *Indications for fibrinolytic therapy in suspected acute myocardial infarction: collaborative overview of early mortality and major morbidity results from all randomised trials of more than 1000 patients. Fibrinolytic Therapy Trialists' (FTT) Collaborative Group. Lancet 1994; 343:311–322.*

3. Hochman JS, Lamas GA, White H. *Thrombolytic therapy in the elderly. Lancet 2000, 356:2028–2030.*

4. Morrison LJ, Verbeek PR, McDonald AC, et al. *Mortality and prehospital thrombolysis for acute myocardial infarction: a meta-analysis. JAMA 2000; 283:2686–2692.*

5. F van de Werf, D.S. Baim. *Reperfusion for ST-Segment Elevation Myocardial Infarction an Overview of Current Treatment Options. Circulation 2002; 105:2813-2816*.

6. Keeley EC, Boura JA, Grines CL. *Primary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomized trials. Lancet. 2003; 361:13-20.*

7. Lansky A, Pietras C, Costa R, et al. *Gender differences in outcomes after primary angioplasty versus primary stenting with and without abciximab for acute myocardial infarction. Circulation. 2005; 111:1611-1618.*

8. Mandelzweig L, Battler A, Boyko V, et al. *The second Euro Heart Survey on acute coronary syndromes: characteristics, treatment, and outcome of patients with ACS in Europe and the Mediterranean Basin in 2004. Eur Heart J 2006; 27:2285–2293.*

9. G. Tatu-Chitoiu, A. Petris. *The first Romanian Registry for ST-elevation Myocardial Infarction (1997-2009) final report. Almateea publishing House 2009, pag 38.*

10. Lee KL, Woodlief LH, Topol EJ et al. for the GUSTO-1 investigators. *Predictors of 30-day mortality in the era of reperfuson for acute myocardial infarction. Results from an international trial of 41021 patients. Circulation 1995, 91: 1659-1667.*

11. Kelly DJ, Gershlick T, Witzenbichler B, et al. *Incidence and predictors of heart failure following percutaneous coronary intervention in ST-segment elevation myocardial infarction: the HORIZONS-AMI trial. Am Heart J. 2011; 162:663-670.*

12. Henkel DM, Witt BJ, Gersh BJ, et al. *Ventricular arrhythmias after acute myocardial infarction: a 20-year community study. Am Heart J. 2006; 151:806-812.*

13. Steg PG, Dabbous OH, Feldman LJ, et al. *Global Registry of Acute Coronary Events Investigators. Determinants and prognostic impact of heart failure complicating acute coronary syndromes: observations from the Global Registry of Acute Coronary Events (GRACE).* Circulation. 2004*;* 109*:*494*–499.*

14. Hasdai D, Topol EJ, Kilaru R, et al. *Frequency, patient characteristics, and outcomes of mild-to-moderate heart failure complicating ST-segment elevation acute myocardial infarction: lessons from 4 international fibrinolytic therapy trials.* Am Heart J. 2003*;* 145*:*73*–79.*

15. Kashani A, Giugliano RP, Antman EM, et al. *Severity of heart failure, treatments and outcomes after fibrinolysis in patients with ST-elevation myocardial infarction.* Eur Heart J.2004*;* 25*:*1702*–1710.*

16. Spencer FA, Meyer TE, Gore JM, Goldberg RJ. *Heterogeneity in the management and outcomes of patients with acute myocardial infarction complicated by heart*

failure: The National Registry of Myocardial Infarction. Circulation. 2002; 105:2605–2610.

17. The International Study Group. *In-hospital mortality and clinical course of 20 891 patients with suspected acute myocardial infarction randomized between alteplase and streptokinase with or without heparin. Lancet 1990; 336:71-79.*

18. Third International Study of Infarct Survival Collaborative Group. *ISIS-3: a randomized comparison of streptokinase vs. tissue plasminogen activator vs. anistreplase and aspirin plus heparin vs. aspirin alone among 41 299 cases of suspected acute myocardial infarction. Lancet 1993;339: 753-770.*

19. The GUSTO-1 Investigators. *An international randomized trial comparing four thrombolytic strategies for acute myocardial infarction. N Eng J Med 1993; 329:673-682.*

20. Sanz G, Castaner A, Bietriu A et al. *Determinants of prognosis in survivors of myocardial infarction. N Eng J Med 1982; 306:1065-1070.*

21. Moss AJ, Decamilla J, Davis H, Bayer L. *The early posthospital phase of myocardial infarction: prognostic stratification. Circulation 1976; 54:58-64.*

22. Nicolau JC, Serrano CV Jr, Garzon SA, Ramires JA. *Prognosis of acute myocardial infarction in the thrombolytic era: medical evaluation is still valuable. Eur J Heart Fail. 2001;3(5):569-576.*

23. Barbash GI, White HD, Modan M, Van de Werf F. *Antecedent angina pectoris predicts worse outcome after myocardial infarction in patients receiving thrombolytic therapy: experience gleaned from the International Tissue Plasminogen Activator/Streptokinase Mortality Trial. J Am Coll Cardiol. 1992;20(1):36-41.*

24. Witeveen SA, Hemker HC, Hollaar L, Hermens WT. *Quantitation of infarct size in man by means of plasma enzyme levels. Br Heart J 1975; 37:795-803.*

25. Paulsen SH, Host NB, Jensen SE, et al. *Quantitative analysis of plasma enzyme levels based on simultaneous determinations of different enzymes. Cardiovasc Res 1982,16:120-131.*

26. Dissman R, Linderer Th, Schroder R. *Estimation of enzymatic infarct size: direct comparison ofthe marker enzymes creatine kinase and hydroxybutyrate dehydrogenase. Am Heart J 1998,135:1-9.*

27. Thygesen K, Alpert JS, White HD. *Universal definition of myocardial infarction. Eur Heart J 2007; 28:2525–2538.*

28. Lee KL, Woodlief LH, Topol EJ et al. for the GUSTO-1 investigators. *Predictors of 30-day mortality in the era of reperfuson for acute myocardial infarction. Results from an international trial of 41021 patients. Circulation 1995; 91:1659-1667.*

29. Cotter G, Cannon CP, McCabe CH et al. *Prior peripheral arterial disease and cerebrovascular disease are independent predictors of adverse outcomes in patients with acute coronary syndromes: are we doing enough? Results from the Orbofiban in Patients with Unstable Coronary Syndromes-Thrombolysis InMyocardial Infarction (OPUS-TIMI) 16 Study. Am Heart J 2003;145: 622-627.*

30. Diehm C, Lange A, Darius H et al. *Risk factors for total and cardiovascular mortality in elderly patients – the german epidemiological trial on ankle brachial index (GETABI).*

    J Am Coll Cardiol 2004;13: 84-85.

31. Hirsch AT, Criqui MH, Treat-Jacobson D et al. *Peripheral arterial disease detection, awareness and treatment in primary care. JAMA 2001; 286:1317-1324.*

32. Hochmann JS, Wess J, Sleeper et al. *Determinants of mortality when primary left ventricular failure complicates acute myocardial infarction. Circulation 1995; 92:776-781.*

33. Herlitz J, Hjalmarson A, Waldenstrom J. *Five-year mortality rate in relation to enzyme-estimated infarct size in acute myocardial infarction. Am Heart J 1987; 114:731-737.*

34. Grande P, Christiansen C, Alstrup K. *Comparison of ASAT, CK, CK-MB, and LD for the estimation of acute myocardial size in man. Clin Chim Acta 1983;128(2-3):329-335.*

35. Willems GH, Visser M.O.. *Quantitative analysis of plasma enzyme levels based on simultaneous determinations of different enzymes. Cardiovasc Res 1982; 16:120-131.*

36. S.H. Paulsen, N.B. Host, S.E. Jensen, K. Egstrup. *Colagen scar formation after acute myocardial infarction. Relationships to infarct size, left ventricular function and coronary artery patency. Circulation 1997; 96:2565-2572.*

37. Grande P, Fischer Hansen B, Christansen C, et al. *Estimation of acute myocardial infarct size in man by serum CK-MB measurements. Circulation 1982; 65:756-764.*

38. Hollman ER, Buller VGM, De Ross A et al. *Detection and quantitation of dysfunctional myocardium by magnetic resonance imaging. Circulation 1997; 95:924-931.*

39. Califf RM, O'Neil WW, Stack RS. *Failure of simple clinical measurements to predict perfusion status after intravenous thrombolysis. An Intern Med 1998; 108:658-662.*

40. Golf SW, Temme H, Kempf KD et al. *Systemic short therm fibrinolysis with high-dose streptokinase in acute myocardial infarction: time course of biochemical parameters. J Chem Clin Biochem 1984;22(11):723-729.*

41. Dissman R, Linderer TH, Schroder R. *Estimation of enzymatic infarct size: direct comparison of the marker enzymes creatine kinase and a hydroxybutyrate dehydrogenase. Am Heart J 1998; 135:1-9.*

42. Willems JL, Willems RJ, George M et al. *Significance of initial ST segment elevation and depression for the management of thrombolytic therapy in acute myocardial infarction. Circulation 1990; 82:1147-1157.*

43. Morrow DA, Antman EM, Charlesworth A, et al. *TIMI risk score for ST-elevation myocardial infarction: A convenient, bedside, clinical score for risk assessment at presentation: An intravenous nPA for treatment of infarcting myocardium early II trial substudy. Circulation. 2000;102(17):2031-2037.*

44. De Luca G, Suryapranata H, van 't Hof AW, et al. *Prognostic assessment of patients with acute myocardial infarction treated with primary angioplasty: implications for early discharge. Circulation. 2004;109(22):2737-2743.*

45. Fox KA, Dabbous OH, Goldberg RJ, et al. *Prediction of risk of death and myocardial infarction in the six months after presentation with acute coronary syndrome: prospective multinational observational study (GRACE). BMJ 2006 ;333(7578):1091*

46. Marchioli R, Avanzini F, Barzi F, et al. *Assessment of absolute risk of death after myocardial infarction by use of multiple-risk-factor assessment equations: GISSI-Prevenzione mortality risk chart. Eur Heart J. 2001; 22:2085-2103.*

47. Barra S, Providência R, Paiva L, Caetano F, Almeida I, Gomes P, Marques AL. *ACHTUNG-Rule: a new and improved model for prognostic assessment in myocardial infarction. Eur Heart J Acute Cardiovasc Care. 2012 ec;1(4):320-36.*

48. Betty R. Kirkwood MA MSc and Jonathan A.C. Sterne MA MSc PhD, *Essential Medical Statistics, Blackwell Publishing 2005, Oxford, UK, Part E, Statistical modelling:297-393.*

49. Petra L. Graham, MS and David A. Cook, MBBS, *Prediction of Risk of Death Using 30-Day Outcome, A Practical End Point for Quality Auditing in Intensive Care, CHEST 2004; 125(4):1458-1466.*

50. Varma C., Brecker S., *Predictors of mortality in acute myocardial infarction. The Lancet 2001; 358:1473-1474.*

51. Pedrazzini G, Santoro E, Latini R, et al. *Causes of death in patients with acute myocardial infarction treated with angiotensin-converting enzyme inhibitors: findings from the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto (GISSI)-3 trial. Am Heart J 2008;155(2):388-394.*

52. Granger CB, Goldberg RJ, Dabbous O, et al. *Global Registry of Acute Coronary Events Investigators. Predictors of hospital mortality in the global registry of acute coronary events. Arch Intern Med 2003;163(19):2345-2353.*

53. *Long-term effects of intravenous thrombolysis in acute myocardial infarction: final report of the GISSI study. Gruppo Italiano per lo Studio della Streptochi-nasi nell'Infarto Miocardico (GISSI). Lancet. 1987;2(8564):871-874.*

54. *Randomized trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction:* ISIS-2. Lancet 1988; 2:349-360.

55. The GUSTO Angiographic Investigators: *The effects of tissue plasminogen activator, streptokinase, or both on coronary artery patency, ventricular function, and survival after acute myocardial infarction. N Engl J Med 1993;329: 1615-1622.*

56. The GUSTO Investigators: *An international randomized trial comparing four thrombolytic strategies for acute myocardial infarction. N Engl J Med 1993;329(10):673-682.*

57. Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto Miocardico (GISSI): GISSI-2: *A factorial randomized trial of alteplase versus streptokinase and heparin versus no heparin among 12,490 patients with acute myocardial infarction. Lancet I990;336 (8707): 65-71.*

58. ISIS-3 *(Third International Study of Infarct Survival) Collaborative Group: ISIS-3: A randomized comparison of streptokinase vs. tissue plasminogen activator vs. anistreplase and or aspirin plus heparin vs. aspirin alone among 41,299 cases of suspected myocardial infarction. Lancet 1992;339: 753-770.*

59. The TIMI Study Group: *Comparison of invasive and conservative strategies after treatment with intravenous tissue plasminogen activator in acute myocardial infarction: Results of the Thrombolysis in Myocardial Infarction (TIMI) phase II trial. N Engl J Med 1989; 320 (10):618 -627.*

60. Jha P, Deboer D, Sykora K, Naylor CD. *Outcomes of thrombolysis trial participants and nonparticipants: a population-based comparison. JACC 1996; 27:1335-1342.*

61. Zeymer U, Senges J – *Why do we need prospective registries in patients with acute myocardial infarction? Eur Heart J 2003; 24:1611–1612.*

62. Meine TJ, Al-Khatib SM, Alexander J H, et al. *Incidence, predictors, and outcomes of high-degree atrioventricular block complicating acute myocardial infarction treated with thrombolytic therapy. Am Heart J 2005;149: 670–674.*

63. Aplin M, Engstrom T, Vejlstrup NG, Clemmensen P, et al. *Prognostic importance of complete atrioventricular block complicating acute myocardial infarction (TRACE Study Group). Am J Cardiol 2003; 92:853–856.*

64. Spencer F A, Jabbour S, Lessard D, et al. *Two-decade-long trends (1975–1997) in the incidence, hospitalization, and long-term death rates associated with complete heart block complicating acute myocardial infarction: a communitywide perspective. Am Heart J 2003; 145:500–507.*

65. Giulio G, Stefanini MD, David R, Holmes MD Jr. *Drug-eluting coronaryartery stents. N Engl J Med 2013; 368: 254–265.*

66. Dangas GD, Claessen BE, Caixeta A, et al. *In-stent restenosis in the drug-eluting stent era. J Am Coll Cardiol 2010;56(23):1897–1907.*

67. Wilber D, Walton J, O'Neill W, et al. *Effects of reperfusion on complete heart block complicating anterior myocardial infarction. J Am Coll Cardiol 1984; 4:1315–1321.*

68. Sgarbossa EB, Pinski SL, Topo EJ, et al. *Acute myocardial infarction and complete bundle branch block at hospital admission: clinical characteristics and outcome in the thrombolytic era. J Am Coll Cardiol 1998; 31:105–110.*

69. Harpaz D, Behar S, Gottlieb S, et al for the SPRINT Study Group and the Israeli Thrombolytic Survey Group. *Complete atrioventricular block complicating acute myocardial infarction in the thrombolytic era. J Am Coll Cardiol 1999; 34:1721–1728.*

70. Kloner RA, Speakman M. *Erectile dysfunction and atherosclerosis. Curr Atheroscler Rep. 2002;4(5):397-401.*

71. Seftel AD, Sun P, Swindle R. *The prevalence of hypertension, hyperlipidemia, diabetes mellitus and depression in men with erectile dysfunction.* J Urol *2004; 171:2341–2345.*

72. Thompson IM, Tangen CM, Goodman PJ, et al. *Erectile dysfunction and subsequent cardiovascular disease.* JAMA *2005; 294:2996–3002.*

73. Chiurlia E, D'Amico R, Ratti C, et al. *Subclinical coronary artery atherosclerosis in patients with erectile dysfunction. J Am Coll Cardiol 2005;46(8):1503-1506.*

74. Montorsi P, Ravagnani PM, Galli S, et al. *Association between erectile dysfunction and coronary artery disease: Matching the right target with the right test in the right patient. Eur Urol. 2006;50(4):721-731.*

75. *http://www.seekwellness.com/mens_sexuality/questionnaire.htm*

76. Jackson G, Boon N, Eardley I, et al. *ED and CHD prediction: evidence-based guidance and consensus.* Int J Clin Pract *2010; 64: 848–857.*

77. Rogers JH, Karimi H, Kao J, et al. *Internal pudendal Artery Stenoses and ED: Correlation with angiographic coronary artery disease.* Cath Cardiovasc Interv *2010;76: 882–887.*

78. A Ponholzer; J Stopfer; G Bayer; et al. Is *Penile Atherosclerosis the Link Between Erectile Dysfunction and Cardiovascular Risk? An Autopsy Study. Int J Impot Res. 2012;24(4):137-140.*

79. *Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. Expert Panel on the Identification, Evaluation, and Treatment of Overweight in Adults. Am J Clin Nutr. 1998; 68:899-917.*

80. Ford ES, Giles WH, Mokdad AH. *Increasing Prevalence of the Metabolic Syndrome Among U.S. Adults. Diabetes Care. 2004; 27:2444-2449.*

81. Barre DE. *The role of consumption of alpha-linolenic, eicosapentaenoic and docosahexaenoic acids in human metabolic syndrome and type 2 diabetes—a mini-review. J Oleo Sci. 2007;56(7):319-325.*

82. Grundy SM, Brewer HB Jr, Cleeman JI, et al for the Conference Participants. *Definition of Metabolic Syndrome Report of the National Heart, Lung, and Blood Institute/American Heart Association Conference on Scientific Issues Related to Definition. Circulation. 2004; 109:433-438.*

83. Barre DE. *The role of consumption of alpha-linolenic, eicosapentaenoic and docosahexaenoic acids in human metabolic syndrome and type 2 diabetes-a mini-review. J Oleo Sci. 2007; 56:319-325.*

84. *Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106: 3143- 3421.*

85. Hotamisligil GS, Shargill NS, Spiegelman BM. *Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259: 87-91.*

86. Zhao G, Etherton TD, Martin KR, et al. *Dietaryalpha-linolenic acid inhibits proinflammatory cytokine production by peripheral blood mononuclear cells in hypercholesterolemic subjects. Am J Clin Nutr 2007; 85:385-391.*

87. Camm AJ, Kirchhof P, Lip GY, et al. *Guidelines for the management of atrial fibrillation: The Task Force for the Management of Atrial Fibrillation of the European Society of Cardiology (ESC). Europace; 12: 1360-1420.*

88. Steinwender C, Honig S, Leisch F, Hofmann R. *One-year follow-up after pulmonary vein isolation using a single mesh catheter in patients with paroxysmal atrial fibrillation. Heart Rhythm 2010; 7: 333-339.*

89. Metzner A, Chun KR, Neven K, et al. *Long-term clinical outcome following pulmonary vein isolation with high-intensity focused ultrasound balloon catheters in patients with paroxysmal atrial fibrillation. Europace; 12: 188-193.*

90. Ahmed H, Neuzil P, Skoda J, et al. *The permanency of pulmonary vein isolation using a balloon cryoablation catheter. J Cardiovasc Electrophysiol 2010; 21:731-737.*

91. Chun KR, Schmidt B, Metzner A, et al. *The 'single big cryoballoon' technique for acute pulmonary vein isolation in patients with paroxysmal atrial fibrillation: a prospective observational single centre study. Eur Heart J 2009; 30: 699-709.*

92. Klein G, Oswald H, Gardiwal A, et al. *Efficacy of pulmonary vein isolation by cryoballoon ablation in patients with paroxysmal atrial fibrillation. Heart Rhythm 2008; 5: 802-806.*

93. Kojodjojo P, O'Neill MD, Lim PB, et al. *Pulmonary venous isolation by antral ablation with a large cryoballoon for treatment of paroxysmal and persistent atrial fibrillation: medium-term outcomes and non-randomised comparison with pulmonary venous isolation by radiofrequency ablation. Heart 2010; 96: 1379-1384.*

94. Kuhne M, Suter Y, Altmann D, et al. *Cryoballoon versus radiofrequency catheter ablation of paroxysmal atrial fibrillation: biomarkers of myocardial injury,*

recurrence rates, and pulmonary vein reconnection patterns. Heart Rhythm 2010; 7:1770-1776.

95. Linhart M, Bellmann B, Mittmann-Braun E, et al. *Comparison of cryoballoon and radiofrequency ablation of pulmonary veins in 40 patients with paroxysmal atrial fibrillation: a case-control study. J Cardiovasc Electrophysiol 2009; 20: 1343-1348.*

96. Neumann T, Vogt J, Schumacher B, et al. *Circumferential pulmonary vein isolation with the cryoballoon technique results from a prospec*tive 3-center study. J Am Coll Cardiol 2008; 52: 273-278.

97. Van Belle Y, Janse P, Rivero-Ayerza MJ, et al. *Pulmonary vein isolation using an occluding cryoballoon for circumferential ablation: feasibility, complications, and short-term outcome. Eur Heart J 2007; 28: 2231-2237.*

98. Boersma LV, Wijffels MC, Oral H, et al. *Pulmonary vein isolation by duty-cycled bipolar and unipolar radiofrequency energy with a multielectrode ablation catheter. Heart Rhythm 2008; 5: 1635-1642.*

99. Duytschaever M, Anne W, Papiashvili G, et al. *Mapping and isolation of the pulmonary veins using the PVAC catheter. Pacing Clin Electrophysiol 2010;33: 168-178.*

100. Fredersdorf S, Weber S, Jilek C, Heinicke N, C VONB, Jungbauer C, Riegger GA, Hamer OW, Jeron A. *Safe and rapid isolation of pulmonary veins using a novel circular ablation catheter and duty-cycled RF generator. J Cardiovasc Electrophysiol 2009; 20: 1097-1101.*

101. Pappone C, Oral H, Santinelli V, et al. *Atrio-esophageal fistula as a complication of percutaneous transcatheter ablation of atrial fibrillation. Circulation 2004; 109:2724–2726.*

102. Scanavacca MI, Davila A, Parga J, Sosa E. *Left atrial-esophageal fistula following radiofrequency catheter ablation of atrial fibrillation. J Cardiovasc Electrophysiol 2004; 15:960–962.*

103. Gaita F, Caponi D, Pianelli M, et al. *Radiofrequency catheter ablation of atrial fibrillation: a cause of silent thromboembolism? Magnetic resonance imaging assessment of cerebral thromboembolism in patients undergoing ablation of atrial fibrillation. Circulation. 2010; 122(17):1667-1673.*

104. Gaita F, Leclercq JF, Schumacher B, et al. *Incidence of silent cerebral thromboembolic lesions after atrial fibrillation ablation may change according to technology used: comparison of irrigated radiofrequency, multipolar nonirrigated catheter and cryoballoon. J Cardiovasc Electrophysiol. 2011; 22(9):961-968.*

105. Kok LC, Mangrum JM, Haines DE, Mounsey JP. *Cerebrovascular complication associated with pulmonary vein ablation. J Cardiovasc Electrophysiol. 2002; 13:764–767.*

106. Gaita F, Leclercq JF, Schumacher B, et al. *Incidence of silent cerebral thromboembolic lesions after atrial fibrillation ablation may change according to technology used: comparison of irrigated radiofrequency, multipolar nonirrigated catheter and cryoballoon. J Cardiovasc Electrophysiol. 2011; 22:961–968*.

107. Trumbo P, Schlicker S, Yates AA, Poos M. *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J Am Diet Assoc. 2002; 102:1621-1630.*

108. Flickinger BD. *Utilizing biotechnology in producing fats and oils with various nutritional properties. J AOAC Int. 2007; 90:1465-1469*.

109. Hadi HA, Carr CS, Al Suwaidi J. *Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. Vasc Health Risk Manag. 2005;1(3):183-198.*

110. Bernatova I. *Endothelial dysfunction in experimental models of arterial hypertension: cause or consequence? Biomed Res Int. 2014:598271. doi:10.1155/2014/598271.*

111. Corretti MC, Anderson TJ, Benjamin EJ, et al. *International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39(2):257-265.*

112. Thijssen DJ, de Groot P, Kooijman M. *Sympathetic nervous system contributes to the age-related impairment of flow-mediated dilation of the superficial femoral artery. Am J Physiol Heart Circ Physiol 2006;291: H3122–H3129.*

113. Marc DT, Joseph W, Ailts JW, et al. *Neurotransmitters excreted in the urine as biomarkers of nervous system activity: Validity and clinical applicability. Neuroscience and Biobehavioral Reviews 2011; 35:635–644.*

114. Kaplon R.E., Walker A.E, Seals D.R. *Plasma norepinephrine is an independent predictor of vascular endothelial function with aging in healthy women. J Appl Physiol 2011; 111:1416–1421.*

115. Murry CE, Jennings RB, Reimer KA. *Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986;74(5):1124-1136.*

116. Brevoord D, Kranke P, Kuijpers M, *Remote ischemic conditioning to protect against ischemia-reperfusion injury: a systematic review and meta-analysis. PLoS One. 2012;7(7):e42179.*

117. Xiong J, Liao X, Xue FS, et al. *Remote ischemia conditioning-an endogenous cardioprotective strategy from outside the heart. Chin Med J 2011;124(14):2209-2215.*

118. Duda M, Czarnowska E., Kurzeewski M. *Ischemic preconditioning prevents endothelial dysfunction, P-selectin expression, and Neutrophil adhesion by preventing endothelin and O2 - generation in the post-ischemic guinea-pig heart. Journal of physiology and pharmacology 2006;57(4):553-569.*

119. Laude K, Thuillez C, Richard V. *Coronary endothelial dysfunction after ischemia and reperfusion: a new therapeutic target? Brazilian Journal of Medical and Biological Research 2001; 34:1-7.*

120. Zhang C, Xu X, Potter BJ.TNF- *Contributes to Endothelial Dysfunction in Ischemia/Reperfusion Injury Arterioscler Thromb Vasc Biol. 2006;26:475-480.*

121. Bøtker HE, Kharbanda R, Schmidt MR, et al. *Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. Lancet 2010;375(9716):727-734.*

122. Raja S.G., Dreyfus G.D. *Modulation of systemic inflammatory response after cardiac surgery. Asian Cardiovasc Thorac Ann 2005; 13:382-395.*

123. M. Schillinger, E. Minar, *Percutaneous Treatment of Peripheral Artery Disease Novel Techniques, Circulation, 2012;126:2433-2440.*

124. Byrne RA, Joner M, Kastrati A. *Stent thrombosis and restenosis: what have we learned and where are we going? The Andreas Grüntzig Lecture ESC 2014. Eur Heart J 2015;36(47):3320-3331.*

125. Garg P, Cohen DJ, Gaziano T, Mauri L. *Balancing the risks of restenosis and stent thrombosis in bare-metal versus drug-eluting stents: results of a decision analytic model. J Am Coll Cardiol. 2008;51(19):1844-1853.*

126. Kapnisis K, Constantinides G, Georgiou H, et al. *Multi-scale mechanical investigation of stainless steel and cobalt-chromium stents. J Mech Behav Biomed Mater. 2014; 40:240-251.*

127. Kapnisis K, Halwani DO, Brott BC, et al. *Stent overlapping and geometric curvature influence the structural integrity and surface characteristics of coronary nitinol stents Journal of the Mechanical Behaviour of Biomedical Materials. 2013; 20:227–236.*

128. Tammareddi S, Sun G, Li Q. *Multiobjective robust optimization of coronary stents, Materials and Design 2016;90:682–692.*

129. Yuan Y, Jin X, Fan Z, et.al. *In vivo degradation of copolymers prepared from L-lactide, 1,3-trimethylene carbonate and glycolide as coronary stent materials Journal of Science Materials in Medicine, 2015;26:139:1-9.*

130. Jorge C, Dubois C.. *Clinical utility of platinum chromium bare-metal stents in coronary heart disease, Medical Devices: Evidence and Research 2015:8;359-367.*

131. Abizaid A, Costa R. *New Drug-Eluting Stents an Overview on Biodegradable and Polymer-Free Next-Generation Stent Systems, Circ Cardiovasc Interv. 2010; 3:384-393.*

132. Kang SH, Park KW, Kang DY, et al. *Biodegradable-polymer drug-eluting stents vs. bare metal stents vs. durable-polymer drug-eluting stents: a systematic review and Bayesian approach network meta-analysis. Eur Heart J 2014;35(17):1147-1158.*

133. Wojakowski W., Pyrlik A., Król M. et al. *Circulating endothelial progenitor cells are inversely correlated with in-stent restenosis in patients with non-ST-segment elevation acute coronary syndromes treated with EPC-capture stents. Minerva Cardioangiologica 2013; 61:301-311.*

134. Taggart DP, Kaul S, Boden WE, et al. *Revascularization for unprotected left main stem coronary artery stenosis stenting or surgery. J Am Coll Cardiol. 2008;51(9):885-892.*

135. Gao LR, Chen Y, Zhang NK, et al. *Intracoronary infusion of Wharton's jelly-derived mesenchymal stem cells in acute myocardial infarction: double-blind, randomized, controlled trial. BMC Med. 2015; 13:162-177.*