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ABSTRACT

EFFECTS OF LYCOPENE SUPPLEMENTATION ON OXIDATIVE STRESS IN CHILDREN AFFECTED BY PRIMARY DYSLIPIDEMIA

F. Abello, P. Cagliero, C. Cena, M. Giorgis, P. Assandro, O. Guardamagna
Department of Pediatrics, University of Turin, Turin, Italy
E-mail: progetto.child@unito.it

Introduction. A protective role against atherosclerosis and cardiovascular disease has been attributed to Mediterranean dietary components, which include carotenoids. Among these lycopene, present in tomatoes and tomato products, has probably the highest antioxidant capacity but contrasting data have been reported about bioavailability and efficacy in humans. The aim of the study was to evaluate the effects of lycopene on oxidative stress in children affected by primary dyslipidemia.
Material and Methods. Twenty-height dyslipidemic children (7 Familial Hypercholesterolemia, 8 Familial Combined Hyperlipidemia, 13 Autosomal Dominant Hypercholesterolemia), aged 10.7±2.4 ys, were enrolled in a double-blind, randomized, placebo-controlled, cross-over trial. Children received a dietary supplement containing lycopene (7 mg) or placebo for 4 weeks, separated by a 4-week washout period. Lipid profile (TC, HDL-C, TG, ApoB and ApoA-I) was assessed at baseline and after each treatment period by automatic analyzer (Olympus AU 2700, Japan). The concentration of OxLDL in plasma samples stored at -80°C were measured by an ELISA procedure using a commercial kit (Biomedica Gruppe). Urine samples were collected and stored at -80°C and assayed for quantification of the 8-iso-PGF2α-Isoprostane Enzyme Immunoassay (Cayman Chemical).
Results. Baseline lipid profile examination showed: TC 230.4±34.7 mg/dl, HDL-C 55.6±15.1 mg/dl, TG 76(38-171), LDL-C 157.8±31 mg/dl, ApoB 111.5±18 mg/dl, ApoA-I 142.1±24.4 mg/dl. Lycopene supplementation did not determine any variation of lipid profile, compared to placebo. Baseline OxLDL did not differ according to sex, age and diagnosis OxLDL levels resulted 633 (172-2524) ng/mL at baseline, 953 (506-3432) ng/mL and 938 (262-3260) ng/mL after lycopene and placebo treatment respectively. Data analysis concerning urinary 8-iso-PGF2α-Isoprostane are in progress.
Conclusions. Lycopene supplementation did not show any significant effect on lipid profile and OxLDL in children affected by primary dyslipidemia.

ISOLATED HYPERLIPOPROTEIN(a) IN CHILDREN WITH FAMILIAL CARDIOVASCULAR EVENTS

F. Abello, P. Assandro, O. Guardamagna
Department of Pediatrics, University of Turin, Italy
E-mail: progetto.child@unito.it

Introduction. Dyslipidemias are one of the major risk factors for atherogenesis and the determination of lipoprotein(a) [Lp(a)] is one of the emerging component to evaluate accurately the cardiovascular risk. HyperLp(a) is often related to hypercholesterolemia but few studies about isolated hyperLp(a) have been reported in pediatrics.
Objective. The first aim is to identify patients with isolated hyperLp(a) in children with a familial history positive for cardiovascular events (CVD+) and/or dyslipidemia. The second is to evaluate the correlation between familial CVD and Lp(a) levels.
Methods. In 53 subjects (24 female, 29 male), 6-18 years, Lp(a) level, lipid profile and a 2-generation genealogic tree to detect CVD were evaluated according to standard methods. Children with isolated hyperLp(a) (>30 mg/dL) were selected. Lp(a) levels were stratified according to onset age, number and type of CVD in children kindreds. CVD occurring <55 years in males and <65 years in females were considered precocious events (pCVD).
Results. Children showed a highly skewed distribution of Lp(a) concentrations [75 (31-261,8) mg/dl]. 37 patients (69.8%) resulted CVD+ and 28 (52.8%) pCVD+, without significant differences in lipid profile and Lp(a) levels. We observed the highest Lp(a) levels in children with a family history for both myocardial infarctions and strokes [103,95 (57,1-261,8) mg/dl]. Moreover patients with relatives and/or grandparents who had both precocious and late events presented an increase of Lp(a) [117,2 (36,6-261,8)mg/dl] but not statistically relevant.
Conclusion. This study showed the high frequency of familiar CVD events, in particular pCVD ones, in children with isolated hyperLp(a). Furthermore we observed a relation between Lp(a) levels and the type of CVD in their families. Long-term follow-up studies are needed to determine whether isolated hyperLp(a) levels in children are associated with increased later cardiovascular risk.

IMPROVEMENT OF ENDOTHELIAL DYSFUNCTION, HYPERCHOLESTEROLEMIA AND MARKERS OF OXIDATIVE STRESS AFTER ALBUMIN INFUSION IN PATIENTS WITH CONGENITAL ANALBUMINEMIA

F. Albanese, L. Loffredo, T. Augelletti, L. Perri, F. Baratta, L. Polimeni, D. Salzano, R. Carnevale, P. Pignatelli, M. Del Ben, F. Angelico, F. Violi
I Clinica Medica, Sapienza University, Rome, Italy
E-mail: francesco.angelico@uniroma1.it

Introduction. Analbuminemia is a rare autosomic recessive inherited disorder in which subjects have little or no plasma albumin. Patients with congenital analbuminemia are essentially asymptomatic. The body is able to compensate for the lack of albumin with the synthesis of immunoglobulins and other serum proteins and notably with an increased secretion of apolipoprotein-B from the liver. Therefore, a severe, compensatory hypercholesterolemia characterized by enhanced LDL-cholesterol levels is the prominent serum alteration in most patients with congenital analbuminemia. However, so far, very few patients with congenital analbuminemia have been reported with a long term follow-up during adult age. Therefore whether patients are at high cardiovascular risk is a major concern, although some investigators believe that the condition does not lead to premature atherosclerosis. Moreover, there is no consensus on the indication for treatment of compensatory hypercholesterolemia.
Patients. Congenital analbuminemia was diagnosed in a 43 yrs old woman and in a 35 yrs old unrelated man. The female patient suffered of severe carotid atherosclerosis and peripheral arterial disease and underwent endoarteriectomy at age 65. The male patient which is the only one in whom a case of compound heterozygosity for novel mutations in the albumin gene was detected, was essentially asymptomatic except for some lipotymic events and the presence of mild ankle oedema treated with pressure stockings. Both patients had less that 1.0 g of serum albumin, slightly

increased serum alpha1-antitripsin, ceruloplasmin, transferrin, C3, IgG and IgM immunoglobulins and severe hypercholesterolemia (496 mg/dl and 387 respectively).
Results. After a single 20 g human albumin infusion both patients showed a remarkable decrease of total and LDL cholesterol, sCD40L, and markers of oxidative stress (urinary 8-isoprostanes and serum NOX2). Treatment with atorvastatin was started in the male patient at the daily dose of 10 mg, which was increased to 20 mg at week 5 and to 40 mg at week 21. Total and LDL-C dropped by 37.7% and by 50.6% respectively and HDL-C increased by 13.4% in response to atorvastatin 40 mg.
A major decrease of total and LDL-C and of apolipoprotein-B (-34.2%, -40.8% and -25.6% respectively) was already present after the first four weeks on treatment at atorvastatin 10 mg. At the end of the drug period, apolipoprotein-B and lipoprotein(a) were decreased by 18.7% and 19.7% respectively and apolipoprotein-A1 was increased by 65.0%.
However, after one year on low-cholesterol treatment, oedema of the ankles and lower legs intensified. Statin therapy was stopped and the patient received replacement therapy with six 20 g human albumin infusions over a period of four weeks. At the end of the first infusion, brachial artery flow-mediated dilation (FMD), a surrogate marker of endothelial dysfunction, increased from 0 to 7% and maintained the same value during the following 30 days, when serum albumin increased from 0.8 g/dl to 1.9 g/dl and a progressive decrease in total and LDL cholesterol was observed (TC from 391 mg/dl to 273 mg/dl; LDL-C from 312 mg/dl to 196 mg/dl). Moreover, a marked increase of sCd40L and of markers of oxidative stress was observed. A major improvement of lower legs oedema was also observed.
Conclusions. This is the first study to demonstrate the improvement of endothelial dysfunction, serum lipids and markers of oxidative stress after infusion of albumin in a patient with congenital analbuminemia. The utility of treatment of hypercholesterolemia in this clinical setting remains still under debate.

EXPERIMENTAL CONDITIONS FOR THE LIPID AND LIPOPROTEIN PROFILING OF DE-NOVO HEART TRANSPLANTED PATIENTS TREATED WITH EVEROLIMUS AND FLUVASTATIN

L. Arnaboldi[1], F. Sala[1], L. Sommariva[1], C. Bugatti[1], R. Guarisco[2], A. Corsini[1]
[1]Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, Italia; [2]Novartis Pharma, Italia
E-mail: lorenzo.arnaboldi@unimi.it

Hyperlipidemia is a preminent complication after organ transplantation that contributes to the development of atherosclerosis. This phenomenon can be exacerbated by immunosuppressants like mTOR inhibitors.
Available data suggest that one of these, everolimus, increases apoB-rich particles and HDL-cholesterol (C), possibly due to an enhanced C biosynthesis or intestinal C absorption. Since despite relevant evidence, the mechanism of this dyslipidemia is not well-described, to assess and control this effect, we will analyze plasma samples of de-novo heart transplanted patients treated with cyclosporine, steroids, mofetil mycophenolate plus everolimus (immediately or after 4-6 weeks MMF) and fluvastatin as lipid-lowering drug.
The aim of this work is to illustrate and validate the methods that will be utilized to analyze these samples. After thawing and adjusting density at 1,21 g/mL, ultracentrifugation will be performed (72 h, 4°C, 40000 rpm), top fractions collected and lipoprotein subfractions separated by HPLC. On these, we will measure C, triglycerides, phospholipids and total proteins, plus characteristic apoproteins (apoB, apoA-I, apoC-II). On selected samples, the fatty acid content of lipid classes will be also determined by GC. On whole plasma we will measure:
1. the activity of key enzymes of lipid metabolism (CETP, LPL);
2. the ratio of C/campesterol and C/lathosterol, after extraction, sylanization and GC analysis, to understand whether the dyslipidemic effect is due to an increase in C biosynthesis or in intestinal absorption;
3. the effect of the treatment on lipid peroxidation (TBARS) and inflammation markers (CD40L, matrix metalloproteases), by zymography and ELISA.

Finally, we will quantify and analyze changes in LDL and HDL size distribution by nondenaturing gradient (2-16%/4-30%) PAGE. By this approach, we should be able to determine the effect of everolimus-induced dyslipidemia and to assess the effectiveness of everolimus-fluvastatin treatment in transplanted patients.
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CHOLESTERYL ESTER STORAGE DISEASE: EXPERIENCES IN PEDIATRICS

P. Assandro, F. Abello, A. De Giorgi, I. Rabbone, L. Pisciotta, S. Bertolini, O. Guardamagna
Department of Pediatrics, University of Turin, Italy
E-mail: progetto.child@unito.it

Introduction. Cholesteryl ester storage disease (CESD) is a rare recessive inborn error of lipid metabolism caused by mutations in the LIPA gene encoding the lysosomal acid lipase which hydrolyzes cholesteryl esters and triglycerides (TG) internalized via receptor-mediated endocytosis of plasma lipoprotein particles. As phenotipic expression variability, CESD sometimes may be underdiagnosed or diagnosed late in life. We recently characterized three novel patients affected by CESD.
Case 1. 3.5-year-old italian female showing persistent liver enzymes elevation, hepatomegaly and combined hyperlipidemia. Blood examination showed: AST 78 UI/l, ALT 92 UI/l, TC 264 mg/dl, TG 178 mg/dl, HDL-C 31 mg/dl, ApoA 80 mg/dl, ApoB 132 mg/dl.
Case 2. 7-year-old italian male with combined hyperlipidemia, low HDL-C levels and liver enzymes elevation. Blood examination showed: AST 44 UI/l, ALT 59 UI/l, TC 306 mg/dl, TG 173 mg/dl, HDL-C 34 mg/dl, ApoA 85 mg/dl, ApoB 186 mg/dl. Abdominal ultrasonography showed normal liver size with initial stage of periportal fibrosis.
Case 3. 18-year-old italian male referred to our clinic after a liver biopsy, performed for hepatomegaly and liver enzymes elevation of unknown origin, which showed periportal fibrosis with foamy histiocytes, suggesting CESD diagnosis. Fasting lipid profile showed: TC 292 mg/dl, TG 148 mg/dl and HDL-C 39 mg/dl. Genetic analysis were performed by sequencing the LIPA gene: Case 1 and 3: both subjects resulted homozygous for LIPA gene mutation c.894 G>A in exon 8 (del p.S275_Q298) Case 2: the subject resulted heterozygous for LIPA gene mutation c.894 G>A (del p.S275_Q298) and c.883 C>T (p.H295Y) in exon 8.
Conclusions. CESD should more often be considered, since childhood, as a differential diagnosis in liver diseases of unknown origin, when hypertransaminasemia and combined hyperlipidemia with low HDL cholesterol levels occur. Awareness

of the disease and efficient diagnostic tools should facilitate the correct diagnosis and therapy.

APOB AND NON-HDL-CHOLESTEROL: CARDIOVASCULAR RISK FACTORS IN CHILDREN

P. Assandro, F. Abello, O. Guardamagna
Department of Pediatrics, University of Turin, Italy
E-mail: paola.assandro@libero.it

Introduction. The correlation between unfavorable lipid profile and the atherosclerosis is well recognized and the vascular damage begins in childhood. The prognostic value of apolipoprotein B (apoB) and non-HDL-cholesterol were demonstrated in adults as accurate index of cardiovascular disease (CVD) risk, greater than LDL-cholesterol. They should be evaluated also in selected children to predict accurately their CDV risk, but few studies about this aim have been reported in pediatrics.
Objective. Estimate apoB and non-HDL-C levels and their possible relation to familial CVD in a cohort of children affected by primary dyslipidemias.
Methods. A healthy and dyslipidemic cohort comprising 351 children and adolescents (2-18 years) was considered. According to international criterias for dyslipidemia's diagnosis, a 2-generation genealogic tree to detect CVD and lipid profile was estimate by standard methods.
Patients resulted. 159 FH, 76 FCHL, 79 Dominant Hypercholesterolemia (ADH), 37 controls. They were also subdivided into 4 age-groups: 0-4.9, 5-9.9, 10-14.9, 15-18.9 years. Statistical analysis were performed by SPSS18.0 software.
Results. Independently of age, apoB and non-HDL-C levels were significantly higher in children affected by FH (133,5±29 and 219,9±53,5 mg/dl respectively) compared to other groups. Levels in FCHL and ADH patients were similar (apoB=92.6±17.6, non-HDL-C=151,1±29,5 mg/dl and apoB=90±30.6, non-HDL-C=142.7±21.3 mg/dl respectively) but they moved away in adolescence. Among patients, 68,6% had a family history of CVD events (CVD+), and 45,5% positive for precocious events (pCVD+). ApoB and non-HDL-C levels were higher in children CVD+ and pCVD+ but this difference was not statistically significant.
Conclusion. The study showed how apoB and non-HDL-C are relevant biochemical markers to identify the diagnosis in a better way. Furthermore their levels represent an advantage over traditional lipid variables for the risk prediction also in childhood and should be routinely added to the standard lipid profile in children affected by primary dyslipidemia.

EVALUATION OF TWO METHOS FOR LIPOPROTEIN(a) DETERMINATION: PRELIMINARY RESULTS

G. Avveduto[1], R. Marcucci[2], A. Terreni[1], A.M. Gori[2,3], A.L. Caldini[1], E. Sticchi[2,3], F. Cesari[2], R. Abbate[2], G. Messeri[1], S. Fedi[2]
[1]Department of Laboratory Diagnosis of Careggi Hospital, Az. Ospedaliero-Universitaria, Careggi, Florence, Italy; [2]Department of Medical and Surgical Critical Care, University of Florence, Italy; [3]Fondazione Don Gnocchi, Centro Santa Maria agli Ulivi, Impruneta, Florence, Italy
E-mail: annamaria.gori@unifi.it

Background. There is clinical evidence for an association between high lipoprotein(a) [Lp(a)] concentrations and atherosclerotic disease. Lipoprotein(a) [Lp(a)] is a complex molecule formed by the assembly of apolipoprotein B100 with apolipoprotein(a) [apo(a)]. Apo(a) is characterized by variable number of repeats of kringle 4 type 2, which accounts for the apo(a) size variation. This size apo(a) variation constitutes a serious challenge for the immunochemical measurement of Lp(a) plasma levels, as the reactivity of the antibodies directed to the repeated antigenic sites of apo(a) K4 type 2 will vary depending on the size of apo(a). Therefore, the Lp(a) plasma concentrations may vary in relation to different determination methods used. The purpose of the present study was to compare two commercial methods for Lp(a) determination in an unselected population of patients referred to Department of Laboratory Diagnosis of Careggi Hospital, Florence.
Methods. We determined Lp(a) plasma levels in 90 patients by using an immunoturbidometric assay, Lp(a) Ultra (Sentinel) and an immunonephelometric system, LPAX IMMAGE (Beckman Coulter).
Results. The analysis of data from of all patients by deming regression showed a slope value 0.79 (95% CI 0.70-0.88) with an intercept of -5.33. Deming regression analysis performed on patients with Lp(a) values <300 mg/L showed a slope value 0.95 (95% CI 0.70-1.20) with an intercept of -32.68. Using 300 mg/L as cut-off value, Lp(a) Ultra detected 33 (36.7%) patients with Lp(a) plasma levels above the cut-off and LPAX IMMAGE detected 37 (41.1%) patients. By using Lp(a) Ultra as reference method 84/90 (93.3%) samples were concordant, with 52/57 (91.2%) patients with Lp(a) <300 mg/L and 32/33 (97.0%) with Lp(a) >300 mg/L, whereas among the 6 discordant results, 1 showed Lp(a) >300 mg/L by Lp(a) Ultra and 5 by LPAX IMMAGE. A significant good agreement between the two assays was observed (κ=0.86, SE 0.05, p<0.0001).
Conclusions. Our results demonstrate that the choose of the assay for Lp(a) determination may affect the identification of patients with high levels of Lp(a). Thus a properly evaluation of the plasma levels of this protein is required in order to ameliorate the clinical risk assessment and the pharmacological approach.

NITRIC OXIDE-RELEASING STATINS DECREASE NEUTROPHILIC INFLAMMATION IN CAROTID ARTERIES OF NORMOCHOLESTEROLEMIC RABBITS

R. Baetta[1], A. Granata[1], D. Miglietta[2], D. Dellavecchia[2], L. Arnaboldi[1], N. Ferri[1], A. Bonomo[1], S. Bellosta[1], P. Pfister[3], A. Corsini[1]
[1]Dept. of Pharmacological Sciences, University of Milan, Italy; [2]Nicox Research Institute, Bresso, Milan, Italy; [3]NicOx SA, Sophia Antipolis, France
E-mail: roberta.baetta@unimi.it

Background. Polymorphonuclear neutrophils (PMN) are ubiquitous effector cells in inflammatory conditions, but their role in human atherosclerosis has long been neglected. Despite this drawback, new evidence has emerged suggesting that PMN can give important contributions to vascular inflammatory processes driving the development of atherosclerosis, thus representing new possible targets for atheroprotection. Recent observations suggest that inhibition of the HMG-CoA/mevalonate pathway by statins may also affect neutrophil recruitment and function. However, this hypothesis is not definitely established and warrant for further study. Another pathway that may represent a potential target for neutrophil-directed therapy is the nitric oxide (NO) - soluble guanylate cyclase - yclic guanosine monophosphate signal transduction pathway, which is involved in the regulation

of a variety of pathophysiological processes in mammals, including leukocyte-vessel wall interactions. NO-releasing statins are a new class of compounds designed with the aim of combining the pleiotropic effects of statins with the atheroprotective properties of NO.
Aim. Herein, we evaluated the effects of a short-term treatment with NO-releasing derivatives of atorvastatin (as compared to atorvastatin) on PMN infiltration in rabbit carotids subjected to perivascular collar placement, a model of acute arterial inflammation related to atherogenesis.
Methods and Results. Two prototype NO-releasing atorvastatins, NCX 6560 (successfully investigated in early clinical development) and NCX 616 (a structural analog of NCX 6560 optimized for NO release), were used. In preliminary in vitro studies, the compounds were shown to retain the inhibitory activity of atorvastatin on HMG-CoA reductase (IC50 =7.7 and 8.7 nM, respectively, vs 6.5 nM for atorvastatin) and to release bioactive NO (EC50 for vasorelaxing activity in isolated rabbit aortas =53.5 and 10.4 microM, respectively). For the in vivo study, chow-fed NZW rabbits (N=10/group) received a daily oral dose of vehicle or experimental compounds (equivalent to 5 mg/kg/day atorvastatin) for 6 days. One hour after the last dose of treatment, blood samples were collected from the central ear artery for evaluation of plasma concentrations of atorvastatin and its active metabolites (2-OH-atorvastin and 4-OH-atorvastatin). Silicone collars were thus implanted around the right common carotid arteries. Twenty-four hours later carotids were removed, immunostained for PMN (MCA805G Mab, AbD Serotec), and measured by image analysis. Total plasma concentration of atorvastatin and its active metabolites measured by UPLC-MS/MS were similar in the 3 treatment groups (5.6, 4.2, and 4.5 nM for atorvastatin, NCX 6560, and NCX 616-treated rabbits, respectively). Treatment with NO-releasing statins was associated with a lower amount of PMN infiltration as compared to control (approx. -39% and -26% for NCX 616 and NCX 6560 respectively; $p<0.05$ vs control for NCX 616). Treatment with atorvastatin did not influence PMN infiltration.
Conclusions. Pharmacological interventions aimed at blocking neutrophil emigration from the blood into the arterial wall is currently not an option for treating atherosclerosis. However, several lines of evidence suggest that limiting neutrophilic inflammation may represent a new therapeutic strategy in cardiovascular diseases. Herein we showed that incorporating NO-releasing moieties into the structure of statins has led to the development of NO-releasing statins, which, in contrast to their parent compounds, were able to limit PMN infiltration in acutely inflamed rabbit carotid arteries. This finding adds to the spectrum of favorable actions of NO-releasing statins and provide further pharmacological rationale for their clinical development.

METABOLIC AND CARDIOVASCULAR PROFILE IN PRE-DIABETIC SUBJECTS IDENTIFIED BY A1C OR OGTT. THE GENFIEV STUDY

C. Bianchi, R. Miccoli, G. Penno, I. Crisci, S. Politi, S. Del Prato, on behalf of the GENFIEV Study Group
Department of Endocrinology and Metabolism, University of Pisa and Azienda Universitaria Ospedaliera Pisana, Pisa, Italy
E-mail: cribianchi@hotmail.com

Background and aims. HbA1c (A1c) has been proposed for the diagnosis of diabetes (T2DM) and identification of individuals at risk of T2DM. Therefore, aims of the current study were to evaluate the performance of these criteria compared with the traditional one, based on OGTT, in respect of β-cell function, insulin action and cardiovascular profile.
Materials and Methods. We have examined 844 consecutive subjects (44% men; age 49.5±11 years; BMI 29±5 kg/m^2) entering into the Genetics Physiopathology and Evolution of Type 2 diabetes (GENFIEV) study. Plasma glucose and C-peptide were determined during OGTT to assess β-function while lipid profile, HOMA-IR and HbA1c (HPLC) were evaluated in fasting condition.
Results. Based on ADA criteria 43% had normal glucose tolerance (NGT), 42% IGR and 15% T2DM on OGTT, while IGR and T2DM were 38 and 11% on A1c with a concordance rate between of 54% and 44%, respectively. A1c specificity was 74% and 95% for IGR and T2DM. Subjects meeting both diagnostic criteria (A1c and OGTT) for pre-diabetes presented greater IR and impairment of insulin secretion and had worse cardiovascular risk profile than those NGT at both diagnostic methods. No significant difference in these parameters has been observed between subjects with pre-diabetes based on A1c or OGTT criteria or in those meeting only one diagnostic criteria for IGR or both the criteria.
Conclusions. A1c identifies a smaller proportion of individual with pre-diabetes and even smaller with T2DM than OGTT, however no significant difference in IR, insulin secretion and cardiovascular risk profile can be detected in subjects identified as pre-diabetic with A1c or OGTT criteria. Subjects fulfilling both diagnostic methods for pre-diabetes or T2DM are characterized by a worse metabolic profile.

RELATIONSHIP OF THYROID-STIMULATING HORMONE WITHIN THE NORMAL REFERENCE RANGE WITH RISK FACTOR FOR ATHEROSCLEROSIS

A. Branchi, A. Boggio[1], F. Muzio[1], M. Fiscella[1], D. Sommariva
Centro per lo Studio e la Prevenzione dell'Aterosclerosi, Department of Internal Medicine IRCCS Cà Granda, Ospedale Maggiore Policlinico Foundation, University of Milan, Italy
[1]Dietetic and Clinical Nutrition Unit, "Luigi Sacco" University Hospital, Milan, Italy
E-mail: adriana.branchi@unimi.it

Introduction. The relationship between overt hypothyroidism and cardiovascular risk has been well documented and some data also suggest an association between cardiovascular risk and subclinical hypothyroidism. The aim of our study was to investigate, in a large cohort of euthyroid subjects, the association of thyroid stimulating hormone (TSH) within the normal reference range with cardiovascular risk factors. Patients. The study was carried out on 978 patients (240 males and 738 females) with normal thyroid function (TSH 0.3 to 4.9 μU/mL).
Results. 495 (51%) patients were obese, 361 (37%) overweight and 122 (12%) normal weight. Central obesity (waist girth >=102 cm in men and >=88 cm in women) was present in 75% of men and in 69% of women. More than half of the patients (55%) were hypertensive, of them 45% was on antihypertensive drug therapy. Patients with TSH above the median (≥2.0 μU/mL) were more obese, had greater waist girth, were more hypertensive and had higher levels of serum triglycerides (TG) and blood sugar (BG) and lower levels of HDL-cholesterol (HDL-C) than patients with TSH below the median. TSH was significantly correlated with body mass index (BMI), waist circumference, total cholesterol (TC), BG, TG, HDL-C and hypertension. Multiple backward

stepwise regression analysis with age, gender, BMI and TSH as independent variables confirmed the strong association of TSH with TG, HDL-C and hypertension. A total of 308 patients (31%) fulfilled the definition criteria of the metabolic syndrome and the prevalence of metabolic syndrome was significantly greater in patients with TSH above than in patients with TSH below the median (38% vs 25%, $\chi 2$=18.543, P<0.001). Results of logistic analysis confirmed the association of TSH with metabolic syndrome (O.R. 1.335, 95% CI 1.174-1.518).
Conclusion. The results of the present study suggest that TSH in the upper limits of the reference range (above 2.0 µU/ml) is associated with a less favourable cardiometabolic profile and consequently with a higher risk of developing cardiovascular diseases

SERUM LIPIDS AND BLOOD GLUCOSE IN PATIENTS WITH AND WITHOUT METABOLIC SYNDROME AFTER MIXED MEALS OF DIFFERENT COMPOSITION

A. Branchi[1], A. Torri[1], C. Berra[1], E. Colombo[2], D. Sommariva[1]
[1]Centro per lo Studio e la Prevenzione dell'Aterosclerosi, Department of Internal Medicine, Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, University of Milan;
[2]Department of Internal Medicine 1, G. Salvini Hospital, Garbagnate Milanese, Milan, Italy
E-mail: adriana.branchi@unimi.it

Aims. To investigate the postprandial changes in serum lipoprotein pattern and blood glucose and to verify whether different nutrient composition of the lunch elicits different response in patients with (MetS+) and without (MetS-) metabolic syndrome. Research design and methods. The study was carried out on 50 patients (25 males and 25 females, 31 to 65 years) with MetS as diagnosed according to the criteria of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII) and on 50 age and sex matched patients without MetS. All meals were prepared by the hospital kitchen and the patients were instructed to choose among 3 different menus what was more similar to their usual diet. The patients did not consume alcoholic beverages. The composition of meals was assessed by an accurate nutritional analysis that was made for each subject by two of us (C.B. and E.C.). Blood samples were collected in the morning at 8:00 after an overnight fast and before the breakfast, at 2:30 PM and at 5:00 PM.
Results. Serum triglycerides increased more in MetS+ than in MetS-, HDL-cholesterol (HDL-C) decreased 2 hours after the meal in both groups and Apo A-I/HDL-C ratio significantly increased only in MetS+. Blood sugar similarly increased in both groups and plasma insulin increased more and remained high longer in MetS+ than in MetS-. Difference in nutrient composition of the meal (carbohydrate 57%, fat 28% vs carbohydrate 45%, fat 35%) was not associated with differences in postprandial levels of serum triglycerides, HDL-C, serum glucose and plasma insulin within each group.
Conclusions. As compared with MetS-, MetS+ show a greater hypertriglyceridemic and hyperinsulinemic response to a regular lunch whatever the carbohydrate or fat content of the meal.

RELATION BETWEEN ASYMMETRIC DIMETHYLARGININE AND ASYMPTOMATIC CAROTID ATHEROSCLEROSIS

V. Bucciarelli[1], C. Rizzo[2], C. Mandurino[2], M.A. Grasso[3], M. Frascaria[3], S. Cusenza[1], G. Della Malva[1], E. Della Malva[1], G. Riccioni[1]
[1]Human Nutrition, University of Chieti, Italy;
[2]Cardiology, University of Bari, Italy; [3]Cardiology Unit, San Camillo de Lellis, Hospital, Manfredonia, Foggia, Italy
E-mail: griccioni@hotmail.com

Objectives. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide (NO) associated with an increased risk of cardiovascular disease (CVD). We assessed the relationship between ADMA and asymptomatic carotid intima-media thickness (CIMT).
Methods. 122 subjects underwent a complete history and physical examination, determination of serum chemistries and ADMA levels, and carotid ultrasound investigation (CUI). All subjects had no symptoms of carotid atherosclerosis and were taking no medications.
Results. Statistical analyses showed that high plasma levels of ADMA were positively correlated to CIMT (p<0.001). Total cholesterol, low density lipoprotein cholesterol, triglycerides and C-reactive protein plasma concentrations were significantly associated with asymptomatic carotid atherosclerosis (p<0.001).
Conclusions. High serum concentrations of ADMA were associated with early carotid atherosclerotic lesions as measured by CIMT and represent an important marker of asymptomatic carotid atherosclerosis.

THE EFFECTS OF ROSUVASTATIN ON INTIMA MEDIA THICKNESS IN ADULT VERSUS ELDERLY PATIENTS. ANALYSES FROM A SUBSET OF HYPERCHOLESTEROLEMIC PATIENTS FROM THE ASYMPTOMATIC CAROTID ATHEROSCLEROTIC DISEASE IN MANFREDONIA (ACADIM) STUDY

V. Bucciarelli[1], C. Rizzo[2], C. Mandurino[2], M.A. Grasso[3], M. Frascaria[3], S. Cusenza[1], G. Della Malva[1], E. Della Malva[1], G. Riccioni[3]
[1]Biomedical Science, University of Chieti, Italy; [2]Cardiology, University of Bari, Italy; [3]Cardiology Unit, San Camillo de Lellis, Hospital, Manfredonia, Foggia, Italy
E-mail: griccioni@hotmail.com

Introduction. The benefits of cardiovascular therapies such as statins for the treatment of atherosclerosis have been well documented. Many studies have demonstrated important benefits in patients with asymptomatic carotid atherosclerosis.
Methods. We have evaluated the effect of low dose of rosuvastatin on asymptomatic carotid atherosclerosis in elderly versus adult subjects. Among 640 participants in the Asymptomatic Carotid Atherosclerotic Disease in Manfredonia Study (ACADIM Study) forty-five patients (21 adults, 24 elderly) with hypercholesterolemia and asymptomatic carotid atherosclerosis on baseline carotid ultrasound investigation (CUI) were examined with repeat CUI after one treatment year (rosuvastatin 10 mg/day).

Results. Total and low density lipoprotein cholesterol decreased significantly (p<0.001) while high density lipoprotein cholesterol increased significantly (p<0.001) during the intervention. Mean decrease in carotid intima media thickness (CIMT) of the right and left common carotid arteries were higher in adult versus elderly subjects (p<0.04 for each), even if in both group there was a significant regression in carotid atherosclerosis respect to baseline values (P<0.001).
Conclusion. These results confirm the reduction in IMT of the CCAs in response to ROS at a low dose in a one-year treatment period, even if in elderly subjects this effect is lower respect to adult. The treatment of asymptomatic carotid atherosclerosis defined by CIMT started in the adult age is more effective.

THE IMPACT OF BMI AND BODY COMPOSITION ON CIRCULATING LEVELS OF OPG, RANKL, MGP, NT-PROCNP IN TYPE 2 DIABETIC PATIENTS AND IN NORMAL SUBJECTS

A. Cadirni, C. Pondrelli, C. Caffarelli, A. Perrone, V. Ghini, B. Franci, B. Lucani, S. Gonnelli, R. Nuti
Department of Internal Medicine, Endocrine-Metabolic Science and Biochemistry, University of Siena, Italy
E-mail: gonnelli@unisi.it

Recent observations have suggested that several cytokines involved in bone formation may also be implicated in diabetic vasculopathy. Among these osteoprogeterin (OPG)/RANKL/RANK axis, vitamin K dependent matrix GLA protein (MGP) and C-type natriuretic peptide (CNP) have been reported to play an important role.
This study aimed to evaluate the impact of BMI and body composition parameters on the serum levels of OPG, RANKL, MGP and NT-proCNP in normal and subjects with type 2 diabetes (DM2). In 150 DM2 patients and 190 controls OPG, RANKL, MGP and NT proCNP were measured. Intima media thickness and carotid plaque echogenicity were assessed by ultrasonography. The body composition was performed by DXA. MGP was significantly lower in the diabetic population than in the normal subjects (7,39±2,2 nmol/l vs 12,9±6,4 nmol/l). In both DM2 and control subjects MGP was inversely associated with carotid stenosis (r=-0,15; p<0,05 and r=-0,17; p<0,05).
A significant correlation between MGP and IMT (r=-0,17; p<0,05) was found in diabetics. MGP was directly correlated with BMI, total fat mass and abdominal fat mass (p<0.01), whereas in controls MGP was inversely correlated with fat percentage (p<0.05). OPG levels were higher in DM2 patients than in normals and associated with atherosclerosis. OPG was also correlated with HbA1c (p<0.05). NT-proCNP was lower in diabetic subjects (p<0,05).
A direct correlation was found between carotid calcification and NT-proCNP in both normals and diabetics (p<0,05). In controls but not in DM2 patients NT-proCNP was positively correlated with BMI. In conclusion, our results seem to confirm that OPG, MGP and NT-proCNP play a role in the carotid atherosclerosis in both DM2 and control subjects. The influence of BMI and body composition on MGP and CNP differs between diabetic and non diabetic subjects.

LOW ESTIMATED GLOMERULAR FILTRATION (eGFR) IN A SOUTHERN ITALIAN SAMPLE (VIP-STUDY): PREVALENCE AND ASSOCIATED RISK FACTORS

V. Capuano, N. Lamaida, M.I. Borrelli, E.R. Capuano, M. Sonderegger, S. Torre, E.D. Capuano
Unità di Cardiologia Mercato S. Severino, Azienda Ospedaliera Universitaria di Salerno, Italia
E-mail: norman.lamaida@libero.it

Background. Screening for chronic kidney disease is recommended in people at high risk, but data on the independent and combined associations of eGFR with all-cause and cardiovascular mortality are limited. The our study investigated on prevalence of low eGFR and associated risk factors in the VIP-Study.
Methods. Data were collected on serum creatinine, blood pressure, other laboratory indices and medical history in the VIP study (2400 partecipants, both sexes, age 25-74 years). Analyses were carried out on eGFR (equation of Cockroft-Gault), and risk factors (hypertension, marker of inflammation disease (PCR, fibrinogen, age, T.cholesterol, LDL-cholesterol, non HDl-cholesterol, uric acid, glycaemia) potentially associated to kidney disfunction. Thus, the present study was designed to further investigate on the prevalence of low kidney function in the population focusing analyses also disorders potentially secondary to kidney disease and other related variables.
Results. The prevalence of low eGFR (<60 ml/min x 1.73 m^2) increased with age in both sexes. In the group with eGFR <60 ml/min x 1.73 m^2, number of risk factors secondary to kidney disfunction was >2 in the majority of persons, was higher than in persons with eGFR >60 ml/min x 1.73 m^2 (p<0.001). Is interesting to note that, in both sexes, the values of age, PAS and marker of inflammation disease (PCR, fibrinogen) increased with decrease of GFR; conversely, T.cholesterol, non HDL cholesterol, LDL cholesterol, uric a., increased only in women with the decrease of GFR.
Conclusions. Low kidney function is frequent in the older population and is associated with numerous risk factors. Thus, detection of patients at risk in the general population is becoming even more important.

PREVALENCE OF LOW ESTIMATED GLOMERULAR FILTRATION (EGFR) IN A POPULATION OF SOUTHERN ITALY (VIP-STUDY)

V. Capuano, N. Lamaida, M.I. Borrelli, E.R. Capuano, R. Capuano, F. Franculli
Unità Operativa di Cardiologia ed UTIC, Ospedale Amico "G. Fucito", Azienda Ospedaliera Universitaria Integrata di Salerno, Italia
E-mail: norman.lamaida@libero.it

Background. Chronic kidney disease (CKD) is a common disorder and its prevalence is increasing worldwide. In Italy the prevalence of CKD, especially the early stages, is still not exactly known. This is also true, for CKD stage 3, when cardiovascular and other major complications generally appear. Detection of these subjects at risk is important to implement measures to slow down progression of CKD and avoid secondary complication.The

our study investigated on prevalence of low eGFR in a southern Italian population sample (VIP-STUDY).
Methods. A rapresentative sample of 2400 non institutionalized adults (age 25-74 years, both sexes) from the VIP study was analyzed. eGFR was used for analysis of kidney disease prevalence. Kidney function (GFR) and stages of CKD (GFR) was estimated from calibrated serum creatinine level, age and sex. eGFR was estimated using the Cockcroft-Gault equation (CGe).
Results. The study reports population-based data on the prevalence of low kidney function defined by the CGe.The prevalence of low eGFR ≤60 ml/min x 1.73 m^2) increased exponentially with ages in both sexes(from rates <1% for ages 25-34 years up to rates >27% for ages ≥65 years, p<0.001).
By stage, the prevalence of stage 2 (eGFR ≥60-90 ml/min x 1.73 m^2) was more increased in women than in men (48% as opposed to 32%, p<0.026).
Conclusions. Screening for CKD in the general population is still not recommended. However, high-risk groups like patients and subjects above age 60 should have their glomerular filtration rate estimated. Better interplay between primary and secondary care is needed for successful implementation of CKD clinical guidelines in general practice.

PRO-ATHEROGENIC POSTPRANDIAL PROFILE: MEAL INDUCED CHANGES OF LIPOPROTEIN SUB-FRACTIONS AND INFLAMMATION MARKERS IN OBESE BOYS

M. Carlon, L. Pinelli, M.G. Surano, S. Cordioli, S. Gasperotti, D. Vianello, C. Maffeis, A. Zambon
Department of Medical and Surgical Sciences, University of Padova, Italy, and Department of Mother and Child, Biology-Genetics, Section of Pediatrics, University of Verona, Italy
E-mail: alberto.zambon@unipd.it

Background and aims. Obesity is a pro-atherogenic condition and postprandial lipoprotein profile and circulating cytokines changes may contribute to promote the process.
The aim of this study is to investigate postprandial metabolic response, lipoprotein oxidation and circulating cytokine levels, after the ingestion of two different meals with different fat/carbohydrate ratio.
Methods and Results. Ten prepubertal obese boys consumed two meals with the same energy and protein content but with a different carbohydrate to fat ratio:
1) moderate fat (MF): 61% carbohydrate, 27% fat;
2) high fat (HF): 37% carbohydrate, 52% fat.
The AUC of glucose and insulin were significantly (p<0.05) lower after the HF meal.
HF meal was followed by a significant decrease in the cholesterol carried in the HDL fractions, while cholesterol in the small, dense LDL and in the VLDL particles increased, as compared to baseline (p<0.05 for all). No differences were found in the cholesterol distribution after the MF meal.
Moreover, HDL-C concentration was lower (p<0.05) at 300 min after HF vs. MF meal. Oxidized LDL (ox-LDL) concentration increased after the HF meal but not after the MF meal [9.3 (2.2) vs 1.8 (2.2)% from baseline, p<0.02].
A positive association (r=0.33, p<0.05) was observed between the densest LDL particles and the ox-LDL plasma levels. A reduction of IL-6 was found at 120 min after the MF [-23.3(5.5)vs-8.4(3.8)% from baseline, p<0.05] compared with the HF meal.
Conclusion. A simple change of ≈25% of energy load from fat to carbohydrate in a meal significantly improves postprandial pro-atherogenic factors in obese boys.

THE NEW EUROPEAN GUIDELINES IN THE ITALIAN POPULATION: ANALYSIS FROM THE CHECK STUDY

M. Casula[1], E. Tragni[1], A. Filippi[2], O. Brignoli[2], C. Cricelli[2], A. Poli[3], A.L. Catapano[1,4]
[1]Centro Interuniversitario di Epidemiologia e Farmacologia Preventiva (SEFAP), Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, Italia; [2]Società Italiana di Medicina Generale (SIMG), Firenze, Italia; [3]Nutrition Foundation of Italy (NFI), Milano, Italia; [4]IRCCS MultiMedica, Sesto San Giovanni (MI), Italia
E-mail: sefap@unimi.it

The recent European guidelines (EGL 2011) on dyslipidaemias recommend risk stratification according to the SCORE algorithm (10-year risk of a fatal atherosclerotic event), but in Italy another algorithm is commonly used: the CUORE risk score (10-year risk of a first fatal and non fatal CV event).
We calculated SCORE (low risk function) and CUORE scores in a sample of Italian subjects aged 40-79 enrolled in the CHECK study, estimated the correlation between the two scores, and evaluated the distribution of our sample into the EGL risk classes. According to EGL, we stratified our cohort (N 4815) into low risk (37.0%), intermediate risk (32.6%), high risk (5.1%), and very high risk (25.3%) classes.
In the subgroup without statin treatment (N 4325), the stratification based on CV risk and LDL-C levels resulted in 8.2% to no intervention, 35.2% to lifestyle intervention, 29.5% to lifestyle intervention + drug if uncontrolled, 1.4% to lifestyle intervention + consider drug, and 25.8% to lifestyle intervention + immediate drug intervention.
The correlation analysis between SCORE and CUORE values (in a subgroup without CV events and/or diabetes, N 3300) showed a strong association (Spearman's rho 0.939). We found a multiplicative factor of 2,1 from SCORE to CUORE, then we redefined low risk (CUORE <2.1%), intermediate risk (2.1-10.4%), high risk (10.5-20.9% or high risk conditions), and very high risk (>=21.0% or very high risk conditions) classes (discordance 13.9%; kappa statistic 0.8).
Therefore, the five therapeutic approaches involved 7.2%, 30.4%, 33.6%, 1.7%, and 27.2% of our cohort. As the CUORE algorithm is derived from Italian data and its use is quite widespread in health care, it is important to find ways of translating the European recommendations in the Italian context.
The current analysis proposes a conversion factor that allows the use of CUORE score, obtaining a classification by risk class and by intervention strategies very similar to that expected with the SCORE algorithm.

LA NUOVA NOTA 13: QUANTI SONO I PAZIENTI TRATTABILI IN REGIME DI RIMBORSO RISPETTO ALLA VERSIONE PRECEDENTE?

M. Casula[1], A. Poli[1,2], E. Tragni[1], A. Filippi[3], O. Brignoli[3], C. Cricelli[3], A.L. Catapano[1,4]
[1]Centro Interuniversitario di Epidemiologia e Farmacologia Preventiva (SEFAP), Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, Italia; [2]Nutrition Foundation of Italy (NFI), Milano, Italia; [3]Società Italiana di Medicina Generale (SIMG), Firenze, Italia; [4]IRCCS MultiMedica, Sesto San Giovanni (MI), Italia
E-mail: sefap@unimi.it

La prescrizione delle statine in regime di rimborso è soggetta, in Italia, a criteri stabiliti dalla Nota 13 AIFA, recentemente modificata (D.M. 13 luglio 2011). Questa analisi è stata condotta con l'obiettivo di applicare i criteri definiti dalla vecchia e dalla nuova Nota a un campione rappresentativo della popolazione nazionale adulta e stimare l'impatto delle recenti modifiche su numero e caratteristiche dei soggetti trattabili in regime di rimborso. I criteri di rimborsabilità sono stati applicati alla coorte CHECK (5.696 soggetti di entrambi i sessi, età 40-79 anni, arruolamento randomizzato presso ambulatori di medicina generale in tutta Italia).
Per i pazienti trattati con statine all'arruolamento sono stati stimati i valori di colesterolo LDL pre-trattamento in base alla molecola e al dosaggio riportati nella cartella rilevamento dati. I criteri della vecchia Nota identificavano 1.260 soggetti (22,1% del campione totale) trattabili con statine in regime di rimborsabilità, i nuovi criteri 1860 soggetti (32,7%). 1.002 soggetti (17,6% della coorte) rientrano in entrambe le Note. I nuovi criteri escludono il 9,1% dei pazienti in prevenzione secondaria e il 18,3% dei diabetici, perché il loro valore di C-LDL è inferiore ai valori target fissati dalla Nota. Dei soggetti che rientrano nella nuova Nota, il 47,8% (15,6% dell'intera coorte) dista dal proprio target meno del 20%, il 33,9% (11,1%) dista il 20-39,9%, il 9,5% (3,1%) dista il 40-49,9% e l'8,8% (2,9%) dista il 50% e oltre. L'adozione della nuova Nota 13 consentirà il trattamento con statine in regime di rimborso di circa 10 milioni di soggetti (considerando anche pazienti con iperlipidemie familiari ed età <40 anni, previsti da entrambe le Note, e un piccolo numero di soggetti in prevenzione primaria con ≥2 fattori di rischio ed età <40 anni). Di questi, 2,15 milioni saranno candidati a statine più efficaci o associazioni farmacologiche per la loro distanza dal target (>40%). Circa 1,3 milioni dovrebbero perdere invece la rimborsabilità loro garantita dalla Nota precedente.

EFFECTS OF ANTIRETROVIRAL TREATMENT ON ENDOTHELIAL DYSFUNCTION AND REGENERATION IN HIV-POSITIVE PATIENTS: 1-YEAR OF FOLLOW-UP

F. Cesari, A. Rogolino, G. Cioni, C. Martinelli[1], A. Carocci[1], P. Corsi, F. Leoncini[1], G.F. Gensini, R. Abbate, A.M. Gori
Department of Medical and Surgical Critical Care, Thrombosis Centre, University of Florence, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy; [1]Infectious Diseases Unit, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy
E-mail: gabriele.cioni@unifi.it

Introduction. The purpose of the present study was to evaluate the effect of HAART on the markers of endothelial dysfunction and regeneration, such as circulating endothelial cells (CECs) and endothelial progenitor cells (EPCs) in a population of HIV naïve positive patients.
Materials and Methods. 16 HIV+ patients naïve for antiretroviral drugs (M 15, F 1) with a median age of 42 (22-65) years and 16 age- and sex-matched control subjects were enrolled in the study. Ten of 16 HIV positive patients were treated with HAART therapy for 1 year. Circulating CECs were defined as CD146+/CD31+/CD45-/CD61-, while EPCs were defined as CD34+KDR+, CD133+KDR+ and CD34+CD133+KDR+.
Results. Before antiretroviral treatment HIV positive subjects showed a significant higher number of CECs and a significant lower number of EPCs with respect to the controls [CD146+/CD31+/CD45-/CD61-8.5 (3-23) cells/106 events vs 3 (0-13) cells/106 events p=0.021; CD34+/KDR+ 7 (0-23) cells/106 events vs 16 (3-40)cells/106 events, p=0.047; CD133+/KDR+ 7(0-23) cells/106 events vs 13 (7-43) cells/106 events p=0.043]. After 1 year, 8/10 (80%) patients in HAART showed a marked decrease in CECs number and 6/10 (60%) in EPCs number, whereas in patients who did not receive antiretroviral therapy the numbers of CECs and EPCs decreased in 3/6 (50%) and 5/6 (83.3%) respectively. After 1-year of follow-up HIV+ patients who received antiretroviral therapy showed lower number of CECs with respect to HIV+ patients who did not receive the treatment [CD146+/CD31+/CD45-/CD61 1.5 (0-15) cells/106 events vs 10 (0-37) cells/106 events p=0.056], whereas EPCs number were similar in both groups.
Conclusions. Our data demonstrate the presence of an endothelial dysfunction, as documented by low EPCs number and high CECs number, in naïve HIV+ patients with respect to a control population. Moreover present results suggest that HHAT treatment is associated with an improvement of the markers of endothelial dysfunction.

THE POTENTIAL ROLE OF THE ENDOCANNABINOID SYSTEM IN ATHEROGENESIS

V. Chiurchiù, M. Lanuti, F. Filomena, M. Maccarrone
IRCCS Fondazione Santa Lucia
E-mail: valchiu@yahoo.it

Atherosclerosis is a chronic inflammatory disease which is the primary cause of morbidity and mortality in the western world and whose identification of promising novel therapeutics is thus of great interest. The endocannabinoid system (ECS) comprises an ever-growing class of bioactive lipids known to exert numerous central effects in the brain as well as peripheral effects in distinct body tissues, including regulation of lipid metabolism, food intake and energy balance. Established evidence also indicate that cannabinoid type-1 receptor (CB1R) is closely associated with cardiometabolic risk factors, including obesity and increased serum lipid production. However, the role of the whole ECS, which comprises the endogenous ligands, the cannabinoid receptors and the enzymatic machinery deputated for endocannabinoids metabolism has yet to be thoroughly elucidated or understood in human atherosclerotic cells. Our data shows that several elements of the ECS are significantly altered in human foam cells and that the endogenous cannabinoids exert differential effects in regulating CD36-mediated intracellular lipid droplets accumulation. Furthermore, we show an unprecedented effect of the ECS in modulating the inflammatory immune responses of such human atherosclerotic cells.
Overall, we provide evidence that the ECS is indeed a novel biomarker of atherosclerosis and that it is implicated in modulating

several key hallmarks of this disease; thus our findings may be of crucial importance for the rational design of new endocannabinoid-based immunotherapeutic strategies for atherosclerosis or cardiovascular diseases.

GENDER DIFFERENCE IN FACTORS ASSOCIATED TO THE LIVER STEATOSIS INDEX IN A LARGE POPULATION SAMPLE: DATA FROM THE BRISIGHELLA HEART STUDY

A.F.G. Cicero, S. D'Addato, B. Gerocarni, M. Rosticci, M. Giovannini, E. Grandi, C. Borghi on behalf of the Brisighella Heart Study Group
Internal Medicine, Aging and Kidney Disease Department, University of Bologna, Italy
E-mail: afgcicero@cardionet.it

Background. Non-alcoholic fatty liver disease (NAFLD) is a largely prevalent emerging cardiovascular risk factor. The Liver Steatosis Index (LSI) is a validated index, useful for epidemiological evaluation of NAFLD. The aim of our study was to evaluate the factors associated to LSI in a large sample of general population.
Methods. We selected the 1638 volunteers (M: 48.2%, W: 51.8%; mean age: 53.04±18.06 years old) attending to the 2008 Brisighella Heart Study survey without a known history of liver disease and a known history of alcohol abuse. Then we calculated the LSI for all subjects (LSI = 8 x GOT/GPT ratio + BMI +2 if women/+2 if type 2 diabetes) and evaluated by linear regression the factors predicting the LSI level. The considered factors included working physical activity, leisure-time physical activity, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, apolipoprotein B100, apolipoprotein AI, uric acid, eGFR.
Results. The selected population sample was representative of the Brisighella Heart Study historical cohort as it regards sex, age and body mass index distribution. Mean LSI was 35.78±6.13, not significantly different in men and women. In an age adjusted model, the best predictor of LSI in men were LogTG (OR 9.53; 95% CI 7.66, 22.43) and leisure-time physical activity (OR -0.939; 95% CI -1.49, -0.39). In women, in an age adjusted model, the best predictor of LSI were LogTG (OR 6.33; 95% CI 4.58, 8.07) and uric acid (OR 1.27; 95% CI 0.91, 1.64).
Conclusion. In a general population sample LogTG are the more strong predictor of high LSI in both genders, while leisure-time physical activity is inversely associated to LSI in men and uric acid directly in women, despite a similar LSI level.

EFFECTS OF A COMBINED NUTRACEUTICAL ON METABOLIC PARAMETERS AND VASCULAR REMODELLING BIOMARKERS IN POST-MENOPAUSAL WOMEN

A.F.G. Cicero, B. Gerocarni, M. Giovannini, E. Grandi, C. Borghi
Internal Medicine, Aging and Kidney Disease Department, University of Bologna, Italy
E-mail: afgcicero@cardionet.it

Background. Menopause is associate to a broad, even if usually not massive, changes in a large number of metabolic parameters. The aim of our study was to evaluate the efficacy and tolerability of a combined nutraceutical approach on a large number of cardiometabolic risk markers and menopause symptoms in otherwise healthy mildly dyslipidaemic post-menopausal women.
Method. 40 women were enrolled in the context of a controlled, randomised, prospective study with parallel groups. They were randomised to treatment with a nutraceuticals containing soy isoflavones 60 mg and berberine 500 mg (Estromineral lipid© - Rottapharm-Madaus, Monza, Italy) or calcium 240 mg + vitamin D3 5 µg at a dosage of 1 tablet daily between meals for 12 weeks.
Results. All patients completed the study without significant side effects. Anthropometric measures, blood pressure, HOMA index, and basal homocysteinemia significantly improved in isoflavone-berberine treated group when compared to the baseline, but not when compared to the calcium-vitamin D3 treated patients. Compared to calcium-vitamin D3 treated patients, the isoflavone-berberine treated ones experienced a significant improvement in plasma lipid and metalloproteinases serum levels, but also in the main menopausal symptoms. In particular, in the isoflavone-berberine treated group MMP-2 improved from 1433.03±60.62 to 1080.68±313.34, MMP-9 from 149.30±53.75 to 96.18±16.70, while TIMPs remained unchanged.
Conclusion. The short-term assumption of a nutraceutical containing isoflavones and berberine was well-tolerated and improved menopausal symptoms, the plasma lipid level, and the level of MMPs in a cohort of mildly dyslipidaemic post-meopausal women, when compared with a neutral control.

RELATIONSHIP BETWEEN BLOOD PRESSURE, CHOLESTEROLEMIA AND SERUM APOLIPOPROTEIN B IN A LARGE POPULATION SAMPLE: THE BRISIGHELLA HEART STUDY

A.F.G. Cicero, S. D'Addato, M. Veronesi, M. Rosticci, F. Santi, A. Dormi, C. Borghi for the Brisighella Heart Study Group
Internal Medicine, Aging and Kidney disease Department, University of Bologna, Italy
E-mail: afgcicero@cardionet.it

Objective. To evaluate the relationship between cholesterolemia, serum apolipoprotein B (apoB) level and blood pressure in a large sample of general population.
Methods. The Brisighella Heart Study (BHS) is a prospective, population-based longitudinal epidemiological investigation. For this study, we analysed the data sampled in the 2008 BHS population survey, excluding those subjects treated with antihypertensive and/or lipid lowering drugs (N: 2473).
Results. In a sex, BMI, smoking habit, physical activity level and serum creatinine adjusted model, LDL-C appears to be significantly related to SBP ($p<0.001$), DBP ($p=0.026$), and PP ($p<0.001$). In subjects aged <52 years, LDL-C was significantly associated to SBP and DBP ($p<0.001$), but not PP. In the same model, apoB appears to be mildly but significantly related to SBP ($p<0.001$), DBP ($p<0.001$), and PP ($p<0.001$). In subjects aged <52 years, apoB was significantly associated to SBP ($p<0.001$), DBP ($p<0.001$), and PP ($p<0.001$). In subjects aged 52 or more, nor LDL-C neither apoB were significantly associated to blood pressure. Including in the same model LDL-C and apoB, apoB excluded the predicting role of LDL-C as it regards the blood pressure either in the whole population sample and in the younger subjects.
Conclusion. On the basis of our observation, either serum LDL-C and apoB are significantly related to the blood pressure level in a large sample of subjects untreated with antihypertensive and

lipid-lowering drugs. This association is stronger in younger subjects than in elderly. ApoB seems to be a stronger predictor of either SBP, DBP and PP than LDL-C.

ACIDO NICOTINICO, COLESTEROLO HDL E LIPOPROTEINA (a) IN UNA POPOLAZIONE DI PAZIENTI AD ALTO RISCHIO CARDIOVASCOLARE SOTTOPOSTI AD AFERESI TERAPEUTICA

G. Cioni[1], R. Marcucci[1], C. Fatini[1], E. Sticchi[1], S. Fedi[1], G. Casola[1], A. Tognaccini[2], R. Abbate[2], G. D'Alessandri[2]
[1]Dipartimento Area Critica Medico-Chirurgica, Università degli Studi di Firenze, Italia; [2]SIMT, Azienda Ospedaliera USL 3, Pistoia, Italia
E-mail: gabriele.cioni@unifi.it

Introduzione. L'LDL-aferesi in associazione a terapie farmacologiche è impiegata per pazienti ad alto rischio cardiovascolare, ma non è tuttavia in grado di aumentare i livelli di colesterolo HDL. Lo scopo dello studio è stata la valutazione dell'effetto dell'acido nicotinico sul profilo lipidico, in particolare sui livelli di colesterolo HDL e di lipoproteina (a) [Lp(a)], in pazienti sottoposti ad aferesi.
Materiali e Metodi. Sono stati studiati 8 pazienti [3 F e 5 M; età 64 (55-68) aa], sottoposti a procedura di LDL-aferesi ogni 7-10 giorni da almeno 2 anni [mediana: 3,5 (2-7) aa] per elevati livelli di colesterolo LDL non corretti da terapia farmacologica, elevati livelli di Lp(a) e precedenti eventi cardiovascolari prima dei 60 anni. In aggiunta alla terapia con statine è stato inserito acido nicotinico/laropiprant (2000 mg/40 mg). Nessun effetto collaterale ha determinato l'interruzione della terapia. Prelievi di sangue venoso periferico (colesterolo LDL, colesterolo HDL, trigliceridi, Lp(a)] sono stati eseguiti al T1 (basale), T2 (3 mesi), T3 (6 mesi) nel corso di sei mesi precedenti all'introduzione nella terapia ipolipemizzante di acido nicotinico/laropiprant ed eseguiti al T1N (basale), T2N (3 mesi), T3N (6 mesi) durante i sei mesi successivi all'introduzione di questa terapia.
Risultati. I livelli di colesterolo HDL sono risultati significativamente più elevati a 3 e 6 mesi dall'inizio della terapia con acido nicotinico [(T1N: 46,5 (26-67) mg/dl; T2N: 57,5 (41-87) mg/dl, p=0,195; T3N: 69,5 (48-91) mg/dl, p=0,013, p for trend=0,041], con un incremento percentuale medio pari al 52,8% dopo 6 mesi. I livelli di coleserolo HDL risultavano invece invariati in assenza di terapia con acido nicotinico [(T1: 52 (33-71) mg/dl; T2: 43 (29-57) mg/dl, p=0,195; T3: 51,5 (26-62) mg/dl, p=0,878, p for trend=0,318]. I livelli di Lp(a) sono risultati significativamente più bassi a 3 e 6 mesi dall'inizio della terapia con acido nicotinico [(T1N: 1455 (710-1870) mg/L; T2N: 1025 (410-1420) mg/L, p=0,021; T3N: 915 (508-1300) mg/L, p=0,013, p for trend 0,016], osservando una riduzione percentuale media del 34,2% dopo 6 mesi, rispetto al periodo in assenza di terapia [(T1: 1510 (610-1840) mg/L; T2: 1620 (580-2190) mg/L, p=0,645; T3: 1430 (650-2300) mg/L, p=0,959, p for trend=0,927]. I livelli di trigliceridi sono risultati significativamente più bassi a 3 e 6 mesi dall'inizio della terapia con acido nicotinico [(T1N: 96 (59-197) mg/dl; T2N: 74 (37-111) mg/dl, p=0,130; T3N: 74 (41-88) mg/dl, p=0,043; p for trend=0,091], con una riduzione percentuale media pari al 33,9% dopo 6 mesi. I livelli di trigliceridi risultavano invece invariati in assenza di terapia con acido nicotinico [(T1: 94 (71-202) mg/dl; T2: 103 (74-203) mg/dl, p=0,382; T3: 97,5 (53-246) mg/dl, p=0,574, p for trend=0,645]. I livelli di colesterolo LDL si sono mantenuti pressochè invariati in corso di terapia con acido nicotinico [(T1N: 84 (74-167) mg/dl; T2N: 66 (43-149) mg/dl, p=0,054; T3N: 90 (57-118) mg/dl, p=0,432; p for trend=0,180] Questo comportamento è sovrapponibile al periodo in assenza di acido nicotinico [(T0: 92,5 (69-161) mg/dl; T2: 93 (66-181) mg/dl, p=0,878; T3: 79,5 (53-180) mg/dl, p=0,505, p for trend=0,751].
Conclusioni. I dati dimostrano che in una popolazione ad alto rischio cardiovascolare, sottoposta a procedura aferetica, la somministrazione di acido nicotinico/laropiprant è in grado di ottimizzare l'assetto lipidico con il significativo aumento dei livelli di colesterolo HDL e la riduzione dei livelli di Lp(a). Lo studio, tuttora in corso, ci consentirà di valutare se la somministrazione di acido nicotinico oltre alla terapia standard possa consentire di allungare il timing fra le procedure aferetiche in pazienti selezionati.

LIPOPROTEIN (a): A MARKER OF SYSTEMIC ATHEROSCLEROTIC BURDEN IN PATIENTS WITH ACUTE CORONARY SYNDROME

G. Cioni, R. Marcucci, G. Degl'Innocenti, M. De Stefano, G. Casola, E. Sticchi, S. Valente, C. Giglioli, S. Berardino, R. Abbate, M. Boddi
Department of Medical and Surgical Critical Care, University of Florence, Azienda Ospadeliero-Universitaria Careggi, Florence, Italy
E-mail: gabriele.cioni@unifi.it

Introduction. Several studies have documented that carotid intima media thickness (c-IMT) is a surrogate measure of atherosclerosis which is associated with cardiovascular risk factors and cardiovascular outcomes. Recent data showed that lipoprotein (a) [Lp(a)] is associated with the presence of carotid stenosis in patients undergoing coronary artery bypass grafting. Aim of our study is to evaluate the possible association between carotid and femoral IMT, as marker of systemic atherosclerotic burden in a group of patients with previous cardiac events (acute coronary syndromes).
Materials and Methods. We have studied 46 patients [M 34 F 12, age 65 (37-91) yrs] in the framework of a study aimed to evaluate vascular function in patients with a previous acute coronary syndrome on statin treatment. Intima-media thickness (IMT) of the right and the left common carotid arteries was measured in the 1-cm segment proximally to the carotid dilation with B-mode ultrasonography, by computerized problem using a 7.5 MHz transducer attached to a MyLab 70 XVision esaote machine. Femoral IMT was measured in the far wall of a 1-cm long arterial segment proximal to the femoral bifurcation IMT. For each subject the maximum carotid IMT value was used for statistical analysis. According to the guidelines of the European Society of Hypertension c-IMT values >0.9 mm and f-IMT values >1.2 mm were considered pathologic. Lp(a) was measured by a nephelometric method (Sentinel Diagnostics, Milan, Italy).
Results. Abnormal c-IMT and f-IMT values were detected in 10/46 patients (21.7%). Lp(a) values were 585±504 mg/L [median 465 (20-1828) mg/L]. Lp(a) values >300 mg/L were detected in 25/46 patients (24,3%). Lp(a) values in patients with impaired c-IMT and f-IMT were significantly higher than in the others (915±612 mg/L vs 493±436 mg/L, respectively; p=0.035 and 811 (128-1828) mg/L vs 364 (20-1610) mg/L, respectively; p=0.038]. ROC curve demonstrated that Lp(a) levels were able to discriminate patients with and without an impaired c-IMT and f-IMT: AUC 0.72 (95% CI, 0.54-0.90), p=0.037. 571 mg/L is the

Lp(a) value associated with the maximun sensitivity and specificity for the detection of intimal thickening.
Conclusions. Our findings indicated that Lp(a) is significantly associated with carotid and femoral intima media thickness, and predicts systemic atherosclerotic burden in the secondary prevention of patients with previous acute coronary syndrome. Lp(a) value can identify high risk subgroup for cardiovascular events recurrence.

ASSESSMENT OF CARDIOVASCULAR RISK BY PERIPHERAL ARTERIAL TONOMETRY AND ULTRASOUND EVALUATION OF INTIMA MEDIA THICKNESS AT COMMON CAROTIDS IN HIV POSITIVE PATIENTS

G. Cioni, C. Martinelli[1], F. Cesari, A. Rogolino, P. Corsi[1], A. Carocci, F. Leoncini[1], M. Marinoni[1], G.F. Gensini, R. Abbate, M. Boddi
Department of Medical and Surgical Critical Care, Thrombosis Centre, University of Florence, Azienda Ospadeliero-Universitaria Careggi Florence, Italy; [1]Infectious Diseases Unit, Azienda Ospadeliero-Universitaria Careggi Florence, Italy
E-mail: gabriele.cioni@unifi.it

Introduction. In this study we evaluated markers of endothelial dysfunction, such as reactive hyperemia, von Willebrand factor (vWF), plasminogen activator inhibitor type 1 (PAI-1), tissue-type plasminogen activator (t-PA) and sub-clinical atherosclerosis by ultrasonographic carotid intima-media thickness (c-IMT) assessment, in HIV positive patients.
Materials and Methods. 16 HIV positive patients [time from diagnosis 4 (1-7) years] [M 15, F 1, median age of 44 (24-65) years; naïve for antiretroviral drugs, with viral load of 9,400 (2,100-100,000) couple/mL, and number of CD4 of 587 (340-1,000) mm3 at the baseline] and 16 age- and sex-matched controls subjects were enrolled in the study. All patients and controls underwent c-IMT ultrasound assessment and peripheral arterial tonometry (PAT) (EndoPAT™). vWF was measured by using miniVidas analyser (BioMerieux, Lyon, France), PAI-1 and t-PA by an ELISA method.
Results. c-IMT ultrasound assessment showed intimal thickening in 56.3% (9/16) of HIV positive patients compared to 12.5 % of controls (2/16) (p=0.023). HIV positive patients showed a lower reactive hyperemia index (RHI) with respect to the control population [1.99 (1.27-2.85); 2.20 (1.48-3.22); p=0.032]. RHI values were significantly lower in subjects with carotid intimal thickening with respect to those without carotid intimal thickening [1.98 (1.39-2.51); 2.20 (1.27-3.22); p=0.007]. In HIV positive patients RHI values were significantly correlated with t-PA (r=-0.65, p=0.007), and a trend towards a significant correlation between RHI and PAI-1 was observed (r=-0.39, p=0.13). No relationship between t-PA, PAI-1, vWF and c-IMT was found.
Conclusions. Our data demonstrate the presence of an increased carotid intima-media thickness in HIV positive patients. The altered endothelial function detected by non invasive peripheral arterial tonometry is associated with soluble markers and sub-clinical atherosclerosis.

STUDIO DELLA FUNZIONE ENDOTELIALE ATTRAVERSO TONOMETRIA ARTERIOSA PERIFERICA IN UNA POPOLAZIONE AFFETTA DA BRONCOPNEUMOPATIA CRONICA OSTRUTTIVA

G. Cioni[1], S. Monti[2], R. Marcucci[1], M. De Stefano[1], G. Casola[1], G. Degl'Innocenti[1], R. Abbate[1], M. Miniati[1]
[1]Dipartimento Area Critica Medico-Chirurgica, Università degli Studi di Firenze, Italia; [2]Fondazione CNR/Regione Toscana "G. Monasterio", Pisa, Italia
E-mail: gabriele.cioni@unifi.it

Introduzione. La broncopneumopatia cronica ostruttiva (BPCO) è associata alla patologia vascolare, principale causa di morte tra i pazienti con alterata funzionalità polmonare. Questa associazione è determinata in parte dai fattori di rischio in comune (primo tra tutti il fumo) ed in parte dall'infiammazione cronica causata dalla patologia.
La tonometria arteriosa periferica (PAT) (EndoPAT™), che misura la risposta iperemica endoteliale all'ischemia come indice di iperemia reattiva (RHI), è una tecnologia non invasiva che offre una registrazione pletismografica battito per battito dell'onda pulsatoria arteriosa digitale attraverso sonde pneumatiche. In questo studio abbiamo valutato la PAT, come indice di aterosclerosi subclinica, in una popolazione affetta da BPCO, asintomatica per malattia aterosclerotica.
Materiali e Metodi. La PAT è stata indagata in 24 pazienti affetti da BPCO [M 20, F 4; età: 73 (51-87) aa] di grado moderato-severo, clinicamente stabili, in sola terapia inalatoria, e 30 controlli [M 13, F 17; età 64,5 (52-81) aa]. In relazione al profilo di rischio vascolare, 9 pazienti (37,5%) erano dislipidemici (4 in terapia con statine), 14 ipertesi (58,3%), 14 ex fumatori (58,3%) e 7 fumatori (2,9%).
Risultati. I valori di RHI ottenuti nei pazienti si sono dimostrati significativamente inferiori rispetto ai controlli [1,56 (1,26-2,92); 1,93 (1,23-3,54); p=0,02]. L'RHI è risultato alterato (<1,50, cut-off ottenuto dal Framingham Heart Study) nel 29,2% (7/24) rispetto al 3,3% (1/30) dei controlli (p=0,016). Nessuna associazione è stata dimostrata tra RHI e la presenza dei fattori di rischio cardiovascolare.
Conclusioni. I risultati di questo studio mostrano che nei pazienti con BPCO di grado moderato è presente una disfunzione endoteliale non correlata ai classici fattori di rischio cardiovascolare. L'elevata prevalenza di disfunzione endoteliale può spiegare l'associazione epidemiologica tra BPCO e malattia cardiovascolare. Lo studio della funzione endoteliale, metodica non invasiva, rapida e di semplice esecuzione, può rappresentare uno strumento per identificare fra i soggetti BPCO asintomatici, quelli a più alto rischio di malattia cardiovascolare.

ECHOCARDIOGRAFIC AND ECO-DOPPLER ABNORMALITIES IN RELATION TO LDL CHOLESTEROL IN FAMILIAL HYPERCHOLESTEROLEMIA

M.G. Coppola[1], F. Jossa[1], M. Nunziata[1], M. Gentile[1], G. De Simone[1], M. De Marco[1], G. Fortunato[2,3], M.N. D'Agostino[2,3], M.D. Di Taranto[2,3], G. Marotta[1], P. Rubba[1]
[1]Department of Clinical and Experimental Medicine, "Federico II" University, Medical School, Naples, Italy; [2]CEINGE S.C.a r.l. Biotecnologie Avanzate, Napoli, Italy; [3]Dipartimento di Biochimica eBiotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli, Italy
E-mail: gabry.cop@libero.it

Familial hypercholesterolemia (FH) is an autosomal, codominant disease primarily caused by mutations in the LDL receptor(LDLR) gene. The aim of this study is to evaluate the impact of LDL-Chol levels on extension of subclinical CVD in FH. Baseline levels of LDL (before starting lipid-lowering therapy) were determined in 46 patients (61% women; 44±15 years) with clinical features of FH (Simon Broome Register criteria) without diabetes or other metabolic disorders. Mutation screening was performed in all patients and 37 result to be heterozygous, 4 compound heterozygous and 1 homozygous, confirming the FH diagnosis in 91% of patients. Patients were defined having higher LDL, when their LDL levels were higher than the median value (285 mg/dL) observed in the whole patient sample. All patients underwent standard transthoracic echocardiogram and carotid artery B-mode and Doppler ultrasound. In the whole sample, prevalent CVD was significant higher in patients with higher compared to those with lower LDL-Chol (48% vs 4%), independently of age (p=0.03). Patients with higher LDL-Chol had increased IMT, higher number of carotid plaques and more severe carotid stenosis (>50%), independently of age, gender hypertension and CVD (all p<0.02). Prevalence of aortic stenosis and severity of mitral or aortic valve calcification was higher in these patients, independently of age, gender and presence of hypertension (p<0.04), but this association was not statistically significant when history of CVD was also taken into account. In a FH population, elevated LDL-Chol levels are strongly and independently related to carotid atherosclerosis. Further studies should be carried out to clarify the association between LDL-Chol levels and cardiac valve abnormalities. Acknowledgements: CEINGE Convenzione Regione Campania, DGRC 1901/2009 and IRCCS Fondazione SDN.

ASSOCIAZIONE TRA PCR E SPESSORE INTIMA MEDIA CAROTIDEA NELLA PREVENZIONE PRIMARIA DELLE MALATTIE CARDIOVASCOLARI: STUDIO CONDOTTO PRESSO L'AMBULATORIO DEL MEDICO DI MEDICINA GENERALE

M. Coronelli, M. Framarin, E. Pavesi
Università degli Studi di Pavia, ASL 312 di Pavia, Italia
E-mail: maurizio.coronelli@fastwebnet.it

La medicina generale è quella disciplina medica che offre assistenza primaria,continua,completa e volta alla prevenzione. Le malattie cardiovascolari rivestono un ruolo di primaria importanza tra le patologie che più frequentemente si riscontrano negli ambulatori del medico di famiglia.

Sappiamo bene come fattori di rischio modificabili e non siano la causa più frequente di insorgenza di malattie cardiocerebrovascolari. Per questo motivo abbiamo intrapreso uno studio per verificare se la contemporanea presenza di Proteina C reattiva ultrasensibile e aumento dello spessore della intima-media carotidea abbiano una qualche relazione come markers predittivi della patologia cardio cerebro vascolare. Nel nostro ambulatorio di Medicina Generale affluiscono circa 1.650 pazienti. Nel periodo Ottobre 2010-Giugno 2011 abbiamo invitato i nostri pazienti a partecipare allo studio. Hanno aderito al progetto 123 soggetti e solo 101 sono stati dichiarati eleggibili.
Abbiamo diviso poi i soggetti eleggibili in 4 gruppi: ipertesi, diabetici, ipertesi-diabetici, apparentemente sani. Tutti sono stai sottoposti a prelievo per la determinazione della PCR Ultrasensibile e all'esame ecocolodoppler TSA per la misurazione dello spessore della intima-media carotidea al bulbo. Al termine della nostra indagini, abbiamo constatato come non esista una relazione specifica e marcata tra l'aumento della PCR e dell'intima media carotidea, in quanto solo nel 28% dei pazienti abbiamo riscontrato questo dato. Abbiamo, invece, notato come il 31% dei pazienti apparentemente sani abbiano presentato un aumento significativo dello spessore intima-media carotidea.
Questo dato ci porta a considerare come la prevenzione delle malattie cardiocerebro vascolari sia di primaria e fondamentale importanza e come il medico di medicina generale sia in grado di poter effettuare questa prevenzione nel proprio ambulatorio. Sarebbe quindi auspicabile una presa di coscienza maggiore da parte di tutti i medici di medicina generale verso il tema della prevenzione,in particolar modo della prevenzione delle malattie cardiocerebrovascolari.

CLINICAL GOVERNANCE DELL'IPERTENSIONE ARTERIOSA: RUOLO DEI MARKERS DI DANNO D'ORGANO IN MEDICINA GENERALE

M. Coronelli, M. Framarin
Università degli Studi di Pavia, ASL 312 di Pavia, Italia
E-mail: maurizio.coronelli@fastwebnet.it

L'ipertensione arteriosa essenziale rappresenta la patologia che più di ogni altra vede nel Medico di Medicina Generale la figura di riferimento per il paziente, dalla prevenzione alla diagnosi alla terapia ed alla gestione delle complicanze. L'ipertensione è in gran parte asintomatica, ma riveste un ruolo di primo piano in quanto capace di portare nel tempo ad un incremento del rischio cardiovascolare globale e quindi a complicanze cardio-cerebro-vascolari estremamente gravi (stroke, infarto del miocardio, ecc.). Tra l'esordio della malattia e le complicanze esiste tuttavia una fase intermedia, di solito della durata di anni, nella quale si sviluppa progressivamente un danno ai cosiddetti organi bersaglio: cuore, encefalo, vasi arteriosi periferici, reni, retina. Per questo motivo, quando viene diagnosticata l'ipertensione vanno immediatamente ricercati (oltre ad altri fattori di rischio e possibili cause secondarie) segni di danno d'organo, in quanto la presenza di quest'ultimo deve indirizzare verso un approccio più aggressivo per ritardare la comparsa delle complicanze.
L'attenzione della ricerca si sta sempre più focalizzando sulla scoperta di indicatori (o markers) in grado di svelare precocemente il danno d'organo, prima cioè che questo diventi clinicamente manifesto. La maggior parte delle tecniche di valutazione del danno d'organo precoce si adattano bene al setting della medicina del territorio e dovrebbero progressivamente entrare a far parte della

routine per il Medico di Medicina Generale. La ricerca di ipertrofia ventricolare sinistra con ECG e/o Ecocardiogramma, la misura dell'intima media thickness con ecocolordoppler dei tronchi sovraortici, la misura dell'indice pressorio caviglia-braccio, la stima del filtrato glomerulare mediante formula di Cockroft-Gault o MDRD e il dosaggio della microalbuminuria su campione estemporaneo di urine sono tecnicamente eseguibili senza un significativo incremento della spesa sanitaria e indubbiamente utili dal punto di vista clinico per guidare al meglio l'inquadramento ed il follow-up del paziente iperteso consentendo di ottimizzarne la terapia.
Documento di riferimento: Linee Guida 2007 per il trattamento dell'ipertensione arteriosa; ESH/ESC; Ipertensione prev. cardiovascolare. Sett 2007.

FATTORI DI RISCHIO E PATOLOGIA CARDIOVASCOLARE: COME PREVENIRLI CON UNA GESTIONE INTEGRATA DEL PAZIENTE NELL'AMBULATORIO DEL MEDICO DI MEDICINA GENERALE

M. Coronelli, I. Coronelli, M. Framarin, C. Villani
Università degli Studi di Pavia, ASL 312 di Pavia, Italia
E-mail: maurizio.coronelli@fastwebnet.it

Nel corso degli ultimi decenni abbiamo assistito, nel mondo, ad enormi cambiamenti. La forte incidenza di malattie e decessi causati da patologia cardio-cerebro-vascolare, l'aumento dei pazienti diabetici, ipertesi ed obesi, hanno evidenziato come abitudini di vita sedentarie e scarso fitness cardiorespiratorio rappresentano il problema di salute pubblica di maggior rilievo in molti paesi.
Una soluzione al problema sta nella cooperazione tra medici, psicologi, dietologi e personal trainer che sono coinvolti nella salute pubblica al fine di motivare e aiutare questi pazienti a diventare più attivi. Nel nostro ambulatorio abbiamo deciso di intraprendere questa strada fornendo ai pazienti diabetici, ipertesi, obesi e affetti da patologie cardiovascolari la consulenza medica del loro medico di famiglia, il supporto psicologico, l'ottimizzazione della dieta e un personal trainer in grado di promuovere l'adozione di uno stile di vita più attivo e personalizzato al paziente. Lo scopo che vogliamo raggiungere è quello di intervenire sui fattori di rischio modificabili, motivare il paziente e responsabilizzarlo e cercare di ridurre, in questo modo, l'insorgere ed il progredire delle malattie cardio-cerebro-vascolari.

KRP-206, A SELECTIVE S1P(1) AGONIST INHIBITS DEVELOPMENT OF ATHEROSCLEROSIS IN LOW-DENSITY LIPOPROTEIN RECEPTOR-DEFICIENT MICE

S. Costa[1], F. Poti[1], V. Bergonzini[1], H. Freise[2], G. Varga[3], M. Simoni[1], J.R. Nofer[1,4]
[1]Department of Medicine, Endocrinology, Metabolism and Geriatrics, University of Modena and Reggio Emilia, Modena, Italy; [2]Department of Anesthesiology and Intensive Care Medicine, University Hospital Münster, Germany; [3]Institute of Immunology, University of Münster, Germany; [4]Center for Laboratory Medicine, University Hospital Münster, Germany
E-mail: costa1sara@gmail.com

Background. Sphingosine 1-phosphate (S1P), a bioactive lysosphingolipid associated with high density lipoprotein (HDL), at least partly accounts for anti-atherogenic properties of this lipoprotein. We previously demonstrated that FTY720 - a synthetic S1P analog targeting all S1P receptors but S1P(2) inhibits development of atherosclerosis in a murine model of disease. The present study addressed the identity of S1P receptor mediating atheroprotective effects of S1P.
Methods and Results. LDL receptor-deficient mice on a cholesterol-rich diet were given KRP-203, a selective S1P(1) agonist, at a dose of 3.0 mg/kg/day for 14 weeks. KRP-203 substantially reduced atherosclerotic lesion formation both in aortic root and arteria thoracica.
Plasma lipids remained unchanged in course of KRP-203 treatment. However, KRP-203 induced marked peripheral blood lymphopenia, reduced total (CD4+, CD8+) and activated (CD69+/CD8+, CD69+/CD4+) T-cells in peripheral lymphoid organs, and interfered with lymphocyte function, as evidenced by decreased splenocyte proliferation and IL-2 and IFN-γ production; in response concanavalin A or phytohaemagglutinin as well as reduced RANTES levels in plasma.
Plasma concentrations of macrophage-derived cytokines TNF-α and IL-6 were reduced by KRP-206 administration. Moreover, peritoneal macrophages from KRP-206 treated mice showed reduced surface expression of activation markers MCH-II and CD86 as well as LPS-elicited production of TNF-α and IL-6. In vitro experiments demonstrated reduced production of TNF-α and IL-6 in LPS-stimulated and IP-10 in INF-γ-stimulated bone marrow macrophages.
Conclusions. Present results demonstrate that activation of S1P signaling pathways inhibit atherosclerosis by modulating lymphocyte and macrophage function and suggest that S1P(1) at least partly mediates anti-atherogenic effects of S1P.

IDENTIFICATION OF SIGNIFICANT SINGLE NUCLEOTIDE POLYMORPHISM (SNPS) IN ACUTE CORONARY SYNDROMES: A CANDIDATE GENE APPROACH

N. Cristell, F. Martinelli Boneschi, F. Ancona, G. Casari, M. Ferrari, A. Durante, G. Peretto, A. Laricchia, M. Spartera, A. Maseri, D. Cianflone on behalf of the FAMI Study investigators
San Raffaele Scientific Institute and Vita-Salute, San Raffaele University, Milan, Italy
E-mail: durante.alessandro@gmail.com

Background. Coronary artery disease (CAD) is a polygenic multifactorial disease with a wide spectrum of manifestations. Previous studies aimed to identify SNPs possibly associated with CAD, in large cohorts of clinically/phenotipically heterogeneous patients.
Materials and Methods. We studied an array of 33 SNPs potentially involved in the inflammatory, thrombotic and atherosclerotic pathways of well characterized and clinically homogeneous groups of patients, with no previous history of CAD: patients with their first STEMI (randomly extracted from the 1.099 patients of the FAMI [First Acute Myocardial Infarction] population) and NSTEMI, and matched controls. DNA was analyzed with SNPStream-BeckmanTMSystem array. For each SNP X2, Odds Ratio, P-value and Bonferroni Correction were calculated.
Results. We studied 75 patients with STEMI (79.49% males, median age 62), 55 patients with NSTEMI (67.19% males, median age 64) and 120 matched controls (70.20% males, median age 64). The allelic frequencies of Natriuretic Peptide Precursor A (rs5065, OR 2.807, p=0.0015) and Arachidonate 5-LipOXygenase Activating Protein (rs17222814, OR 4.11, p=0.0011) were significantly higher

in patients vs controls. We observed a different genetic profile in STEMI vs NSTEMI: rs5065 NPPA (OR 3.134 p=0.0011 vs OR 2.368 p=0.031), rs1800629 TNFA (OR 2.285 p=0.0080 vs OR 1.90 p=0.07),rs 17222814 ALOX5AP (OR 5.646 p=0.00007 vs OR 2.21 p=0.17),rs 1800795 IL6 (OR 1.781 p=0.0089 vs OR 1.52 p=0.09); and conversely in NSTEMI vs STEMI: rs1978331 LTA4H (OR 0.4669 p=0.0026 vs OR 0.76 p=0.21), rs2383206 chr9p21 (OR 0.5571 p=0.0169 vs OR 0.90 p=0.91), rs10507391 ALOX5AP (OR 0.5397 p=0.0166 vs OR 0.73 p=0.73).
Conclusion. The observed differences might be related to possible different prevailing pathogenic components of STEMI and NSTEMI. Our study differs from previous studies because of the distinct groups of patients, thus providing newer insights for future research in the development of a genetic risk score. Further validation on a larger population will be performed on the entire FAMI population.

ROLE OF THE LONG PENTRAXIN PTX3 IN ATHEROTROMBOSIS

L. Cutuli[1], S.S. Barbieri[4], P. Amadio[1], S. Gianellini[4], G. Porfirio[1], C. Garlanda[2], A. Mantovani[1], E. Tremoli[1], G.D. Norata[1,3], A.L. Catapano[1,3]
[1]Università degli Studi di Milano, Italy; [2]Istituto Clinico Humanitas, Rozzano (MI), Italia; [3]Centro per lo Studio dell'Aterosclerosi, Ospedale Bassini, Cinisello Balsamo (MI), Italia; [4]Centro Cardiologico Monzino, Milano, Italia
E-mail: lucia.cutuli@guest.unimi.it

Pentraxins are a superfamily of acute-phase proteins that include short pentraxins such as C-reactive protein, and long pentraxins such as PTX3, an essential component of the humoral arm of innate immunity. PTX3 in humans is a marker of atherosclerosis and correlates with the risk of cardiovascular events.
Mice lacking of PTX3 and apolipoprotein E fed a western-type diet develop larger atherosclerotic lesions compared to wild-type mice, and show a pronounced inflammatory profile in the vascular wall. Recently, it was shown that PTX3 selectively binds P-selectin thus inhibiting the leukocytes rolling process on endothelium. Here, we investigated the potential role of PTX3 in the thrombosis. PTX3 KO mice show a grater thrombus formation induced by carotid artery ferric chloride injury compared to wild-type mice, indeed, following the application of FeCl3, a 70% of reduction in carotid artery blood flow was observed in the PTX3 KO mice comparated to a 20% reduction observed in wild type mice ($p<0.01$).
Of note, the administration of human recombinant PTX3 strongly prevented the arterial thrombus formation induced by FeCl3, both in PTX3 KO and in wild-type mice, suggesting that PTX3 influences arterial thrombosis. In contrast, no differences between wild-type and PTX3 KO were observed after intravenous injection of collagen and epinephrine, a model of lung thrombo-embolism (disseminated thrombosis).
This suggest that PTX3 deletion does not predispose to thrombosis by direct modulation of platelets and/or coagulation factors. Indeed, the expression of adhesion molecules (e.g. P-selectin and integrin $\alpha 2\beta 3$), and platelets aggregation induced by collagen, thrombin o U46619 (an analogue of thromboxane), such as bleeding time and fibrinogen plasma levels were similar between the two groups of animals.
We can conclude that PTX3 regulates arterial thrombus formation, but further studies are needed to better understand of the molecular mechanisms underlying this event.

DIFFERENCES OF GENDER IN PERIPHERAL ARTERY DISEASE

A. Da Porto, V. Pavan, E. Fabbro, D. Gasparini, L.A. Sechi, A. Cavarape
Clinica Medica, Azienda Ospedaliero-Universitaria di Udine, Italia
E-mail: andrea_daporto@tin.it

Objectives. Atherosclerosis is a multifactorial disease and thus its clinical manifestations are likely to present gender specific differences, with respect to their development course symptom complexies and prognosis. The present study aimed to examine sex differences in peripheral arterial disease (PAD)its risk factors and clinical correlates.
Methods. PAD severity (clinical and angiographical), traditional and novel risk factors an comorbidities were valued in 382 men and 158 Women who underwent to limb percutaneous revascularization between years 2001-2010.
Results. Compared to men , women showed a higher prevalence of critical limb ischemia (P=0,007) and less frequently have involvement of iliac vessels (P<0,00001). Women tends to be older (P<0,0001), and more frequently obese (p=0,01), they show lower prevalence of smokers (p<0,0001) and patients with microalbuminuria (p=0,006). Coronary artery disease were more frequent in men (P<0,0001) such as prevalence of multivascular atherosclerosis (Contemporary coronary, carotid and peripheral artery disease p<0,0001). Futhermore women reveal higher levels of Apo A1, Lp(a), LDL and HDL but lower levels of TG (p<0,001). Among coagulation parameters women present higher levels of platelets, ATIII, Protein C Activated and lower levels of Protein S (p<0.0001). In Women but not in men Activated Protein C and ATIII correlates with Apo A (r=0,212 p 0,017; r=0,221 p 0,015).
Conclusions. In PAD there are many sex differences: women have more severe disease at presentation, less frequent involvement of iliac vessels, and less diffuse atherosclerosis compared to men. Many differences were found in the lipid profile and coagulation that appear to be more favourable in women. Knowledge of these sex specific differences may help to achieve an optimal gender specific cardiovascular risk prevention.

IDENTIFICATION OF LDLR MUTATIONS IN PATIENTS WITH FAMILIAL COMBINED HYPERLIPIDEMIA

M.N. D'Agostino[1,2], M.D. Di Taranto[3], E. Bloise[1,2], A. D'Angelo[1,2], I. Altieri[2], G. Marotta[2], M. Gentile[4], F. Jossa[4], A. Iannuzzi[5], P. Rubba[4], G. Fortunato[1,2]
[1]CEINGE S.C. a r.l. Biotecnologie Avanzate, Napoli, Italy; [2]Dipartimento di Biochimica e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli, Italy; [3]IRCCS Fondazione SDN, Napoli, Italy; [4]Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Napoli Federico II, Napoli, Italy; [5]Centro per la Diagnosi e la Terapia delle Dislipidemie Familiari, Azienda Ospedaliera Antonio Cardarelli, Napoli, Italy
E-mail: dagostino@dbbm.unina.it

Introduction. Familial Combined Hyperlipidemia (FCH) is associated with the increase of total cholesterol, triglycerides or both and has a polygenic and multifactorial basis not yet completely clarified. Familial Hypercholesterolemia (FH) is a disease characterized by high levels of total and LDL cholesterol, showing a clinical picture overlapping with that of FCH, in some instances. Mutations in the LDL receptor (LDLR) gene are the main cause

of FH. More than 1100 mutations in the LDLR have been reported although mutation clusters are present in different countries. We aim to identify LDLR mutations in patients with a clinical diagnosis of FCH.
Patients and Methods. Based on a clinical diagnosis, we enrolled 142 FH patients and 137 FCH patients from Southern Italy. The promoter and 18 exons of the LDLR gene were amplified by PCR and directly sequenced.
Results. Direct sequencing analysis revealed mutations in the LDLR gene in 106/142 unrelated FH patients (mutation rate 74.6%). Among the 41 different mutations found, 6 mutations (2 splicing alterations and 4 missense mutations) account for more than 50% of cases. We screened the exons with the most frequent mutations (hot spot exons) and found mutations in 13/137 patients (mutation rate 9.5%). Through the ROC curve analysis we identified the threshold values of LDL cholesterol (184.2 mg/dL) and ApoB (151 mg/dL), for selecting a group of FCH patients with an high mutation rate (20%).
Conclusions. The screening protocol based on the 6 hot spot exons allowed to identify 9.5% of FCH patients with a molecular diagnosis of FH. The evaluation of lipid parameters such as LDL cholesterol and ApoB is useful to select FCH patients with high probability of LDLR mutations.
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PATTERNS OF EXPRESSION OF NUCLEAR RECEPTORS IN PERIPHERAL MONONUCLEAR BLOOD CELLS OF PATIENTS WITH METABOLIC SYNDROME

S. D'Amore[1,2], M. Vacca[2], G. Graziano[2], A. D'Orazio[2], M. Cariello[3], R. Salvia[1], G. Grandaliano[4], A. Moschetta[1,2], G. Palasciano[1]
[1]Clinica Medica "Augusto Murri", University of Bari 'A. Moro', Italy; [2]Department of Translational Pharmacology, Consorzio Mario Negri Sud, Santa Maria Imbaro (CH), Italy; [3]Nephrology, Dialysis and Transplantation Unit Urology and Renal Transplant Unit, Department of Emergency and Organ Transplantation, University of Bari 'A. Moro', Italy; [4]Nephrology, Dialysis and Transplantation Unit, Department of Biomedical Sciences, University of Foggia, Italy
E-mail: simodamo@hotmail.it

Introduction. Nuclear receptors (NRs) are a class of 48 ligand-activated transcription factors identified as key players of metabolic and developmental events. Most of these receptors are potential target for pharmacological strategies in metabolic diseases, including the Metabolic Syndrome (MS). We present here a comprehensive atlas of expression of NRs in peripheral blood mononuclear cells (PBMCs) of patients with MS.
Methods. We enrolled 11 healthy controls (5F:6M) and 20 naïve patients (10F:10M; >3 criteria for MS upon Adult Treatment Panel III) without organ damage. We collected clinical and biochemical features, and blood samples for PBMCs isolation. Using quantitative real-time PCR (RTqPCR), we assessed the expression patterns of all members of the NR family in PBMCs.
Results. MS patients showed increased BMI, abdominal circumference, blood pressure, insulinemia/insulin-resistance, triglyceridemia, erythrocyte sedimentation rate (ESR), cardiovascular risk, and decreased levels of HDL cholesterol ($p<0.05$). 38/48 NRs where expressed in PBMCs.
We performed a statistical analysis with classification threes and correlation studies: several hits were identified and a predictive core of expression patterns was depicted for MS subjects. MS patients showed increased expression of peroxisome proliferator activated receptor α (PPAR α), liver X receptors α/β (LXR α/β), estrogen receptor-related receptor α (ERR α), and small heterodimer partner (SHP). On the other hand, the retinoic acid-related orphan nuclear receptor γ (RORγ) showed decreased expression. Interestingly, no changes were observed in VDR and LRH-1. Furthermore, intriguing correlations were found between PPAR α, LXR α and HDL cholesterol.
Conclusions. Our data show for the first time the expression chart of nutrient-/hormone-regulated NRs in PBMCs of MS patients. Future prospective studies are needed to support the role of NRs as predictive biomarkers of disease, as well as putative targets in the management of lipid disorders, inflammation and atherosclerosis.

RISCHIO CARDIOVASCOLARE RESIDUO NEI DIABETICI DOPO INFARTO MIOCARDICO ACUTO TRATTATO CON ANGIOPLASTICA PRIMARIA

A. De Monte[1], M. Chiuch[1], M. De Roia[2], G. Barbati[3], A. Perkan[2], G. Sinagra[2], L. Cattin[1]
[1]Dipartimento di Medicina Interna, Servizio Diabetologia, Azienda Ospedaliero-Universitaria di Trieste, Italia; [2]Dipartimento di Cardiologia, Azienda Ospedaliera-Universitaria di Trieste, Italia; [3]Università degli Studi di Trieste, Italia
E-mail: ariellademonte@alice.it

Introduzione. Il diabete mellito e le relative complicanze cardiovascolari sono in continuo aumento. La malattia coronarica è la più frequente causa di morte e di morbilità nei diabetici tipo 2. Dopo infarto miocardico acuto nei diabetici si manifesta un eccesso di mortalità. Le linee guida ESC/EASD raccomandano di trattare con angioplastica primaria i soggetti con infarto miocardico acuto ad ST sopraelevato (STEMI) con particolare attenzione ai diabetici. Scopo del lavoro è studiare l'impatto del diabete e dei fattori di rischio cardiovascolari ad esso associati sulla mortalità e sugli eventi cardiovascolari a 30 giorni e al follow up di sette anni dopo STEMI trattato con angioplastica coronarica primaria e con trattamento farmacologico ottimale.
Materiali e Metodi. Dal dicembre 2003 stiamo conducendo uno studio osservazionale su tutti i soggetti con STEMI trattati con angioplastica primaria nella UTIC della Cardiologia di Trieste. Per ogni soggetto abbiamo raccolto i dati anamnestici per fattori di rischio e storia cardiovascolare, la stratificazione del rischio cardiovascolare all'ingresso in ospedale, i dati coronarografici e della procedura di angioplastica, il decorso clinico intraospedaliero, la terapia in dimissione, i dati di mortalità e degli eventi cardiovascolari a 30 giorni e durante il follow up di sette anni. Tra gli esami di laboratorio abbiamo raccolto la HbA1c, l'assetto lipidico completo e la funzione renale prima e dopo angioplastica. Per l'analisi statistica abbiamo utilizzato il test ANOVA per le variabili continue a distribuzione di varianza omogenea tra i due gruppi ed il test robusto di Brown-Forsythe in alternativa. I dati sono stati sintetizzati come media e deviazione standard o con mediana e range interquartile. Per il confronto tra variabili discrete è stato utilizzato il test del Chi-quadro. La curva di sopravvivenza stratificata per presenza di diabete è stata stimata con il metodo di Kaplan-Meier, applicando il test Log-rank. Abbiamo applicato il modello di sopravvivenza multivariato ad azzardi proporzionali di Cox a una lista di variabili scelta in base a criteri clinici, sia di parametri cardiovascolari che metabolici e tramite procedura di selezione di tipo stepwise

backward-conditional abbiamo selezionato l'insieme di predittori indipendenti più significativi sulla prognosi.
Risultati. la nostra coorte comprende 794 soggetti di cui 192 (24%) con diabete noto o diagnosticato durante la degenza. I soggetti diabetici risultano più ipertesi (P≤0.0001) e con più arteriopatia degli arti inferiori (P≤0.007), si presentano clinicamente con una classe prognostica Killip più elevata (P≤0.001) e con frequenza cardiaca più elevata (P≤0.011); hanno trigliceridemia più elevata (P≤0.004) e più basse HDL(P≤0.027). Dopo rivascolarizzazione hanno più scompenso cardiaco (P≤0.0001), disfunzione ventricolare sinistra severa (P≤0.004) e shock (P≤0.012), creatininemia più elevata(P≤0.020). Entrambi i gruppi hanno ricevuto lo stesso trattamento riperfusivo meccanico e farmacologico anche alla dimissione.
Lo studio della curva di sopravvivenza indica che i diabetici hanno maggior mortalità sia intra che extraospedaliera (P≤0.001) a 7 anni, con amplificazione del fenomeno a lungo termine. Nella nostra coorte i predittori di morte dopo angioplastica risultano la classe Killip 3-4 (HR3.565; P≤0.0001) lo scompenso cardiaco (HR2.055; P≤0.011), l'età (HR1.301; P≤0.0001) e la creatinina (HR1.301; P≤0.0001).
Conclusioni. Nonostante un trattamento riperfusivo meccanico e farmacologico ottimale dello STEMI, i diabetici continuano ad avere un eccesso di mortalità sia nel breve che nel lungo termine, con un decorso intra ed extraospedaliero caratterizzato da maggiore instabilità emodinamica cardiorenale attribuibile a un rischio cardiovascolare residuo non azzerato dalla terapia medica. Numerosi studi hanno già confermato che il grado di insufficienza renale rappresenta un potente fattore di rischio cardiovascolare indipendente. Nel nostro campione il controllo glicemico non risulta predittivo di mortalità laddove emergono invece fattori cardiovascolari associati al diabete che probabilmente hanno agito per anni prima dell'infarto e che condizionano la prognosi anche dopo la loro correzione farmacologica. Il maggior sforzo terapeutico deve essere fatto quindi all'esordio del diabete non solo per mantenere un adeguato controllo glicemico ma per prevenire le alterazioni molecolari che condizionano la malattia cardiovascolare in modo indipendente dalla correzione dei fattori che le hanno determinate.

RUOLO PROGNOSTICO DI HDL NELL'INFARTO MIOCARDICO ACUTO CON ST SOPRASLIVELLATO

A. De Monte[1], G. Sinagra[2], G. Barbati[3], M. Chiuch[1], M. De Roia[2], A. Perkan[2], L. Cattin[1]
[1]Dipartimento di Medicina Interna, Azienda Ospedaliero-Universitaria di Trieste, Italia; [2]Dipartimento di Cardiologia, Azienda Ospedaliera-Universitaria di Trieste, Italia; [3]Università degli Studi di Trieste, Italia
E-mail: arielladem onte@alice.it

Introduzione. Le concentrazioni plasmatiche delle HDL sono inversamente correlate all'incidenza di eventi coronarici acuti.Il loro ruolo protettivo cardiovascolare deriva non solo dalla attività di trasporto del colesterolo ma anche dalle funzioni antinfiammatoria e antiossidante. Recenti studi indicano però che stimoli infiammatori acuti come l'infarto miocardico alterano le normali funzioni delle HDL indipendentemente dalla loro concentrazione plasmatica. L'end-point principale di questo studio è valutare il ruolo prognostico delle HDL sulla mortalità intra ed extra-ospedaliera nei soggetti con infarto miocardico acuto con ST sopraslivellato (STEMI). L'end-point secondario composito comprende gli eventi cardiovascolari (ictus, reinfarto o nuova angioplastica coronarica o by-pass aortocoronarico) nel follow-up di 7 anni.
Materiali e Metodi. Dal dicembre 2003 stiamo conducendo uno studio osservazionale su tutti i soggetti con STEMI trattati con angioplastica primaria nella UTIC della Cardiologia di Trieste. Per ogni soggetto abbiamo raccolto i dati anamnestici per fattori di rischio e storia cardiovascolare, la stratificazione del rischio cardiovascolare all'ingresso in ospedale, i dati coronarografici e della procedura di angioplastica, il decorso clinico intraospedaliero, la terapia in dimissione, i dati di mortalità e degli eventi cardiovascolari a 30 giorni e durante il follow up di sette anni. Per ogni soggetto sono stati rilevati in particolare colesterolemia tot., LDL, HDL e Trigliceridemia entro le prime 24 ore dall'ingresso in ospedale. Il target lipidico individuale è stato stabilito applicando lo SCORE (Systemic Coronary Risk Estimation) secondo le recenti linee guida ESC/EAS. Per l'analisi statistica abbiamo utilizzato il test ANOVA per le variabili continue a distribuzione di varianza omogenea tra i due gruppi ed il test robusto di Brown-Forsythe in alternativa. I dati sono stati sintetizzati come media e deviazione standard o con mediana e range interquartile. Per il confronto tra variabili discrete è stato utilizzato il test del Chi-quadro. Abbiamo applicato il modello di regressione logistica binaria uni e multivariato.
Risultati. La nostra coorte comprende 794 soggetti con STEMI, in prevalenza maschi (75%) di età media di 66±12 anni e i cui fattori di rischio prevalenti sono: ipertensione arteriosa (65%), fumo di sigaretta (50%) e diabete mellito (24%). Nel 30% dei casi vi è un'anamnesi positiva per angina e il 15% ha avuto un precedente infarto miocardico. Per quanto riguarda i valori lipidici mediani all'ingresso in ospedale: colesterolemia totale 191 (162;221) mg/dl, LDL 120 (95;147) mg/dl, HDL 43 (37;52) mg/dl, trigliceridemia 109 (83;150) mg/dl. Il target lipidico raccomandato dalle attuali linee guida risulta raggiunto nel 18% dei casi per LDL, nel 54% per HDL, nell'86% per la trigliceridemia. Il 39% dei casi di dislipidemia risultano misconosciuti. Le classi di rischio cardiovascolare sono significativamente associate alla mortalità extraospedaliera (p<0.001) ma perdono la significatività sulla morte intraospedaliera. A parità di rischio cardiovascolare l'aumento di HDL si associa ad un maggior di rischio di mortalità (p=0.04), mentre non rileviamo effetti significativi di HDL sull'end-point secondario composito. Inoltre a parità di rischio cardiovascolare e di valori di LDL, HDL conferma l'effetto negativo sul rischio di mortalità (p=0.005). Nella fase acuta dell'infarto HDL diventa un fattore di rischio per mortalità intraospedaliera dove LDL assume invece un ruolo protettivo. Tali effetti si esauriscono nella fase extraospedaliera .Confrontando i parametri metabolici con i parametri di gravità dell'infarto riscontriamo nella nostra coorte una correlazione positiva significativa tra HDL e il valore massimo CPK-MB (Spearman's rho=0.1, p=0.01); una correlazione significativa e negativa tra classe Killip e LDL (rho=0.1, p=0.01).
Conclusioni. Il principale risultato che emerge dal nostro studio è che in corso di STEMI le HDL perdono l'effetto protettivo cardiovascolare, laddove le LDL risultano assumere invece una funzione protettiva. Queste modificazioni sono temporalmente e quantitativamente in relazione alla flogosi acuta determinata dal danno miocardico, che comporta una alterazione strutturale e quindi funzionale delle HDL e una rapida caduta delle concentrazioni di LDL nelle prime 24-48 ore dallo STEMI.
Le concentrazioni plasmatiche di HDL non possono quindi essere utilizzate nello score di classificazione del rischio cardiovascolare in corso di STEMI, se non per la classe di rischio più elevato che non considera fattori metabolici.
Nello STEMI i fattori metabolici non sono indipendenti ma variano con il grado di flogosi acuta e quindi con l'estensione dell'area infartuale.

A SILENT MUTATION OF NPC1L1 MODULATES CHOLESTEROL ABSORPTION IN PRIMARY HYPERLIPEMIAS

S. De Vuono, G. Lupattelli, L. Pisciotta[1], D. Siepi, A. Bellocchio[1], F. Melis, M.R. Mannarino, S. Bertolini[1], E. Mannarino
Department of Clinical and Experimental Medicine, University of Perugia, Italy; [1]Department of Internal Medicine, University of Genoa, Italy
E-mail: insect3@tiscali.it

Cholesterol absorption efficiency differs among individuals and different hyperlipemias; non-cholesterol sterols, which reflect cholesterol absorption, are influenced by diets rich in plant sterols or genetic variants of enzyme/receptors involved in this process: Nieman Pick C1 Like 1 (NPC1L1), ATP Binding Cassette G5 (ABCG5), apo E.
The G allele of c.816 C>G polymorphism (L272L) of NPC1L1 influences the response to ezetimibe, probably related to higher cholesterol absorption, in patients with familial hypercholesterolemia; the role of apoprotein E on cholesterol absorption is still controversial.
Our purpose was to investigate the role of the silent mutation c.816 C>G (L272L) of NPC1L1 and of apo E alleles on cholesterol absorption in subjects with primary hyperlipemias. In 80 patients with primary hyperlipemias we determined lipid profile, sitosterol, campesterol and lathosterol, apoprotein A1 and apoprotein B. Patients were also genotyped for NPC1L1 gene and for APOE polymorphism.
NPC1L1 variant genotype distribution was as follows: 45 patients were CC, 33 were CG, 2 patients were GG. After stratification for the presence of G allele, patients with G allele showed significantly higher concentrations of campesterol (1.84±0.3 vs 1.59±0.2 102μ; mol/mmol cholesterol, p.001) and sitosterol (2.03±0.2 vs 1.94±0.2 102 μmol/mmol cholesterol, p<.05).
The ratio between lathosterol/campesterol, a variable reflecting synthesis and absorption simultaneously, was significantly lower in the group with G allele (1.87±1.4 vs 2.8±1.8, p 0.11). Apo E alleles were distributed as follows: 10 patients were E2E3, 48 E3E3, 21 E3E4, one E4E4.
Patients with at least one E4 allele showed values of campesterol and sitosterol higher than those carrying E3E3 or E3E2, with a nearly significantly difference. Stepwise regression analysis showed G allele (β=.379, p<0.01, p of the model <0.001)). and HDL-Cholesterol (β=.309, p=0.05, p of the model <0.001) as independent predictors of campesterol values. HDL-C was the only independent predictor of sitosterol values (β=.290, p=0.12, p of the model <0.001).
The silent mutation c.816 C>G (L272L) of NPC1L1 plays a central role in the multistep regulation of cholesterol absorption as soon as carriers of this mutation have higher cholesterol absorption and this polymorphism is an independent predictor of cholesterol absorption.
This polymorphism could account for the inter-individual variability in cholesterol absorption and for the therapeutic response also in subjects with primary hyperlipemias.

LDL CHOLESTEROL GOAL ATTAINMENT IN REAL PRACTICE. FINDINGS FROM THE STAR STUDY (STATINS TARGET ASSESSMENT IN REAL PRACTICE)

L. Degli Esposti[1], D. Sangiorgi[1], M. Arca[2], G.B. Vigna[3], S. Buda[1]
[1]CliCon S.r.l. Health, Economics and Outcomes Research, Ravenna; [2]Centro Aterosclerosi, Dipartimento di Medicina Interna e Specialità Mediche, Università Sapienza, Roma, Italia; [3]Medicina Interna Universitaria, Az. Ospedaliera di Ferrara, Italia
E-mail: luca.degliesposti@unife.it

Introduction. The objective of this study was to analyze LDL cholesterol goal attainment in newly treated patients with statins and with a gap to LDL cholesterol goal ≥50%.
Material and Methods. A retrospective observational study including 5 Local Health Units (LHUs) was conducted. Administrative databases were linked to laboratory tests databases in order to collect cholesterol values. Patients were included if they had filled at least one prescription for statins between January 1st, 2007 and June 30th, 2008, if they had a gap to LDL cholesterol goal ≥50% and if they had no prescriptions of statins during the 12 months before the first prescription in the accrual period; patients had been followed for 12 months.
Results. A total of 912 patients had a gap to target LDL cholesterol ≥50% (28.2% of newly treated patients with statins), (age 63.2±11.8, male 39%). Mostly prescribed statins were simvastatin (36.5%), atorvastatin (23.7%), and rosuvastatin (23.6%). Among those treated with atorvastatin or rosuvastatin, only 17.6% were prescribed for recommended dosages (40 or 80 mg for atorvastatin, 20 or 40 mg for rosuvastatin). Statins with highest proportion of LDL cholesterol goal attainment were rosuvastatin (14.9% of treated patients), simvastatin+ezetimibe (13.0%), atorvastatin (8.8%) and simvastatin (3.9%). When only adherent patients were considered, percentages were 28.6%, 14.3%, 14.3% and 3.4%, respectively. At the multivariable logistic regression analysis, only significant predictors of LDL cholesterol goal attainment were rosuvastatin use (OR=4.95 compared to simvastatin, 95%CI 2.42-10.13) and adherence >80% (OR=1.81, 95%CI 1.04-3.15). Among patients treated with highly-effective therapies, those maintaining same therapy and dosage were 76% for rosuvastatin 20 mg and 41% for atorvastatin 80 mg and average LDL-cholesterol reduction was 52.0% for rosuvastatin 20mg and 41.6% for atorvastatin 40 mg.
Conclusions. In real practice setting, the percentage of patients with a gap to LDL cholesterol goal ≥50% is relevant and not adequately treated since only few patients are prescribed for recommended statins and dosages (8.3%). In these highest cardiovascular risk patients, rosuvastatin use and adherence with treatment are key factors in goal attainment.
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AMP-ACTIVATED PROTEIN KINASE GENETIC VARIANTS AND THE RISK OF MYOCARDIAL INFARCTION IN THE STOCKHOLM HEART EPIDEMIOLOGY PROGRAM

R. Del Giorno, I. Moreno Velasquez[1], P. Frumento[2], M. Vikström[1], K. Leander[1], M. Pirro, M.R. Mannarino, E. Mannarino, U. de Faire[1], B. Gigante[1]
[1]Unit of Internal Medicine, Angiology and Arteriosclerosis Diseases, University of Perugia, Italy; [2]Division of Cardiovascular Epidemiology and Unit of Biostatistics, Institute of Enviromental Medicine, Karolinska Institutet, Stockholm, Sweden
E-mail: delgiornorosaria@yahoo.it

Introduction. AMP-activated protein kinase (AMPK) has an important role in balancing energy demand and supply through the activation of catabolic pathways and the inhibition of anabolic pathways. Acting on multiple cellular targets, activation of AMPK influences blood glucose homeostasis, lipid profile, and blood pressure. We have investigated the role of AMPK genetic variants in the occurrence of myocardial infarction (MI) in the Stockholm Heart Epidemiology Program (SHEEP).
Methods. Thirteen SNPs, two of the α-catalytic subunit, eleven of the γ 2 and β1 non catalytic subunits, within the AMPK loci were genotyped in the SHEEP (n=2,774). SHEEP was designed as a population (n=2774) case-control study (1213 cases; 1561 sex and age-matched controls) to dissect both genetic and environmental factors underlying the occurrence of myocardial infarction (MI) in men and women. A logistic regression was runned for obtaining the odds ratios, adjusting for age, sex and residential area in the crude estimate, and for age, sex, BMI, smoking, hypertension, hypercholesterolemia and diabetes mellitus in the adjusted model.
Results. Three genetic variants mapping in the γ 2 non-catalytic subunit (rs7806619; rs17643028; rs2538038) were associated with a reduction or with a significant increase in MI risk. Specifically, rs7806619 was associated with an odds ratio (OR) (95% confidence interval [CI]) of 0.72 (0.52-0.99) in a dominant adjusted model, rs17643028 with an OR (95%CI) of 3.17 (1.2-8.5) in a recessive adjusted model, and rs2538038 with an OR (95%CI) of 1.16 (1.01-1.33) in an additive adjusted model.
Conclusions. Our preliminary results demonstrate that AMPK genetic variants may have an influence on the risk of MI, although the mechanism underlying this association remains still to be defined. A further understanding of the association between AMPK genetic variants and MI risk might arise from the analysis of haplotypes and their interaction with cardiovascular risk factors.

URIC ACID LEVELS IN METABOLIC SYNDROME, TYPE 2 DIABETES AND FAMILIAL COMBINED HYPERLIPIDEMIA: A COMPARISON

L. D'Erasmo, E. Grieco, P. Coletta, G. Barbarossa, A. Renzi, A. Gallo, C. Favoccia, G. Pigna, A. Montali, M. Colotto, S. Da Ros, F. Vinci, D. Pergolini, M. Santilli, M. Rossetti, E. Mandosi, M. Fallarino, S. Morano, M. Arca, M. Maranghi
Dipartimento di Medicina Interna e Specialità Mediche, Sapienza Università di Roma, Italia
E-mail: derasmolaura@gmail.com

Background and aims. Hyperuricemia-associated increased cardiovascular risk has been identified in epidemiological studies. Interestingly, this relationship has been also observed for uric acid levels in the high-normal range (5.2-5.5 mg/dl) after correction for age, male gender and antihypertensive therapy. Furthermore, a positive association between hyperinsulinemia and elevated uric acid levels has been shown.
Metabolic Syndrome (MS), Type 2 Diabetes Mellitus (T2DM) and Familial Combined Hyperlipidemia (FCHL) are high prevalent diseases characterized by insulin resistance, hyperinsulinemia and elevated cardiovascular risk. At present there are limited data about the prevalence of hyperuricemia in these three conditions and its role as cardiovascular risk factor.
The aim of our study was to compare uric acid levels and the prevalence of hyperuricemia in subjects with MS, T2DM and FCHL according to gender.
Material and Methods. Consecutively recruited 422 outpatients: 244 subjects with MS (145 males, 99 females), 112 with T2DM (72 males, 40 females) and 66 with FCHL (45 males, 21 females).
Anthropometric and clinical parameters were measured and information on medication use was obtained by self-report. Venous blood samples were drawn after an overnight fast.
Uric acid was measured by standard assays. Subjects with glycated hemoglobin (HbA1c) of more than 9% and with previous cardiovascular disease were excluded.
Hyperuricemia was defined as serum uric acid >7 mg/dl among male and >6 mg/dl among female. Data are expressed as mean ± SD for normally distributed variables and as frequency for categorical variables. Non-normally distributed variables were log transformed.
The ANOVA analysis was used for comparison between groups and a generalized linear model was performed for multivariate analysis (adjusting for age, sex, log BMI, log triglycerides, glomerular filtration rate [GFR] and use of antihypertensive therapy). Pearson's Chi square test was used for comparison frequency distribution between groups. A p value <0.05 was considered significant.
Results. Mean age was 51.01 yrs (±10.96 SD), 59.35 yrs (±10.34 SD) and 48.24 yrs (±9.9 SD) in MS, T2DM and FCHL subjects respectively [48.58 (±10.43), 58.14 (±11.23) and 45.71 (±8.45) among males; 54.57 (±10.81), 61.53 (±8.19) and 53.67 (±10.79) among females].
Age and BMI were significantly lower in FCHL subjects as compared to MS and T2DM. Mean serum uric acid levels was 5.65 mg/dl (±1.37 SD) in MS subjects [6.22 (±1.28) among males and 4.82 (±1.05) among females]; 5.66 mg/dl (±1.2 SD) in FCHL subjects [6.03 (±0.94) among males and 4.89 (±1.33) among females] and 5.72 mg/dl (±1.34 SD) in T2DM subjects [5.93 (±1.23) among males and 5.34 (±1.45) among females].
In the multivariate analysis uric acid levels were not different between the three groups (adjusted p=0.06). Similar results were found in the female group and in the male one (female p=0.067 and male p=0.43).
Hyperuricemia was present in 19.7% of patients with MS, in 20.5% of T2DM patients and in 18.2% of FCHL patients. When compared according to gender, the prevalence of hyperuricemia between groups was significantly different among females (X2=7.37 p=0.025), but not in males (p=0.135).
Conclusion. In our pilot study a tendency towards higher uric acid levels and higher prevalence of hyperuricemia was observed among female diabetic patients as compared to subjects with MS and FCHL.

QUERCETIN-3-O-GLUCURONIDE AFFECTS GENE EXPRESSION PROFILE OF M1 AND M2A MACROPHAGES EXHIBITING ANTI-INFLAMMATORY EFFECTS

E. Derlindati[1], M. Dall'Asta[2], D. Ardigò[1], I. Zavaroni[1], F. Brighenti[2], D. Del Rio[2]
[1]Department of Internal Medicine and Biomedical Science, University of Parma; [2]The ϕ Laboratory of Phytochemicals in Physiology, Human Nutrition Unit, Department of Public Health, University of Parma, Italy
E-mail: derlindati.ele@libero.it

Background. Macrophages are an heterogeneous cell population that plays a central role in atherogenesis. Classical macrophage activation (M1), induced by IFN γ and LPS, exerts inflammatory activities, while alternative activation (M2a) is promoted by IL-4 and/or IL-13 and shows immunoregulatory properties. Quercetin is an ubiquitous plant-derived flavonoid with antioxidant and anti-inflammatory properties.
Quercetin-3-O-glucuronide, derived from phase II metabolism, is the main circulating form in humans, after consumption of quercetin, but it is still unknown to what extent this metabolite affect macrophages functions.
Aim. Evaluation of the effects induced by quercetin-3-O-glucuronide at physiological concentrations on macrophages activation, trough the investigation of whole human gene expression profile.
Material and Methods. Quercetin-3-O-glucuronide (500 nmol/L) was added to M1 and M2a stimulated macrophages. Resting macrophages (no stimuli) were used as a control. A transcriptome analysis using whole genome dual color microarray technology (Agilent technologies) was applied for each condition. Gene ontology (GO) and pathway analysis were performed using Gorilla (http://cbl-gorilla.cs.technion.ac.il/) and David Functional Annotation Tools (http://david.abcc.ncifcrf.gov/).
Results. The presence of quercetin-3-O-glucuronide on M1 slightly reduced the expression of inflammatory GO terms (immune system process, p-value = 4.26E-7), maintaining however their inflammatory properties. Differently, M2a macrophages with quercetin-3-O-glucuronide significantly reduced the expression of inflammatory GO terms (cellular response to type I interferon, p-value: 4.07E-20; cytokine-mediated signaling pathway, p-value: 9.01E-18), also compared to M2a alone. In both cases, the addiction of quercetin-3-O-glucuronide was associated to a down-regulation of cell cycle related GO terms (M phase, p-value: 5.48E-10).
Discussion. Quercetin-3-O-glucuronide appeared to exert anti-inflammatory effects synergistically with IL-4. Moreover, the small reduction in inflammatory GO observed in M1 showed that the anti-inflammatory properties of quercetin-3-O-glucuronide does not affect the role of these cells in the immune response. The significant down regulation of cell cycle GO terms suggested a role of quercetin-3-O-glucuronide in regulation of macrophage differentiation that deserves further investigation.

INFLUENCE OF 25-OH-VITAMIN D AND PARATHYROID HORMONE LEVELS ON ARTERIAL STIFFNESS IN POSTMENOPAUSAL WOMEN WITH EITHER VITAMIN D INSUFFICIENCY OR DEFICIENCY

F. Di Filippo, M. Pirro, M.R. Manfredelli, M.R. Mannarino, R.S. Helou, G. Schillaci, A.M. Scarponi, E. Mannarino
Unit of Internal Medicine, Angiology and Arteriosclerosis Diseases, University of Perugia, Italy
E-mail: francescodifilippo@hotmail.com

Introduction. Vitamin D insufficiency and increased parathyroid hormone (PTH) levels have been suggested as prognostic indices for cardiovascular disease.
Arterial stiffness, a surrogate marker for cardiovascular disease, is often increased in patients with primary hyperparathyroidism. PTH levels increase in patients with low 25-OH-vitamin D levels, but the influence of such an increase on arterial stiffness has not been investigated in postmenopausal women with reduced 25-OH-vitamin D levels.
We investigated the association between PTH and aortic stiffness in postmenopausal women with reduced 25-OH-vitamin D levels.
Methods. In a cross-sectional study, 150 postmenopausal women with either 25-OH-vitamin D insufficiency (10-30 ng/mL) or deficiency (<10 ng/mL) were compared. Aortic pulse wave velocity (aPWV), a measure of arterial stiffness, PTH and 25-OH-vitamin D levels were measured.
Assessment of traditional cardiovascular risk factors and of markers of bone formation was evaluated.
Results. 25-OH-vitamin D levels were negatively associated with aPWV (rho=-0.23, p=0.006), but the association was not significant when controlling for PTH levels.
Other significant correlates of aPWV included age, body mass index, mean arterial pressure and PTH (rho=0.39, p<0.001). Arterial stiffness was positively and significantly predicted by PTH levels (b=0.23, p=0.007), independent of traditional cardiovascular risk factors and factors involved in bone formation. Increased PTH levels (>62 pg/mL) were associated with a 3.0-5.4-fold increased probability of having mild-severe increase in aortic stiffness, irrespective of confounders.
Conclusions. Among postmenopausal women with reduced 25-OH-vitamin D levels, elevated PTH levels were a significant predictor of aortic stiffness, irrespective of cardiovascular risk factors and of factors involved in bone formation. Also, PTH accounted for the association between 25-OH-vitamin D levels and aortic stiffness.

SNP ASSOCIATED TO FAMILIAL COMBINED HYPERLIPIDEMIA: APPLICATION OF AN ENSEMBLE OF DECISION TREES PROTOCOL

M.D. Di Taranto[1], E. Bloise[2], M.N. D'Agostino[2,3], A. D'Angelo[2,3], G. Marotta[4], M. Gentile[4], F. Jossa[4], A. Iannuzzi[5], P. Rubba[4], G. Fortunato[2,3], A. Staiano[6]
[1]IRCCS Fondazione SDN, Napoli, Italy; [2]CEINGE S.C. a r.l. Biotecnologie Avanzate, Napoli, Italy; [3]Dipartimento di Biochimica e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli, Italy; [4]Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Napoli Federico II, Napoli, Italy; [5]Centro per la Diagnosi e la Terapia delle Dislipidemie Familiari, Azienda Ospedaliera Antonio Cardarelli, Napoli, Italy; [6]Dipartimento di Scienze Applicate, Università degli Studi di Napoli Parthenope, Italy
E-mail: ditaranto@dbbm.unina.it

Introduction. Familial Combined Hyperlipidemia (FCH) is characterized by the increase of total cholesterol, triglycerides or both. Its molecular causes have not yet completely clarified although a polygenic and multifactorial basis is considered. Genes encoding enzymes involved in lipid metabolism, apolipoproteins or transcription factors are candidate genes for the association with FCH.
In order to identify susceptibility markers for FCH we perform the analysis of 22 Single Nucleotide Polymorphisms (SNP) in 10 genes associated with high cardiovascular risk (such as Lipoprotein Lipase, Cholesteryl ester transfer protein, HMG-CoA reductase, PCSK9, ApoA5, ApoC3).
Materials and Methods. We enrolled 123 patients with a clinical diagnosis of FCH (after exclusion of misdiagnosis of familial hypercholesterolemia) and 142 healthy controls. After genomic DNA extraction from peripheral blood samples, the TaqMan assay was performed for the SNP typing.
We then analysed the data through a wrapper feature selection procedure using an ensemble learning paradigm based on a set of 150 Decision Trees (DT) combined by bagging. In this way we found a subset of SNPs localized in different genes and their association with FCH.
Results. Four out of ten genes were identified as containing 6 out of 22 significant SNPs in predicting the presence of FCH: ApoA5 (S19W and -1131T>C), PCSK9 (25467958T>C and R46L), GJA4 (P319S), USF1 (9996G>A).
After the feature selection procedure, the ensemble of DT was used to predict the presence of FCH, obtaining a global prediction accuracy of 67%.
Conclusions. The ensemble of bagged DT appears to be a useful tool in identifying gene polymorphisms involved in FCH and also efficient from the computational point of view. This modern machine learning approach confirms the role of the above mentioned SNP typically evaluated by other elaboration methods, such as haplotype analysis.
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IN NON DIABETIC PATIENTS WITH ACUTE CORONARY SYNDROMES FATTY LIVER DISEASE IS ASSOCIATED TO MULTIVESSEL CORONARY ARTERY DISEASE

S. Dolenti[1], R. Tarquini[2], M. Chiostri[1], G. Kadiu[2], F. Marra[2], S. Valente[3], C. Giglioli[3], G.F. Gensini[3,4], R. Abbate[1], M. Boddi[1]
[1]Department of Medical and Surgical Critical Care, Florence, Italy; [2]Department of Internal Medicine, University of Florence, Italy; [3]Intensive Cardiac Coronary Unit, Heart and Vessel Department, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy; [4]Don Carlo Gnocchi Foundation, IRCCS, Florence, Italy
E-mail: maria.boddi@unifi.it

Introduction. Non-alcoholic fatty liver disease (NAFLD) is considered a relevant risk factor for atherosclerosis, but its contribution to acute coronary syndromes (ACS) is unclear. In this study, we investigated the prevalence of NAFLD in non-diabetic patients with ACS and its possible role as an independent risk factor.
Methods. Forty-six consecutive non-diabetic patients admitted to a cardiac ICU for ST-segment elevation myocardial infarction (STEMI) were studied by liver ultrasound within 72 hours from admission. All had glycated hemoglobin <6.5%. The presence of NAFLD was evaluated and graded according to a semiquantitative severity score and classified as mild (score <3) or moderate-severe (≥3 score). We evaluated the prevalence of other cardiovascular (CV) risk factors and the presence of the metabolic syndrome (MS) on the basis of the most recent revision of the National Cholesterol Education Program Adult Treatment panel III (ATP III).
Results. Twenty-three patients showed moderate-severe NAFLD (SFL group). Seventeen patients had mild NAFLD and 6 patients showed no NAFLD (all belonging to MFL group). Patients of SFL group were younger (59±12 vs 66±10 years, $p<0.01$) and showed higher prevalence of multi-vessel (i.e. ≥2) coronary artery disease (CAD) than patients of MFL group (75 vs 45%, $p<0.01$). Conventional CV risk factors did not differ between the 2 groups. SFL patients also showed higher triglyceride levels and a higher prevalence of MS ($p<0.05$). Among components of the MS, waist circumference showed the strongest association with SFL ($p<0.05$). At logistic regression analysis SFL was associated with a 4-fold risk of multi-vessel CAD, even after adjustment for age, presence of MS, triglyceride levels and waist circumference at bivariate analyses.
Conclusions. The presence of SFL independently identifies a subgroup of non-diabetic patients with STEMI who are younger and with a higher prevalence of multi-vessel CAD.

INFLUENCE OF OLEIC ACID ON VASCULAR ENDOTHELIAL GROWTH FACTOR SYNTHESIS AND SECRETION IN AORTIC VASCULAR SMOOTH MUSCLE CELLS: SIGNALLING MOLECULES INVOLVED AND ROLE INSULIN RESISTANCE

G. Doronzo, M. Viretto, I. Russo, L. Mattiello, C. Barale, E. Ceraolo, G. Anfossi, M. Trovati
Internal Medicine and Metabolic Disease Unit, Dept. of Clinical and Biological Sciences, S. Luigi Gonzaga Faculty of Medicine of the Turin University, S. Luigi Gonzaga Hospital, Orbassano, Turin, Italy
E-mail: gabriella.doronzo@unito.it

Introduction. Impaired collateral vessel formation occurs in diabetes, a condition characterized by both defective insulin secretion

and insulin resistance. The role of insulin resistance in this phenomenon is poorly clarified as yet. Vascular Endothelial Growth Factor (VEGF) plays a major role in the arteriogenetic process. It is regulated not only by hypoxia but also by hormones and metabolites. We previously observed that insulin activates VEGF synthesis and secretion in arterial Vascular Smooth Muscle Cells (VSMC) via the interplay between PI3-K and MAPK signalling pathways, and that this effect is deeply impaired in conditions of insulin resistance.
Among the molecules able to induce insulin resistance, a key role is played by Free Fatty Acids (FFA), since they inhibit insulin signalling at different steps. At this purpose, a peculiar role is played by oleic acid: this mono-unsaturated FFA is the more represented in blood, with concentrations increased in the insulin resistant states, and in particular in obesity. Aim of the present study is to evaluate:
a) whether oleic acid influences VEGF synthesis and secretion occurring in VSMC and whether PI3-K and MAPK signalling molecules are involved in this phenomenon;
b) whether this putative effect of oleic acid interferes with the similar action exerted by insulin and is modified by the presence of insulin resistance.

Materials and Methods. The study has been carried out in VSMC obtained as primary culture in our laboratory from aortas of insulin sensitive lean Zucker fa/+ rats and of insulin resistant obese Zucker fa/fa rats, a classical model of obesity-related insulin resistance. In these VSMC, we measured the influence of a 24-hour incubation with 50-100 microM oleic acid and/or with 2 nM insulin on VEGF synthesis and secretion (western immunoblotting and ELISA) (n=6). To evaluate the signalling molecules involved in the putative effects of oleic acid, experiments were repeated after 60 min pre-incubation with:
1. LY294002 (50 microM) and wortmannin (100 nM), specific PI3-K inhibitors;
2. rapamycin (100 nM), a specific inhibitor of mTOR, a molecule activated also (but not only) by Akt;
3. specific inhibitors of molecules of the MAPK pathway, i.e.
a) PD98059 (30 microM), inhibitor of ERK-1/2;
b) SP600125 (15 microM), inhibitor of JNK-1/2;
c) SB203580 (10 microM), inhibitor of p38 MAPK.

Results are expressed as mean±SEM.

Results. In aortic VSMC from lean insulin sensitive Zucker fa/+ rats, oleic acid 50-100 microM:
1. dose-dependently increased VEGF synthesis and secretion (p=0.003-0.0001);
2. did not modify the influence of insulin on VEGF synthesis and secretion.

Furthermore, in VSMC from Zucker fa/+ rats the effects of oleic acid on both synthesis and secretion of VEGF:
a) are mediated by PI3-K/Akt-pathway activation, being blunted by LY294002 and wortmannin (p=0.04-0.0001), and are partially modulated by mTOR, being reduced - but not blunted - by rapamycin;
b) are mediated by ERK-1/2 and JNK 1/2 activation, being blunted by PD98059 and SP600125 (p=0.05-0.027), and are partially modulated by p38MAPK, being reduced - but not blunted - by SB203580.

In aortic VSMC from obese Zucker rats oleic acid did not increase synthesis and secretion of VEGF.

Conclusions. This study demonstrates that oleic acid stimulates VEGF synthesis and secretion in aortic VSMC from lean, insulin-sensitive Zucker rats with a mechanism mediated by molecules of PI3-K/Akt and MAPK pathways, without interfering with the similar action exerted by insulin. The effects of oleic acid are lost in aortic VSMC from obese, insulin resistant Zucker rats, that are therefore resistant to the effects of both insulin and oleic acid as far as VEGF synthesis and secretion in VSMC is concerned. These results provide a further piece of information concerning the interplay between insulin resistance and molecules involved in vascular damage and repair.

ELECTROCARDIOGRAM AND ON-LINE CARDIOLOGIST IN THE PHARMACIES, AN ADVANCE IN TELECARDIOLOGY

A. Durante, A. Laricchia, G. Peretto, F. Ancona, M. Spartera, A. Mason, A. Stefani, A. Sichera, S. Bersani, D. Cianflone
San Raffaele Scientific Institute, Vita-Salute San Raffaele University and Centro Servizi Telemedicina, Telbios
E-mail: durante.alessandro@gmail.com

Introduction. The idea of a "multi-service pharmacy" is slowly spreading among people thanks to the availability of variable services including tele-managed ECG recording. We evaluated referral reason, cardiovascular risk factors profile and clinical history of patients who referred to pharmacies to record an ECG, managed with our cardiological support.

Materials and Methods. We prospectively evaluated 891 consecutive patients (pts) from September 2009 to August 2011. They presented to one out of 24 partecipating pharmacies to record an ECG. ECG were recorded by a 12-lead, 10-wire tele-ECG device HeartView 12L. They were transmitted trans-telephonically to the service center. We recorded the referral reason, clinical history and risk factors (RF) of each patient through a questionnaire (Patient Health Record - PHR). The remote cardiologist spoke with the patient to integrate the ECG findings in the clinical context.

Results. We divided patients into 2 groups according to the referral reason: 89% were control ECG (Gr.A) while the remaining recorded the ECG for an ongoing symptom (Gr.B). Most frequent complaints were: palpitations (29), chest pain (29), and dyspnoea (12) followed by other symptoms (25). Median age was lower in Gr.A (45.72) as compared to Gr.B (51.88), $p<0.05$, whereas sex distribution was similar among groups for men and women. In Gr.A 8.5% of pts reported a previous cardiovascular disease (CVD), vs 26.3% of pts in Gr.B

Discussion. Most of the pts recorded an ECG as a control. While 90.8% of patients were reassured, 9.2% were referred to a medical evaluation (10% of patients in Gr.B were suggested to go an emergency department). These data indicate that the use of an integrated telecare delivery system is a quick diagnostic and monitoring tool, but also an attractive mean for screening in an apparently health population.

NOVEL MUTATIONS IN SAR1B AND MTP GENES IN CHYLOMICRON RETENTION DISEASE AND ABETALIPOPROTEINEMIA

T. Fancello[1], A.L. Magnolo[1], M. Najah[2], A. Jelassi[2], E. Di Leo[1], M.N. Slimane[2], P. Tarugi[1]
[1]Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy; [2]Research Unit of Genetic and Biological Factors of Atherosclerosis, University of Monastir, Tunisia
E-mail: lucillamag@libero.it

Object of the Study. Identification of mutations in candidate genes in two patients with primary hypobetalipoproteinemia as-

sociated with severe fat malabsorption, suggesting the clinical diagnosis of Abetalipoproteinemia (ABL), Chylomicron retention disease (CMRD) or homozygous Familial Hypobetalipoproteinemia (FHBL).
Methods and Results. We investigated two Tunisian children with a similar clinical phenotype characterized by diarrhea, retarded growth, and the presence of lipid droplets in enterocytes. Proband #805, a 7-year old girl, had a moderate hypobetalipoproteinemia suggesting the diagnosis of heterozygous FHBL. However, she had no mutations in APOB or PCSK9 genes. The sequence of MTP gene was negative. She was found to be homozygous for a novel mutation (c.184 G>A) in exon 4 of SAR1B gene resulting in non conservative amino acid substitution (p.Glu62Lys). This mutation is likely to be pathogenic and the cause of CMRD, as it affects a highly conserved amino acid residue in the SAR1B protein and is "probably damaging" in silico. Proband #AB5, a 8-month old girl, had very low plasma cholesterol level and undetectable LDL-C and apoB, suggesting the diagnosis of ABL. MTP gene sequencing showed that she was homozygous for a single nucleotide substitution in the donor splice site of intron 16 (c.2342+1G>A), predicted to abolish the function of this site. To investigate the effect of the intronic mutation on pre-mRNA splicing, COS1 cells were transfected with wild-type and mutant MTP minigenes encompassing exon 16/exon 18 region. In the transcript of the mutant minigene exon 16 joined to the 5' end of intron 16, as the result of the activation of an intronic cryptic donor site in intron 16. The translation product of the mutant MTP cDNA is a truncated protein of 781 amino acids.
Conclusion. This study demonstrates that patients with ABL and CMRD sometime show a similar clinical phenotype despite a different lipid profile.

OXIDIZED-LDL AND APO B48 IN DIABETIC KIDNEY DISEASE

N. Fanton, T. Moreno, M.J. Alférez, M. Jiménez, I. Coca, D. Vianello, P. Valdivielso, A. Zambon
Department of Medical and Surgical Sciences, University of Padova, Italy; Medicina Interna and Nefrologia, Hospital Virgen de la Victoria, Malaga, Spain
E-mail: alberto.zambon@unipd.it

Background and aims. Post-prandial hyperlipidemia is a risk factor for atherosclerosis. Fasting plasma apo B48 is a promising marker of post-prandial lipid metabolism. We investigated the association of apo B48 with oxidized LDL (ox-LDL), and the presence of peripheral arterial disease (PAD) in diabetics with and without kidney disease (DKD).
Patients and Methods. 40 age and sex-matched subjects were enrolled, 20 with DKD and 20 without, defined by albumin excretion rate and estimated glomerular filtration rate (eGFR by Cockroft-Gault formula). Lipoproteins, subclinical PAD by ankle-brachial index (ABI), post-prandial lipaemia by fasting apo B48 (ELISA),levels of ox-LDLs (ELISA), albuminuria and eGFR were evaluated.
Results. Demographic and anthropometric data, glycaemic control and lipid profile were similar between patients with and without nephropathy. Apo B48 was significantly associated with TG content in VLDL, LDL and HDL (all $p<0.01$), with plasma ox-LDL ($p=0.016$), and glycated haemoglobin A1c ($p<0.01$). Apo B48 ($p<0.01$) and ox-LDL ($p<0.05$) were higher in patients with eGFR<60 ml/min vs. those with eGFR ≥60. Progressively lower eGFR was associated with increasing apo B48 ($p=0.014$,non-parametric ANOVA). Estimated-GFR <60 ml/min was associated with significantly lower ABI vs eGFR >60 ml/min. Levels of ox-LDL were significantly lower in patients on statin vs off statin ($p<0.05$) regardless of eGFR.
Conclusions. This study provides evidence of a significant association between apoB48, ox-LDL and a proatherogenic lipid phenotype in patients with DKD, suggesting also a significant effect of statins on LDL oxidation in these patients.

CHOLESTEROL EFFLUX POTENTIAL OF SERA FROM DIABETIC PATIENTS: CLINICAL APPLICATIONS

E. Favari[1], M.P. Adorni[1], F. Zimetti[1], D. Ardigò[2], S. Calandra[3], A.F.G. Cicero[4], G.B. Vigna[5], I. Zavaroni[2], F. Bernini[1] and [2]DiAL-ER (ADSL) Group
[1]Dept. of Pharmacological and Biological Sciences and Applied Chemistries, University of Parma, Italy; [2]Dept. of Internal Medicine, University of Parma, Italy; [3]Dept. Biomedical Sciences, University of Modena & Reggio Emilia, Italy; [4]Dept. Medicina Clinica e Biologia applicata Policlinico S. Orsola-Malpighi, Bologna, Italy; [5]Medical Dept., Azienda Ospedaliero-Universitaria di Ferrara, Italy
E-mail: elda.favari@unipr.it

High-density lipoprotein (HDL) may provide cardiovascular protection by promoting reverse cholesterol transport (RCT) from macrophages. The ability of serum HDL to promote RCT by accepting cholesterol from peripheral cells, termed "cholesterol efflux potential", plays a key role in this process. The determination of this parameter may provide a novel biomarker for cardiovascular risk in human, independently of HDL concentrations. The aim of this study was to functionally characterize the sera of subjects with diabetic dyslipidemia (SDD) and to correlate the capacity of promoting cholesterol efflux with plasma level of lipoproteins. Sera from type 2 diabetic patients (n=113) with 'atherogenic dyslipidemia' were compared with those sera from 98 healthy volunteers (HV). Cholesterol efflux potential of whole sera was assessed through a cell cultured-based and isotopic assay: J774 macrophages in basal condition release cholesterol to extracellular acceptors mainly by aqueous diffusion. In a second set of experiments we measured cholesterol efflux after stimulation of J774 murine macrophages with cAMP. Under these conditions, total release of cholesterol occurs mainly by ABCA1 and aqueous diffusion pathways. The difference between efflux from cAMP stimulated cells and J774 in basal condition specifically represents the ABCA1-mediated efflux. Results showed that the total efflux process from cells correlates with HDL plasma concentration in HV subjects ($r2=0.0935$; $p=0.005$) but this correlation was missed in SDD, suggesting the presence of dysfunctional HDL in sera from diabetic subjects. ABCA1-mediated efflux showed slight or no correlation with HDL plasma concentrations in both HV and SDD, confirming the observation that the ABCA1 efflux pathway is a measure of plasma HDL functionality and quality rather than quantity. In SDD, ABCA1-driven efflux correlated with the triglycerides plasma levels ($r2=0.2406$; $p<0.0001$), and this link was not observed in HV. This result may suggest that the high triglycerides levels observed in the plasma of SDD may increase the cholesterol ester transfer protein (CETP) activity, through a substrate-driven mechanism, leading to an increased formation of pre-beta HDL particles, the specific acceptors for ABCA1 efflux. In conclusion SDD show dysfunctional mature HDL particles compared to HV and display an induced ABCA1-mediated efflux possibly due to an increased plasma concentration of specific ABCA1 acceptors (pre-beta HDL) related to the increased levels of plasma triglycerides in these patients.

PROPROTEIN CONVERTASE SUBTILISIN KEXIN TYPE 9

N. Ferri, G. Tibolla, A. Pirillo, F. Cipollone, A. Mezzetti, S. Pacia, A. Corsini, A.L. Catapano
Department of Pharmacological Sciences, University of Milan, Italy
E-mail: nicola.ferri@unimi.it

Introduction. Proprotein convertase subtilisin kexin type 9 (PCSK9) is an important regulator of hepatic low-density lipoprotein (LDL)-cholesterol levels. Although PCSK9 is mainly of hepatic origin, extra-hepatic tissues significantly contribute to PCSK9 production and, potentially, local regulation of LDL receptor expression.
Methods and Results. In the present study we show that PCSK9 is expressed in smooth muscle cells (SMCs) and fibroblasts, but not in endothelial cells, macrophages and monocytes. PCSK9 was also detectable in human atherosclerotic plaques. Conditioned media from SMC affected LDLR expression and cholesterol uptake from β-VLDL in the macrophage cell line J774. Co-cultured experiments also demonstrated the influence of SMCs on LDLR expression in J774. By retroviral overexpression or knockdown with small interfering RNA, we demonstrated that PCSK9 released from SMCs directly regulated LDLR expression in J774. Stimulation of SMCs with PDGF-BB induces both PCSK9 expression and SMC migration. PCSK9 overexpression significantly increased SMC migration in response to PDGF-BB, while PCSK9 knock down led to a significant reduction of cell migration, suggesting a possible effect of PCSK9 on SMC motility.
Conclusions. Taken together our data indicate that PCSK9 secreted by human aortic SMCs is functionally active and capable to reduce LDLR expression in macrophages and to influence SMC migration, suggesting a possible role for this protein in atherogenesis.

CHEMOTACTIC EFFECT OF PRORENIN ON HUMAN AORTIC SMOOTH MUSCLE CELLS: A NOVEL FUNCTION OF THE PRORENIN RECEPTOR

N. Ferri, C.M. Greco, M. Camera, L. Facchinetti, M. Brambilla, S. Pellegrino, M.L. Gelmi, E. Tremoli, A. Corsini
Department of Pharmacological Sciences, University of Milan, Italy
E-mail: nicola.ferri@unimi.it

The discovery of a specific prorenin receptor has opened to a possible biological function of prorenin independently from angiotensin I production. In the present study we show that prorenin receptor is expressed in normal human vessels (mammary arteries and saphenous veins) and in cultured human aortic smooth muscle cells, by western blotting, quantitative real time PCR and immunocytochemistry analysis. Prorenin (10 nmol/L) exerted a 3 fold induction of smooth muscle cell migration assessed by Boyden chamber chemotaxis assay after 6h stimulation and a 30% increase of smooth muscle cell random motility determined by video microscopy. The prorenin decoy peptide (10 μmol/L) almost completely inhibited smooth muscle cell migration in response to prorenin, while no effect was observed with a scrambled peptide. Knock down of prorenin receptor by small interfering RNA completely affected the migratory response of smooth muscle cells to prorenin. Prorenin increased focal adhesion size (+42.8±23.0%) and RhoA activation (+15.0±5.0%) after 10 minutes stimulation associated with cleavage of the focal adhesion kinase (pp125FAK) at 60 minutes. The generation of a 50kDa fragment of pp125FAK was suppressed by the calpain inhibitor ALLN (100 μmol/L), which also inhibited smooth muscle cell migration in response to prorenin. Thus, in the present report we show that prorenin is expressed in human vessels and in cultured smooth muscle cells where it exerts a chemotactic action. This effect is associated with a profound cytoskeleton and focal adhesion re-organization, RhoA activation and calpain-mediated pp125FAK cleavage.

ALISKIREN REDUCES PRORENIN RECEPTOR EXPRESSION AND ACTIVITY IN CULTURED HUMAN AORTIC SMOOTH MUSCLE CELLS

N. Ferri, C.M. Greco, G. Maiocchi, A. Corsini
Department of Pharmacological Sciences, University of Milan, Italy
E-mail: nicola.ferri@unimi.it

Introduction. The recent discovery of a specific receptor for renin/prorenin (PRR) has added new interest to the potential pharmacological actions of aliskiren, the first direct renin inhibitor.
Materials and Methods. In the present study, to gain new insights on the pharmacological properties of aliskiren, we investigated the effect of aliskiren on PRR expression and activity in cultured human smooth muscle cells (HSMCs).
Results. Co-incubation of HSMCs with ANG (1.5±10-7M) and prorenin (10-8÷10-7M) determined an efficient production (within 4h) of angiotensin I, almost completely inhibited by 10-5M aliskiren (-86.0±14.0%). In HSMCs stimulated with both ANG and prorenin, a 24h incubation with aliskiren (10-6÷10-5M) determined a concentration-dependent reduction of PRR mRNA levels (IC50 4.6±10-6M). The cell surface expression of PRR determined by flow cytometry analysis was also reduced after incubation with aliskiren in a concentrations dependent manner. The lower levels of PRR were also associated with a reduced expression of TGF-b, PAI-1 and type I collagen mRNA.
Conclusions. These results suggest a direct pharmacological action of aliskiren on PRR expression and its signaling pathway in human HSMCs. This reported action of aliskiren may open to a new scenario on the pharmacological properties of aliskiren.

PERSISTENZA A 2 ANNI ALLA PRESCRIZIONE DI TERAPIA CON STATINE IN PAZIENTI AD ALTO RISCHIO CARDIOVASCOLARE

A. Ferroni, S. D'Addato, L. Caiazzo, A. Spighi, A.F.G. Cicero, A. Dormi, C. Borghi
Dip. Medicina Interna dell'Invecchiamento e Malattie Nefrologiche, Policlinico S. Orsola Malpighi, Università di Bologna, Italia
E-mail: alienor.ferroni@yahoo.it

Background. Hypercholesterolemia is a major risk factor for atherosclerosis and cardiovascular disease, the leading cause of death. Lipid-lowering medication can significantly reduce the risk for cardiovascular events. However many patients do not achieve their lipid goals and remains at high risk for cardiac events because of poor adherence to therapy.
Objective. The aim of this work was to investigate persistence to statin therapy during 2 years following discharge from a hospital recovery and its clinical effects.

Methods. This retrospective cohort study was conducted among a population of patients who were discharged from the S. Orsola-Malpighi Hospital between 1 January 2008 and 31 December 2008 with a statin therapy. Our cohort consisted of a total of 556 patients aged 40-70 years in primary prevention, but ad high risk, and secondary-prevention of cardiovascular diseases. Persistence was measured more than 2 years after the hospital discharge with telephone calls. We examined the persistence and the association between persistence and multiple outcomes among which total HDL and LDL-cholesterolemia.
Results. Persistence at the study end point was 89.7% for women and 92.9% for men. Persistence was associated with medium levels of cholesterol HDL higher than at the discharge. Levels of cholesterol LDL, total cholesterol and triglycerides weren't significantly correlated with the persistence. Among the statins used most often in this study, persistence was higest with the Atorvastatin, Rosuvastatin and with the combination Simvastatin/Ezetimibe.
Conclusions. This study shows a very high level of persistence to statin therapy after hospital discharge, despite that previously reported in the literature. A statistically significant increase of cholesterol HDL in patients who adhere to statins.

GLUCOSE HOMEOSTASIS AND POSTPRANDIAL LIPOPROTEIN METABOLISM IN NONALCOHOLIC STEATOHEPATITIS (NASH) ARE AFFECTED BY LECTIN-LIKE OXIDIZED LDL RECEPTOR-1 (LOX-1) POLYMORPHISM

R. Gambino[1], G. Musso[2], M. Cassader[1], F. Saba[1], N. Alemanno[1], M. Potenza[1], S. Bo[1]
[1]Department of Internal Medicine, University of Turin, Italy;
[2]Gradenigo Hospital, Turin, Italy
E-mail: roberto.gambino@unito.it

Background. Nonalcoholic steatohepatitis (NASH) affects 3-5% of general adult population. It encompasses a histological spectrum ranging from simple steatosis to steatosis plus necroinflammation, the latter with different severity of fibrosis, and predisposes to cirrhosis, cardiovascular disease (CVD) and diabetes through unclear mechanisms.
Lectin-like oxidized LDL receptor-1 (LOX-1) has been connected to cardiovascular risk in the general population and to insulin resistance in experimental models. Several LOX-1 functional single nucleotide polymorphisms (SNPs) have been recently linked to CVD susceptibility in humans. The IVS4-14 A>G SNP is one of these functional SNPs. Objective. To assess the impact of the common functional IVS4-14 A>G LOX-1 polymorphism on adipokines, oxidative stress, lipoprotein metabolism and glucose homeostasis in NASH.
Methods. Forty nonobese, nondiabetic, normolipidemic biopsy-proven NASH patients and 40 age, sex, BMI and IVS4-14 A>G LOX-1 polymorphism-matched healthy controls underwent an oral fat load test (OFT), with measurement of plasma triglyceride-rich lipoprotein subfractions, oxidized LDL, total antioxidant status (TAS), adipokines (resistin and adiponectin). Subjects underwent also an oral glucose tolerance test (OGTT), with Minimal Model analysis to yield parameters of glucose homeostasis.
Results. LOX-1 IVS4-14 A>G genotype distribution was in Hardy-Weinberg equilibrium. In patients and controls, the prevalence of LOX-1 AA genotype was 34%, of AG genotype was 33%, of homozygous GG genotype was 33%. LOX-1 polymorphism affected both lipoprotein and glucose metabolism; during the OFT, the G allele was associated with small triglyceride-rich lipoprotein accumulation, lower TAS levels, adipokine imbalance (higher resistin and lower adiponectin). The G allele was also independently associated with insulin resistance, impaired pancreatic β-cell function and incretin effect during the OGTT.
Conclusions. We provided evidence that LOX-1 polymorphism may predispose to CVD through modulation of postprandial small triglyceride-rich lipoproteins and adipokine balance and to diabetes by affecting both insulin secretion and insulin sensitivity. The G allele was associated with postprandial small TRLPs accumulation. As small TRLPs are represented by the atherogenic remnants, this may be a novel mechanism connecting LOX-1 polymorphism to CVD risk. The independent association of LOX-1 IVS4-14 A>G SNP with β-cell function and insulin resistance suggests that a more effective oxLDL internalization by LOX-1 in G-allele carriers may impair glucose metabolism, regardless of circulating oxLDL levels. Further in vitro and adequately-powered prospective studies need to elucidate underlying mechanisms and to assess the usefulness of LOX-1 IVS4-14 A>G SNP for screening and treatment of patients at higher metabolic and cardiovascular risk.

THE MEMBRANE-BOUND MERTK PROTEIN IS CLEAVED IN THE EXTRA CELLULAR DOMAIN VIA ADAM17 IN THE AREA SURROUNDING THE LIPID CORE OF HUMAN CAROTID PLAQUES

U. Garbin, E. Baggio, C. Stranieri, S. Manfro, A. Pasini, G. Lipari, A. Fratta Pasini, C. Mozzini, L. Cominacini
Azienda Ospedaliera Universitaria Integrata Verona, Dipartimento di Medicina, Sezione di Medicina Interna D, Verona, Italia
E-mail: ulisse.garbin@univr.it

Introduction. Tyro3, Axl and Mer tyrosine kinase (Merkt) (TAM receptors) are surface receptors that have been implicated in the process of apoptotic cell recognition and engulfment.
Materials and Methods. in the present study, we investigated the relationships between apoptosis, Mertk and metallopeptidase domain 17 (Adam17) in the area surrounding the lipid core (LC) and in the periphery (P) of human carotid plaques. Further, in macrophages-like THP-1 cells we studied the effect of plaque extract (PE) on the expression of Adam17 and Merkt and on the cleavage of its extra cellular domain (sMer) that antagonizes the grown arrest-specific protein 6 (Gas6), a bridging molecule between TAM receptors and apoptotic cells.
Results. among the TAM receptors only Merkt resulted significantly higher in LC than in P ($p<0.01$). Also Adam17 but not Gas6 was higher in LC than in P ($p<0.01$). By immunoistochemistry, there was an opposite trend of Merkt and Adam17 expression from the outer edge of LC out, Adam17 being higher in the area closest to the edge of LC. While the incubation of THP-1 with PE increased the mRNA and protein of ADAM17 ($p<0.001$), the rise of Merkt mRNA was followed by a reduction of its extra cellular domain ($p<0.01$).
This phenomenon was associated with the increase of sMer in the culture medium ($p<0.01$).
Conclusions. The ex vivo and in vitro results suggest that the area closest to the lipid core may be a strong inducer of ADAM17 which in turn may release the extra cellular domain of Merkt producing sMer, an inhibitor of Gas6 activity.

ASSOCIATION BETWEEN SMALL DENSE LDL AND PSORIATIC ARTHRITIS

M. Gentile, S. Iervolino, R. Peluso, Dario di Minno, B. de Simone, G. Iannuzzo, R. Scarpa, P. Rubba
Department of Clinical and Experimental Medicine, University "Federico II", Medical School, Naples, Italy
E-mail: margenti@unina.it

Psoriatic arthritis (PA) is an inflammatory rheumatic disorder of unknown etiology occurring in patients with psoriasis. Several studies have shown an association between psoriasis and traditional atherosclerotic risk factors. We evaluated the relationship between small dense LDL and PA in a group of 50 patients with clinical diagnosis of PA. Serum Cholesterol, HDL-cholesterol, LDL-cholesterol, Triglycerides (TG), Insulin, HOMA, Apo B and small dense LDL have been measured in 50 patient with PA and in 100 healthy subjects (control group). LDL particle separation was performed by Lipoprint System: 7 LDL subfractions were obtained, LDL score (% of sd-LDL particles) and mean LDL particle size were calculated. PA patients and control group differ significantly ($p<0.001$) in TG values (119.3±52.0 vs 90.7±40.7 mg/dL); Apo B (1.1±0.2 vs 0.9±0.1 g/L); Insulin (8.9±4.9 vs 5.8±3.2 mU/L); Homa (2.2±1.7 vs 1.3±0.8); BMI (27.7±3.3 vs 25.8±3.8). LDL score is significantly ($p<0.001$) higher in PA as compared to control (9.0 ±10.7 vs 2.9±4.7 mg/dL); and mean LDL size is significantly lower ($p<0.001$) in PA than control (268.1±4.6 vs 271.2±2.7). These findings show a novel relationship between small dense LDL score and mean LDL size with PA diagnosis. Sd-LDL gives potentially useful information in the risk assessment for atherosclerotic disease in PA patients.

THE IMPROVED BIOCHEMICAL DIAGNOSTICS OF THE LIPID PROFILES IN THE FRAMEWORK OF REGIONAL NETWORK FOR INHERITED LIPID DISORDERS

M. Gentile[1], F. Rubba[2], F. Verdesca[3], F. Jossa[1], M. Sodano[1], E. La Fata[1], L. Maresca[1], S. Ubaldi[1], E. Giancotti[2], P. Rubba[1]
[1]Network Regionale Dislipidemie, Dipartimento di Medicina Clinica e Sperimentale, A.O.U. Federico II, Napoli, Italia;
[2]Direzione Medica di Presidio, A.O.U. Federico II, Napoli, Italia;
[3]CEINGE, Napoli, Italia
E-mail: margenti@unina.it

Familial combined hyperlipidemia (FCHL) is a common and severe inherited hyperlipidemia, with a prevalence in European populations of 0.5-2%, The main clinical feature of FCHL is the development of premature atherosclerotic lesions, with a very high cardiovascular mortality. Affected individuals have elevated cholesterol or trygliceride concentrations or both. Such a lipid profile is frequently associated with an unfavourable decrease in high density lipoprotein concentration, an elevated apolipoprotein B and an increased prevalence of atherogenic, small, dense low-density lipoprotein (LDL) subfractions. The exact cause of this metabolic disorder is presently unknown.
Furthermore, no model proposed so far is able to fully explain the genetic bases of FCHL. Due to the absence of a specific genetic or metabolic marker for the disorder and to the characteristic variability in the presenting lipid phenotype, family studies are necessary to establish the diagnosis of FCHL in each patient. Since it is not always possible to get some biochemical data from first-degree relatives, the dosage of small dense LDL can be performed in these cases. LDL particles separation is performed by Lipoprint System.
The proportion of sdLDL particles to the whole LDL area is calculated (LDL score). An LDL score higher than 10.0 is related in multivariate analysis to FCHL diagnosis, sensitivity 78% and specificity 89% (Atherosclerosis 2009).
The improved biochemical diagnostics of the lipid profiles which include the dosage of the LDL sub-fractions has a precise organizational importance regarding the appropriateness of the prescription. In fact, it allows to value, in absence of a familiar history, whether a drug can be refundable or not with a clear feedback and efficacy both on the appropriateness of the A.O.U Federico II and on the regional induced cost.

CIGARETTE SMOKE EXTRACT IMPAIRS HUMAN MONOCYTES MIGRATION AND MODULATE THE EXPRESSION OF PRO-INFLAMMATORY GENES INVOLVED IN THE ATHEROSCLEROTIC PROCESS

I. Giunzioni, A. Bonomo, A. Corsini, S. Bellosta
Department of Pharmacological Sciences, University of Milan, Milan, Italy
E-mail: ilaria.giunzioni@unimi.it

Cigarette smoke is widely accepted as an environmental factor that aggravates atherosclerosis, but the pro-atherogenic processes affected by cigarette smoke and which mechanism(s) play(s) the major role are still unknown. In particular, it is not clear how cigarette smoke affects macrophage recruitment and the expression of inflammatory factors such as metalloproteinases (MMPs) and cytokines in the atherosclerotic lesions.
Based on these premises, the aim of our study was to evaluate in vitro the effect(s) of cigarette smoke extracts (total particulate matter, TPM) on cell migration and the expression of MMPs and cytokines. Human monocytes (HM) have been isolated from peripheral blood withdrawn from healthy volunteers (non-smoker) using the Ficoll-Paque.
HM have been used right after isolation by cell adhesion to culture dishes; further studies have been performed in HM-derived macrophages (HMDM) following differentiation for 10-14 days in culture medium.
Preliminary experiments showed that TPM has a cytotoxic effect on HM and HMDM at concentration higher than 62.5 ug/ml or 250 ug/ml, respectively.
At non toxic concentrations, TPM treatment reduces, in a concentration-dependent and statistically significant manner, cell migration stimulated by different chemotactic agents (fMLP, MCP1 and RANTES). This is a late effect, since it is evident after 24 hours of incubation, while at 1 and 3 hours TPM is ineffective. The addition of TPM does not have any statistically significant effect on MMP-9 activity or expression.
On the contrary, TPM increases in a concentration-dependent manner MMP-1 expression (4-fold increase at 50 ug/ml), while it does not show any significant effect on the expression of TIMP-1. Finally, TPM reduces MCP1 expression in a concentration-dependent manner (up to 70% reduction at 50 ug/ml).
These results indicate that TPM might affect the atherogenic process by reducing monocyte/macrophage mobility and inducing a pro-inflammatory milieu. Study funded by British American Tobacco, Southampton, UK.

LOW-DENSITY LIPOPROTEIN-RELATED RECEPTOR 5 GENE POLYMORPHISMS AND ABDOMINAL AORTIC ANEURYSM GENETIC SUSCEPTIBILITY

B. Giusti, S. Galora, C. Saracini, G. Pratesi, R. Pulli, C. Pratesi, G.F. Gensini, R. Abbate
Department of Medical and Surgical Critical Care, University of Florence, Atherothrombotic Diseases Center, A.O.U. Careggi, Florence, Italy; Vascular Surgery Unit, Department of Surgery, University of Rome "Tor Vergata", Italy; Department of Vascular Surgery, University of Florence, Italy
E-mail: betti.giusti@unifi.it

Previous data of our group showed decreased low-density lipoprotein-related receptor 5 (LRP5) gene expression in the peripheral blood cells of abdominal aortic aneurysm (AAA) patients. The LRP5 gene is involved in bone metabolism as well as lipoprotein and glucose metabolism.
Experimental studies have shown that apoE;Lrp5 double knockout mice developed multiple atheromatous aortic lesions manifesting a hump structure, which were associated with cholesterol deposits, fibrosis, and elastolysis, some even showing the destruction of internal elastic lamina and the degenerative changes in aortic smooth muscle.
Moreover, in our previous paper, we demonstrated an association between decreased expression levels of LRP5 gene and increased levels of lipoprotein (a) [Lp(a)] in AAA patients.
The importance of LRP5 for the regulation of bone mass has recently been established, where loss of function mutations is followed by severe osteoporosis and gain of function is related to increased bone mass.
On the bases of these evidences, the aim of this study was to evaluate the role of polymorphisms in the LRP5 gene in regulating susceptibility to AAA.
A total of 423 AAA patients and 423 control subjects comparable for sex and age were genotyped for seven different single nucleotide polymorphisms (SNPs) within the LRP5 gene (rs667126, rs3736228, rs4988300, rs3781590, rs312016, rs556442, rs627174) by TaqMan approach.
All the polymorphisms were in Hardy-Weinberg equilibrium. Two polymorphisms were significantly associated with AAA: rs667126, in AAA TT=50.2%, TC=41.0%, CC=8.8% vs in controls TT=37.7%, TC=45.2%, CC=17.1%, p=0.002; rs3781590, in AAA CC=34.4%, TC=47.2%, TT=18.4% vs in controls CC=49.3%, TC=39.6%, TT=12.1%, p=0.003.
At the multivariable logistic regression analysis adjusted for age, gender, dyslipidemia, hypertension and smoking habit, rs667126 and rs3781590 polymorphisms remained significant and independent determinants of AAA (OR=0.7, 95%CI 0.5-0.9, p=0.021 and OR=1.6, 95%CI 1.1-2.2, p=0.006, respectively).
In conclusion, present data have identified polymorphisms in LPR5 gene as genetic markers of AAA, and underline the need to concentrate our effort in studying the role of these markers in the aneurysmal disease.
The identification of genetic susceptibility factors and the evaluation of their role is fundamental to design gene-based clinical studies in the future to validate diagnostic or prognostic scores to be applied in the everyday clinical practice.

NIACIN EXTENDED-RELEASE AND FENOFIBRATE IMPROVE HDL ABILITY TO PRESERVE ENDOTHELIAL CELL HOMEOSTASIS: RESULTS FROM THE HIFUN STUDY

M. Gomaraschi[1], S. Pozzi[1], K. Coutant[2], S. Rossomano[2], F. Lamour[2], R. Benghozi[2], G. Franceschini[1], L. Calabresi[1]
[1]Center Enrica Grossi Paoletti, Dept. of Pharmacological Sciences, Università degli Studi di Milano, Italy; [2]F. Hoffmann-La Roche Ltd, Basel, Switzerland
E-mail: Monica.Gomaraschi@unimi.it

Background and aim. Niacin and fenofibrate are the most effective available therapies to increase HDL-C levels. A multicenter, randomized, open-label, cross-over study was performed to compare drugs' effects on HDL composition/distribution and on HDL atheroprotective functions.
Methods and Results. 66 dyslipidemic patients with low or normal HDL-C levels received 6 weeks' treatment with niacin extended-release (niacin ER, 0.5 g/d then 1.0 g/d) and fenofibrate (160 mg/d), with 4 weeks' wash-out period.
Niacin ER and fenofibrate similarly increased plasma levels of HDL-C and apoA-I; apoA-II increased only after fenofibrate treatment. Niacin ER shifted HDL distribution towards large particles, while fenofibrate increased the percentage of both medium- and large-sized HDL.
In a subset of 37 patients, HDL ability to preserve endothelial cell homeostasis was assessed by incubating endothelial cells with HDL isolated at baseline and after treatment. Niacin ER and fenofibrate both increased the anti-inflammatory potential of HDL, tested as their ability to inhibit TNFalpha-induced VCAM-1 expression, with a significantly higher effect of niacin ER. Post-niacin HDL anti-inflammatory potential was also higher than that of HDL from healthy controls with high HDL-C levels. Niacin ER and fenofibrate similarly improved HDL ability to induce the expression of endothelial nitric oxide synthase.
Conclusion. Niacin ER and fenofibrate improved HDL ability to preserve endothelial cell homeostasis. The superior effect of niacin ER on HDL ability to inhibit VCAM-1 expression suggests that increasing large apoA-I-containing HDL could be a possible therapeutic approach to improve HDL anti-inflammatory potential.

EVALUATION OF ENDOTHELIAL DYSFUNCTION AND REGENERATION IN ULTRANONAGENARIANS: THE MUGELLO STUDY

A.M. Gori[1,2], F. Cesari[1], F. Vannetti[2], R. Molino Lova[2], F. Sofi[1,2], R. Abbate[1], G.F. Gensini[1,2], C. Macchi[2]
[1]Department of Medical and Surgical Critical Care, University of Florence, Italy; [2]Fondazione Don Gnocchi, Centro Santa Maria agli Ulivi, Impruneta, Florence, Italy
E-mail: annamaria.gori@unifi.it

Background. Several studies have documented that advanced age is a risk factor for cardiovascular diseases both in women and in men. However, the precise mechanisms underlying the relationship between increasing age and increased risk for vascular diseases are not completely elucidated.
Dysfunction of the vascular endothelium has been documented in conditions that are associated with the development and pro-

gression of atherosclerosis. Endothelial damage and repair may be assessed by cell-based approaches, which evaluate ex vivo the circulating endothelial cells (CEC) and the endothelial progenitor cells (EPC).
CEC derived from the vascular wall, by sloughing of resident endothelial cells into the circulation, as part of their normal turnover process or as effect of damaging factors and may be a marker of the ongoing vascular damage.
EPC are bone marrow-derived cells able to enhance angiogenesis, promote vascular repair and improve endothelial function. It has been demonstrated that a low number of EPC represents an independent risk factor for ischemic events.
Clinical studies have demonstrated in young and middle-aged population that EPCs inversely correlated with the presence of traditional cardiovascular risk factors and with circulating pro-inflammatory moleculess, but scarce data in the elderly are available. At this purpose, we used data from the Mugello Study, a prospective study which enrolled ultranonagenarian subjects, who lived in the Mugello Area to evaluate EPC, CEC in ultragenarians.
Methods. We investigated CEC and EPC number in 191 ultranonagenarians enrolled in the Mugello study.
Circulating CEC and EPCs were assessed by flow cytometric analysis and were defined as CD146+/CD31+/CD45-/CD61- (CEC) and CD34+/KDR+, CD133+/KDR+, CD34+/CD133+/KDR+ (EPC) respectively. We also evaluated circulating levels of C-Reactive Protein (CRP).
Results. We enrolled 191 ultranonagenarians (148 females/43 males) with a median age of 92 years (90-103 yrs). In the Mugello population, women were slightly, but significantly older than men [92 yrs (89-103) vs 91 (89-100 yrs), p<0.01)].
Ultranonagenarians showed a significant higher number of CEC [3 (0-187) cells/106 events vs 1 (0-23) cells/106 events, p<0.001] and a lower number of EPC [CD34+/KDR+: 2 (0-28) cells/106 events vs 10 (3-43) cells/106 events; CD133+/KDR+: 3 (0-23) cells/106 events vs 10 (4-45) cells/106 events; CD34+/CD133+/KDR+: 2 (0-70) cells/106 events vs 9 (3-43) cells/106 events, p<0.001] with respect to younger (median age 65 yrs, 45-78 yrs) control population. In the Mugello population, CEC and EPC number did not differ between women and men.
Furthermore, no significant relationships between CEC, EPC number and age, total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides levels were found. In this population we found a significant relationship between CEC, EPC number and CRP serum levels [CEC: r=0.142, p=0.050; CD34+/KDR+: r=-0.317, p<0.001; CD133+/KDR+: r=-0.306, p<0.001; CD34+/CD133+/KDR+: r=-0.312, p<0.001 respectively]. CEC and EPC number was not affected by traditional cardiovascular risk factors. Furthermore, no significant difference in CEC and EPC number was found according to the presence of cardiovascular and cerebrovascular diseases.
Conclusions. Our results demonstrate the presence of an endothelial dysfunction, as documented by high CEC and low EPC number in ultranonagenarians.
Moreover the negative relationship between CEC and CRP and the positive relationship between EPC and CRP suggest that an inflammatory state, which is commonly present in the elderly, significantly affects the endothelial function by promoting vascular damage and by reducing the endothelial regenerative capacity.

N-3 POLYUNSATURATED FATTY ACIDS TREATMENT REVERSES ENDOTHELIAL DYSFUNCTION AND OXIDATIVE STRESS IN AORTAS FROM OVARIECTOMIZED RATS

G. Gortan Cappellari[1], P. Losurdo[2], E. Panizon[2], M. Jevnicar[2], L. Macaluso[3], R. Barazzoni[1], L. Cattin[4], R. Carretta[2], M. Zanetti[1]
[1]U.C.O. Clinica Medica Generale e Terapia Medica; [2]U.C.O. Medicina Clinica; [3]U.C.O. I Medica; [4]U.C.O. III Medica, DSMCS University of Trieste, Italy
E-mail: gigici2@iol.it

Introduction. Menopause is associated with endothelial dysfunction, oxidative stress and cardiovascular disease. n-3 polyunsaturated fatty acids (n-3 PUFA) are cardioprotective and decreased concentrations of n-3 PUFA are associated with cardiovascular disease in postmenopausal women. Since the impact of n-3 PUFA on menopause-associated vascular disease is largely unknown we investigated the effects of chronic n-3 PUFA treatment on endothelial dysfunction and oxidative stress in an animal model of surgical menopause.
Materials and Methods. 30 six-months old female Wistar rats were subdivided into 3 groups: 1) control: sham-surgery (n=10); 2) bilateral ovariectomy (OVX, n=9); 3) bilateral ovariectomy + n-3 PUFA (EPA+DHA 0.8 g/kg/day by gavage, ratio 0.9-1.5, OVX + n-3 PUFA, n=11). 60 days after surgery the following measurments were performed: ex-vivo assessment of aortic vasomotility with acetylcholine (ACh, 10-9 to 10-6 mol/l), DEA-NONOate (DEA, 10-10 to 10-6 mol/l) with or without Tiron preincubation (200 µmol/l, 20 min); arterial superoxide production by SOD-inhibitable cytochrome C reduction assay; aortic nitrotyrosine by ELISA; endothelial nitric oxide synthase (eNOS), NADPH-oxidase 4 (NOX4) and p22phox protein concentration in aorta by Western blot; NOS activity by the conversion of oxyhemoglobin to methemoglobin by NO. Statistical analysis was performed by one way ANOVA or Student's t-test as appropriate.
Results. Endothelium-dependent (ACh 10-6 mol/l, 31.1±4.7 vs. 55.1±6.5 relaxation %) but not-independent (DEA 10-6 mol/l, 90.6±6.3 vs. 97.3±1.3 %) vasodilation was impaired (p<0.05) in OVX and this finding was abolished (p<0.05) by Tiron (48.1±4.6 vs. 52.0±6.5 %), a superoxide scavenger. Ovariectomy did not significantly reduce total NOS activity (422.8±6.7 vs 441.3±22.1 a.u.) and eNOS protein expression (1.1±0.3 vs. 1.4±0.3 a.u.) but resulted in enhanced (p<0.05) vascular superoxide production (142.3±14.6 vs. 97.4±8.3 nmol/min/mm^2), nitrotyrosine concentration in aortic tissue (2.2±0.3 vs. 1.0±0.3 µg BSANT eq./mg protein) and aortic NOX 4 expression (3.4±0.3 vs. 2.5±0.4 arbitrary units, a.u.). Treatment with n-3 PUFA, reversed (p<0.05) endothelial dysfunction (Ach 10-6, 57.4±4.6 %), superoxide anion generation (105.0±9.8 nmol/min/mm^2), aortic nitrotyrosine (1.1±0.3 µg BSANT eq./mg protein) content and NOX 4 protein expression (1.1±0.7 a.u.). Morover, although total NOS activity did not change (451±24.1 u.a.), n-3 PUFA also markedly increased (p<0.05) eNOS protein expression (2.2±0.2). p22phox levels were not significantly modified by ovariectomy (1.0±0.2 vs 0.8±0.1 a.u.) nor by n-3 PUFA (0.8±0.0 a.u.).
Conclusions. These findings show that in a surgical model of menopause, endothelial dysfunction is strictly linked to an increase in oxidative stress and consequent reduction in nitric oxide bioavailability. Morover they demonstrate a significant therapeutic effect of n-3 PUFA treatment on vascular complications in postmenopause.

ROLE OF APOLIPOPROTEIN E IN CELLULAR CHOLESTEROL EFFLUX

D. Greco, G. Lusardi, F. Bernini, I. Zanotti
Dip. Scienze Farmacologiche Biologiche e Chimiche Applocate, Università degli Studi di Parma, Italia
E-mail: daniela.greco@nemo.unipr.it

The apolipoprotein E expressed in macrophages modulates lipid metabolism, while systemic apoE regulates the plasma lipoprotein profile. The role of apoE in Reverse Cholesterol Transport (RCT) was recently defined, demonstrating that macrophage but not systemic apoE contributes to macrophage RCT in vivo, thus showing an antiatherosclerotic effect.
In this study we focused on the role of systemic and macrophage apoE in the first rate limiting step of RCT, cellular cholesterol efflux. In order to quantify the influence of apoE on exchange of cholesterol between cells and plasma we performed in vitro experiments of cholesterol flux, using peritoneal macrophages extracted from WT and apoE-/- mice and whole plasma from WT and apoE-/- animals as extracellular lipid acceptors/donors. In these experiments apoE-/- plasma, characterized by high levels of apoB-containing lipoproteins and low levels of HDL, and WT plasma, promoted a similar cholesterol efflux from WT MPM: 14.29%±6.41 vs 12.40%±0.61 respectively.
Consistent with efflux data, exposition of WT MPM to apoE-/- plasma induced a reduction in the amount of intracellular cholesterol similar to that induced by WT plasma (-20.40 µg cholesterol/mg proteins ±0.36 vs -24.53 µg cholesterol/mg proteins ±0.80). The deficiency of apoE in macrophages reduced cholesterol efflux in comparison with WT macrophages: 8.5%±1.0 vs 12.40%±0.5 ($p<0.01$).
Consistently after exposition to WT plasma, ApoE-/- MPM show a less significant reduction of cholesterol content compared to MPM (-3.41 µg cholesterol/mg protein ±0.36 vs -20.40 µg cholesterol/mg protein ±0.36). This study indicates that apoE specifically expressed in macrophages is relevant for lipid exchange between cells and extracellular acceptors.
Moreover we demonstrated that the deficiency of systemic apoE despite the effect on plasma lipoprotein composition, is not likely to show pro-atherogenic potential in vitro, as it does not impair the athero-protective process of cholesterol efflux.

LIPIDOMICS OF FATTY LIVER IN NAFLD AND HCV INFECTION: LIVER SPHYNGOLIPIDS AND FATTY ACIDS

M. Greco[1], D. Noto[1], S. Petta[2], M. Minà[1], F. Fayer[1], I. Altieri[1], V. Valenti[1], R. Spina[1], A. Craxi[2], M. Averna[1]
[1]Department of Internal Medicine and Specialties (DIMIS); [2]Department of Gastroenterology, University of Palermo, Italy
E-mail: massimiliano.greco@unipa.it

Lipids are used by the liver mainly for energy storage and membrane building, but they also contribute to cell signaling. Fatty liver is characterized by activation of metabolic and inflammatory pathways. In this work differences in lipidomic profiles of sphyngolipids (SL) and fatty acids (FA) have been evaluated in biopsies from 10 NAFLD patients, 22 (14 FL, 8 w/o FL) HCV patients and 13 healthy controls.
FL is characterized by increased content of the main FA as myristic, palmitic, oleic, linoleic, saturated and monounsaturated FA in both model. FL of HCV is characterized by a more severe degree of cell damage and fibrosis associated with an increase of palmitoleic acid, long chain SL and desaturase (SCD) activity, measured as oleic/stearic ratio.
FL of NAFLD shows a higher degree of metabolic disturbances as increased total cholesterol, triglycerides, HOMA, some long chain FA (C21:0, C22:2, C24:1) and n9 FA. A combination of 6 FA and 4 SL completely resolved the two model of FL (NAFLD, HCV) from non-FL by discriminant analysis. HCV vs healthy non-FL patients showed higher levels of long chain SL.
In conclusion FL is characterized by accumulation of the most abundant FA, but only FL of HCV is characterized by increased long chain SL. The accumulation of SL, in particular ceramides, might be responsible of the more severe features of FL due to HCV infection.

EFFICACY OF EZETIMIBE COADMINISTERED WITH ROSUVASTATINA 40 mg IN A PATIENT WITH FAMILIAL HYPERCHOLESTEROLEMIA

E. La Fata, G. Marotta, M. Sodano, L. Maresca, F. Jossa, M. Gentile, P. Rubba
Department of Clinical and Experimental Medicine, "Federico II" University, Naples, Italy
E-mail: ersilia.lafata@libero.it

Introduction. Familial Hypercholesterolemia is an autosomal dominant disease caused by LDL receptor's mutation with LDL particles reduced catabolism.
The heterozygous form has a prevalence of 1:500 in the general population. Severe and premature coronary atherosclerosis is often the first clinical manifestation. When CHD history or clinical evidence of atherosclerosis or a CHD risk equivalent is present in the patient's medical history, the LDL cholesterol goal now suggested is <70 mg/dl.
Case report. A 44 years male with a positive history of hypertension, Primary Hypercholesterolemia, CAD and stroke was admitted to the outpatient Lipid Clinic of the "Federico II" University of Naples. The patient was both on a low-salt, low-fat diet regimen and on simvastatin 40/Ezetimibe.
With this therapy the LDL cholesterol was 371 mg/dl, transaminases and Creatine-phospho-kinase were in normal range; the patient was asymptomatic for myalgia.
We shifted the patient from Simvastatin 40/Ezetimibe to Rosuvastatin 20 mg. This treatment was well tolerated by the patient so we switched to Rosuvastatin 40 mg. After one month's therapy, transaminases and CPK were still normal and the patient didn't complaint of any symptom.
After three months therapy, the LDL cholesterol was 255 mg/dl. At this time, Ezetimibe was added on. The LDL cholesterol was reduced to 130 mg/dl by this new drug scheme, without any side effect.
Conclusions. Rosuvastatin 40/Ezetimibe combination reduces LDL cholesterol significantly more than Simvastatin 40/Ezetimibe association and more than Rosuvastatin 40 alone. Rosuvastatin 40/Ezetimibe combination should be an effective and safe therapy for patients with Familial Hypercholesterolemia at high risk, especially when other drugs do not reach target values. In this type of patients there is need of more effective drug intervention to achieve the goal of 70 mg/dL.

CEREBRAL VENOUS THROMBOSIS(CVT): A CASE REPORT AND LITERATURE REVIEW

N. Lamaida[1], E. Lamaida[2], V. Pizza[2], A. Rocco[3],
L. Galasso[4], E.R. Capuano[1], E. Capuano[1], R. Capuano[1]
[1]Unità Operativa Cardiologia Mercato S. Severino (SA), Italia; [2]Unità Operativa Neurochirurgia, Ospedale S. Luca Vallo della Lucania (SA), Italia; [3]Clinica Malzoni, Avellino (AV), Italia; [4]Unità Operativa Neuroradiologia, Ospedale S. Luca Vallo della Lucania, Italia
E-mail: norman.lamaida@libero.it

Introduction. CVT is a very rare disorder and can be associated with poor outcome. The incidence of CVT is three to four cases per one million in adults, with 75% of adult patients being women, and up to seven cases per one million among children. The majority of adult patients, up to 80%, will have a good neurologic outcome if diagnosed correctly. Among childhood cases of CVT, neonates are the most commonly affected are group. CVT may be associated with variety of causes. These include use of oral contraceptives, pregnancy, pueperium, dehydration, hypercoagulable states and infection specially mastoiditis.
Case Report. We discuss a case of CVT in a 18 years-old woman with a hystory of migraine headaches, drowsiness and vomiting of a short duration. Neurological examination was non focal. EEG showed generalized delta waves suggestive of encephalitis. MRI images showed hyperintense signal involving the thalami bilaterally. Venous infarction was suspected and immediately gadolinium enhanced MR Venography was performed. This showed non visualization of internal cerebral veins, vein of Galen and straight sinus. These findings were consistent with deep cerebral venous thrombosis. Treatment with heparin gave completed recovery.
Conclusions. Early diagnosis of CVT is vital for prompt treatment. The diagnosis of CVT based on clinical symptoms is diffucult but modern technology of MRI is very useful for its diagnosis. When edema or infarcts which may be hemorrhagic, are present in thalami or basal ganglia, one should suspect CVT. If detected immediate therapy should be started to avoid a devastating outcome. With prompt treatment prognosis can be favourable.

AUMENTATA ESPRESSIONE DI ACVRL1/ ALK1 ED ENDOGLINA IN PLACCHE ATEROSCLEROTICHE DI CAMPIONI AORTICI

G.M. Lenato[1], B. Buttari[2], M. Neri[3], G. Di Giammarco[4],
E. Profumo[2], V. Fineschi[3], C. Bernabeu[5], L.M. Botella[5], F. Resta[1],
C. Sabbà[1], R. Riganò[2]
[1]Centro Sovraziendale Malattie Rare, U.O.C. Geriatria, Policlinico, Università di Bari; [2]Dipartimento di Malattie Infettive, Parassitarie e Immuno-mediate, Istituto Superiore di Sanità; [3]Dipartimento di Scienze Chirurgiche, Sezione di Medicina Legale, Università di Foggia; [4]Dipartimento di Neuroscienze ed Imaging, Unità di Cardio-Chirurgia, Policlinico, Università di Chieti-Pescara; [5]Centro Investigaciones Biologicas, CSIC, Madrid
E-mail: c.sabba@dimimp.uniba.it

Introduzione. Diversi dati indicano un rilevante ruolo svolto da fattori di crescita del supergruppo TGFBeta/BMP nel processo di aterogenesi. Abbiamo studiato l'espressione di diversi componenti della famiglia di recettori TGFBeta/BMP in placche aterosclerotiche di campioni aortici.
Metodi. Lo studio è stato condotto mediante immunoistochimica, su tre campioni di placche aterosclerotiche aortiche e tre campioni controllo di aorta proveniente da soggetti sani. Abbiamo studiato l'espressione di diversi componenti del sistema TGFBeta/BMP (ALK1/ACVRL1, Endoglina, ALK5/TGFBR1, TGFBR2, TGFB1) e di Fibrillina-1, proteina della matrice extracellulare vascolare.
Risultati. Nelle aorte dei controlli, l'espressione di Endoglina e ALK1/ACVRL1 è risultata piuttosto ridotta, limitata essenzialmente agli strati endoteliali di intima e avventizia. Nella tunica media non si è osservata alcuna espressione di Endoglina e solo una modesta espressione di ALK1/ACVRL1. Al contrario, i due recettori ALK5/TGFBR1 e TGFBR2, così come la Fibrillina-1, sono risultati espressi ad alti livelli nella tunica media. Nelle sezioni derivate da placche aterosclerotiche, abbiamo riscontrato un notevole aumento dell'espressione di ALK1/ACVRL1 ed Endoglina, esteso anche alla fibrocellule vascolari lisce della tunica media. Questo aumento è particolarmente pronunciato nelle regioni delle placche associate a iperproliferazione cellulare, mentre è meno evidente nelle placche a bassa densità cellulare, essenzialmente necrotiche. È stato possibile anche notare un lieve aumento di espressione a carico di ALK5/TGFBR1 e TGFBR2, osservazione che suggerisce una generale up-regolazione dell'intero sistema recettoriale del TGFB. L'espressione di Fibrillina-1 è risultata ridotta, a causa della disorganizzazione della matrice cellulare associata alle lesioni aterosclerotiche.
Conclusioni. I nostri risultati confermano che ALK1/ACVRL1 ed Endoglina sono overespressi nelle placche aterosclerotiche, quindi le osservazioni della letteratura sono valide anche per le placche aortiche. Questi dati sembrano suggerire che ALK1/ACVRL1 ed Endoglina sono overespressi in risposta ad eventi di lesione vascolare, probabilmente legati ad un pathway di risposta allo shear stress, attivato da elevato flusso sanguigno.

CORRELAZIONI TRA SEVERITÀ DELLE APNEE OSTRUTTIVE NOTTURNE E IMC, COMPOSIZIONE CORPOREA, FATTORI DI RISCHIO CARDIOVASCOLARE E MORFOLOGIA CARDIACA

C. Lubrano, P. Specchia, M. Tenuta, D. Costantini, E. Addessi,
G. Di Lorenzo, G. Barbaro, M. Saponara, L. Gnessi
Dipartimento di Medicina Sperimentale, Sezione di Fisiopatologia Medica, "Sapienza" Università di Roma, Italia
E-mail: Carla.Lubrano@uniroma1.it

Introduzione. Nei paesi occidentali oltre il 4% della popolazione maschile e il 2% di quella femminile in età adulta è affetta da OSAS (sindrome delle apnee ostruttive notturne). È stato documentato che l'OSAS è un fattore di rischio indipendente per malattie cardio e cerebro-vascolari. L'obesità è un fattore predisponente per lo sviluppo di malattie cardiovascolari e di OSAS.
Materiali e Metodi. In questo studio abbiamo voluto valutare le correlazioni esistenti tra severità delle apnee notturne, composizione corporea, fattori di rischio cardio-metabolici, markers surrogati di aterosclerosi e morfologia cardiaca in 151 pazienti sovrappeso/obesi (IMC 39, 18±8,32 kg/m^2) di età compresa tra 16 e 76 anni, 103 femmine e 48 maschi.
Risultati. L'indice apnea/ipopnea (AHI) valutato tramite polisonnografia mostra significative correlazioni con l'IMC (r=0,4079 p<0,001) e con la circonferenza vita (r=0,5135 p<0.001), con la percentuale di massa grassa di testa/collo (valutata tramite DXA) (r=0,3314 p<0.001) e il rapporto grasso del tronco/grasso delle gambe (r=0,3592 p<0,001). La densità ossea lombare corretta per IMC mostra una significativa correlazione inversa con l'AHI (r=-

0,3569 p<0,001). L'AHI mostra inoltre correlazioni significative con la pressione arteriosa sia diastolica che sistolica, con i livelli di trigliceridi circolanti e con HOMA-I (p<0,001). I parametri ecocardiografici sia morfologici che funzionali così come lo spessore medio-intimale carotideo sono correlati alla gravità delle apnee: in particolare lo spessore del grasso epicardico, marcatore di adiposità viscerale/ectopica, mostra una relazione estremamente significativa con AHI (r=0.4602 p<0,001).
Conclusioni. La gravità delle apnee notturne è correlata ad importanti alterazioni della funzione diastolica e della massa ventricolare indicizzata, all'aumento dello spessore medio-intimale e alla prevalenza di sindrome metabolica.
La distribuzione centripeta e viscerale del tessuto adiposo, come evidenziato dall'aumentata percentuale di massa grassa di testa/collo, dall'alterato rapporto grasso tronco/grasso gambe da un lato e dall'aumentato spessore del grasso epicardico dall'altro caratterizza i soggetti con maggior AHI e più evidente rischio cardiovascolare.

IN VIVO TREATMENT WITH CYCLOSPORINE A INCREASES EFFLUX POTENTIAL OF MOUSE PLASMA VIA ATP-BINDING CASSETTE TRANSPORTER ABCA1

G. Lusardi, D. Greco, G. Stomeo, F. Potì, F. Bernini, I. Zanotti
Dipartimento di Scienze Farmacologiche, Biologiche e Chimiche Applicate, Università degli Studi di Parma, Italia
E-mail: giulia.lusardi@nemo.unipr.it

Treatment with immunosuppressant drug Cyclosporine A (CsA) has been demonstrated to increase cardiovascular risk in humans. Macrophage Reverse Cholesterol Transport (RCT) process, by which cholesterol is removed from the foam cells in the arterial wall and transported by lipoproteins (HDL) to the liver for elimination into the feces, is recognized as protective against atherosclerosis. CsA is able to affect this process, especially fecal excretion through the inhibition of CYP27A1, a key enzyme of the biliary acids synthesis pathway.
This mechanism could not be the only one by which CsA exerts its pro-atherogenic activity. In this study we investigated if CsA treatment can affect the first step of macrophage RCT, evaluating the drug influence on the capacity of macrophages to release cholesterol, and on plasma capacity to stimulate lipid efflux from cells. J774 macrophages were treated with CsA (1-5 µM) and efflux was stimulated by incubation with mouse plasma, but no significant alterations by the drug were observed. Plasma was isolated from mice treated or not with CsA (50 mg/kg/die for 14 days), and used as lipid acceptor in efflux experiments.
Plasma from mice treated with CsA showed an increased potential of efflux via passive diffusion (mean±sd: 4,5%±0,7 vs 3,0%±0,3; P<0,01) and ATP Binding Cassette transporter A1 (ABCA1) (mean±sd: 4,4%±0,3 vs 2,6%±1,2; P<0,05).
No significant differences were found in ATP Binding Cassette transporter G1 and SR-B1-mediated efflux. From this data we can exclude an effect of CsA treatment on RCT first step, cholesterol efflux. Interestingly the in vivo CsA treatment, despite not showing any quantitative modification in total cholesterol, HDL and triglycerides, seems to increase efflux potential of plasma via ABCA1. This result can suggest CsA influence on HDL quality, potentially increasing the pre βHDL-HDL fraction and apolipoprotein AI, specific acceptors of ABCA1.
This hypothesis and the mechanism accounting for this effect deserve further investigations.

HYPERTENSIVE PATIENTS WITH DIABETES MELLITUS AND NORMAL ARTERIAL STIFFNESS SHOW AN EARLY INCREASE IN RENAL RESISTIVE INDEX

V. Maestripieri, G. Pacciani, N. Zaffarana, I. Tassinari, M.C. Cavallini, A. Berni, E. Ciani, M. Bernetti, R. Abbate, L. Poggesi, M. Boddi
Dipartimento di Area Critica Medico-Chirurgica, Università di Firenze, Azienda Ospedaliero-Universitaria Careggi, Firenze, Italia
E-mail: berniandrea@inwind.it

Background. Renal resistive index (RRI) detected by Doppler ultrasound is influenced by intra- and extra-renal factors. Increased RRI in patients with normal renal function detects tubulo-interstitial damage, which may be present before glomerular damage. Arterial wall stiffness evaluated by estimation of pulse wave velocity (PWV) is one of the most significant extra-renal factors. Both RRI and PWV are significantly higher in patients with hypertension and diabetes mellitus (DM) than in age-matched control subjects. However, the role of intra- and extra-renal factors in determining the RRI increase is still under debate. This study was aimed to evaluate whether high RRI values of patients with hypertension and DM reflect tubulo-interstitial damage or increased arterial stiffness.
Methods. We studied hypertensive patients between 45 and 75 years, in chronic antihypertensive therapy, with or without DM, with conserved renal function (creatinine clearance >60 mL/min). RRI [(peak systolic velocity - end - diastolic velocity]/peak systolic velocity) was calculated by the analysis of the Doppler flow wave obtained at the renal interlobar arteries and considered pathologic when ≥0.70 or >95% of upper confidence limit expected for age decade.
Results. We evaluated 21 patients (58±8 years, 15M/6F). Ten were affected by DM. Patients with DM were older (62±8 vs 54±4 years, P=0.01) and had significantly higher RRI values (0.69±0.05 vs 0.65±0.07, P<0.05) and prevalence of pathologic RRI (6/10 vs 2/11, P<0.05), compared to patients without DM. There was no significant difference in PWV values between hypertensive patients with or without DM (approximately 8 m/sec). DM resulted a significant independent predictor for pathologic RRI (crude O.R.6.8; CI95%1.2-49.2; P<0.05), even after adjustment for age (O.R.8.1; CI95%1.1-85.8; P<0.05) and PWV (O.R.5.1; CI95%1.1-40.0; P<0.05).
Conclusions. In our hypertensive patients with DM, increased RRI values may reflect a reduction in intra-renal compliance due to tubulo-interstitial damage, rather than an increase in systemic arterial stiffness.

FUNCTIONAL EFFECT OF NOVEL AMINO ACID VARIANTS OF APOLIPOPROTEIN B IN FAMILIAL HYPOBETALIPOPROTEINEMIA

A.L. Magnolo[1], T. Fancello[1], E. Pinotti[1], V. Valenti[2], A.B. Cefalù[2], M.R. Averna[2], P. Tarugi[1]
[1]Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy; [2]Department of Internal Medicine and Medical Specialities (DIMIS), University of Palermo, Italy
E-mail: lucillamag@libero.it

Introduction. Familial Hypobetalipoproteinemia (FHBL) is a codominant disorder characterized by reduced plasma levels of LDL-C and apolipoprotein (apo) B. In 50% of cases FHBL is due to mutations in APOB gene resulting in truncated apoBs of various

size. Some mutations in APOB gene resulting in non-conservative amino acid substitutions were reported to cause FHBL. In vitro, these mutations induce the retention of the mutant apoB in the endoplasmic reticulum (ER) and impair the secretion of apoB-containing lipoproteins. In two FHBL subjects we identified two novel amino acid variants (Thr26_27delinsAsn and Tyr102Cys) located in the N-terminal 1000 amino acids of mature apoB.
Methods. To investigate the functional effect of these mutations we constructed plasmids containing human apoB-48 cDNAs harbouring the mutations. McA-RH7777 rat hepatoma cells were transiently and stably transfected with wild type or mutant human apoB-48. The secretion efficiency of human apoB-48 was determined by immunoblotting. To evaluate whether the mutant apoB-48 was able to form apoB-containing lipoproteins, the incubation media were ultracentrifuged to separate the lipoprotein classes. Immunocytochemistry was used to assess the intracellular localization of the mutant proteins.
Results. The mutation Thr26_27delinsAsn strongly reduces the secretion of apoB-48 from the transfected cells. The mutant apoB-48 appears to be retained in ER as demonstrated by the confocal images showing co-localization of the mutant apoB with the ER marker. In stably transfected cells the defect of mutant apoB-48 secretion was confirmed by the absence of apoB-48 containing lipoproteins in the medium. These observations suggest that Thr26_27delinsAsn alters the structure of the beta-barrel of N-terminal domain of apoB (the first 267 amino acids of mature protein) preventing the secretion of apoB-containing lipoproteins. By contrast the mutation Tyr102Cys had no effect on apoB-48 secretion.
Conclusions. This finding supports the notion that Thr26_27 delinsAsn is the cause of FHBL.

TREDAPTIVE: EFFECTS ON SUSCEPTIBILITY TO OXIDATION OF LOW DENSITY LIPOPROTEIN AND ON ACTIVITY OF PARAOXONASE TYPE-1 AND PLATELET ACTIVATING FACTOR ACETYLHYDROLASE BOUND TO HIGH DENSITY LIPOPROTEIN

F. Mamone, S. Riggio, G. Mandraffino, A. D'Ascola, M. Cinquegrani, R. Mandraffino, M. Castaldo, M.A. Sardo, A. Saitta
University of Messina, Dept. of Internal Medicine and Medical Therapy, Messina, Italy
E-mail: federicamamone@msn.com

The use of statins is the most widely accepted lipid lowering strategy, and tested for primary and secondary prevention of cardiovascular disease in dyslipidemic subjects. Additional drugs, such as niacin (nicotinic acid), have been proven to have important clinical effects. Niacin decreases low density lipoprotein-cholesterol (LDL-C) and triglycerides (TG) and increases high-density lipoprotein-cholesterol (HDL-C) levels, reducing CV morbidity and mortality; non-lipid effects of niacin have also been suggested. Increased oxidation of LDL-C is a feature of atherosclerosis. Paraoxonase type 1 (PON1), an enzyme bound to HDL-C, and Platelet Activating Factor Acetylhydrolase (PAF-AH), an enzyme bound both to LDL-C and HDL-C, work in concert to contrast lipid oxidation and atherosclerosis. The present study aimed to investigate the effects of niacin therapy on PON1 and PAF-AH activities and LDL-C susceptibility to oxidation in patients with familial combined hyperlipidemia (FCHL) with no additional risk factor for cardiovascular disease. Forty-five untreated patients with diagnosis of FCHL (25 men and 20 women, mean age 39±11 years) were consecutively included. Patients received niacin/laropiprant at the standard dose of 1000/20 mg per day for 4 weeks. Lipid profile, glucose and fibrinogen levels, serum PON1 and PAF-AH activities and the susceptibility of LDL-C to oxidation were evaluated at baseline and after treatment. PON1 and PAF-AH activities were measured by the spectrophotometric method. The susceptibility of LDL-C to oxidation was determined as the production of conjugated dienes (LDL-C lag phase). After 4 weeks of treatment, plasma total cholesterol (CT), TG and LDL-C levels were significantly lower compared with baseline levels (TC: 179.72±40.81 vs 239.81±58.03 mg/dl, $p<0.001$; TG: 118.18±44.22 vs 350±97.19 mg/dl, $p<0.001$; LDL-C: 110.45±34.36 vs 131.63±51.84 mg/dl, $p<0.05$; NHDL-C: 134.09±40.14 vs 201.63±57.96, $p<0.01$), while HDL-C levels, PON1 and PAF-AH activities were significantly increased, as the LDL-C lag-phase was slowed (HDL-C: 45.63±13.27 vs 38.18±15.07 mg/dl, $p<0.001$; PON1: 239.6±97.1 vs 170.7±107 U/L, $p<0.001$; PAF-AH: 411.5±58 vs 345.26±105 U/L, $p<0.001$; LDL-C lag phase: 70.3±24.3 vs 57.3±12.6 minutes, $p<0.001$). HDL-C and LDL-C levels were not correlated with PON1 and PAF-AH activities and the LDL-C -lag phase, while the LDL-C -lag phase was significantly correlated with PON1 and PAF-AH activities (both $p<0.001$). The results of the present study show that niacin/laropiprant significantly improves lipid profile and lipoprotein antioxidant activities. The increase of PON1 and PAF-AH activities appears to be independent of the changes in plasma HDL-C and LDL-C levels, thus confirming niacin as a lipid lowering agent with additional non-lipid effects.

MICRORNAS INVOLVEMENT IN ABCA1 PATHWAY MODULATION IN HUMAN ATHEROSCLEROTIC PLAQUES

C. Mandolini[1], D. Santovito[1], P. Marcantonio[1], S. Ucchino[2], A. Mezzetti[1], F. Cipollone[1]
[1]Center of Excellence on Atherosclerosis, Hypertension and Dyslipidemia, "G. d'Annunzio" University and Clinical Research Center, Center of Excellence on Aging (Ce.S.I.); [2]Vascular Surgery Unit, "G. d'Annunzio" University, Chieti, Italy
E-mail: c.mandolini@unich.it

Background. The uptake of oxidized LDL by macrophage is a key initial event in atherosclerosis and the removal of oxidized lipids is considered anti-atherogenic. ATP-Binding Cassette transporter A1 (ABCA1) is a gene encoding for a transmembrane protein involved in cholesterol efflux pathway and its mutation is responsible for Tangier disease, a disease characterized by cholesterol accumulation. Together with another LXR-alpha responsive transporter, known as ABCG1, ABCA1 promotes cholesterol efflux to HDL. We have recently demonstrated a higher ABCA1 mRNA expression in human hypercholesterolemic atherosclerotic plaques correlated to intracellular cholesterol levels, but not followed by similar protein syntesis. Because microRNAs are small non-coding RNAs of 20-22 nt of length, strongly resistent to degradation and largely conserved in species with a post-transcriptional activity, the aim of this study was to investigate the modulation of cholesterol efflux pathway in human hypercholesterolemic and normocholesterolemic atherosclerotic plaques and the involvement of microRNAs in this modulation.
Materials and Methods. Plaques from both patients who underwent carotid endarterectomy were collected and stored at -80°C. Plaques were subdivided in equal part in order to exctract RNA with TRIZOL method and proteins by using RIPA buffer. Proteins were runned on an acrylammide gel, transferred on a nitrocellu-

lose membrane and immunoblotted with specific primary antibodies for ABCA1, ABCG1, PPAR gamma and Beta Actin for the normalization. Bands density was quantified by densitometric analysis. Total RNA was quantified and 1 µg was retrotranscribed with Archive Kit (Applied Biosystem). The cDNA was used to analyze by RealTime PCR the expression of ABCA1, ABCG1, LXRalpha mRNA. On the other side 75 ng of the total RNA was retrotranscribed and cDNA was used to study microRNAs expression by RealTime PCR . MicroRNAS probes were purchased from Exiqon. ABCA1 and CD68 Immunohistochemistry were performed on frozen plaques slides of 5 µM of thickness for ABCA1 immunolocalization on macrophages.
Results. RealTime PCR analysis shows a significant upregulation of ABCA1 (p=0.03) and ABCG1 (p=0.02) mRNA expression in plaques of hypercholesterolemic patients versus normocholesterolemic ones, and a not statistical significative difference between the two groups in LXR alpha mRNA expression (p=ns). Moreover in both groups, ABCA1 is significantly more upregulated at mRNA level compared to ABCG1 (hypercholesterolemic plaques, p=0.03; normocholesterolemic plaques, p=0.007).Western blot analysis doesn't show a significative difference in ABCA1 and ABCG1 protein levels between the two groups (p=ns). In contrast protein analysis in normocholesterolemic and hypercholesterolemic groups show a switch in ABCA1/ABCG1 ratio, infact there is a significative more ABCG1 expression in each group compared to ABCA1 (normocholeterolemic p=0.027, hypercholesterolemic, p=0.0002). Immunohistochemistry confirmed the co-localization between ABCA1 protein and CD68 macrophage scavenger receptor. Finally preliminary data on microRNAs shows a slight significative downregulation of has-mir-27b in hypercholesterolemic patients (p= 0.05) and has-mir-145 (p= 0.03).
Conclusions. Our preliminary data show a strong post transcriptional regulation of ABCA1 but not ABCG1 in atherosclerotic plaques. At least in part, this may be consequence of specific microRNAs activation. This is responsible for a re-organization of this cholesterol efflux pathway. The clinical and pharmacological rule of this phenomenon should be investigated by further studies.

MICRORNAS PROFILING IN HUMAN ATHEROSCLEROTIC PLAQUES

C. Mandolini[1], D. Santovito[1], P. Marcantonio[1], S. Ucchino[2], F. Magnacca[2], A. Mezzetti*, F. Cipollone[1]
[1]Center of Excellence on Atherosclerosis, Hypertension and Dyslipidemia, "G. d'Annunzio" University and Clinical Research Center, Center of Excellence on Aging (Ce.S.I.); [2]Vascular Surgery Unit, "G. d'Annunzio" University, Chieti, Italy
E-mail: c.mandolini@unich.it

Background. Atherosclerosis is a progressive inflammatory disease whose characteristic lesion is the atherosclerotic plaque mainly composed of vascular smooth muscle cells, oxidized LDL and macrophages surrounded by a dense collagenous stroma. Plaque formation, progression and rupture is caused by interaction of environmental and genetic factors. MicroRNAs are small non-coding RNAs of 20-22 nt of length with an inhibitory activity on the translation of an mRNA target. Currently microRNAs involvement have been demonstrated in many diseases such as cancer and cardiac remodeling; however their involvement in atherosclerosis is still unclear. Thus, the aim of this study was to realize the microRNA profiling of the atherosclerotic lesions in patients with hypercholesterolemia.
Materials and Methods. Carotid atherosclerotic plaques were surgically removed from 22 patients underwent carotid endarterectomy and divided according to LDL cholesterol levels (normocholesterolemic = LDL ≤100 mg/dL, n: 11; hypercholesterolemic = LDL ≥160 mg/dL, n: 11). RNA and proteins were extracted according with previously validated methods. Then RNA was quantified and pooled in order to have one sample for each group (normocholesterolemic vs hypercholesterolemic). Finally, retrotranscritpion was performed by using MiRCURY LNA kit and the cDNA was dispensed on the Human Panel 1 platform (Exiqon). Data analyses were performed with deltadelta ct method.
Results. Microarray analysis of 384 microRNAs on normocholesterolemic plaques (LDL ≤100) vs hypercholesterolemic ones (LDL ≥160) showed that 8 microRNAs are strongly upregulated (≥20 fold induction), 7 microRNAs are upregulated (from 7 to 15 of fold induction) and 7 are strongly downregulated or almost absent (≤0,01 fold induction). Of particular interest appear the data regarding hsa-miR-302c (which is one of the 7 upregulates miRNAs) and hsa-miR-302d (which is strongly downregulated), as both have ABCA1 as target gene. On the other side, hsa-miR-208 which target ApoE was the most upregulated miRNA in these plaques.
Conclusions. To the best of our knowledge, this is the first report describing the miRNA profiling in human atherosclerotic plaques. Futhermore, this study demonstrates that hypercholesterolemia leads to activation or inactivation of specific microRNAs that are directly involved in cholesterol efflux pathway. Further studies on these specific miRNAs targets are required to better understand this modulation.

MICRO RNA 221/222 LEVELS IN CD34+ CELLS FROM HYPERTENSIVE SUBJECTS WITH ARTERIAL STIFFNESS AND WITH OR WITHOUT LEFT VENTRICULAR HYPERTROPHY

G. Mandraffino, S. Riggio, F. Mamone, E. Imbalzano, C. Tomasello, M. Creazzo, A. Versace, M.A. Sardo, A. Saitta
University of Messina, Dept. of Internal Medicine and Medical Therapy, Messina, Italy
E-mail: gmandraffinomd@libero.it

Circulating cardiovascular progenitor cells (CPCs), including CD34+ cells and endothelial progenitor cells (EPCs), contribute to endothelial repair and angiogenesis. In CPCs, NADPH oxidase (Nox)–derived Reactive Oxygen Species (ROS) have a central role as signalling messengers in driving angiogenesis. "In vitro" experiment suggest that redox control of angiogenesis is regulated by micro RNA (miR) 221/222; miR 221/222 are a class of small RNAs that negatively regulate protein expression at the post-transcriptional level. Although ROS have been shown to exert modulating features, as proliferative and antiapoptotic signals, they may have detrimental effects when in excess. Cellular defences against excessive ROS production include manganese superoxide dismutase (MnSOD), catalase (CAT) and glutathione peroxidase-type1 (GPx-1). The aim of the present study was to investigate the relationship between circulating CD34+ cells and expression of intracellular miR-221/222, Nox-containing subunit gp91phox, MnSOD, CAT, GPx-1 and ROS level, arterial stiffness (AS) and left ventricular mass (LVM) in patients with arterial hypertension. We included 74 hypertensives; 34 patients (31.5±11 years; 24 men and 10 women) with increased AS (group A), and 41 (34.7±10.3 years 26 men and 15 women) with increased both AS and LVM (group B). As a control group, 29 normotensive subjects matched for sex and age were also included. All patients were newly diagnosed, untreated

hypertensives with no additional risk factors for atherosclerosis or clinical and instrumental signs for cardiovascular disease (CVD). Fresh blood flow cytometry was used to identify and count circulating CD34+cells. Immunomagnetic sorting was used to enrich the sample of CD34+ cells. In the enriched sample, the expression of miR-221/222 and, gp91phox, MnSOD, CAT and GPx-1-mRNA was measured by real-time RT-PCR. ROS generation was measured by flow cytometry using a fluorescent compound. Carotid intima media thickness (cIMT), AS and LVM were evaluated by ultrasound methods. Circulating CD34+ cells were significantly higher in group A ($p < 0.001$) and lower in group B ($p < 0.05$) as compared with controls. Expression of miR-221/222, gp91phox, MnSOD, CAT and GPx-1-mRNA in CD34+ cells were higher in hypertensives than in controls ($p < 0.001$). No difference in miR-221/222 expression was found between group A and controls. Group B showed higher values of miR-221/222, gp91phox, MnSOD, CAT and GPx-1-mRNA ($p<0.001$) with respect to group A. ROS generation was slightly increased ($p=0.04$) in group A and significantly higher in group B ($p < 0.001$) with respect to controls. In group A the number of CD34+ cells correlated with gp91phox, MnSOD, CAT and GPx-1 and with ROS, fibrinogen, and AS. In group B CD34+ cells correlated negatively with miR-221/222, ROS and LVM. Our data suggest that in the early stages of hypertension, the CD34+ cell count can be associated with molecules, such as gp91phox and ROS, and with miR-221/222, which may contribute to the protective function of CPCs on the vascular wall. Conversely, with the progression of the hypertension related lesions, the highest increase of these molecules may be associated with an impairment in cell number and vascular repair capability.

PREDICTORS OF CARDIOVASCULAR MORTALITY AND CARDIOVASCULAR PREVENTION IN PATIENTS ADMITTED TO AN INTERNAL MEDICINE WARD

M.R. Mannarino, F. Di Filippo, R. Del Giorno, C. Fattori, P.G. Mariani, R. Colella, A. Pedicino, M. Pirro, G. Lupattelli, L. Pasqualini
Internal Medicine, Angiology and Arteriosclerotic Diseases, University of Perugia, Italy
E-mail: m.mannarino@libero.it

Pharmacological drug therapy for cardiovascular prevention is frequently underused among elderly patients hospitalized in the internal medicine wards. Our study analyzed the prevalence of cardiovascular risk factors, predictors of mortality and adequacy of preventive cardiovascular drug therapy in 707 patients, aged 50 years or older, admitted to our Department of Internal Medicine. Among the enrolled patients 81% had a history of hypertension, 45% were smokers, 26% had diabetes and 23% were dyslipidemic. Twenty-eight percent suffered for a previous myocardial infarction, 8% had a previous stroke and 8% had peripheral arterial disease. Thirty-four percent was under antiplatelelet treatment and 15% was taking statins. Particularly, only 19% patients at high cardiovascular risk was taking a statin. Among high risk patients 41% had LDL-cholesterol above the conservative recommended target (<100 mg/dL) and only 27% reached the more aggressive LDL-cholesterol target (<70 mg/dL). Among patients with previous cardiovascular events, antiplatelets were prescribed in 53% patients. The main diagnosis at the hospital discharge was an acute cardio- and cerebrovascular event in 30% of the patients (AMI 15%, angina 3%, Stroke 9%, TIA 3%). During a mean follow-up period of 1.6 years a total of 163 patients died, 63 of whom from cardiovascular causes (39%). The patients who died were older and more frequently diabetic, had a lower BMI and total cholesterol and higher creatinine. This group of patients also exhibited an higher prevalence of ECG abnormalities and previous ischemic events. Alive patients were more frequently under statins treatment (17% vs 8%; $p=0.006$), while antiplatet treatment did not affected survival. In conclusion our results suggest that despite high cardiovascular morbility and mortality, the rates of cardiovascular preventive therapies we recorded in our patients are low. Hospital stay for any reason could be therefore an opportunity to detect cardiovascular risk and to initiate appropriate preventive actions.

EFFECT OF ROSUVASTATIN ON ABCA1 AND ABCG1 EXPRESSION IN HUMAN ATHEROSCLEROTIC PLAQUES: THE QUASAR STUDY

P. Marcantonio[1], G. Desideri[2], D. Santovito[1], C. Mandolini[1], G. De Blasis[3], A. Mezzetti[1], F. Cipollone[1]
[1]Center of Excellence on Atherosclerosis, Hypertension and Dyslipidemia, "G. d'Annunzio", University and Clinical Research Center, Center of Excellence on Aging (Ce.S.I.), "G. d'Annunzio" University Foundation, Chieti, Italy; [2]University of "L'Aquila", L'Aquila, Italy; [3]Vascular Surgery Unit, Avezzano Hospital, Italy
E-mail: p.marcantonio@unich.it

Background. Statins are the leading therapy for the treatment of hypercholesterolemia and ischemic artery disease. Although lowering of plasma low-density lipoproteins (LDL) is the main explanation for their cardioprotective effect, however other mechanisms may be involved. Growing evidences suggest that statins may induce a series of biological effects which are not directly correlated to cholesterol levels (so-called "pleiotropic" effects). Increasing attention is being given to the role of ATP-binding cassette transporter A1 (ABCA1) and G1 (ABCG1) in atherosclerosis. These membrane proteins act through distinct, yet synergistic, mechanisms to promote the removal of cholesterol from lipid-laden macrophages and their expression is modulated by intracellular cholesterol levels. However, the mechanism linking lipid-lowering drugs and ABCA1/ABCG1 expression need further analysis. Thus, in this study we investigated whether a short-time treatment with low or high dose of rosuvastatin may affect ABCA1/ABCG1 expression in human atherosclerotic plaques.
Material and Methods. 70 patients with severe stenosis of the extracranial tract of the internal carotid artery were randomized to receive a 12 week low (10 mg/day) or high (40 mg/day) doses of rosuvastatin before the elective endoarterectomy. Then, plaques were collected, appropriately dissected and stored for the subsequent analysis. A control group of 9 plaques collected from subjects with normal cholesterol levels (LDL<130 mg/dL) and a reference group of 6 plaques from subjects with high cholesterol levels (LDL>160 mg/dL) without statin treatment were selected. Patients with previous atherothrombotic events were excluded. ABCA1 and ABCG1 protein expression was evaluated by Western Blot analysis.
Results. At this time, we have completed the analysis of 9 plaques in the high dose (40 mg) rosuvastatin group, 14 plaques in the low dose (10 mg) rosuvastatin group and 6 and 9 plaques in hypercholesterolemic and normocholesterolemic groups, respectively. We found a trend toward increased ABCA1 protein expression in patients treated with high dose rosuvastatin, in contrast no differences in ABCA1 protein levels were observed among plaques from patients treated with low-dose rosuvastatin as compared to normo-

cholesterolemic or hypercholesterolemic groups. On the contrary, both rosuvastatin doses significantly reduced ABCG1 protein levels compared to hypercholesterolemic patients. The magnitude of ABCG1 realized by statin therapy was comparable with the different observed in normocholesterolemic vs hypercholesterolemic subjects (p=0,007 by ANOVA).
Conclusions. These preliminary data suggest that short-term rosuvastatin treatment may reduce ABCG1 protein levels in atherosclerotic plaques at both high and low doses. The magnitude of this reduction was similar to the differences observed in normocholesterolemic plaques compared to hypercholesterolemic ones, suggesting that ABCG1 reduction realized by statin therapy is the direct consequence of cholesterol reduction. In contrast, ABCA1 increase in atherosclerotic plaques observed by high dose but not by low doses of rosuvastatin is not related to drug induced cholesterol reduction, but could represent a pleiotropic effect of high dose rosuvastatin. Further investigations will be needed in order to understand mechanisms behind statin ABCA1/ABCG1 modulation in human atherosclerotic plaques.

ATERO E ARTERIOSCLEROSI NELL'INSUFFICIENZA RENALE CRONICA IN EMODIALISI: RUOLO DELL'INFIAMMAZIONE

A. Marinelli[1], V. Pistolesi[2], P. Luciano[3], G. Baudena[1], M.T. Ferrazzano[1], M. Golisano[4], F. Della Grotta[1]
[1]U.O.C. Nefrologia e Dialisi, Osp. Riuniti di Anzio, Roma, Italia; [2]Centro Dialisi "Villa dei Pini", Anzio, Italia; [3]Servizio di Radiologia, Osp. Riuniti di Anzio, Italia; [4]Centro Dialisi "Annunziatella", Roma, Italia
E-mail: annibalemarinelli@virgilio.it

Introduzione. Le calcificazioni vascolari (CV) sono associate ad aumentata mortalità cardiovascolare nell'insufficienza renale cronica e nel diabete, in particolare in studi radiologici condotti a livello dell'arteria femorale superficiale. I due tipi di CV, le calcificazioni arteriose dell'intima (AIC) e della media (AMC) possono interessare indipendentemente determinate regioni della parete arteriosa. Le complicazioni associate a queste due tipi di CV sono differenti: la AIC si associa soprattutto con stenosi od occlusione del vaso interessato, la AMC con rigidità vascolare, aumento della pressione arteriosa differenziale e ridotta perfusione periferica. Scopo del lavoro è stato quello di valutare la presenza di AIC e AMC in soggetti non diabetici in emodialisi da almeno cinque anni, un tempo sufficiente per sviluppare CV, e di valutare la loro associazione con fattori di rischio cardiovascolare e non.
Materiali e Metodi. Sono stati studiati 34 soggetti non diabetici, in emodialisi cronica (17M/17F, età 59±17 anni) da almeno 5 anni (140±69 mesi). I soggetti sono stati sottoposti ad ecografia con sonda lineare da 7,5 MHz (Toshiba Corevision SSA-350A) dell'arteria femorale comune e superficiale per la ricerca di calcificazioni dell'intima e della media. L'arteria femorale comune è stata studiata in prossimità della biforcazione, l'arteria femorale superficiale dall'origine sino al terzo inferiore della coscia. La calcificazione dell'intima è stata considerata presente in caso di lesione iperecogena con cono d'ombra posteriore e con spessore >50% rispetto ai tratti di parete vascolare limitrofa; la calcificazione della media come deposizione lineare iperecogena nello spessore medio-intimale nei segmenti senza placche sovrapposte. Come controllo è stata eseguita una radiografia del femore bilaterale, valutata come positiva in presenza un aspetto "a binario" secondo quanto riportato in letteratura.
Risultati. Le CV, espresse come AIC e/o AMC erano presenti nel 79% dei casi. Le calcificazioni dell'intima e della media erano presenti rispettivamente nel 59% e nel 62% dei soggetti. Alla radiologia, la calcificazione arteriosa della media era presente solo nei pazienti con severa deposizione lineare calcifica rilevabile all'ecografia (26%) mentre era negativa nei rimanenti casi. Considerando i due tipi di calcificazione separatamente, i soggetti con AIC erano significativamente più anziani (67±12 vs 47±1 anni, p <0,001), mentre non vi erano differenze di età tra soggetti con/senza AMC (61±12 vs 55±20 anni p=NS). La proteina C reattiva era più alta nei soggetti con AMC rispetto ai negativi (12,2±11,3 vs 2±1,7 mg/l) e sebbene in modo non significativo, una stato microinfiammatorio era osservabile anche in caso di AIC (PCR 9,6±9,8 vs 6,5±10,9 p=NS). Il tempo di dialisi, il sesso, il fumo, l'ipertensione arteriosa, la dislipidemia, l'insulino-resistenza, l'albumina, il calcio, il fosforo, il paratormone e i trattamenti farmacologici associati, non differivano tra soggetti con AIC/AMC e assenza di calcificazioni. All'analisi multivariata, l'età anagrafica >50 anni e valori di PCR >5 mg/l si associavano alla presenza di AIC (p=0,007) e AMC (p=0,003).
Conclusioni. L'ecografia può essere impiegata non solo per la ricerca delle calcificazioni intimali, ma anche per la diagnosi di calcificazioni della media, in una fase più precoce rispetto alla radiologia, che attualmente rappresenta il "gold standard" per questo tipo di calcificazione. In dialisi, l'infiammazione sembra giuocare un ruolo importante soprattutto nel determinismo delle calcificazioni della media che rappresenta la vera lesione vascolare in questi tipi di pazienti e che a differenza delle calcificazioni dell'intima, tende a manifestarsi nei soggetti più giovani.

CARDIOVASCULAR RISK FACTORS SURVEILLANCE BY THE ITALIAN BEHAVIOURAL RISK FACTOR SURVEILLANCE SYSTEM (PASSI)

V. Mastrilli, S. Vasselli, R. D'Elia, G. Laurendi, G. Filippetti, Gruppo Tecnico Passi[1] (S. Salmaso, S. Baldissera, N. Bertozzi, G. Carrozzi, B. De Mei, G. Ferrante, S. Campostrini, P. D'Argenio, A. D'Argenzio, P. Fateh-Moghadam, V. Minardi, E. Quar)
Ministero della Salute, Italia; [1]Istituto Superiore di Sanità, Italia
E-mail: v.mastrilli@sanita.it

Introduction. Chronic diseases are the leading cause of death in the Western world and are mainly attributable to a few and modifiable risk factors. The adoption of unhealthy lifestyle is an epidemiological emergency in relation to risk of cardiovascular disease, cancer, diabetes, which are the major causes of mortality and morbidity in adult population. Surveillance systems have a strategic role in addressing public health policies, providing health-related data essential to the planning, monitoring, and evaluation of programmes and actions. In Italy, since 2007, Behavioural Risk Factor Surveillance System (PASSI) monthly collects, at local level, representative and well-performing data on behavioral risk factors and preventive measures in the adult population (18-69 years). The topics explored by PASSI are: perceived health, physical activity, smoking, diet, alcohol consumption, road safety, cardiovascular risk factors, cancer screening, adult immunizations, mental health, domestic accidents, sociodemographic aspects. Purpose To describe up-to-date results of indicators connected to cardiovascular risk factors, related from the interviewee population.
Methods. Monthly telephone interviews, by standardized questionnaire, are conducted by personnel of the Local Health Units

(LHUs) to a random sample of the resident general population 18-69 years. More than 140.000 interviews have been collected (2007-May 2011). In 2010, 138 LHUs participated to surveillance with an eligibility rate of 96%, response rate: 87%, substitution rate: 13%. We consider data collected on the pool of ASL in 2010 and related to cardiovascular risk factors: hypercholesterolemia, hypertension, diabetes.
Results. Cholesterol was measured at least once in their lifetime in 79% of the population. High cholesterol diagnosis is 24%. The interregional differences are statistically significant, both for the cholesterolemia measure at least once in lifetime (from 67% of Molise to 89% Basilicata), and for the related diagnosis of hypercholesterolemia (16% in Campania, 29% Calabria). 32% of adults interviewed are treated with a drug, a General Practitioner (GP) recommended a proper diet to the 85%, suggested to control body weight and to practice regular physical activity to the 78%. In the last 2 years blood pressure was controlled to 83% of the population. The percentage of population with hypertension diagnosis was 20.3%. The interregional differences are statistically significant, both for the blood pressure control (from 65% of Basilicata to 88% of Liguria and Lombardia) and for the related diagnosis of hypertension (16% in Lombardia, 27% in Calabria). The 79% refer to be treated with a drug, a GP recommended a salt consumption reduction to the 87% and weight control and physical activity to the 81%. Over the last three years, the prevalence of hypertensive patients who received a medical prescription or advice about lifestyle is increasing. People who report having diabetes are 5% of the pool of ASL with a statistically significant north-south gradient (from 4% of the northern regions to 10% of Basilicata).
Conclusions. PASSI provides public health authorities with useful information to plan and evaluate interventions for chronic diseases prevention. Data from PASSI surveillance confirm the idea that the progress of a health care system requires greater interaction between service supply and demand, between users and providers of care. Data show that only 3% of adults 18-69 have not cardiovascular risk factors, 97.5% at least 1 risk factor and about 40% more than 3 (prevalence could be underestimated, due to self-reported data). Public health have to enable strategies that incorporate measures to promote healthy lifestyles at the population level with an appropriate clinical approach (early diagnosis and appropriate treatment of single and multiple risk factor).

FATTY-ACID BINDING PROTEIN-4 AND LIPOCALIN-2 CORRELATION WITH HIV-RELATED METABOLIC ABNORMALITIES

M.L. Morieri, J.M. Sanz, E. Dalla Nora, A. Minzoni, F. Di Vece, E. Lodi, L. Sighinolfi[1], G. Zuliani, A. Passaro
Department of Clinical and Experimental Medicine, Section of Internal Medicine, Gerontology and Clinical Nutrition, University of Ferrara, Italy; [1]Division of Infectious Diseases, Ospedale Sant'Anna, Ferrara, Italy
E-mail: psn@unife.it

Introduction and aim. HIV infection is frequently associated with a typical pattern of metabolic abnormalities recalling the metabolic syndrome. Lipokalin family proteins are novel adipokines associated with obesity diabetes and metabolic syndrome. Aim of this study was to determine circulating levels of fatty-acid binding protein 4 (FABP-4) and lipocalin-2 in a cohort of HIV-infected patients (n=43) compared to uninfected controls in order to evaluate their possible association with HIV-related metabolic abnormalities.
Methods. 43 HIV-infected (HIV+) patients and 10 uninfected controls (CTR) were enrolled. FABP-4 and Lipocalin-2 were measured by ELISA Kit (BioVendor-Laboratory Medicine, Palackeho, Czech Republic). All subjects underwent a medical examination and a blood sampling for biochemical analysis. Body composition was estimated by bioelectrical impedance analysis.
Results. HIV+ patients showed higher glycaemia (106±21 vs 87±11 p=0.01), triglyceridemia (193±176 vs 73±20 p=0,04) and number of metabolic syndrome criteria (ATPIII) =2,1±1,4 vs 0,6±0,8 p=0,02). Lipocalin-2 levels were significantly higher in HIV+ patients compared to controls while FABP4 concentrations did not differ. Among HIV+ patients those receiving HAART therapy showed highest Lipocalin 2 concentration (CTR 31±8; HAART 49±19; Naïve 40±12; Anova between groups p=0.01). In both CTR and HIV+ FABP 4 correlates adiposity index.
Conclusion. As expected HIV patients have a worse metabolic profile. HIV+ subjects showed higher lipocalin-2 levels compared to controls while HIV infection does not seem to influence FABP4 concentration which seems to mainly depend on adiposity. Whether Lipocalin-2 plays a role in HIV associated "metabolic syndrome" has to be elucidated.

INCREASING OXIDATIVE STRESS MARKERS AND REPRESSION OF NRF2/ANTIOXIDANT RESPONSE ELEMENT IN PATIENTS WITH ACUTE VERSUS CHRONIC CORONARY ARTERY DISDASE

C. Mozzini. A. Fratta Pasini, U. Garbin, L. Cominacini
Azienda Ospedaliera Universitaria Integrata Verona, Dipartimento di Medicina, Sezione di Medicina Interna D, Verona, Italy
E-mail: chiaramozzini@libero.it

Introduction. Evidence has been generated that oxidative stress and a weakened antioxidant defence are strongly implicated in atherogenesis. Experimental studies have suggested that the transcription factor NF-E2-related factor 2 (Nrf2) induces the expression of antioxidant genes and reduces oxidative stress. Moreover, since monocyte/macrophage plays key roles both in initiation and progression of atherosclerosis, Circulating Peripheral Blood Mononuclear Cells (PBMC) are a particularly fitting surrogate for atherosclerotic tissue in vivo.
We have recently demonstrated the activation of Nrf2 in PBMC of moderate smokers, whereas in heavy smokers it was repressed. On the basis of these previous results, the present study was aimed to evaluate oxidative stress and the expression of Nrf2 in PBMC of patients with Acute Coronary Syndrome (ACS) compared to patients with Chronic Coronary Artery Disease (CAD), in order to define if oxidative stress affects the expression of the protective Nrf2 in different clinical manifestations of the same pathology: coronary atherosclerosis.
Materials and Methods. 22 patients with ACS, 29 patients with CAD and 23 age-matched healthy controls were enrolled. ACS patients underwent percutaneous coronary angioplasty revascularization within 24 hours from the onset of symptoms, while in CAD patients, asymptomatic at the time of the enrolment, coronary heart disease was previously diagnosed by angiographic examination or by Cardiovascular Computed Tomography. Oxidative stress was evaluated measuring plasma oxidized phospholipid 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphorylcholine (PGPC) and GSH concentrations (HPLC/mass spectrometry and HPLC respectively). Nrf2 (mRNA and protein expression) was evaluated in circulating PBMC by Real Time PCR and Western blotting.

Results. There were no statistical differences among traditional cardiovascular risk factors, drug treatments and total, LDL, HDL cholesterol and plasma glucose between ACS and CAD group. PGPC concentrations resulted significantly higher in ACS and CAD patients than in controls ($p<0.01$). CAD and ACS had lower GSH concentrations than controls ($p<0.01$). Interestingly PGPC concentrations resulted significantly higher and GSH significantly lower in ACS compared to CAD patients ($p<0.01$). The expression of Nrf2 in PBMC was significantly lower in ACS and CAD than in controls ($p<0.01$). Noteworthy, in ACS patients the expression of Nrf2 was significantly lower than in CAD ones ($p<0.01$).
Conclusions. The higher levels of PGPC and the lower concentrations of GSH in ACS compared to CAD patients indicate that oxidative stress was at the most in the former group. Moreover the protective Nrf2 was abolished in ACS patients suggesting that the behaviour of this system is similar in heavy smokers and in ACS patients, making a link between heavy smoking and acute coronary event in term of oxidative stress response.

METHYLENETETRAHYDROFOLATE REDUCTASE C677T GENE POLYMORPHISM AND HOMOCYSTEINE LEVELS IN THE SARDINIAN POPULATION

Sa. Muntoni[1,2], V.P. Leoni[1], L. Atzori[1], R. Rossino[3], Se. Muntoni[2]
[1]Toxicology Department, Oncology and Molecular Pathology Unit, University School of Cagliari, Italy; [2]Centre for Metabolic Diseases and Atherosclerosis, The ME.DI.CO. Association, Cagliari, Italy; [3]Biomedical Science and Biotechnologies Department, University School of Cagliari, Italy
E-mail: smuntoni@unica.it

Introduction. Higher Levels of homocysteinemia (Hcy) are associated with several clinical conditions, such as risk of ischaemic heart disease (IHD), deep vein thrombosis with or without pulmonary embolism, and stroke. Hyper-Hcy results from either genetic or environmental factors. The lack of one of the three principal enzymes in homocysteine metabolism, may cause extremely high serum homocysteine levels, while the role of C677T mutation in the gene coding methylenetetrahydrofolate reductase (MTHFR) is controversial. Environmental factors and in particular dietary folate, which reduces homocysteine levels, influence the expression of the mutation. The aim of the present study was to measure plasma Hcy levels in the Sardinian population in relation to C677T genotype.
Materials and Methods. Random samples of free living populations were collected during 2006-2009 in South Sardinia (Pula). Overall, 182 subjects (70 males and 111 females) aged 30-80 years have been recruited. Each individual has been submitted to clinical visit, measure of BMI, WHR, systolic and diastolic blood pressure. A food frequency questionnaire and lifestyle questions has been administered. Plasma Hcy (normal values <12.0 µmol/L) was determined by chemiluminescence. DNA samples for MTHFR C677T polymorphism was PCR amplified and genotyped using pyrosequencing technology.
Results. The C677T genotype allele frequency was similar in both sexes (%): 22.8 (M) vs 28.8 (F) CC; 57.1 (M) vs 50,4 (F) CT; 20.0 (M) vs 20.7 (F) TT. Hcy levels appear to increase by ageing in the entire population examined (≤70 years =9.37 vs ≥70 years =13.02; $p<0.0001$). Considering data by sex, a significant increase in HCy was found in older female compared to younger (≤70 years =8.45 vs ≥70 years =13.20; $p <0.0001$); on the contrary, no differences were observed in the male group (≤70 years =11.09 vs ≥70 years =12.82; p=n.s.). Regarding Hcy levels and C677T genotype, no differences were observed in males; on the contrary, in females a significant increase in Hcy was found for the genotype TT compared to CC (11.59 vs 8.78; $p=0.036$).
Conclusion. In the Sardinian population, HCY levels appear to be a potential IHD risk factor in older women and with T677T genotype. All together, Hcy levels appear to be the result of gene-environment (folic acid in Mediterranean diet) interactions.

INVECCHIAMENTO E ARTERIOSCLEROSI: IMPORTANZA CLINICA DELL'INDICE DI WINSOR IN UNA POPOLAZIONE ANZIANA AMBULATORIALE

C. Mussi, M.A. Ferri, R. Scotto, G. Resta, G. Lancellotti, M. Bertolotti
Unità Operativa di Geriatria, Dipartimento di Medicina Endocrinologia Metabolismo e Geriatria, Università di Modena e Reggio Emilia, Italia
E-mail: marco.bertolotti@unimore.it

Scopo. Gli effetti sistemici dell'arteriosclerosi sono noti. L'indice di Winsor (Ankle-Brachial Index, ABI) rappresenta un test di screening insostituibile nell'esame obiettivo del paziente geriatrico in quanto consente di individuare soggetti con arteriopatia obliterante degli arti inferiori anche asintomatica (AOAI), monitorarne la progressione, valutare la capacità funzionale degli arti inferiori, predire il rischio di mortalità e morbilità cardiovascolare, predire il rischio di ictus, monitorare il decorso clinico di pazienti ad alto rischio.
Materiali e Metodi. Sono stati studiati 453 pazienti (età media 75,7 6,3 anni, 44,4% maschi). I soggetti sono stati sottoposti a valutazione cardiogeriatrica con valutazione dell'ipotensione ortostatica e della variabilità della frequenza cardiaca (HRV), misura della Cumulative Illness Rating Scale (CIRS), misure antropometriche, ECG in 12 derivazioni. Per la determinazione dell'ABI è stata utilizzata metodica MiniDoppler a flusso continuo. I soggetti sono stati suddivisi in due gruppi, in base al valore dell'ABI (normale, >0,90, N=401, patologico, <0,90, N=52).
Risultati. I pazienti con ABI patologico sono maggiormente affetti, in modo statisticamente significativo ($p<0.05$), da cardiopatia ischemica, ipertensione arteriosa, insufficienza venosa, diabete di tipo II, ictus e depressione.
Essi presentano inoltre punteggio CIRS più elevato, HRV più bassa e più importanti alterazioni elettrocardiografiche rispetto ai pazienti con ABI nella norma. All'analisi multivariata, l'ABI patologico è indipendentemente correlato ad elevati valori pressori, all'anamnesi positiva per cardiopatia ischemica e depressione, ad alti valori del punteggio CIRS, e a segni di ischemia laterale all'ECG.
Conclusione. L'ABI è un metodo semplice, rapido e poco costoso per identificare pazienti anziani con caratteristiche di fragilità. Consente di escludere la presenza di un'arteriopatia periferica senza ricorso ad accertamenti più dispendiosi e rivela la presenza di danno vascolare più diffuso, sia a livello cardiaco che cerebrale. Siamo convinti che tale misura dovrebbe essere inserita nella routine clinica per la valutazione multidimensionale dell'anziano.

MANAGEMENT OF SEVERE HYPERCHOLESTEROLEMIA IN A FAMILY BEARING C.974G>A LDL RECEPTOR MUTATION

M. Nunziata[1], G. Marotta[1], M.G. Coppola[1], M.F. Coppola[1], M. Gentile[1], G. Fortunato[2,3], M.N. D'Agostino[2,3], M.D. Di Taranto[4], P. Rubba[1]
[1]Dept. of Clinical and Experimental Medicine, "Federico II" Univ., Medical School, Naples; [2]CEINGE S.C. a r.l. Biotecnologie Avanzate, Napoli; [3]Dip. di Biochimica e Biotecnologie Mediche, Univ. di Napoli Federico II, Napoli, Italy; [4]IRCCS Fondazione SDN, Napoli, Italy
E-mail: mrnunziata@inwind.it

A 76 years old women with history of premature coronary artery disease, peripheral arterial disease and previous right carotid endarterectomy was followed in our outpatient clinic together with her daughter of 46 years old and her nephew 23 years old (both without history of cardiovascular (CV) disease). All patients had a diagnosis of family hypercholesterolemia (FH) according to the Simon Broome Register Criteria. At the first visit, the woman (proband) had high basal LDL-cholesterol (LDL-C 291 mg/dL) levels, she had been treated by statin therapy, only in the last 10 years, after CV events. Also her daughter, despite of being free of prevalent CV disease, had, at the first visit, high baseline LDL-C levels (294 mg/dL), and clinical signs of hypercholesterolemia, as Achilles tendons xanthomas and bilateral xanthelasma. Carotid echo-color-Doppler showed an increased intima-media thickness (1.2 mm). She underwent treatment with Rosuvastatin (20 mg/day) in the next 8 years reaching, after only three months a therapeutic target for LDL- C of 100 mg/dL. Low dosage of Rosuvastatin (10 mg/day) was prescribed after the first visit, also in the nephew, which showed high basal LDL-C levels (296 mg/ dL), despite the young age, and absence of signs of atherosclerosis. A genetic analysis was carried out for the detection of LDL-receptor mutations and a mutation (c.974G>A, p.Cys325Tyr) was identified at heterozygous status. In conclusion, early diagnosis and treatment of high LDL levels represent an important primary prevention strategy. Detection of mutations in members of family affected by FH may help to identify and treat patients at high CV risk, even before clinical evidence of atherosclerosis. In order to achieve adequate LDL-C target (<70 mg/day) more effective drug intervention, possibly including antisense nucleotides should be considered.
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VASCULOPROTECTIVE FUNCTION OF HDL FROM CETP-DEFICIENT SUBJECTS

A. Ossoli[1], S. Pozzi[1], P. Nilsson[2], W. Jessup[3], J.A. Kuivenhoven[4], R. Spina[5], V. Valenti[5], A.B. Cefalù[5], M.R. Averna[5], C.R. Sirtori, G. Franceschini[1], L. Calabresi[1], M. Gomaraschi[1]
[1]Center E. Grossi Paoletti, Department of Pharmacological Sciences, University of Milano, Italy; [2]Department of Clinical Sciences, Lund University, University Hospital, Malmö, Sweden; [3]Center of Vascular Research, University of New South Wales, Sydney, Australia; [4]Department of Experimental Vascular Medicine, Academic Medical Center, University of Amsterdam, the Netherlands; [5]Department of Clinical Medicine and Emerging Diseases, Policlinico "Paolo Giaccone", University of Palermo, Italy
E-mail: alice.ossoli@unimi.it

Objective. Cholesteryl ester transfer protein (CETP) is a plasma glycoprotein that catalyses the transfer of cholesteryl esters from HDL to the other plasma lipoproteins. Genetic deficiency of CETP is one of the known causes of primary hyperalphalipoproteinemia and represents a unique tool to evaluate how structural HDL alterations impact on HDL atheroprotective activity. Aim of the present study was to assess the vasculoprotective activity of HDL isolated from carriers of genetic CETP deficiency.
Subjects and Methods. HDL and HDL subfractions were isolated from carriers of the R37X, Q165X and IVS7+1 CETP mutations. HDL and HDL subfractions from carriers were tested for their protein/lipid composition and for their anti-inflammatory and NO-promoting activity in endothelial cells.
Results. HDL and HDL3 from carriers proved to be as effective as control HDL in down-regulating cytokine-induced VCAM-1 and in enhancing eNOS expression in endothelial cells. Carriers HDL2 were instead more effective than control HDL2 in inhibiting VCAM-1 and enhancing eNOS expression with a gene-dose dependent effect. These findings appear to be related to the peculiar lipid composition and the very high content in apoE of the HDL2 particles isolated from carriers of CETP deficiency. On the contrary, carrier HDL2 were less effective than control HDL2 in stimulating eNOS activation, likely because of a reduced S1P content.
Conclusions. Large, apoE enriched HDL that accumulate in genetic CETP deficiency are very efficient in maintaining endothelial cell homeostasis, supporting the use of pharmacological CETP inhibition to increase HDL levels and enhance HDL-mediated atheroprotection.

NUTRACEUTICAL COMBINATION (RED YEAST RICE, BERBERINE AND POLICOSANOLS) IMPROVES AORTIC PULSE WAVE VELOCITY IN LOW-MODERATE RISK HYPERCHOLESTEROLEMIC PATIENTS

F. Paciullo, M. Pirro, R. Del Giorno, M.R. Mannarino, S. Berisha, R.S. Helou, G. Lupattelli, F. Bagaglia, F. Melis, E. Mannarino
Unit of Internal Medicine, Angiology and Arteriosclerosis Diseases, University of Perugia, Italy
E-mail: Fpaciullo1986@mail.com

Introduction. Hypercholesterolemia is important in the pathogenesis of arterial stiffness. Treatment with a combination of red yeast rice, berberine and policosanols reduces cholesterol levels in hypercholesterolemic patients. We sought to determine whether the same nutraceutical combination would improve aortic stiffness in hypercholesterolemia.
Methods. Fifty-four hypercholesterolemic patients were assigned to oral nutraceutical combination (NC, red yeast rice 200 mg, berberine 500 mg and policosanols 10 mg) or no active treatment (noNC). Lipid levels and aortic pulse wave velocity (aPWV) were assessed before and after treatment.
Results. NC reduced total cholesterol by 13.4% and low-density lipoprotein (LDL) cholesterol by 18.7%. NC was associated with a reduction in aPWV (from 9.1±1.9 to 8.4±1.6 m/sec, p=0.003), whereas any change was observed in the noNC arm (from 9.0±1.9 to 9.2±2.0 m/sec, p=NS). LDL cholesterol reduction was associated with improvement in aPWV (rho=0.32, p=0.02). In regression analyses, NC was associated with the presence of aPWV amelioration (OR and 95% CI, 3.9, 1.2-12.7) and the degree of aPWV reduction (b=0.33, p=0.02).
Conclusions. In patients with hypercholesterolemia, combined therapy with red yeast rice, berberine and policosanols reduced cholesterol levels and improved aortic stiffness. An association between cholesterol reduction and aortic stiffness was also found.

CONTROLLO DEI FATTORI DI RISCHIO CARDIOVASCOLARE NELLA COMUNE PRATICA CLINICA IN UN CAMPIONE DI PZ. IN PREVENZIONE SECONDARIA RAPPRESENTATIVO DELLA POPOLAZIONE ABRUZZESE

C. Paganelli, D. Santovito, M. Pagnottella, F. Cipollone, A. Mezzetti, M. Bucci
Centro di Eccellenza Europeo e di Riferimento Regionale per lo Studio dell'Arteriosclerosi, dell'Ipertensione e delle Dislipidemie, Università "G. d'Annunzio", Chieti, Italia
E-mail: camilla.paganelli@gmail.com

Background. Molta attenzione è stata posta nei confronti dei pazienti in prevenzione primaria ma poco è noto sui pazienti in prevenzione secondaria. Questi vengono classificati ad alto/altissimo Rischio Cardiovascolare Globale con target terapeutici sempre più restrittivi. Scopo di questo lavoro è la valutazione dell'aderenza alle raccomandazioni delle attuali linee guida per il trattamento dei pazienti in prevenzione secondaria.
Materiali e Metodi. dati relativi ai vari fattori di rischio cardiovascolare, alla terapia in atto e alla sua efficacia (raggiungimento del target) di un campione di pazienti in prevenzione secondaria, sono stati estratti (software Quick-Openetica) dai database di un gruppo di MMG (n= 25) rappresentativo della popolazione della provincia di Chieti.
Risultati. 369 schede (su 400) risultavano complete e analizzabili. I maschi erano 261 (70,7%), le femmine 108; l'età media era di 71,7±9,84 anni. I diabetici erano 149 (40,4%): 125 (83,9%) avevano un dato recente di HbA1c (media =7,11%; ±1,19) che non era a target (<7%) in 69 pazienti (46,3%). Nonostante il pregresso evento CV, 32 pazienti (8,7%) continuavano a fumare. 310 pazienti (84%) avevano un dato recente relativo alla PA; 241 (65,3%) erano in trattamento ma solo il 54,8% aveva una PAS adeguatamente trattata (PAS <130 mmHg). Tra i pazienti ultraottantenni (n=88) il 30,7% non era sufficientemente trattato (PAS >140 mmHg). 334 pazienti (90,5%) avevano dati relativi al profilo lipidico: 313 (84,8%) risultavano in trattamento con statina, solo 189 (=60,4%) avevano un valore di LDL-C <100 mg/dL. Dati relativi a BMI (305 pazienti) e circonferenza vita (263 pazienti) hanno documentato una significativa prevalenza di sovrappeso/obesità (=79,3%) con disposizione centrale del tessuto adiposo (media: M=102,25±11,86 cm; F=95,18±12,09 cm).
Conclusioni. I risultati dimostrano che, sebbene i vari fattori di rischio non siano ancora adeguatamente controllati, l'adozione di schede informatizzate sta migliorando la qualità della gestione di tali pazienti. Una maggiore consapevolezza dei MMG nei confronti di tale problematica potrà ulteriormente migliorare la gestione del RCG nei pazienti in prevenzione secondaria.

LA GESTIONE DEL PROFILO LIPIDICO NEI PZ IN PREVENZIONE SECONDARIA SUL TERRITORIO: LO STUDIO ENSURING A LONGER LIFE

M. Pagnottella, D. Santovito, C. Paganelli, A. Mezzetti, F. Cipollone, M. Bucci
Centro di Eccellenza Europeo e di Riferimento Regionale per lo Studio dell'Arteriosclerosi, dell'Ipertensione e delle Dislipidemie, Università "G. d'Annunzio", Chieti, Italia
E-mail: donato.santovito@email.it

Background. Secondo le recenti linee guida, il target di LDL-colesterolo (LDL-C) previsto per i pazienti in prevenzione secondaria in virtù del rischio cardiovascolare globale (RCG) molto elevato, dovrebbe essere <70 mg/dL. Il nostro studio ha indagato l'efficacia del controllo lipidico su una popolazione in prevenzione secondaria nella comune pratica clinica.
Materiali e Metodi. Lo studio ELL si compone di:
1. Fase retrospettiva: utilizzando l'applicativo gestionale QUICK-Openetica, 25 medici di medicina generale (MMG) abruzzesi hanno fornito informazioni sul profilo lipidico e sul trattamento ipolipidemizzante di 400 pazienti in prevenzione secondaria.
2. Fase prospettica (tuttora in corso): valuterà l'efficacia di un approccio integrato specialista-MMG nella gestione dei pazienti ad elevato RCG.

Risultati. 332 delle 400 schede totali sono risultate complete e valide per l'analisi. La colesterolemia totale media era 176 mg/dL (±39,01), la colesterolemia HDL era 50 mg/dL (±12,95), la trigliceridemia era 136 mg/dL (±69,09). Il 78,6% dei pazienti assumeva un trattamento ipolipidemizzante e i valori medi di LDL-C sono risultati di 98 mg/dL (±33,02). Solo il 20,7% risultava a target terapeutico (<70 mg/dL). Anche considerando un target meno restrittivo (<100 mg/dL, secondo ATP-III), solo il 60,5% dei pazienti risultava efficacemente trattato.
Tra i pazienti in trattamento, il 60,1% risultava in terapia con statine di II livello (come da attuale nota 13), il 5,8% con l'associazione ezetimibe+simvastatina mentre i restanti con statina di I livello. I pazienti trattati con statine di II livello o con l'associazione raggiungevano il target complessivamente nel 26,9% dei casi (63,3%, considerando il target <100 mg/dL) con una distanza media residua dal target del 29,2% (15,3%) mentre i pazienti trattati con statine di I livello raggiungevano il target nel 12,9% (54,1% se target <100 mg/dL) con distanza media residua dal target del 31,7% (14,6%). Solo il 22,5% dei pazienti non in trattamento aveva valori di LDL-C <70 mg/dL, mentre per i pazienti con valori di LDL-C >70 mg/dL la distanza media dal target era del 39,7% (13%).
Conclusioni. I dati retrospettivi del nostro studio dimostrano come, nei pazienti in prevenzione secondaria, il controllo del profilo lipidico non sia ancora soddisfacente.

MAY THE IMBALANCED ALLELIC EXPRESSION OF LDLR GENE HAVE A ROLE IN FH PHENOTYPIC EXPRESSION?

L. Parlati[1], A. Cantafora[1], M. Siciliano[1], I. Blotta[1], S. Bertolini[3], E. Poli[1], F. Maldarelli[1], G. Mennini[2], M. Rossi[2], S. Ginanni Corradini[1]
[1]Department of Clinical Medicine, Division of Gastroenterology; [2]Department of General Surgery "Paride Stefanini", Sapienza University, Rome, Italy; [3]Department of Internal Medicine, University of Genoa, Italy
E-mail: lucia_parlati@yahoo.it

The concept of Allelic Expression Imbalance (AEI) arises from the recent cognition that the parentally derived copies of genes display differences of expression that contribute to human variability and susceptibility to genetic diseases.
We developed a simple method for assessing the AEI of LDLR gene in RNA samples heterozygote for the common c.1959T>C SNP on exon 13. Briefly, we amplified a 125 bp section of LDLR messenger encompassing the polymorphism.
The PCR product was digested with Ava II enzyme and analysed by microchip electrophoresis.
The molar concentration of residual 125 bp product was considered proportional to the amount of T wild allele, while the average concentration of the digestion fragments of 79 and 45 bp was as-

sumed to be proportional to the amount of C mutant allele. Therefore, the molar fraction of the mutant allele was calculated by Fmut = [C allele] / [C allele + T allele], and the allele imbalance by R = [C allele] / [T allele].
The method was validated with mixtures of known ratios of the alleles prepared by mixing precise amounts of CC and TT homozygote genotypes. The accuracy of Fmut determination ranged between -4.1% and +2.5% when the C allele fraction raised from 0.20 to 0.80.
The repeatability of the method was below the 4%, as evaluated by repeated analyses of samples with different R values. The analysis of 24 liver biopsies taken from deceased hearth-beating organ donors showed that the 12 heterozygotes found among them had Fmut values of 0.3297+0.0995 (CV= 30.2 %) with a range spanning 3 times from minimum to maximum values (0.1612 and 0.4783, respectively).
The evident AEI of LDLR gene may affect the severity of heterozygote FH phenotype, especially when the LDLR mutation cause of FH co-segregates with the more expressed allele.

ATHEROSCLEROTIC PLAQUES INVESTIGATION IN APOE-/- MICE USING MRI AND FOLLOWING ADMINISTRATION OF B22956/1

C. Parolini[1], M. Busnelli[1], C. Cabella[2], L. Miragoli[2], G. Valbusa[2], F. Tedoldi[2], C.R. Sirtori[1], G. Chiesa[1]
[1]Department of Pharmacological Sciences, Milan, Italy;
[2]CRB Bracco Imaging SpA, Colleretto Giacosa, Italy
E-mail: giulia.chiesa@unimi.it

The brachiocephalic artery is a site where ruptured plaques have been reported to occur in apoE-/- mice. Aim of the study was to identify, by MRI, plaques occurring at the brachiocephalic artery. ApoE-/- mice were maintained for 8 or 12 weeks on a high fat diet. At the end of the dietary treatment, MR images were acquired before and after administration of the contrast agent B22956/1, in order to enhance the lesions.
Animals were then sacrificed and brachiocephalic arteries were removed. Immunohistochemical staining was used to characterize the atherosclerotic plaques. The MRI signal intensity of the plaques was calculated using the post- versus pre-contrast injection signal enhancement in a defined region of interest (ROI) and the percent of contrast between the plaque and muscle ROIs (Plaque to Muscle Contrast).
All the images were also scored by experts (Consensus Vote). No differences were observed between plaque volumes measured in apoE-/- mice after 8 and 12 weeks of diet. A correlation was observed between Plaque to Muscle Contrast and Consensus Vote. Moreover, the Plaque to Muscle Contrast was significantly higher in histologically confirmed plaques compared to that of non-histologically confirmed plaques.
A weak correlation was instead found between Consensus Vote and histologically confirmed plaques. Finally, the % of Extracellular Matrix Area was significantly lower and the % of macrophage area was significantly higher in detected plaques compared to that measured in not-detected plaques.
Our results showed that the contrast agent B22956/1 is able to enhance the MRI sensitivity of detecting atherosclerotic plaques characterized by a low content of extracellular matrix, i.e. plaques at elevated risk of rupture.

IPERLIPEMIA FAMILIARE COMBINATA (IFC) E DANNO RENALE: NOSTRE RILEVAZIONI IN DUE GRUPPI FAMILIARI

M. Pasquale[1], A. Bruzzese[2], F. Nasso[1], A. Persichini[3], G. Rondanini[3], G. Bellinghieri[2], V. Savica[2], D. Santoro[2]
[1]U.O. Medicina Interna, P.O. Polistena, ASP 5, RC, Italia;
[2]Università di Messina, Italia; [3]Università di Perugia, Italia
E-mail: maria_pasquale@alice.it

Introduzione. Nel corso di uno studio osservazionale condotto su 1748 pazienti afferenti ad un ambulatorio specialistico dedicato alla cura delle dislipidemie e mirato alla raccolta di prove per assodare se un primitivo disordine del metabolismo lipidico può essere considerato una causa per lo sviluppo e per la progressione della Malattia Renale Cronica (CKD), ci siamo proposti di rilevare la tipologia del danno renale eventualmente riscontrato nei soggetti dimostratisi "primitivamente" dislipidemici.
Materiali e Metodi. Dopo aver rilevato che la Iperlipemia Familiare Combinata (IFC) rappresentava la forma di più frequente riscontro, con una prevalenza del 50%, abbiamo selezionato due gruppi familiari con IFC nei quali si constatava la concomitante presenza di nefropatia cronica, analizzando gli elementi caratterizzanti il danno renale rilevato.
Risultati. Abbiamo selezionato, in particolare due famiglie colpite da IFC. Nell'ambito della prima famiglia, la madre e tutti i suoi quattro figli sono risultati interessati da un danno renale di tipo "glomerulare". La precisazione diagnostica della glomerulopatia, a seguito dell'effettuazione della biopsia renale, è stata di IgAN (Nefropatia con depositi di IGA) per tre dei cinque soggetti valutati. Un quarto soggetto ha fatto rilevare la presenza GSF (Glomerulosclerosi Focale). Il quinto componente della famiglia, pur presentando "anomalie urinarie associate" ed un iniziale declino del Filtrato Glomerulare (GFR), non è stato ancora sottoposto alla biopsia renale. Nella seconda famiglia abbiamo osservato tre generazioni di soggetti. In alcuni componenti di tutte e tre le generazioni, laddove era stata eseguita la precisazione diagnostica istologica, veniva diagnosticata la IgAN. In molti altri componenti del gruppo familiare, sia attraverso la rilevazione diretta, sia attraverso la rilevazione anamnestica, risultavano presenti "anomalie urinarie associate" e/o una franca Malattia Renale Cronica (CKD) con riduzione del GFR, anche con Insufficienza Renale Cronica evoluta fino alla fase uremica terminale (ESRD).

DISLIPIDEMIE GENETICHE E DANNO RENALE: RISULTATI DI UNO STUDIO OSSERVAZIONALE

M. Pasquale[1], A. Bruzzese[2], F. Nasso[1], A. Persichini[3], G. Rondanini[3], G. Bellinghieri[2], V. Savica[2], D. Santoro[2]
[1]U.O. Medicina Interna, P.O. Polistena, ASP 5 RC, Italia;
[2]Università di Messina, Italia; [3]Università di Perugia, Italia
E-mail: maria_pasquale@alice.it

Introduzione. La dislipidemia costituisce un disordine metabolico altamente frequente nei soggetti con Insufficienza Renale Cronica. È ancora in fase di sviluppo la ricerca di prove per assodare se essa, oltre ad essere una conseguenza della IRC, può essere considerata la causa di momenti fisiopatologici che correlano con lo sviluppo e con la progressione della malattia renale cronica.
Materiali e Metodi. Abbiamo condotto uno studio osservazionale su una coorte di 1.748 pazienti afferenti ad un ambulatorio specialistico dedicato alla cura delle dislipidemie, con l'intento di rilevare eventuali associazioni tra il dismetabolismo lipidico e la patologia renale.

Risultati. Abbiamo osservato che la prevalenza della Malattia Renale Cronica (CKD) nella coorte di soggetti dislipidemici da noi osservata è risultata aumentata rispetto alla popolazione generale, interessando più del 20% di essi. Nei soggetti osservati, la Iperlipemia Familiare Combinata (IFC) è risultata il disordine dell'assetto lipidico più frequente, interessando più del 50% dei soggetti valutati. In alcune famiglie in cui era presente la IFC, avendo avuto modo di osservare un numero di soggetti adeguato per fare una ricostruzione del cosiddetto "albero genealogico familiare", è stata rilevata, accanto alla "dislipidemia familiare" la concomitanza di una patologia renale cronica, anch'essa con caratteristiche di "nefropatia familiare". La patologia renale presente nelle suddette "famiglie", in base all'esito degli esami istologici post-biopsia, si è delineata di tipo "glomerulare". Nell'ambito delle stesse famiglie con IFC e "danno renale" la patologia glomerulare maggiormente rappresentata, a seguito della puntualizzazione diagnostica con l'esame istologico, si è rivelata la IgA Nephropathy (IgAN), seguita dalla Glomerulosclerosi Focale e Segmentaria (GSFS).

ELEVATED SERUM URIC ACID LEVELS ARE ASSOCIATED WITH AN INCREASED RISK OF CHRONIC KIDNEY DISEASE IN TYPE 2 DIABETIC PATIENTS

I. Pichiri[1], G. Zoppini[1], V. Ortalda[2], C. Negri[1], E. Bonora[1], G. Targher[1]
[1]Section of Endocrinology; [2]Section of Nephrology, Department of Medicine, University of Verona, Italy
E-mail: giovanni.targher@univr.it

Background. Recent studies have suggested a significant association between hyperuricemia and adverse renal outcomes in non-diabetic populations. Data on the relationship between hyperuricemia and risk of chronic kidney disease (CKD) in patients with type 2 diabetes are lacking. We determined whether baseline serum uric acid levels predict the risk of incident CKD in type 2 diabetic patients with preserved kidney function.
Materials and Methods. We followed 1,449 (888 men, mean age 66 years) type 2 diabetic patients with normal or near-normal kidney function and without overt proteinuria for 5 years for the occurrence of incident CKD (defined as overt proteinuria or estimated glomerular filtration rate [e-GFR] <60 ml/min/1.73 m^2). Hyperuricemia was defined as allopurinol use or serum uric acid level >7.0 mg/dl in men and >6.5 mg/dl in women.
Results. During a 5-year follow-up period, 194 (13.4%) patients developed incident CKD (148 developed an e-GFR<60 ml/min/1.73 m2, 29 persistent macroalbuminuria and 17 both conditions). The cumulative incidence of CKD was greater in patients with hyperuricemia than in those without hyperuricemia (29.5% vs 11.4%, p<0.001). In univariate logistic regression analysis, the presence of hyperuricemia roughly doubled the risk of developing CKD (odds ratio 2.55, CI 1.71-3.85; p<0.001). After adjustment for age, gender, body mass index, smoking, hypertension, diabetes duration, insulin treatment, HbA1c, e-GFR and albuminuria, hyperuricemia was associated with increased risk of incident CKD (adjusted-odds ratio 2.31, CI 1.30-4.05; p=0.004). In continuous analyses, 1-SD increment (i.e., 1.2 mg/dl) in uric acid level was independently associated with 22% increased risk of incident CKD.
Conclusions. In type 2 diabetic individuals with normal kidney function, hyperuricemia appears to be an independent risk factor for the development of incident CKD. The advantage(s) to treat hyperuricemia in type 2 diabetes for reducing the risk of adverse renal outcomes remains to be demonstrated.

UPREGULATION OF LECTIN-LIKE OXIDIZED LOW-DENSITY LIPOPROTEIN RECEPTOR-1 (LOX-1) BY 15-LIPOXYGENASE-MODIFIED LDL IN ENDOTHELIAL CELLS

A. Pirillo[1,2], A. Reduzzi[2], N. Ferri[2], H. Kuhn[3], A. Corsini[2], A.L. Catapano[1,2,4]
[1]SISA Center for the Study of Atherosclerosis, Bassini Hospital, Cinisello Balsamo, Italy; [2]Department of Pharmacological Sciences, University of Milan, Milan, Italy; [3]Institute of Biochemistry, University Medicine Berlin-Charité, Berlin, Germany; [4]IRCCS Multimedica Milan, Italy
E-mail: angela.pirillo@guest.unimi.it

Objective. Lectin-like oxidized LDL receptor-1 (LOX-1), the endothelial receptor for OxLDL, is believed to be responsible for a number of OxLDL-induced effects in the endothelium. In the present study we aimed at investigating whether other forms of modified LDL could modulate the expression of LOX-1 in endothelial cells.
Methods and Results. We showed that LDL modified by 15-lipoxygenase (15LO-LDL), a form of minimally modified lipoprotein, beside their ability to induce pro-inflammatory responses such as oxidative stress and the expression of adhesion molecules, significantly increases LOX-1 expression in endothelial cells, both at transcriptional and at protein level. Such effect is likely to be mediated by p38 MAPK and NF-kB pathways. We then permanently overexpressed LOX-1 in an endothelial cell line and showed that 15LO-LDL were a ligand for LOX-1; furthermore, the overexpression of LOX-1 markedly increased the amount of ICAM-1 expressed at cell surface in cells incubated with 15LO-LDL, indicating that 15LO-LDL can mediate its effects through LOX-1 activation.
Conclusion. The main finding of the present study is that a very low degree of modification obtained by LDL exposure to 15LO generates a lipoprotein with pro-inflammatory features, able to induce LOX-1 expression in endothelial cells and capable of promoting endothelial cell activation through interaction with LOX-1. These results suggested that during the early phases of atherogenesis, when 15LO is overexpressed by activated macrophages, the enzyme-mediated modification of LDL might initiate endothelial cell dysfunction and sustain it by promoting LOX-1 receptor expression.

CHARACTERIZATION OF THREE KINDRED WITH FAMILIAL COMBINED HYPOLIPIDEMIA DUE TO LOSS OF FUNCTION MUTATIONS OF ANGPTL3

L. Pisciotta[1], E. Favari[2], L. Magnolo[3], S. Simonelli[4], M.P. Adorni[2], R. Sallo[1], T. Fancello[3], I. Zavaroni[5], D. Ardigò[5], F. Bernini[2], L. Calabresi[4], P. Tarugi[3], S. Calandra[3], S. Bertolini[1]
[1]Department of Internal Medicine, University of Genoa, Italy; [2]Deparment of Pharmacological and Biological Sciences and Applied Chemistries, University of Parma, Italy; [3]Department of Biomedical Sciences, University of Modena and Reggio Emilia, Italy; [4]Center E. Grossi Paoletti, Department of Pharmacological Sciences, University of Milan, Italy; [5]Deparment of Internal Medicine and Biomedical Sciences, University of Parma, Italy
E-mail: livia.pisciotta@unige.it

Background. Angiopoietin-like protein 3 (ANGPTL3) affects lipid metabolism by inhibiting the activity of lipoprotein and endothelial lipases. Angptl3 knock out mice have a marked hypolipidemia

and heterozygous carriers of ANGPLT3 loss of function (LOF) mutations were found among individuals in the lowest quartile of plasma triglyceride in population studies. Recently four related individuals with primary hypolipidemia were found to be compound heterozygotes for ANGPTL3 LOF mutations.
Methods and Results. We resequenced ANGPTL3 in four members of three unrelated kindred originally identified for low plasma LDL-C and HDL-C levels (0.97±0.16 and 0.56±0.20 mmol/L), in whom no mutations of known candidate genes for monogenic hypobeta - and hypoalpha-lipoproteinemia had been detected. These subjects were found to be homozygous or compound heterozygous for ANGPTL3 LOF mutations (p.G400VfsX5, p.I19LfsX22/p.N147X), associated with the absence of ANGPTL3 in plasma. They had a marked reduction of plasma LpA-I and preβ-HDL particles. In addition, their apoB-depleted sera had a reduced capacity to promote cell cholesterol efflux through the various pathways (aqueous diffusion, ABCA1-, SR-B1- and ABCG1-mediated efflux). However, these subjects had no evident manifestations of premature atherosclerosis. Heterozygous carriers of the ANGPTL3 mutations had low plasma ANGPTL3, moderately reduced LDL-C (2.52±0.38 mmol/L) but normal plasma HDL-C.
Conclusions. Complete ANGPTL3 deficiency caused by LOF mutations of ANGPTL3 is associated with a recessive hypolipidemia characterized by a reduction of apolipoprotein B and apolipoprotein A-I containing lipoproteins, changes in HDL subclasses and reduced cholesterol efflux potential of serum. Partial ANGPTL3 deficiency is associated only with a moderate reduction of LDL.

HETEROZYGOUS VARIANTS OF LPL GENE IN TWO PATIENTS WITH SEVERE HYPERTRIGLYCERIDEMIA (HTG)

L. Pisciotta, R. Fresa, A. Bellocchio, V. Guido, R. Sallo, S. Bertolini
Department of Internal Medicine, University of Genoa, Italy
E-mail: livia.pisciotta@unige.it

Lipoproteinlipase (LPL), encoded by LPL gene, is a glycoprotein of 448 amino-acids which hydrolyses triglycerides (TG) in chylomicrons and Very Low Density Lipoproteins (VLDL).
Aim of our study was the molecular characterization of patients with severe chronic hypertriglyceridemia (HTG) associated with family history of HTG (TG levels above 10 mmol/L) or clinical history of recurrent pancreatitis. LPL gene was the first candidate gene we sequenced in 90 cases of HTG patients.
Two subjects with rare variants previously described were identified: Proband F.E. was a 47 year-old overweight male (BMI 25.5 kg/m^2) with plasma TG 11.7 mmol/L, total cholesterol (TC) 5.58 mmol/L, HDL cholesterol (HDL-C) 0.72 mmol/L, in absence of pancreatitis, diabetes or alcohol consumption; his brother suffered from HTG. In this proband we identified the c.829 G>A, p.Asp277Asn (Asp250Asn) mutation, previously described as pathogenic variant; the APOE genotype was e3e4.
Proband C.M. was a 48 year-old male (BMI 24.0 kg/m^2) with plasma TG 19.21, TC 7.00, HDL-C 0.67 mmol/L, ApoAI 96 mg/dl, ApoB 102 mg/dl, hepatic steatosis, absence of clinical history of pancreatitis, diabetes or alcohol abuse. He was heterozygous for the c.590G>T, p.Arg197Leu (Arg170Leu) mutation of LPL and c.56 C>G (p.S19W) of APOA5. The father, 79 years-old, BMI 29.0 kg/m^{12}, had severe HTG (TG 10.17, TC 7.03, HDL-C 0.72 mmol/L, ApoAI 109, ApoB 110 mg/dl), hepatomegaly due to hepatic steatosis and was a carrier of both mutations; a 2V-CAD was documented at the age of 67. In silico analysis of this missense mutation was performed by Poly-Phen, PANTHER, SIFT, SNPs3D, Pmut; the mutation resulted to be pathogenic with all programmes. Arginine at position 197 was highly conserved in the evolution of the species. This variant was described by Brites et al. (Acta Paediatr 2003. 92: 621-633) in a 4 years-old compound heterozygote with chylomicronaemia syndrome.

A THREE MONTH-OLD BABY WITH SEVERE HYPERCHYLOMICRONAEMIA: MOLECULAR DIAGNOSIS AND TREATMENT

L. Pisciotta[1], C. Stefanutti[2], M. Gozzer[2], C. Morozzi[2], G. Bosco[3], P. D'Eufemia[4], F. Papadia[5], S. Bertolini[1]
[1]Department of Internal Medicine, University of Genoa, Italy; [2]Department of Molecular Medicine; [3]D.A.I. Pediatrics and infant Neuropsychiatry, University of Rome 'La Sapienza', Umberto I Hospital; [4]Regional Presidio of Rare Disease, Lazio Region; [5]of Metabolic-Genetic Diseases, Pediatric Hospital Giovanni XXIII, Bari, Italy
E-mail: livia.pisciotta@unige.it

The patient was a 3 months-old female from Apulia (IT), born at 37° week of a twin pregnancy with caesarean section (Apgar 8/9, weight 2.490 kg, lenght 47 cm). Biochemical analysis was performed during hospitalisation for occasional haematemesis. The plasma lipid profile resulted to be: Total Cholesterol 20.38 and Triglycerides 359.49 mmol/L. After cardiological and nephrological evaluation, the patients underwent therapeutic plasmapheresis in Rome: blood cell separator used for apheresis was Fresenius AS TEC 204®; the extracorporeal line was primed with a mixture of irradiated leukocyte-poor packed red blood cell and 5% albumin solution adjusted to the desired haematocrit (38%). Vital signs, coagulation and electrolyte changes were monitored during all the procedure and calcium gluconate i.v. was used to maintain the ionised calcium level at 1.0-1.2. For anticoagulation a bolus of heparin (30 UI/Kg)+ ACD-A (1:30) was used.
A sample of blood in K3-EDTA was shipped to Genoa for DNA extraction and molecular characterization: by sequencing LPL gene c.242 G>A, p.Gly81Asp (Gly54Asp) mutation in exon 2 in homozygous form was identified. The mutation, a non polar amino acid substitution with a negative charged polar, was novel. In silico analysis of the the mutation was performed by Poly-Phen, Poly-Phen 2, SNAP, SNP Analyzer, SNPs3D; the mutation was predicted to be pathogenic by all programme. Gly at position 81 is highly conserved during evolution.
After the first session of plasmapheresis a controlled diet (Protein 11.8%, Lipids 22.4% with 100% MCT, CHO 66.3%) was prescribed and a optimal control of the lipid profile was obtained during the follow-up; no further plasmapheresis sessions were required.

PRENATAL GENETIC DIAGNOSIS IN A FOETUS CONCEIVED BY TWO CARRIERS OF A NON SENSE MUTATION OF THE LIPA GENE

L. Pisciotta[1], C. Rabacchi[2], R. Fresa[1], S. Calandra[2], S. Bertolini[1]
[1]Department of Internal Medicine, University of Genoa, Italy; [2]Department of Biomedical Science, University of Modena and Reggio Emilia, Italy
E-mail: livia.pisciotta@unige.it

Wolman Disease (WD) is a recessive disorder caused by the complete deficiency of Lysosomal Acidic Lipase (LAL). This enzyme,

encoded by the LIPA gene, hydrolyses cholesteryl esters derived from cell internalisation of plasma lipoproteins. WD is a rapidly progressive and lethal disease characterized by malabsorption, hepatic and adrenal failure. Two years ago we performed genetic characterization of the LIPA gene in a patient born from consanguineous parents and deceased at 4 months of age; he was reported to have hepato-splenomegaly, diarrhea, vomiting, abdominal distention, failure to thrive and adrenal calcification, consistent with the diagnosis of WD. We identified a nonsense mutation (p.W140X) in exon 4 in homozygous form and, as expected, both the parents resulted heterozygous carriers of this mutation. Three months later on the genetic diagnosis, we received the communication of a new pregnancy of the partners and the request for a prenatal genetic diagnosis. At the 10th week of pregnancy, the chorionic villous were drawn and shipped for DNA extraction and sequencing. The exon 4 of LIPA gene was amplified with the following oligonucleotides: forward 5'-ATG TGA GTA CAT CAC TAT GTC-3', reverse 5'-CTC ATA CAA CTT CAG AGT TAC-3', PCR conditions were: 94 °C for 3 minutes, 33 cycles at 94°C for 30 sec, 58°C for 30 sec, 72°C for 1 min, 72°C per 7 min. The PCR product of 255 bp was purified with EXO-SAP (GE Healthcare Europe GmbH) and sequenced with CEQ8000 Beckman Coulter Analysis System. The sequencing was performed at the same time in Genoa LAB and in Modena (LABGEN laboratory). In both laboratories the chorionic DNA resulted to be negative for the mutation and the diagnosis of a healthy foetus was passed on the parents.

ARTERIAL DYSFUNCTION AND OXIDATIVE STRESS IN OBSTRUCTIVE SLEEP APNOEA SYNDROME AND RESPONSE TO CONTINUOUS POSITIVE AIRWAY PRESSURE TREATMENT

L. Polimeni, M. Del Ben, M. Fabiani, L. Loffredo, M. Brancorsini, R. Carnevale, F. Baratta, M. Brunori, T. Augelletti, F. Angelico, F. Violi
I Clinica Medica, Sapienza University, Rome, Italy
E-mail: francesco.angelico@uniroma1.it

Introduction. Obstructive sleep apnoea syndrome (OSAS) is a common nocturnal disorder characterized by the presence of repetitive apnea and hypopnea during sleep, daytime sleepiness and cardiopulmonary dysfunction. Several studies have provided evidence supporting an increase of oxidative stress in OSAS. Endothelial dysfunction is markedly reduced in patients with moderate/severe OSAS. Aim of our study was to assess the association between OSAS, endothelial dysfunction and oxidative stress. Further aim was to evaluate the effect of nasal continuous positive airway pressure (CPAP) on oxidative stress and arterial dysfunction.
Material and Methods. The study group consisted of 138 consecutive patients who were referred to our metabolic outpatients clinic because of suspected metabolic disorders with heavy snoring and possible OSAS. Patients underwent unattended overnight home polysomnography. Furthermore, 10 patients with severe OSAS were revaluated after 6 months of nCPAP therapy. To assess oxidative stress in vivo, we measured urinary 8-iso-PGF2α, which is a reliable marker of lipoperoxidation and serum levels of soluble NOX2-derived peptide (sNOX2-dp), a ROS generating enzyme implicated in arterial function via oxidative-stress mediated NO inactivation. Nitric oxide generation by serum levels of nitrite/nitrate (NOx) was also determined. Flow-mediated brachial artery dilation (FMD) was measured to asses endothelial function.
Results. As compared to non-OSAS, patients with severe OSAS had statistically significant higher urinary 8-iso-PGF2α ($p<0.001$) and higher serum NOX2 and lower NOx, although not at a statistically significant extent. Furthermore, a statistically significant negative association was observed between FMD and OSA severity. Apnea/hipopnea index was significantly correlated with the indices of central obesity and with urinary 8-isoprostanes ($r=0.298$, $p<0.001$).
In a multiple regression analysis, the metabolic syndrome ($t= -4.63$, $p<0.001$) and urinary 8-isoprostanes ($t= -2.02$, $p<0.05$) were the only independent predictors of FMD. After 6 months of nCPAP treatment, a statistically significant decrease of mean serum GP-91(Phox), ($p<0.005$) and of urinary 8-iso-PGF2α ($p<0.01$) was observed, while serum NOx showed only a minor statistically non significant increase.
A statistically significant increase of FMD was also observed (from 3.6% to 7.0%). During the six months treatment, no significant change in body weight or cardiovascular risk factors was observed.
Conclusions. The results of our study indicate that patients with OSAS and cardiometabolic comorbidities have an increased oxidative stress and arterial dysfunction that are partially reversed by nCPAP treatment.
This is the first study to demonstrate the efficacy of nCPAP therapy on oxidative stress and arterial dysfunction in OSA patients with cardiometabolic risk factors, independent from weight loss and risk factor management.

CARDIOPATIA ISCHEMICA E ANGINA MICROVASCOLARE: FOCUS SULLO STRESS OSSIDATIVO

B. Porro, V. Cavalca, I. Squellerio, A. Garlaschè, S. Eligini, F. Veglia, L. Boccotti, A. Lualdi, E. Tremoli
Centro Cardiologico Monzino IRCCS, Milano, Italia
E-mail: benedetta.porro@ccfm.it

Introduzione. La maggior parte dei pazienti con sintomatologia anginosa presenta lesioni aterosclerotiche significative a carico di almeno uno dei principali vasi coronarici epicardici. Esistono tuttavia pazienti con angina e positività ai test di valutazione della riserva coronarica che presentano un albero coronarico indenne da stenosi angiograficamente documentabili, associato ad un'alterata vasomotilità del microcircolo. Tale condizione patologica è definita "angina microvascolare" (AM). Lo stress ossidativo induce disfunzione endoteliale diminuendo la biodisponibilità di monossido di azoto (NO) ma, a tutt'oggi, non è del tutto chiaro se tale fenomeno sia coinvolto nella stessa misura nelle due condizioni patologiche. Scopo. Valutare il ruolo dello stress ossidativo e della biodisponibilità di NO nei pazienti affetti da coronaropatia (CAD) e AM.
Materiali e Metodi. 39 pazienti anginosi con ridotta riserva coronarica sono stati suddivisi in 2 gruppi: gruppo I: 21 pazienti con lesioni aterosclerotiche a livello coronarico (CAD) e gruppo II: 18 pazienti con normalità dell'albero coronarico epicardico (AM); I due gruppi sono stati confrontati con un terzo gruppo di controllo (Ctrl), sovrapponibile ai precedenti per età e sesso, composto da 16 volontari senza sintomi e segni strumentali di ridotta riserva coronarica. Lo stato di stress ossidativo è stato analizzato mediante la determinazione su sangue in toto della forma ridotta e ossidata del glutatione (GSSG/GSH), mentre la biodisponibilità di NO è sta-

ta misurata mediante analisi del substrato (arginina: Arg) e degli inibitori della sintesi (dimetilarginina asimmetrica e simmetrica: ADMA e SDMA).
Risultati. Rispetto ai controlli, i gruppi con AM e CAD presentavano un rapporto GSSG/GSH significativamente aumentato, mentre la biodisponibilità di NO (espressa come Arg/ADMA-Arg/SDMA) risultava significativamente ridotta. Una relazione lineare veniva riscontrata tra i valori di GSSG e ADMA (r=0,32; p=0,016).
Conclusioni. Questi dati evidenziano che l'aumento di stress ossidativo misurato nei CAD è presente anche negli AM. La correlazione tra ADMA e GSSG depone inoltre per un'associazione tra stress ossidativo e ridotta produzione di NO. Questo potrebbe giustificare l'alterato comportamento del microcircolo in soggetti con AM.

SPHINGOSINE KINASE (SK) INHIBITION INDUCES BOTH PRO- AND ANTI-ATHEROGENIC EFFECTS IN LOW-DENSITY LIPOPROTEIN RECEPTOR-DEFICIENT MICE

F. Poti[1], M. Bot[2], S. Costa[1], V. Bergonzini[1], L. Maynes[3], G. Varga[4], M. Simoni[1], J.R. Nofer[1,5]
[1]Department of Medicine, Endocrinology, Metabolism and Geriatrics, University of Modena and Reggio Emilia, Modena, Italy; [2]Division of Biopharmaceutics, Gorlaeus Leiden/Amsterdam Center for Drug Research, Gorlaeus Laboratories, Leiden University, The Netherlands; [3]Apogee Biotechnology Corporation, Hershey, PA, USA; [4]Institue of Immunology, University of Münster, Germany; [5]Center for Laboratory Medicine, University Hospital Münster, Germany
E-mail: fra.poti@libero.it

Background. Sphingosine 1-phosphate (S1P), a lysosphingolipid associated with high-density lipoprotein (HDL), contributes to the anti-atherogenic potential attributed to this lipoprotein. This study examined whether a reduction of S1P plasma levels affects atherosclerosis in a murine model of disease.
Methods and Results. LDL-R-/-mice on Western diet were given ABC294640, an inhibitor of sphingosine kinases 1 and 2 for 16 weeks at a dose of 50 mg/kg/d. ABC294640 decreased plasma S1P by approx. 30-40%. However, ABC294640 failed to affect atherosclerotic lesion formation. Plasma triglycerides were reduced whereas total and HDL-cholesterol remained unchanged in course of ABC294640 treatment. ABC294640 increased plasma IL-12p70 and IL-12p70 and IFN-gamma production by peritoneal cells and this was paralleled by enhanced activity of peritoneal and spleen dendritic cells as evidenced by up-regulation of CD68 and MHC-II on CD11c+ cells. As a consequence, increased T-cell activation was noted in ABC294640-treated mice as evidenced by enhanced CD4+ splenocyte proliferation, IFN-gamma and IL-2 production, and CD69 expression. Concomitantly, however, ABC294640 treatment redistributed CD4+ and CD8+ cells from blood to lymphatic organs and reduced T-cell number within atherosclerotic lesions. In addition, plasma sVCAM-1, sICAM-1, and MCP-1 levels as well as in vivo leukocyte adhesion and CCL19-induced T-cell penetration into peritoneum were lower in ABC294640-treated animals. In vitro experiments demonstrated reduced VCAM-1 and ICAM-1 expression and lymphocyte adhesion to TNFalpha-stimulated endothelial cells.
Conclusions. Treatment with SK inhibitor leads to both pro- and anti-atherogenic effects in LDL-R-/- mice. As a consequence, SK inhibition fails to affect atherosclerosis despite significant S1P reduction in plasma.

ROLE OF SAPHENOUS VEIN PROGENITORS (SVPS) IN VENOUS BYPASS ARTERIALIZATION

F. Prandi[1], M.C. Vinci[1], A. Guarino[2], G.L. Polvani[3], P. Madeddu[4], M. Pesce[1]
[1]Lab. di Ingegneria Tessutale Cardiovascolare Centro Cardiologico Monzino, Milano, Italy; [2]Banca dei Tessuti Cardiovascolari della Regione Lombardia, Centro Cardiologico Monzino, Milano, Italy; [3]Dip. di Scienze Cardiovascolari, Università degli Studi di Milano, Italy; [4]Bristol Heart Institute, University of Bristol Medical School, UK
E-mail: maurizio.pesce@ccfm.it

Introduction. Saphenous vein (SV) segments are routinely used to generate coronary aorto-coronary bypass grafts (CABG) to revascularize the ischemic myocardium. Between 10 and 15 yrs after implantation more than 50% of vein bypasses undergo lumen narrowing (vein graft disease, VGD) due to development of intimal hyperplasia (IH).
IH is the thickening of SV intima layer due to recruitment and proliferation of smooth muscle cells (SMCs). Mechanical damage associated to SV segments transplantation as well as exposure to arterial flow is one of the main causes for SMCs proliferation and CABG failure.
Recently, vascular progenitors have been identified in the adventitia layer of the SV in close proximity with vasa vasorum; these cells, named saphenous vein progenitors (SVPs) express mesenchymal antigens and differentiate into mesenchymal-derived lineages. Since cyclic strain is reported to induce mesenchymal cells differentiation into SMC-like phenotype we hypothesized that SVPs represent a SMCs reservoir contributing to GABG IH. In the present study we address this hypothesis by a mechanobiology approach.
Matherials and Methods. SV segments were provided by CCM Surgery Department following the end of CABG implantation in patients with chronic myocardial ischemia. Identification of SVPs in vivo was performed by four colors confocal microscopy analysis of paraffin-embedded SV segments sections stained with anti CD34-CD31-vWF and DAPI.
For the isolation and culture of SVPs, a protocol of magnetic isolation is followed (Milteny biotec). Briefly, cell suspensions obtained by enzymatic digestion of SV fragments are positively selected for CD34 antigen and negatively selected for CD31, after which they are plated on fibronectin (BD Biosciences) in endothelial growth medium (EGM-2, Cambrex) plus 2% fetal bovine serum (FBS). At passage 5, SVPs are evaluated by flow cytometry for CD90, CD44, CD73, CD105 (BD Bioscience) expression, immunofluorescence and western blot for α-SMA, SM22α, calponin (abcam) expression before being exposed to ramps of physiologic to pathologic uni/equiaxial strain (Flexcell Technology) for different times.
Results. Studies conducted thus far showed that SVPs are localized in the adventitia layer.
In our study, these cells are correctly identified, often circularly disposed around vasa vasorum, by their CD34+/CD31-/vWF- phenotype. For in vitro studies, are isolated using magnetic beads by consecutive positive selection for CD34 and negative selection for CD31. After isolation, SVPs are grown up to passage 4 (P4) to perform immunophenotyping.
In agreement with a previous study, they show high expression of CD90, CD44, CD73, CD105 mesenchymal antigens. We are currently performing mechanical strain experiments using ramps of uniaxial/equiaxial pathologic strain (10%-15%), in order characterize the molecular pathways activated by mechanical stress in SVPs, and assess their possible commitment into SMCs.
Conclusion. Our results show that we can reproduce SVPs identi-

fication in vivo and their isolation, expansion and characterization protocol as described in literature. In current work we are evaluating possible SVPs differentiation under conditions that are known to induce CABG failure.
These studied will be instrumental to clarify the role of vein resident progenitors in the initiation and vein graft disease and will be of importance to devise therapeutic strategies to limit its progression in patients undergoing CABG.

AMPLIFICAZIONE PRESSORIA CENTRO-PERIFERIA: VALIDAZIONE INVASIVA DI DUE APPARECCHI (SPHYGMOCOR E OMRON HEM9000AI)

G. Pucci, G. Schillaci, E. Mannarino
Medicina Interna, Angiologia e Malattie da Arteriosclerosi, Università di Perugia, Italia
E-mail: giacomo.pucci@gmail.com

Introduzione. Mentre la pressione arteriosa (PA) diastolica e la PA media sono costanti lungo l'albero arterioso, la PA sistolica (PAS) e differenziale (PP) possono aumentare dal centro alla periferia (amplificazione). L'amplificazione della PAS (PAS-amp) e della PP (PP-amp) è un importante predittore di eventi cardiovascolari. Vi sono pochi dati sull'accuratezza della stima non invasiva della PA centrale basata sull'analisi dell'onda pressoria periferica. Obiettivo: confrontare i valori di PAS-amp e della PP-amp calcolati dai sistemi tonometrici Omron HEM9000AI e SphygmoCor con i valori di PAS-amp e PP-amp invasiva.
Materiali e Metodi. Durante coronarografia, la PA invasiva è stata misurata in aorta ascendente e in arteria brachiale in 20 pazienti (64±15 anni, 75% maschi). Contemporaneamente è stata acquisita la forma d'onda radiale con i due sistemi, e la PA brachiale è stata misurata oscillometricamente. L'onda pressoria radiale è stata calibrata alla PA brachiale. La PA centrale è stata derivata tramite funzione di trasferimento generalizzata (SphygmoCor) o calcolo del secondo picco sistolico a livello dell'onda radiale (Omron HEM9000AI). L'amplificazione è stata calcolata come PA brachiale/PA centrale.
Risultati. La PA invasiva era 140/72 mmHg (DS 18/6 mmHg) a livello aortico e 149/69(16/8)mmHg a livello brachiale. Sia Omron HEM9000AI che SphygmoCor sottostimavano la PAS centrale (rispettivamente deltaPAS -9(9) mmHg e -19(6) mmHg, entrambi p<0,001). La PAS-amp invasiva era 1,08(0,10) mentre la PP-amp invasiva era 1,23(0,25). L'amplificazione stimata da Omron HEM9000AI non differiva da quella invasiva (deltaPAS-amp 0,01(0,11), deltaPP-amp 0,04(0,29), entrambe le p=n.s.), mentre SphygmoCor sovrastimava sia PAS-amp (deltaPAS-amp 0,07(0,10), p=0.03) che PP-amp (deltaPP-amp 0,13(0,20), p=0.04).
Conclusioni. La stima di PAS-amp e PP-amp fornita da Omron HEM9000AI è in linea con i valori misurati invasivamente. SphygmoCor sovrastima i valori di PAS-amp e PP-amp. La sottostima della PAS centrale da parte di entrambi gli apparecchi può essere causata da errori sistematici di misurazione o dall'effetto di altre variabili biologiche.

ROLE OF FIBRONECTIN EXTRA DOMAIN-A IN RESTENOSIS

V.K. Pulakazhi Venu[1], R. Baetta[1], A. Bonomo[1], G.D. Norata[1,2], A.F. Muro[3], A. Corsini[1], A.L. Catapano[1,2]
[1]Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, Milano; [2]Centro SISA per lo Studio della Aterosclerosi, Ospedale Bassini, Cinisello Balsamo, Milano, Italy; [3]International Center for Genetic Engineering and Biotechnology, Trieste, Italy
The authors made equal contributions
E-mail: pvvivekkrishna@gmail.com

Background. Fibronectins (FN) are multifunctional glycoproteins present in the plasma and in the extracellular matrix (ECM) of the tissues. The primary transcript undergoes alternative splicing to generate isoforms, namely Fibronectin Extra Domain A (FN-EDA), Fibronectin Extra Domain-B (FN-EDB) and Type III Connecting Segments (III-CS). FN-EDA expression is regulated spatially and temporarily during development and ageing. FN generally exists in two major forms namely plasma FN (pFN) and cellular FN (cFN). Plasma FN is soluble and lacks both EDA and EDB, while cellular FN contain these fragments. Previous evidences indicated that FN-EDA levels are increased during a series of pathophysiological condition including thrombosis, lung fibrosis, Inflammation. Aim of this work was to investigate the role of FN-EDA in neointima formation in murine models.
Materials and Methods. Specific gene targeting allowed to generate mice that express fibronectin constitutively containing EDA or not. At 4 months of age, male mice were used for the study. In order to investigate the role in neointima formation a non-obstructive collar was placed on the right carotid artery of FN-EDA+/+, FN-EDA-/- and C57BL/67 mice for a period of 9 weeks. Formalin-fixed paraffin embedded carotid and sham operated sections were evaluated for haematoxylin and eosin staining was characterized for the intima-media thickness (IMT). Furthermore, the cellular composition of the neointima was analyzed for smooth muscle actin, CD3 for T-cells and F4/80 for macrophages. The extent of vascular remodelling was calculated,
Results. Our data showed that the mice containing the fibronectin-EDA had a reduced neo-intimal hyperplasia, corresponding an IMT of 0.84±0.11, closer to the controls with 1.00±0.35. While the mice lacking the fibronectin-EDA had an higher IMT of 1.42±0.21,with a statistical significance of P≤0.05. The remodelling index of mice containing fibronectin-EDA was 0.56±0.20, which was lower than the controls and the mice lacking fibronectin-EDA of remodelling index of 1.20±0.27 and 0.83±0.11 respectively.
Conclusion. These data suggest a possible role for fibronectin extra domain-A in the process of intimal hyperplasia, further studies are warranted to elucidate the molecular mechanisms and the pathways observed.

NIACIN/LAROPIPRANT THERAPY REDUCES SERUM LIPOPROTEIN (A) LEVELS

M. Puntoni[2], F. Bigazzi[1], F. Sbrana[1], M. Pianelli[1], R. Luciani[1], A. Ragusa[1], S. Vicari[1], E. Grisanti[1], T. Sampietro[1,2]
[1]Fondazione CNR, Regione Toscana "Gabriele Monasterio", Pisa, Italy; [2]CNR Institute of Clinical Physiology of Pisa, Italy
E-mail: federicobigazzi@ifc.cnr.it

Background. High plasma levels of lipoprotein(a) [Lp(a)] are risk factor for ischemic heart disease, stroke and are associated with the severity of coronary atherosclerosis. We sought to see

whether extended-release Niacin/Laropiprant (ERN/LRPN) could lower Lp(a) levels.
Material and Methods. We followed-up for 8 weeks a group of 16 patients (mean age 55±12 years, 66% male) with high Lp(a) levels and chronic coronary artery disease. ERN/LRPN was added on top of maximally tolerated lipid-lowering therapy, administered at the dose of 1 gr/day for the first 4 weeks and then at 2 gr/day for the remaining period. Clinical examination and blood sampling (including lipid profile, renal and hepatic function) were performed at baseline, after 4 and 8 weeks, and in the case of adverse manifestations.
Results. During follow-up, 4 patients discontinued therapy due to side effects (headache, asthenia, and gastrointestinal disorders in 2 patients, eruptive skin rash in 1 patient, onset of diabetes mellitus in 1 patient). In the remaining 12 patients, a significant reduction of Lp(a) serum concentrations was observed: Lp(a) levels was reduced of 20.6% after 4 week ($p<0.0005$) and of 36.0% after 8 week ($p<0.005$) respect to basal serum concentrations. After two months of therapy, in 36% of patients Lp(a) values were normalized (i.e <60 mg/dl). These results are obtained in the absence of substantial changes in other laboratory analyses (with the exception of a non-significant increase in uric acid).
Conclusions. These results show that the ERN/LRPN therapy significant reduces Lp(a) levels, thus representing a new treatment option in patients with high Lp(a) levels and coronary artery disease, although the high frequency of collateral effects may be a limitation.

IL FENOTIPO HYPERTG-WAIST: IMPATTO SUL RISCHIO CARDIOVASCOLARE E SULLA MORTALITÀ NELLA POPOLAZIONE ANZIANA

F. Resta[1], G. Barile[1], M. Benigno[1], L. Marasciulo[1], P. Suppressa[1], P. Lastella[1], V. Triggiani[2], E. Guastamacchia[2], C. Sabbà[1]
[1]U.O.C. di Geriatria e Centro Sovraziendale per le Malattie Rare, Università di Bari "Aldo Moro", Italia; [2]U.O.C. di Endocrinologia, Università di Bari "Aldo Moro", Italia
E-mail: f.resta@geriatria.uniba.it

Introduzione. In letteratura, sono diversi i lavori che hanno dimostrato una correlazione tra fenotipo HyperTG-Waist e rischio cardiovascolare.
Scopo del nostro studio è stato quello di valutare, in una popolazione anziana, la prevalenza del fenotipo HyperTG-Waist ed il suo impatto sul rischio cardiovascolare e sulla mortalità.
Materiali e Metodi. Sono stati randomizzati 282 pazienti (91 maschi, 191 femmine), di età 65-83 anni, affetti da obesità addominale (waist circumference >102 cm nei maschi e >88 cm nelle femmine), e tra questi gli ipertrigliceridemici (TG >150 mg/dL). Abbiamo, poi, accertato le seguenti patologie: coronaropatie, ipertensione arteriosa, diabete mellito e sue complicanze, scompenso cardiaco, ictus cerebrale, arteriopatia periferica. È stata, infine, considerata la comorbilità (compresenza di almeno 2 patologie) e, dopo tre anni dall'inizio dello studio, sono stati rilevati i pazienti deceduti.
Risultati. Confrontando gli HyperTG-Waist con gli obesi puri, è stata osservata una maggiore prevalenza negli HyperTG-Waist di ipertensione arteriosa (NS), diabete mellito ($p<0.003$) e sue complicanze (NS), scompenso cardiaco ($p<0.02$), arteriopatia periferica (NS), comorbilità ($p<0.01$) e mortalità ($p<0.05$). Non è stata osservata alcuna correlazione di tale fenotipo con coronaropatie e ictus.
Confrontando gli HyperTG-Waist con gli ipertrigliceridemici puri, nel primo gruppo abbiamo riscontrato una maggiore frequenza di coronaropatia ($p<0.05$), ipertensione arteriosa ($p<0.05$), diabete mellito ($p<0.05$) e sue complicanze (NS), scompenso cardiaco (NS), ictus (NS), comorbilità (NS) e mortalità ($p<0.05$). Al contrario, si è osservata una maggiore prevalenza dell'arteriopatia periferica fra gli ipertrigliceridemici puri rispetto agli HyperTG-Waist ($p<0.05$).
Conclusioni. In conclusione, nei soggetti anziani il fenotipo HyperTG-Waist risulta un marker di rischio cardiovascolare e di mortalità. Pertanto, lo screening dei pazienti affetti da HyperTG-Waist potrebbe essere utile anche in età geriatrica per identificare i soggetti a più elevato rischio cardio-metabolico.

TWO-YEAR TREATMENT WITH LOW DOSE OF ROSUVASTATIN REDUCES INTIMA-MEDIA THICKNESS IN HYPERCHOLESTEROLEMIC SUBJECTS WITH ASYMPTOMATIC CAROTID ARTERY DISEASE

G. Riccioni, D. Santovito[1], C. Rizzo[2], C. Mandurino[2], M.A. Grasso[3], M. Frascaria[3], S. Cusenza[3], G. Della Malva[3], E. Della Malva[4], V. Bucciarelli[4], L. Scotti
[1]Cardiology Unit, San Camillo de Lellis, Hospital, Manfredonia, Foggia, Italy; [2]Internal Medicine, University of Chieti, Italy; [3]Cardiology, University of Bari, Italy; [4]Human Nutrition, University of Chieti, Italy
E-mail: griccioni@hotmail.com

Objectives. Recent evidences suggest that rosuvastatin 40 mg may exert beneficial effect on both in carotid and coronary atherosclerosis progression. In particular, two-years rosuvastatin treatment reduced the progression of carotid intima-media thickness (cIMT) in patients with low cardiovascular risk. However, despite in clinical practice lower dose of rosuvastatin are usually administered at this time no clear data exist about its effect on cIMT. Thus, the aim of this study was to evaluate the effect of rosuvastatin 10 mg/day on cIMT over a two-year follow-up.
Methods. Forty-three patients with hypercholesterolemia and asymptomatic carotid atherosclerosis on baseline carotid ultrasound investigation (CUI) were treated with rosuvastatin 10 mg/day for 24 months. cIMT and lipid profile were assessed after 12 months and at the end of the study.
Results. Total cholesterol, LDL-cholesterol and triglycerides decreased significantly ($p<0.001$) while HDL cholesterol increased significantly ($p<0.001$) after 12 and 24 months of treatment. cIMT significantly decreased (right CCA cIMT: -0.27 mm and left CCA cIMT -0.25 mm vs baseline, $p<0.001$).
Conclusion. Two-years treatment with 10 mg/day rosuvastatin in hypercholesterolemic adults with evidence of subclinical atherosclerosis establishes significant reduction of cIMT and improves lipid and lipoprotein levels with a good tolerability profile.

CONSIDERAZIONI SULLA INFIAMMAZIONE NELLA ATEROSCLEROSI. PROPOSTA DI UNA TERAPIA EFFICACE

R. Ripa
Casa di Cura Villa Maria, Rimini, Italia
E-mail: rinaldoripa@libero.it

Il ruolo e l'importanza dei processi infiammatori nella genesi e nella progressione della arteriopatia aterosclerotica sono noti e dimostrati. Alla base attivazione dei macrofagi nelle pareti vasali e nei tessuti, con iperproduzione di citochine proinfiammatorie (TNF, IL6). Ogni processo aterosclerotico comporta infiammazione della parete: la noxa iniziale provoca il passaggio in posizione subendoteliale di componenti plasmatiche, proteiche e/o lipidiche, che provocano l'attivazione macrofagica locale, con l'avvio al perdurante e progressivo processo aterosclerotico con esisti e complicanze varie.
Va chiaramente ribadito che allo stato attuale non esiste una vera terapia antiinfiammatoria da adoperare, controindicati essendo gli steroidi e i FANS. Per questo, basandomi su occasionali osservazioni cliniche favorevoli,occorse nella mia ultracinquantennale attività clinica, ho pensato di proporre una terapia della infiammazione vascolare in ogni sua fase evolutiva, da quelli con quadro clinico silente a quelli clinici eclatanti (ictus, infarto miocardico, gangrena). A tal fine propomgo uno schema terapeutico a base di colchicina (1 mg/os/die) per almeno 6 mesi e da protrarre nel tempo, a giudizio clinico, in tutti i casi di processo aterosclerotico di diagnosi clinica o strumentale (IMA, cardiopatia ischemica cronica; ictus ma anche TIA; vasculopatie ateromasiche carotidee; vasculopatie arti inferiori sintomatiche o non, con gangrena o no).
Importante in questa terapia è la durata nel tempo e importanti sono le regole da seguire per una terapia sicura, in genere ben tollerata, se si seguono appunto alcuni semplici acciorgimenti, qui di seguito riportati. È noto che la colchicina è da tempo adoperata dai clinici, per trattamenti a lungo termine, con risultati eccellenti, nella terapia e profilassi della gotta acuta, della Febbre Mediterranea Familiare e nella terapia del m. di Behcet. La colchicina è principalmente escreta per via biliare.
L'eliminazione per via renale e l'inattivazione attraverso la via metabolica del citocromo P 450 CYP 3A4 hanno un ruolo meno importante. La colchicina è anche il substrato della P-glicoproteina, un trasportatore coinvolto nell'efflusso cellulare e nella eliminazione di svariate sostanze. Da quanto detto sono comprensibili i casi segnalati di intossicazione comparsi in p. che assumevano cronicamente contemporanea terapia con macrolidi (claritromicina ed eritromicina), noti inibitori della P-glicoproteina e degli enzimi CYP450 3A4, con conseguente ridotta inattivazione della colchicina, quando co-somministrati, e ridotta sua escrezione per via biliare. Analogamente tossicità quando colchicina è co-somministrata con inibitoir del CYP450 3A4 e della P-glicoproteina, come la ciclosporina o statine (tranne pravastatina).
Devono essere evitati tutti gli altri farmaci noti inibitori del CYP450 3A4. Regola generale: valutare di ogni farmaco co-somministrato con colchicina la possibile interferenza a livello enzimatico e di escrezione(epatica o renale); se queste esistono ricorrere ad altri farmaci, ad azione analoga, non interferenti. Sono questi processi decisionali che ogni buon clinico dovrebbe seguire sempre, quando imposta qualsiasi terapia di associazione in qualunque paziente.
Conclusione. La colchicina è controindicata nella severa insufficienza epatica e renale. Non va somministrata in gravidanza e durante l'allattamento. Cautela in corso di maffezioni gastrointestinali gravi e nei pazienti ematologici. Controllare sempre possibili interferenze con farmaci co-somministrati al paziente, ricorrendo sempre a farmaci sicuri, privi di ogni possibile interferenza. Con queste regole, e questa è la mia esperienza clinica, la terapia dell'infiammazione nell'arteriopatia aterosclerotica con colchicina, al dosaggio consigliato, risulta sicura e costantemente efficace.

ATHEROSCLEROSIS SEVERITY BUT NOT OCCULT DIABETES PREDICTS NEW CARDIOVASCULAR EVENTS OF SUBJECTS IN SECONDARY CARDIOVASCULAR PREVENTION

S. Rizza, M. Copetti, M. Cardellini, Ot. Porzio, A. Luzi, I. Cardolini, N. Senese, E. Martelli, A. Ippoliti, D. Lauro, R. Lauro, M. Federici
Department of Internal Medicine, University of Rome Tor Vergata, Italy
E-mail: rizza@uniroma2.it

Background and aims. Type 2 diabetes mellitus is associated with an increased risk for cardiovascular (CV) disease. Therefore, if its diagnosis remains unknown (occult diabetes), especially in individuals that have still experienced a major atherosclerosis vascular event, it could secretly increase the risk of development of new major cardiovascular event. Nevertheless, few data are available concerning the impact of occult diabetes on CV outcome. To this purpose, we investigated the role of diabetes developed after a major CV event and diagnosed by fasting glucose, oral glucose tolerance test (OGTT) or glycosilated hemoglobin (A1c), on CV disease recurrence in high risk individuals.
Material and Methods. 383 subjects without known diabetes and with an history of a precedent major CV accident (myocardial infarction [MI] or stroke or peripheral artery surgery) included in the Athero-Vascular-Diabetes [AVD) study were followed up for a second major CV event (fatal and non fatal MI, fatal and non fatal stroke or any arterial revascularization procedure). At the baseline each individual carried out a clinical interview, a physical and biochemical examination, performed an oral glucose tolerance test and dosed A1c. In addition, we evaluated the severity of atherosclerosis and patients were then classified as having mono- or poly-vascular disease. The average follow up duration was 30 months.
Results. Incidence of CV events per 100 person-year was 10.01 (98 events/970 person-year). The diagnosed of an occult diabetes was not associated with major CV events, either using glycated hemoglobin values of 6.5% or greater, or fasting glucose value of 126 mg/dl or greater or 2h post load glucose of 200 mg/dl or greater (p-value not significant for all). Among risk factors only poly-vascular disease was correlated with a new major CV events. After adjustment for age, BMI, smoking status, systolic blood pressure, high-density and low-density lipoprotein cholesterol and high sensitivity C-reactive protein levels, poly-vascular disease was a strong significant predictor of a second major CV events (HR 2.48 [95% CI 1.58-3.88]).
Conclusion. Diabetes developed after an established vascular atherosclerosis disease did not increase the risk of new major CV events in subjects in secondary prevention using either fasting glucose, A1c or OGTT. In our population, among clinical CV risk factors, only the poly-vascular disease was able to identify the subjects at high risk to develop a second major cardiovascular events. Thus, in individuals with an history of CV disease an accurate evaluation of its severity may help in identifying subjects at higher risk.

PROFILO GENETICO DI PREDISPOSIZIONE ALLA STENOSI CAROTIDEA: RUOLO DEL SISTEMA RENINA ANGIOTENSINA (SRA)

I. Romagnuolo[1,3], E. Sticchi, F. Sofi[1], G. Pratesi[2], R. Pulli[2], C. Pratesi[2], R. Abbate[1], C. Fatini[1]
[1]Dipartimento di Area Critica Medico Chirurgica, Università di Firenze, Malattia Aterotrombotiche, Azienda Ospedaliero-Universitaria Careggi; [2]Unità di Chirurgia Vascolare, Università di Firenze; [3]Fondazione Fiorgen, Firenze, Italia
E-mail: ilaria_romagnuolo@yahoo.it

Introduzione. La stenosi carotidea rappresenta una manifestazione comune dell'aterosclerosi sistemica. Oltre i tradizionali fattori di rischio, fattori genetici, come i polimorfismi del sistema renina angiotensina (SRA), possono svolgere un ruolo rilevante nella modulazione del processo che porta alla stenosi carotidea. In questo studio abbiamo analizzato il ruolo dei polimorfismi ACE I/D e -240A>T, AGT M235T, e AGTR1 1166A>C nel modulare la suscettibilità alla malattia.
Materiali. Sono stati analizzati 821 pazienti consecutivi con stenosi carotidea severa (≥70%) e 847 soggetti di controllo.
Metodi. La valutazione dei polimorfismi genetici è stata effettuata mediante tecniche di biologia molecolare.
Risultati. È stata osservata una significativa differenza sia nella distribuzione dei genotipi (p<0.0001) che nella frequenza allelica (p<0.0001) tra i pazienti e i controlli per il polimorfismo ACE I/D, ma non per gli altri SNPs investigati. L'allele D del gene ACE era significativamente associato alla stenosi carotidea sia all'analisi di regressione logistica univariata (p<0.0001) che multivariata (p<0.0001). Considerando l'effetto combinato degli alleli sfavorevoli relativi ai singoli polimorfismi del SRA, abbiamo osservato che pazienti che presentavano meno di 3 alleli sfavorevoli avevano un minor rischio [OR=0.79 (0.63-0.99), p=0.05], mentre pazienti con più di 4 alleli presentavano una maggiore predisposizione alla malattia [OR=1.44 (1.12-1.84), p=0.004]. Nei pazienti senza fattori di rischio tradizionali (n=63) la frequenza dell'allele ACE D era significativamente maggiore rispetto a quella osservata nei pazienti con almeno un fattore di rischio (n=758) (0.71 vs 0.61; p=0.04). La frequenza dell'allele ACE D risultava simile tra pazienti con (n=282) e senza (n=559) ulteriori localizzazioni aterosclerotiche. (0.61 vs. 0.62, rispettivamente).
Conclusioni. I nostri risultati evidenziano il ruolo del polimorfismo ACE I/D nell'influenzare la suscettibilità alla stenosi carotidea, anche in assenza dei tradizionali fattori di rischio, ed il contributo di tutti i polimorfismi del SRA nella modulazione del processo aterotrombotico.

PREVALENZA DELLA DISLIPIDEMIA ATEROGENA IN RAPPORTO ALLA STATO DI TOLLERANZA GLICIDICA. CONFRONTO FRA I DUE SESSI

L. Rossi, R. Mella, G.P. Fra, G.P. Carnevale Schianca
Azienda Ospedaliero Universitaria Maggiore della Carità di Novara, Facoltà di Medicina e Chirurgia del Piemonte Orientale Amedeo Avogadro, Divisione di Clinica Medica Generale, Novara, Italia
E-mail: luca.rossi_1981@yahoo.it

Nel soggetto affetto da diabete mellito di tipo 2 (DM) sono presenti modifiche dell'assetto lipidico che risultano in gran parte secondarie alla presenza di insulinoresistenza e/o di iperinsulinemia. Tali alterazioni riguardano principalmente l'aumento dei trigliceridi (TG) e la riduzione del colesterolo-HDL (HDL) che, quando associate, configurano il quadro della dislipidemia aterogena (DIAT); tale assetto lipidico, risulta strettamente correlato con gli eventi cardiovascolari, anche in presenza di colesterolo-LDL (LDL) non aumentato.
L'utilizzo delle statine, il cui "goal" terapeutico è la riduzione del colesterolo-LDL (LDL), non sempre normalizza i valori dei TG e HDL.
Scopo del nostro studio è stato di valutare la prevalenza di DIAT in soggetti stratificati in base alla tolleranza glicidica e di verificare eventuali differenze in rapporto al sesso.
Abbiamo considerato 1.602 soggetti non noti per DM (♀=880) suddivisi in tre gruppi in base all'esito di una curva da carico orale di glucosio (OGTT). Il 52,7% sono risultati con normale tolleranza glicidica (NGT), il 36,8 % sono risultati affetti da pre-DM ed il 10,5% da DM di nuova diagnosi.
In tutti i soggetti abbiamo valutato i parametri lipidici e, in particolare la presenza di DIAT in accordo con NCPET ATP III. Considerando il sesso maschile, il 10.7% (n=39) dei soggetti con NGT (n=362) è risultato portatore di DIAT, tale percentuale era del 15.3% (n=42) nei soggetti pre-DM (n=274) e del 13.9% (n=12) dei soggetti risultati affetti da DM all'OGTT (n=86) (X2=2.98, n.s.). Considerando il sesso femminile, il 9,9% (n=48) dei soggetti con NGT (n=482) è risultato portatore di DIAT, tale percentuale era del 20.7% (n=65) nei soggetti pre-DM (n=316) e del 29.4% (n=24) dei soggetti risultati affetti da DM all'OGTT (n=82) (X2=29.3, p<0.0001).
Nel confronto tra i due sessi, considerando le tre categorie di gluco-tolleranza, abbiamo evidenziato, solo nei soggetti diabetici, una percentuale significativamente aumentata di donne con DIAT rispetto ai maschi (66.7% vs 33.3%, X2=5.8, p<0.02).
L'analisi statistica dei dati ottenuti ci ha consentito di mettere in evidenza che le donne diabetiche presentano una percentuale di dislipidemia aterogena significativamente maggiore rispetto ai maschi diabetici; inoltre, nei soggetti di sesso femminile, la percentuale di soggetti con dislipidemia aterogenica aumenta significativamente con il peggiorare della tolleranza glicidica; tale andamento, non risulta essere presente nella popolazione maschile.
Si può affermare che le donne nel diventare diabetiche acquisiscono un rischio cardiovascolare tale che finisce per annullare il fattore protettivo nei confronti di eventi cardiovascolari legati al sesso; tale quadro parrebbe essere associato all'aumento di prevalenza di dislipidemia aterogena.

HYPERLIPIDEMIAS HOSPITAL PATHWAY MANAGEMENT

F. Rubba, G. Mancini, F. Verdesca, M. Gentile, G. Marotta, F. Jossa, G. Fortunato, G. Canfora, P. Cuccaro, P. Rubba, E. Giancotti
AOU Federico II, Naples, Italy
E-mail: fabiana.rubba@unina.it

In the patient population served by the Department of Federico II University Hospital, cardiovascular disease is an important and common cause of morbidity and mortality and hyperlipidemia is a reversible risk factor for cardiovascular disease. In an effort to deal with the problem of treating this extremely common risk factor in a cost-effective and efficacious manner, a clinical pathway unit was created inside the ccordination center of regional network. This unit employs written protocols and goals derived from the Panel Guidelines. The clinical pathway unit has been created all around

the multi building hospital facilities. Out patient facilities Clinical ambulatory Sharing medicine with general practitioner Dietetic and health style counseling. In patient facilities Hospital care including dysmetabolic and cardiologic specificities Diagnostic facilities Biochemical Lab including LDL sixe measurements Genetic diagnosis of familiar hyperlipidemias. The clinic staff emphasize diet and lifestyle changes, and they follow rational treatment protocols, information data ware house and general practioner front-office is able to integrate clinical report and diagnosis. Preliminary data indicate that the unit is very successful in improving clinical outcomes, patient satisfaction, and costs and reducing hospital organizational waste.

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF A NEW MUTATION LEADING TO DEFECTIVE UPTAKE OF LDL-LDLR COMPLEX

A. Ruotolo[1], M. Romano[1,2], M.D. Di Taranto[3], P. Mirabelli[1,2], M.N. D'Agostino[1,2], A. Iannuzzi[4], G. Marotta[5], M. Gentile[5], R. Di Noto[1,2], L. Del Vecchio[1,2], P. Rubba[5], G. Fortunato[1,2]
[1]CEINGE S.C.a r.l. Biotecnologie Avanzate, Napoli, Italy; [2]Dipartimento di Biochimica e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli, Italy; [3]IRCCS Fondazione SDN, Napoli, Italy; [4]Centro per la Diagnosi e la Terapia delle Dislipidemie Familiari, Azienda Ospedaliera Antonio Cardarelli, Napoli, Italy; [5]Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Napoli Federico II, Napoli, Italy
E-mail: ruotolo.anna@hotmail.it

Introduction. Familial Hypercholesterolemia (FH), the most common form of autosomal co-dominant hypercholesterolemia, is mainly due to mutations in the LDLR gene (70% of cases). The LDLR mutations are classified in 5 different functional classes depending of the functional defects: synthesis, translocation of receptor to plasma membrane, binding to LDL, internalization of complex LDLR-LDL, recycle of receptor.
Materials and Methods. The mutation screening was performed by direct sequencing of the promoter and the 18 exons of the LDLR gene.
To define the causative role of the new mutation, we verified its absence in 150 chromosomes from normocholesterolemic individuals as well as the conservation of substituted amino acid residues across homologous proteins. The functional assay was performed incubating mitogen stimulated T-lymphocytes with fluorescent LDL (DiI-LDL) at 2 temperatures:
1. 37°C to evaluate the binding and the uptake of DiI-LDL;
2. 4°C to inhibit the endocytosis and measure only the binding.
Results. We identified the new mutation c.2476<A in the exon 17 together with the c.1130<707;T (exon 8) in a compound heterozygote.
The functional characterization reveals a residual activity at 37°C (28%) lower than that at 4°C (62%). Since internalization defects lead to a reduced activity at 37°C and a normal activity at 4°C, experimental results indicate that the patient shows a defect in the endocytosis. Since this defect was observed in a compound heterozygote we were unable to distinguish which of the above mutations is responsible for reduced internalization.
Conclusions. We demonstrated that the patient's functional activity is lower than 50% thus being compatible with a compound heterozygous status.
By comparing the two experiments carried out at two different temperatures we concluded for the presence of an internalization defect, although it cannot be attributed to one of the two mutations. Acknowledgements. CEINGE Convenzione Regione Campania, DGRC 1901/2009 and IRCCS Fondazione SDN.

HIGH GLUCOSE REDUCES THE ANTI-AGGREGATING EFFECT OF ASPIRIN BY IMPAIRING THE ASPIRIN-INDUCED ACTIVATION OF THE NITRIC OXIDE/CGMP/PKG PATHWAY

I. Russo, C. Barale, L. Mattiello, M. Viretto, G. Doronzo, E. Ceraolo, M. Trovati, G. Anfossi
Internal Medicine and Metabolic Disease Unit, Dept. of Clinical and Biological Sciences, S. Luigi Gonzaga, Faculty of Medicine of the Turin University, S. Luigi Gonzaga Hospital, Orbassano, Turin, Italy
E-mail: isabella.russo@unito.it

Introduction. Atherothrombotic events are the leading cause of mortality in patients affected by type 2 diabetes and platelet hyperactivity plays a major role in this phenomenon. As a consequence, aspirin treatment has been recommended for both primary and secondary prevention of cardiovascular events in diabetic patients: some intervention studies, however, demonstrated that the percentage of diabetic patients who benefit from aspirin with a reduction of cardiovascular events is significantly lower than that observed in non diabetic subjects. These results induced to consider diabetes mellitus as a condition of "aspirin resistance". Despite the fact that hyperglycaemia is involved in the pathogenesis of diabetes vascular complications, the role played by high glucose on the impaired platelet sensitivity to aspirin, has not been fully investigated as yet.
Aim. To evaluate whether in vitro exposure of platelets to high glucose reduces the anti-aggregating effects of aspirin, and to clarify whether high glucose interferes with the two anti-aggregating effects of aspirin: i.e., the inhibition of thromboxane production and activation of the nitric oxide (NO)/cGMP/cGMP-dependent protein kinase (PKG) pathway.
Methods. The study has been carried out in 53 healthy volunteers (30 M/23 F; age: 23.5±0.3 years; body mass index: 22.4±0.7 kg/m2). In platelet-rich plasma (PRP) and, when appropriate, washed platelets incubated for 60 min with 5-25 mmol/l D-glucose we evaluated the influence of a 30-min incubation with lysine acetylsalicylate (LAS, 1-300 micromol/l) on the following parameters:
1. platelet reactivity in high-shear-stress condition measured by PFA-100;
2. aggregation induced by sodium arachidonate (NaA, 1 mmol/l) or by ADP (10 micromol/l) measured by the Born's method;
3. NaA and ADP-induced thromboxane production (RIA); iv) activation of the NO/cGMP/PKG pathway, evaluated by the measurement of:
   a) NO synthase (NOS) activity (arginine/citrulline conversion);
   b) cGMP concentrations (RIA);
   c) phosphorylation of the Vasodilator-Stimulated Phosphoprotein (VASP) at Ser 239 (western blot), used as a marker of PKG activation.

Experiments were repeated in the presence of the anti-oxidant agent amifostine and of the osmotic control mannitol.
Results. LAS reduced platelet aggregation measured by PFA-100 and Born's method, inhibited thromboxane synthesis and activated the NO/cGMP/PKG pathway in the presence of normal glucose concentrations (5 mmol/l). Platelet exposure to 25 mmol/l glucose:

a) did not modify platelet reactivity in response to 100 micromol/l LAS measured through PFA-100 collagen/epinephrine cartridge (time closure: 283.3±9.9 vs 260.3±11.8 seconds, ns);
b) decreased LAS ability to inhibit platelet aggregation to both NaA (LAS IC-50: 49.2±8.3 vs 31.4±5.2 micromol/l, p<0.01) and ADP (percent inhibition exerted by 300 micromol/l LAS: 25.5±2.9% vs 43.7±3.1% p<0.0001);
c) did not modify LAS-induced inhibition of thromboxane synthesis elicited by both NaA (percent inhibition with 100 micromol/l LAS: 83.8±2.1% vs 81.7±2.8 %, n.s) and ADP (percent inhibition with 150 micromol/l LAS 83.0±2.0 % vs 84.8±0.9 %, ns);
d) completely prevented the stimulation of the NO/cGMP/PKG activation induced by 300 micromol/l LAS:
   1) NOS activity: 15.4±0.11 vs 27.9±1.5 fmol 3H-citrulline/min/mg protein, p<0.0001,
   2) cGMP concentrations: 13.1±1.9 vs 27.5±2.4 pmol/109 platelets, p<0.0001,
   3) VASP phosphorylation: 75.3±5.3 vs 196.8±7.9 A.U., p<0.0001.

In the presence of glucose 25 mmol/l, these three parameters did not differ from values without LAS. Preincubation with amifostine reverted the inhibition exerted by 25 mmol/l D-glucose on the LAS effects on platelet aggregation and on the NO production. Iso-osmolar mannitol did not interfere with the LAS effects on platelets.

Conclusions. In platelets from healthy subjects a short-time exposure to high glucose reduces the antiaggregating effect of aspirin: this effect is independent on the modulation of thromboxane production and is attributable to the inhibition of the aspirin-induced activation of the NO/cGMP/PKG pathway. These results identify a new mechanism potentially involved in the resistance to aspirin in diabetic patients.

DETECTION OF ABCA1 GENE MUTATIONS IN A THREE PROBANDS AFFECTED BY HYPOALPHALIPOPROTEINEMIA

R. Sallo, L. Pisciotta, R. Fresa, A. Bellocchio, V. Guido, S. Bertolini
Department of Internal Medicine, University of Genoa, Italy
E-mail: raffaella.sallo@unige.it

Hypoalphalipoproteinemia (HPA), defined by plasma HDL-C concentrations below the 10th percentile of the distribution in the population, either alone or associated with hypertriglyceridemia (HTG), is the most prevalent lipoprotein abnormality found in patients with premature coronary artery disease. Aim of this study was the searching for causative mutations in HPA subjects by sequencing some major candidate genes (APOA1 and LCAT), and, in absence of mutations, by analysing of ABCA1 gene by Denaturing High Performance Liquid Chromatography (DHPLC) followed by sequencing of the fragments showing abnormal DHPLC profile.

We analyzed ABCA1 gene in 30 selected HPA patients and we identified rare mutations in three probands. Proband P.C. was a 52 year-old male, BMI 25.2 kg/m^2, smoker of 30 cigarettes/day, with Total cholesterol (TC) 5.90, HDL-C 0.49, Triglycerides (TG) 3.48 mmol/L, ApoAI 80 and ApoB 137 mg/dl. ECG stress test was negative, however, echographic evaluation of the carotid arteries revealed fibrocalcific plaques at internal carotid arteries. The patient resulted to be heterozygous for a previously described variant c.5398 A>C (p.N1800H) in exon 40 of ABCA1 gene. Proband R.M.D. was a 49 year-old female from Ecuador, BMI 20.8 kg/m^2, carotid IMT 0.8 mm, TC 4.52, HDL-C 0.70, TG 3.11 mmol/L, ApoAI 100 and ApoB 98 mg/dl. Her sister had HDL-C 0.88 mmol/L. Both subjects were carriers of the mutation c.688 C>T (p.R230C) in exon 7, which was previously described by Wang et al. (ATVB 2000; 20 : 1983-9).

The third mutation was novel and consisted in one adenine insertion in exon 5 (c.396-397 ins A, Q133Tfs*20); it was identified in a 60 year-old female (Proband R.I.), BMI 22.3 Kg/m2 with TC 5.61, HDL-C 0.77, TG 3.51 mmol/L, ApoAI 108 and ApoB 116 mg/dl. Carotid ultrasound examination revealed IMT 1.3 mm at bifurcation, IMT 0.98 mm at common carotid, abdominal echograpy revealed hepatosteatosis.

MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) ANALYSIS IN FAMILIAL HYPERCHOLESTEROLEMIA (FH) PATIENTS NEGATIVE FOR MUTATIONS AT DIRECT SEQUENCING OF LDLR GENE

R. Sallo, L. Pisciotta, A. Bellocchio, S. Bertolini
Department of Internal Medicine, University of Genoa, Italy
E-mail: raffaella.sallo@unige.it

Familial Hypercholesterolemia (FH) is mainly due to mutations in LDLR gene. Diagnosis is based on family history, patient's clinical history, physical examination and high level of plasma LDL cholesterol. In absence of minute or point mutations in LDLR gene, we used Multiplex Ligation-dependent Probe Amplification (MLPA) to detect major rearrangements. With MLPA technique we genetically characterized two FH kindred. Proband A.L. was a 47 year-old female, BMI 23 kg/m^2 with LDL-C 6.15 mmol/L and ApoB 150 mg/dl, without tendon xanthomas and arcus cornea, carotid IMT 0.6 mm.

The proband's 79 year-old mother had LDL-C 6.08 mmol/L and was apparently free of cardiovascular disease.

The proband's 20 year-old daugther, BMI 18.3 kg/m^2, carotid IMT 0.5 mm, had LDL-C 5.12 mmol/L and ApoB 110 mg/dl. MLPA analysis revealed the presence of a deletion of Exon 2 (p.Val23-Leu64 in frame deletion). This mutation was detected for the first time in an Italian FH patient.

Proband G.C. was a 44 year-old male, BMI 25.5 kg/m^2 with tendon xanthomas and the following lipid profile: TC 9.90, HDL-C 1.00, TG 1.36, LDL-C 8.27 mmol/L, Apo AI 125 and Apo B 154 mg/dl. At the age of 40 coronary angiography showed a 3V-CAD and the patients underwent PTCA.

The proband's 62 year-old mother, BMI 32.9 kg/m^2, had LDL 7.08 mmol/L, Apo B 167 mg/dl, xanthomatosis, arcus cornea, and suffered from stroke at 53 years associated with a complete occlusion of the right internal carotid. A deletion of exons 13-15 was identified in this proband by MLPA analysis.

As previously demonstrated this mutation caused 3 abnormal mRNAs: Ex12>Ex16 (p.Glu615fs*17), Ex12>Ex17 (p.Glu615fs*134), Ex11>Ex16 (p.Asp569_Gln770 del) (Lelli N et al. J Lipid Res 1993; 34: 1347-54).

In conclusion, MLPA is a suitable rapid and reproducible alternative to southern blot analysis, a technique not simple to perform and requiring radioactive reagents.

ABCA-1 EXPRESSION IS REDUCED IN ATHEROSCLEROTIC PLAQUES OF HYPERTENSIVE PATIENTS: A NEW LINK BETWEEN HYPERTENSION AND ATHEROSCLEROSIS

D. Santovito[1], C. Mandolini[1], P. Marcantonio[1], M. Bucci[1], S. Ucchino[2], F. Magnacca[2], A. Mezzetti[1], F. Cipollone[1]
[1]ESH Excellence Center on Atherosclerosis, Hypertension and Dyslipidemia and Clinical Research Center, Center of Excellence on Aging (Ce.S.I.) "G. d'Annunzio" University, Chieti-Pescara, Italy; [2]Vascular Surgery Unit, "G. d'Annunzio" University, Chieti, Italy
E-mail: donato.santovito@email.it

Background. ATP-binding cassette transporter A1 (ABCA1) and ABCG1 are involved in reverse cholesterol transport (RCT) and atherosclerosis pathophysiology. ABCA1 mediates cholesterol efflux from macrophages to lipid-free/poor apolipoprotein AI (apoAI), whereas ABCG1 mediates the macrophage cholesterol efflux to phospholipids containing HDL particles. ABCA1 is abundantly expressed in cholesterol-loaded macrophages and its expression is modulated by intracellular cholesterol levels. Few data are still available about the relationship between RCT and hypertension in humans. In particular, recent data seem to suggest that ABCA1 expression is reduced in blood monocytes of hypertensive patients, but nothing is known about the expression of ABCA1 and ABCG1 in human atherosclerotic plaques in the setting of hypertension. Thus, the aim of this study was to investigate the expression of ABCA1 and ABCG1 in atherosclerotic plaques of hypertensive patients.
Material and Methods. We collected atherosclerotic plaques from 17 hypercholesterolemic (LDL cholesterol >100 mg/dL) patients who underwent carotid endarterectomy for high-grade stenosis of extracranial tract of internal carotid artery. Plaques were subdivided into hypertension group (n=10) and control group (n=7) according to presence or absence of hypertension (as defined by blood pressure >140/90 mmHg or concomitant antihypertensive therapy). All the patients have no history for previous cardiovascular or cerebrovascular events. In study plaques, ABCA1 and ABCG1 mRNAs were analyzed by real-time PCR whereas ABCA1 and ABCG1 proteins were investigated by western blot analysis.
Results. We found a slight significant association between hypertension and lower ABCA1 protein expression in atherosclerotic plaques (0,206±0,060 vs 0,438±0,097, protein level±SEM; p: 0.05); a similar trend, however not statistically significant, was found also for mRNA expression (11,22±3,28 vs 42,40±18,49, fold induction±SEM). In contrast, no significant differences in ABCG1 expression were found between hypertensive and control group at both protein (0,501±0,026 vs 0,585±0,056, protein level±SEM; p: 0.164) and RNA level (2,93±1,12 vs 11,03±4,36 fold induction±SEM; p: 0.123).
Conclusions. These results suggest that hypertension is associated with a downregulation of ABCA1 expression in human atherosclerotic plaques. ABCA1 downregulation may contribute to the development of accelerate atherosclerosis in hypertensive patients. Further analysis with a larger sample size will be needed in order to confirm this data and to evaluate the effect of different antihypertensive drugs on ABCA1 expression.

PHARMACOGENETICS OF CLOPIDOGREL: COMPARISON BETWEEN A STANDARD AND A RAPID GENETIC TESTING

C. Saracini, A. Vetrini, S. Galora, A. Armillis, R. Abbate, B. Giusti
Department of Medical and Surgical Critical Care, University of Florence, Atherothrombotic Diseases Center, A.O.U. Careggi, Florence, Italy
E-mail: claudiasaracini@hotmail.it

Background. CYP2C19 variant alleles are independent predictors of clopidogrel response and occurrence of major adverse cardiovascular events in high risk vascular patients on clopidogrel treatment. Increasing evidence suggests as combination of platelet function testing with CYP2C19 genetic testing may be more effective in identifying high-risk individuals for alternative antiplatelet therapeutic strategies. A crucial point in evaluating the use of these polymorphisms in the clinical practice, besides test accuracy, is the cost of the genetic test and rapid availability of the results.
Methods. One hundred venous blood samples were genotyped for CYP2C19*2, *3, *4, *5, and *17 polymorphisms with two platforms: rapid Verigene® and classical TaqMan® system. We compared the performance of the two approaches in terms of accuracy, turnaround time and cost.
Results. Genotyping results obtained by the classical TaqMan® approach and the rapid Verigene® approach showed a 100% concordance for all the 5 polymorphisms investigated. The Verigene® system had shorter turnaround time with respect to the TaqMan®. The cost of reagents for TaqMan® genotyping resulted lower than Verigene® system, but the effective manual staff involvement and the relative cost resulted higher for TaqMan® than for Verigene®.
Conclusions. The Verigene® system demonstrated good performance in terms of turnaround time and cost for the evaluation of the clopidogrel poor metabolizer status, giving genetic information in suitable time (206 minutes) for therapeutic strategy decision to be introduced in the clinical management of high risk vascular patients.

EFFICACY AND SAFETY OF NIACIN/ LAROPIPRANT THERAPY IN FAMILIAL HYPERCHOLESTEROLEMIC PATIENTS WITH CORONARY ARTERY DISEASE

F. Sbrana[1], M. Puntoni[2], F. Bigazzi[1], S. Vicari[1], E. Grisanti[1], A. Ragusa[1], M. Pianelli[1], R. Luciani[1], T. Sampietro[1]
[1]Fondazione CNR, Regione Toscana "Gabriele Monasterio", Pisa, Italy; [2]CNR Institute of Clinical Physiology of Pisa, Italy
E-mail: ifcfsbrana@ifc.cnr.it

Background. Cardiovascular disease is the principal cause of premature mortality and morbidity in Europe. Patients with familial hypercholesterolemia are at particularly increased risk and, despite lipid-lowering therapy, continue to experience cardiovascular events. Currently, for these patients a new treatment option is represented by extended-release niacin/laropiprant (ERN/LRPN).
Material and Methods. We followed-up for 16 weeks a group of 23 familial hypercholesterolemic patients (mean age 61±7 years, 74% male) with chronic coronary artery disease and ERN/LRPN added on top of maximally tolerated lipid-lowering therapy. ERN/LRPN was administered at the dose of 1 gr/day for the first 4 weeks and then at 2 gr/day for the remaining period. Clinical ex-

amination and blood sampling (including lipid profile, renal and hepatic function) were performed at baseline, after 4 weeks, at the end of follow-up, and in the case of eventual clinical manifestations.
Results. During follow-up, 14 patients discontinued therapy due to side effects (headache, asthenia, and gastrointestinal disorders in 4 patients, muscle aches and CK increase in 3 patients, eruptive skin rash in 2 patients, onset of diabetes mellitus in 2 patients, dizziness associated with inability to drive in 1 patient, acute hepatitis in 1 patient and palpitations in 1 patient) and 2 patients voluntarily interrupted the therapy. In the remaining 7 patients, an improvement in lipid profile was observed (total cholesterol -14%, HDL cholesterol +7%, LDL cholesterol -16%, Triglycerides -53%, Apolipoprotein A1 +8%, Apolipoprotein B -21%, Apolipoprotein E -31%) in the absence of substantial changes in other laboratory analyses (with the exception of a non-significant increase in uric acid). Intolerable skin flushing was not observed in any patient. In addition, among patients who did report flushing, a reduction in the incidence of the episodes was observed after the first month of therapy.
Conclusions. In our population, ERN/LRPN therapy improved lipid profile but was poorly tolerated in the majority of patients (61%). In this latter group, we recorded an unexpectedly high incidence of both common (less than 1 in 10 patients) and uncommon (less than 1 in 100 patients) side effects. Key words: familial hypercholesterolemia, extended-release niacin, laropiprant, chronic coronary artery disease.

POLIDISTRECTUAL VASCULAR INVOLVEMENT IN FAMILIAL HYPERCHILOMICRONEMIA

F. Sbrana[1], M. Puntoni[2], F. Bigazzi[1], M. Margelli[3], C. Petersen[1], R. Bonini[3], T. Sampietro[1,2]
[1]Fondazione CNR, Regione Toscana "Gabriele Monasterio", Pisa, Italy; [2]CNR Institute of Clinical Physiology of Pisa, Italy; [3]S.I.M.T. Lucca, Ospedale di Lucca, AUSL 2 di Lucca
Email: mpuntoni@ifc.cnr.it

A 72-year-old man was referred to our clinic with a lipid profile, under combination therapy with a statin and ezetimibe, characterized by severe hypertriglyceridemia (7230 mg/dl), hypercholesterolemia (374 mg/dl), low HDL-cholesterol (17 mg/dl), and normal circulating Lp"a". The patient had undergone post-traumatic splenectomy and presented a history of systemic hypertension treated medically with well-controlled blood pressure. The patient had also presented in the past abdominal pain with subsequent diagnosis of chronic pancreatitis complicated by diabetes mellitus, well compensated when he came to our attention. Following a pathological exercise test, a coronary angiogram was performed which showed a significant stenosis of the right coronary artery, which was treated successfully with percutaneous coronary intervention. Clinical work-up revealed ectasia of the abdominal aorta (28 mm), non significant bilateral carotid artery disease, and peripheral artery disease of the femoral-popliteal axis symptomatic for intermittent claudication. A lipidogram was also performed and electrophoretic lipoprotein patterns did not vary 2 hours after heparin infusion, pointing to the existence of lipoprotein lipase deficit. Electrophoresis also showed a broadband of chylomicrons at baseline, at the beginning, and at the end of heparin infusion. Hyperchilomicronemia is a rare genetic disorder with an incidence of 1 per 1000000. Following diagnosis, our patient began plasma exchange therapy with subsequent improvement of his lipid profile. At the present time, he is regularly followed up at our clinic and non invasive imaging has excluded any significant progression of atherosclerosis after 2 years of therapy.

LA STRATIFICAZIONE DEL RISCHIO DI SVILUPPARE DIABETE MELLITO COME STRUMENTO DI DIAGNOSI PRECOCE: LO STUDIO DIGA

R. Seller[1], D. Santovito[2], M. Bucci[2], C. Paganelli[2], A. Mezzetti[2], F. Cipollone[2]
[1]Nucleo Cure Primarie "Galassia Medica", Cepagatti, Pescara, Italia; [2]Centro di Eccellenza Europeo e di Riferimento Regionale per lo Studio dell'Arteriosclerosi, dell'Ipertensione e delle Dislipidemie, Università "G. d'Annunzio", Chieti, Italia
E-mail: donato.santovito@email.it

Background. Le proiezioni epidemiologiche tracciano un quadro che pone il Diabete Mellito tipo 2 (DMT2) tra le grandi emergenze sanitarie. In Italia sono circa 3 milioni i diabetici noti, ma si stima che ogni 2 pazienti diabetici noti ce ne sia uno non ancora diagnosticato. La prevalenza di condizioni che incrementano il rischio di DMT2 (Impaired Glucose Tolerance, IGT; Impaired Fasting Glucose, IFG) è vicina al 10%. Diagnosi precoce e trattamento adeguato e tempestivo del profilo glicemico e dei fattori di rischio cardiovascolare concomitanti condizionano favorevolmente la prognosi. Diversi approcci sono stati proposti per valutare il rischio di sviluppare DMT2. Nel nostro studio abbiamo analizzato la prevalenza di DMT2 e di condizioni di elevato rischio di diabete nella popolazione di un distretto territoriale della provincia di Pescara.
Materiali e Metodi. Un gruppo di Medici di Medicina Generale ha somministrato un questionario (Diabetes Risk Score, Diabetes Care 2005) a pazienti senza alterazioni note del profilo glicemico né una determinazione della glicemia nei precedenti 2 anni. Il questionario, composto da 6 domande a risposta multipla, consentiva una stratificazione del rischio di sviluppare DMT2 in 5 categorie (basso - lievemente aumentato - moderatamente aumentato - alto - molto alto). I pazienti appartenenti alle 3 categorie superiori sono stati sottoposti alla determinazione della glicemia.
Risultati. Su 800 questionari, ben 760 (95%) risultavano utili per lo screening. 391 pazienti (51.46%) sono stati inclusi nelle categorie a rischio maggiore e sottoposti alla determinazione della glicemia a digiuno. Di questi, 139 (18.28%) presentavano un'alterata glicemia a digiuno (IFG) mentre 26 pazienti (3.42%) presentavano valori di glicemia diagnostici per DMT2.
Conclusioni. Lo studio dimostra l'utilità di uno screening di popolazione atto a individuare pazienti con rischio elevato di sviluppare DMT2 che consente una razionalizzazione delle spese per test diagnostici da effettuarsi in maniera più assidua su tali pazienti.

CHOLESTEROL METABOLISM AFTER STATIN THERAPY IN DIFFERENT HYPERLIPEMIAS

D. Siepi, G. Lupattelli, S. De Vuono, A.R. Roscini, F. Crisanti, D. Covelli, M. Pirro, E. Mannarino
Internal Medicine, Angiology and Atherosclerosis, Department of Clinical and Experimental Medicine, University of Perugia, Italy
E-mail: dsiepi@unipg.it

The quantification of non-cholesterol sterols in human blood allows the study of cholesterol metabolism; plant sterols (sitosterol

and campesterol) reflect cholesterol absorption, while cholesterol precursor (lathosterol) are a surrogate markers of cholesterol synthesis. Statins reduce cholesterol synthesis, usually with a corresponding rise in cholesterol absorption. Common hyperlipemias, such as polygenic hypercholesterolemia (PH) and familial combined hyperlipemia (FCH) have shown different patterns of cholesterol metabolism.
The purpose of our study was to evaluate whether cholesterol absorption and synthesis may differ after statin therapy according to the type of hyperlipemia. We determined lipid profile, apoprotein B and serum sterols (lathosterol, sitosterol, campesterol, by gas chromatography/mass spectrometry) before and after statin therapy in 80 untreated hyperlipemic patients, 40 with polygenic hypercholesterolemia (PH) and 40 with familial combined hyperlipemia (FCH). At baseline lathosterol values were significantly higher in FCH comparing to PH (p=0,04); on the contrary, campesterol and sitosterol were significantly lower in FCH than in PH (p=0,0001). After statin therapy the reduction in LDL-C did not significantly differ between the two groups; in PH there was a significant decrease of lathosterol from 96.1 to 52.6 102 μmol/mmol cholesterol (p=0.0001), with no significant modifications in campesterol and sitosterol plasma levels; on the opposite, in FCH lathosterol decreased from 117 to 43 102 μmol/mmol cholesterol (p=0.0001), campesterol significantly increased from 38 to 48 102 μmol/mmol cholesterol, (p. 0.0001) and sitosterol from 75 to 86 102 μmol/mmol cholesterol, (p. 0.022).
After statin therapy only in FCH Δ-LDL-C showed a significant inverse correlation with Δ-sitosterol (ρ=-0,491, p=0,001) and with Δ-campesterol (ρ=-0,565, p<0,001). In conclusion primary hyperlipemias show different patterns of response to statins: in PH LDL reduction appears completely "synthesis inhibition" dependent, while in FCH LDL decrease appears to be synthesis dependent, partially limited by absorption increase. Studying cholesterol metabolism in dyslipidemias might be useful in identifying the best tailored treatment.

IN VITRO EFFECTS OF RECOMBINANT HUMAN LCAT ON CHOLESTEROL ESTERIFICATION IN LCAT DEFICIENT PLASMA

S. Simonelli[1], C. Tinti[2], L. Salvini[2], L. Tinti[2], G. Orsini[2], V. Sousa[3], G. Franceschini[1], L. Calabresi[1]
[1]Center E. Grossi Paoletti, Department of Pharmacological Sciences, University of Milano, Italy; [2]Toscana Life Sciences Foundation, Siena, Italy; [3]Areta International, Gerenzano, Italy
Email: sara.simonelli76@gmail.com

Background. Genetic lecithin: cholesterol acyltransferase (LCAT) deficiency is an inherited disorder of lipid metabolism characterized by defective cholesterol esterification, HDL deficiency and accumulation in plasma of small dense pre-beta migrating particles. No therapy exists for LCAT deficiency. Early successful attempts to correct the biochemical LCAT deficient phenotype through the infusion of normal plasma provide the basis for the development of enzyme replacement therapy (ERT) as a therapeutic strategy for LCAT deficiency.
Methods. rhLCAT was produced in HEK cells; fasting plasma from 2 subjects with familial LCAT deficiency (FLD) belonging to the Italian families, and 2 controls, was added with rhLCAT (30 units in 100 μl), or an equal volume of saline. Plasma samples were incubated at 37°C for 6 hrs, and then placed on ice. Plasma total and unesterified cholesterol, HDL-C, apoA-I and apoB levels were measured by certified enzymatic methods. Plasma lipoprotein profile was analyzed by FPLC and HDL subpopulations were separated by 2D- electrophoresis.
Results. After rhLCAT incubation with plasma from FLD subjects, total cholesterol didn't change, but free cholesterol markedly decreased and cholesteryl esters increased. HDL-C doubled in FLD subject 1, while didn't change in subject 2. No major changes were observed in controls, except for an increase in HDL-C (from 60 and 50 mg/dl to 66 and 58 mg/dl). FPLC analysis confirmed the normalization of the lipoprotein profile after incubation, showing the appearance of a peak corresponding to normally sized HDL and a shift to larger LDL particles. In control subjects no major changes were observed. The separation of HDL subpopulations by 2D-electrophoresis showed the conversion of small pre-beta HDL into large, spherical, alfa HDL after incubation. In control subjects, the incubation produced minor modification in HDL subpopulations.
Conclusion. The present data show that the incubation of FLD plasma with rhLCAT normalizes the lipid/lipoprotein profile in carriers.

LDL RECEPTOR ALTERATIONS IN A PATIENT WITH CLINICAL DIAGNOSIS OF FAMILIAL COMBINED HYPERLIPIDEMIA

M. Sodano[1], G. Marotta[1], M.N. D'Agostino[2,3], M. Romano[2,3], M. Gentile[1], G. Fortunato[2,3], P. Rubba[1]
[1]Department of Clinical and Experimental Medicine, University "Federico II", Medical School, Naples, Italy; [2]CEINGE S.C. a r.l. Biotecnologie Avanzate, Napoli, Italy
E-mail: martasodano@libero.it

Introduction. Familial Combined Hyperlipidemia (FCHL) is a polygenic multifactorial disease characterized by variable lipid phenotypes, both in patient and in family members. The clinical criteria for diagnosis are: Cholesterol LDL level >160 mg/dL and/or triglycerides >200 mg/dL in patient with hypercholesterolemia and hypertriglyceridemia in the same family. Small dense LDL level >10 mg/dL are also associated with FCHL (Atherosclerosis 2009).
Case report. A 28 years old patient, smoking 10 cigarettes/die , without other cardiovascular risk factors and not in therapy for dyslipidemia, shows a clinical picture compatible with both FCHL and Familial Hypercholesterolemia (FH): cholesterol 310 mg/dL; triglycerides 161 mg/dL; HDL-cholesterol 35 mg/dL; LDL-cholesterol 243 mg/dL; ApoB 1.76 g/L (v.n. 0.5-1.3 g/L). Small dense LDL dosage shows a level of 33.8 mg/dL(normal range in healthy controls 0-10 mg/dL); Carotid Eco-Color-Doppler is normal. At present no data is available on lipid concentration in relatives. Molecular study reveals the mutation p.Pro685Leu in the exon 14 of LDLr gene, also confirming heterozygous FH diagnosis.
Conclusions. This patient bears a mutation of LDL-r gene, responsible for FH. Familial Hypercholesterolemia was diagnosed in the absence of information on lipids of relatives; sdLDL levels >10% are associated with Familial Combined Hyperlipidemia, however their determination power in the presence of Familial Hypercolesterolemia is possibly limited. Acknowledgements. CEINGE Convenzione Regione Campania, DGRC 1901/2009.

SIMILAR EPA+DHA CONTENT WITH DIFFERENT N-6/N-3 RATIO FISH FLESH INTAKE AFFECTS DIFFERENTLY THE ATHEROSCLEROTIC RISK PROFILE: A SINGLE-BLINDED RANDOMIZED CROSS-OVER TRIAL

F. Sofi, F. Cesari, A.M. Gori, L. Mannini, G. Giorgi, G. Parisi, G.F. Gensini, R. Abbate, B.M. Poli
Department of Medical and Surgical Critical Care, Department of Agricultural Biotechnology, Section of Animal Sciences, University of Florence, Italy
E-mail: francescosofi@gmail.com

Aim. Aim of this study was to evaluate the effect of consuming gilthead sea bream fillets with different n-6/omega-3 ratio on biomarkers related to the atherosclerotic process.
Materials and Methods. Twenty healthy subjects (12 M; 8 F) were included in a randomised single-blinded cross-over trial. Participants were randomized into 2 groups receiving for a period of 10 weeks about 630 g per week of fillets with known lipid and fatty acid composition from gilthead sea bream fed with either 100% fishmeal (FM) or partial replacement with plant proteins (PP). Group A received firstly FM fillets and then PP, while group B received firstly PP fillets and then FM. Before and after each intervention, lipid, inflammatory, and hemorheological profiles were measured.
Results. Group A reported to have significant amelioration with regard to lipid variables such as total cholesterol, LDL cholesterol and triglycerides. Indeed, total cholesterol significantly decreased by 29.3% (Δ=-26 mg/dL) in the first phase of intervention, returning to similar values of baseline after the dietary intervention with fishes fed with PP. Similarly, in group A LDL-cholesterol significantly decreased by 21.6% (Δ=-19 mg/dL), increasing afterward as well as triglycerides, that decreased by 11.7% (Δ=-10.7 mg/dL). Improvement resulted also for inflammatory cytokines, interleukin-6 and -8. Moreover, whole blood viscosity appeared significantly improved in group A, with the erythrocytes' filtration rate, which showed a significant increase by 7.59% (Δ=+4.59 mPA).
Conclusions. Similar EPA+DHA content with different n-6/n-3 ratio fish flesh intake affects differently lipid, inflammatory and haemorheological parameters in a group of healthy subjects. A modulation of the farmed fish diet in terms of healthy eating quality of products, with particular reference to both propter EPA+DHA content and favourable n-6/n-3 ratio is needed.

EFFECTS OF THE REGULAR CONSUMPTION OF BUCKWHEAT PASTA ON CARDIOVASCULAR RISK FACTORS: AN INTERVENTION STUDY

F. Sofi, A.M. Gori, L. Mannini, L. Ghiselli, S. Benedettelli, V. Vecchio, A. Casini, R. Abbate, G.F. Gensini
Department of Medical and Surgical Critical Care, Department of Agricultural Sciences, University of Florence, Italy
E-mail: francescosofi@gmail.com

Objective. Buckwheat cereals have unique amino acid composition with special biological activities of cholesterol-lowering and antioxidant effects. Aim of this study was to evaluate the influence of a regular consumption of buckwheat pasta on cardiovascular risk factor.
Methods. Twenty subjects (9 F; 11 M) with a median age of 39.5 years (range: 21-61) were studied. After a run-in period, the subjects followed for 10 weeks a diet containing 80 g/die of buckwheat pasta (Test period) and for the same period a diet containing a placebo pasta of the same quantity (Placebo period). We evaluated lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides), circulating and red blood cells' folate, and haemorheological profile [whole blood viscosity (WBV), plasma viscosity, erythrocytes' filtration rate (EF)] before and after dietary intervention.
Results. A general linear model for repeated measurements after adjustment for age, and gender was conducted. The test period determined a significant improvement of total cholesterol (pre: 211.1±44.9 vs. post: 196.5±44.4 mg/dL; p=0.01), and LDL-cholesterol levels (pre: 133.7±33.1 vs. post: 120.9±36.6 mg/dL; p=0.02), whereas no significant changes during the placebo period have been observed. With regard to haemorheological parameters, the test period significantly decreased all the parameters investigated, namely WBV at high (pre: 26.1±2.2 vs. post: 24.8±3.3; p=0.01) and low shear rates (pre: 5.9±0.4 vs. post: 5.7±0.4), as well as EF (pre: 8.4±3.1% vs. post: 9.1±2.8%; p=0.009) with respect to the placebo period that showed no significant changes.
Conclusions. Dietary short-term intake of buckwheat pasta naturally rich in antioxidants compounds seems to impose favourable biochemical changes, with regard to lower circulating levels of markers of atherosclerosis, such as lipid parameters, and haemorheological variables.

THE EVALUATION OF PHARMACOLOGICAL TREATMENT ON PLASMATIC ADMA/ SDMA AND ENDOTHELIAL NITRIC OXIDE SYNTHASE EXPRESSION IN PATIENTS WITH ACUTE HEART FAILURE

L. Speranza[1], L. Scotti[1], S. Franceschelli[1], M. Pesce[1], V. Bucciarelli[1], M. Felaco[1], A. Grilli[1], G. Riccioni[2]
[1]Department of Human Movement Sciences, University "G. D'Annunzio" Chieti, Italy
[2]Cardiology Unit, San Camillo de Lellis, Hospital, Manfredonia, Foggia, Italy
E-mail: griccioni@hotmail.com

Introduction. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide (NO) which plays an important role in controlling vascular tone and regulates the contractile properties of cardiac myocytes. The aim of this study is to investigate the effect of acute pharmacological treatment on plasmatic production of L-arginine, asymmetrical dimethylarginine (ADMA) and symmetrical dimethylarginine (SDMA) and the related effects on endothelial nitric oxide synthase (eNOS) expression and activity and citochrome c activities in primary blood mononuclear cells (PBMC) isolated from patients with acute congestive heart failure (ACHF).
Methods. 24 hospitalized patients in Cardiology Unit with symptomatic ACHF (NYHA Class III-IV) and impaired left ventricular (LV) function (ejection fraction <35%) were included in the study. ADMA, SDMA, arginine plasma concentrations and levels of eNOS expression and activity were assessed before and after pharmacological treatment by high performance liquid chromatography.
Results. ADMA SDMA and L-arginine plasma levels were significantly higher after pharmacological treatment respect to pre-treatment values (ADMA 0.82 vs 0.43; SDMA 1.52 vs 1.12; L-arginine 1.78 vs 1.29 p<0.01). In addition, the levels of eNOS expression and activity were decreased after pharmacological treatment and

determination of citochrome c oxydase activity resulted in higher O2- production in (PBMC) of post-treated patients.
Conclusions. In patients with ACHF with renal function impairment, high SDMA and ADMA levels are more evident after therapy, with reduced expression and activity of eNOS. Increased O2- production after treatment may be involved in reduced recovery of cardiac function associated with higher plasmatic level of SDMA.

PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF ENDOTHELIAL PROGENITOR CELLS AND EFFECTS OF N-3 PUFA

V. Spigoni[1,2], A. Picconi[1], V. Ridolfi[1], M. Cito[1], C. Casali[1], S. Bonomini[1], L. Gnudi[3], M. Metra[2], I. Zavaroni[1], A. Dei Cas[1]
[1]Department of Internal Medicine and Biomedical Sciences, University of Parma, Italy; [2]Department of Experimental and Applied Medicine, University of Brescia, Italy; [3]Cardiovascular Division, King's College London, UK
E-mail: lspigon@tin.it

Impairment in endothelial progenitor cell (EPC) number and function is associated to an increased cardiovascular (CV) risk. N-3 polyunsaturated fatty acids (PUFA) retain beneficial CV effects due to their antioxidant and anti-inflammatory properties and to their ability to improve endothelial function. Aim. We first carried out a phenotypical and functional ex vivo EPC characterization. We subsequently investigated whether the CV beneficial effects of PUFA are mediated by an improvement in EPC biology.
Methods. We conducted experiments for ex vivo isolation of early and late-outgrowth EPC. Early EPC were obtained culturing lymphomonocytes on fibronectin-coated dishes in EGM-2 for 7 days. In the same culture conditions late-outgrowth EPC were obtained after 3 weeks. Early and late-outgrowth EPCs were phenotypically characterized by immunofluorescence and FACS analyses. AcLDL-uptake and lectin-binding capacity were tested. Tube-formation assay was performed by co-plating cells on Matrigel with HUVEC to test EPC function. PUFA (10 µM) (DHA:EPA=1.5:0.9) were added to the medium to test the effects on EPC viability, apoptosis and function.
Results. Early EPC showed the co-expression of monocyte-macrophage (CD14, CD11b, CD44) and endothelial (CD31, KDR) markers and co-operated to tube formation. Late outgrowth EPC showed an higher expression of endothelial markers. Late outgrowth EPCs were able to form colonies and were capable of autonomously forming tubular-like structures. The addition of PUFA improved early and late-outgrowth EPC viability by 65% and 150% respectively.
No difference in apoptosis was observed. Of note, PUFA prevented H2O2-induced cell death of EPCs.
In the presence of PUFA, EPCs showed a significantly higher expression of endothelial markers and a lower expression of inflammatory molecules (ICAM1 and ICAM2) (We are analyzing data from tube-formation assay).
Conclusions. Our data show a direct beneficial effect of PUFA on EPC biology suggesting a protective role of PUFA on the vascular system possibly mediated by the reduction of inflammation and/or oxidative stress.

PREVALENCE OF ANGPTL3 AND APOB GENE MUTATIONS IN SUBJECTS WITH COMBINED HYPOLIPIDEMIA

R. Spina[1], A.B. Cefalù[1], D. Noto[1], V. Valenti[1], F. Fayer[1], E. Pinotti[2], M. Ditta[1], G. Vigna[3], P. Yue[4], G. Schonfeld[4], S. Kathiresan[5], P. Tarugi[2], M.R. Averna[1]
[1]Department of Internal Medicine and Medical Specialties, University of Palermo, Italy; [2]Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy; [3]Department of Clinical and Experimental Medicine, University of Ferrara, Italy; [4]Washington University, School of Medicine, St. Louis, MO, USA; [5]Division of Cardiology, Massachusetts General Hospital, Boston, Massachusetts
E-mail: rossella.spina@libero.it

Introduction. Mutations of the ANGPTL3 gene have been found responsible for a novel form of primary hypobetalipoproteinemia (pHBL), the combined hypolipidemia, characterized by low total cholesterol (TC) and low HDL-cholesterol (HDL-C) levels. The aim of this work is to define the role of ANGPTL3 gene as determinant of the combined hypolipidemia phenotype in two large cohorts of 913 American and Italian subjects with primary hypobetalipoproteinemia (TC <5th percentile).
Materials and Methods. The cut-offs adopted to define the combined hypolipidemia phenotype were chosen taking into account the TC and HDL-C levels reported in the ANGPTL3 kindred described to date and are as follows: TC levels <2nd percentile and HDL-C levels <20th percentile. We selected seventy-eight subjects with the combined hypolipidemia and analyzed the ANGPTL3 and the APOB genes.
Results. We identified nonsense and/or missense mutations in ANGPTL3 gene in eight subjects; no mutations of the APOB gene were found in the seventy-eight subjects with the combined hypolipidemia phenotype ANGPTL3 homozygous/compound heterozygous subjects showed a more severe biochemical phenotype compared to heterozygous or ANGPTL3-negative subjects with combined hypolipidemia. Lipid profile of ANGPTL3 heterozyotes did not differ from ANGPTL3-negative subjects.
Conclusion. These results demonstrated that in a cohort of subjects with severe pHBL the prevalence of ANGPTL3 gene mutations responsible of a combined hypolipidemia phenotype is high (about 10%) while mutations of APOB gene are absent.

THE CLASS II PHOSPHOINOSITIDE 3-KINASE ISOFORM BETA REGULATES PLATELET FUNCTION AND ARTERIAL THROMBOSIS IN MICE

G. Tibolla[1,2,3], S.S. Barbieri[4], S. Gianellini[4], L. Arnaboldi[1], A. Corsini[1], E. Tremoli[1,4], M. Falasca[3], G.D. Norata[1,2], A.L. Catapano[1,2,5]
[1]Department of Pharmacological Sciences, Università degli Studi di Milano, Italy; [2]Centro per lo Studio dell'Aterosclerosi Ospedale Bassini, Cinisello Balsamo, Milano, Italia; [3]Queen Mary University of London, Barts and The London School of Medicine and Dentistry, Blizard Institute of Cell and Molecular Science, Centre for Diabetes, London E1 2AT, UK; [4]I.R.C.C.S. Centro Cardiologico Monzino, Milano, Italia; [5]I.R.C.C.S. MultiMedica Hospital, Milano, Italia
E-mail: gianpaolo.tibolla@unimi.it

Phosphoinositide 3-kinases (PI3Ks) are crucial components of many signaling pathways involved in platelet activation. A key

role is played by the class I enzymes and especially by the p110β isoform that regulates sustained αIIb β 3 integrin activation and modulates the outside-in signaling properties of activated αIIb β 3 integrin. By contrast very little is known about the role of the class II PI3Ks on platelet function, though the absence of phosphatidylinositol-3,4,5 triphosphate, the main lipid product of class I PI3Ks, during late phases of platelets aggregation and the activation of the class II PI3K isoform β (PI3K C2 β) via activated αIIb β 3 integrin suggest the involvement of these enzymes in the regulation of platelet functional responses. Using a PI3K C2 β knockout mouse model we showed for the first time that PI3K C2 β activity is required for normal collagen and thrombin induced platelets aggregation. Interestingly absence of PI3K C2 β does not affect inside-out signaling, indeed the activation of intracellular kinases, the α-granules release and the activation of αIIb β 3 integrin are similar in PI3K C2 β deficient and wild type platelets. On the other hand collagen induced thromboxane A2 (TxA2) production and clot retraction are significantly impaired in PI3K C2α knockout platelets, suggesting that PI3K C2 β is required for outside-in signaling transduction and regulates the activation of phospholipase A2, possibly downstream activated αIIb β 3 integrin. In vivo studies show that PI3K C2 β knockout mice have platelets count and bleeding time similar to wild type mice, but are protected from collagen induced pulmonary thromboembolism and show a delayed thrombus formation after FeCl3 injury of the carotid artery. Taken together our data indicate the PI3K C2 β enzyme as an interesting new target for antithrombotic drugs development.

GENETIC POLYMORPHISMS OF ANTIOXIDANT ENZYMES SOD1, SOD2, SOD3, CAT AS RISK FACTORS FOR OXIDATIVE STRESS ASSOCIATED COMPLICATIONS IN PRETERM INFANTS

A. Vestrini, B. Giusti, C. Poggi, E. Pasquini, A. Magi, R. Abbate, C. Dani
Department of Medical and Surgical Critical Care, Section of Neonatology, Careggi University Hospital, Florence, Italy; Department of Medical and Surgical Critical Care, University of Florence, Atherothrombotic Diseases Centre, Careggi University Hospital, Florence, Italy; Metabolic and Muscular Unit, Clinic of Pediatric Neurology, Meyer Children's Hospital, Florence, Italy
E-mail: annaves78@libero.it

Oxidative stress occurs when the production of damaging reactive oxygen species (ROS) and other oxidative molecules exceed the capacity of the body's antioxidant defenses. There is increasing evidence that oxidative stress is implicated in the development of many complications of preterm birth, grouped as 'free radical-related diseases' (FRD), and including respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), intraventricular hemorrhage (IVH) and retinopathy of prematurity (ROP). This presumably occurs due to the fact that the antioxidant system of preterm infants is at the same time highly stressed and incompletely developed. Several data exist showing that genetic variants may influence the activity of the encoded enzymes and the susceptibility of developing ROS-induced complications conferred by genes involved in the regulation of oxidative stress, such as the superoxide dismutases (SOD1, SOD2, SOD3) and catalase (CAT). On the basis of these considerations, we hypothesized that the occurrence of critical ROS-induced complications in preterm infants - RDS requiring mechanical ventilation (MV), BPD, IVH and ROP - might be related to the polymorphisms in genes coding the important antioxidant enzymes, SOD1, SOD2, SOD3 and CAT. We studied 11 single nucleotide polymorphisms (SNPs) in 4 candidate genes (SOD1: rs17880135, rs204732; SOD2: Ala-16Val, rs5746136; SOD3: E1, Thr10Ala, Arg213Gly; CAT: -844C/T, -262C/T; -20T/C, C111T) involved in oxidative stress according to their demonstrated or putative function based on literature data, localization in the functional regions in a cohort of 152 of preterm neonates with gestational age (GA) >=28 weeks. Genotyping was performed by Taqman technology.
At the logistic regression analysis adjusted for GA and birth weight, we found that the rs8192287 (E1) SOD3 polymorphism decreased the risk of developing IVH [OR=0.22 (95% CI 0.05-0.98), p=0.047]. With regard to the association between studied polymorphisms and gestational age and birth weight, we found that homozygous infants for Ala16Val SOD2 polymorphism had a lower gestational age (p=0.018) and birth weight (p=0.003) compared to heterozygous and homozygous wild-type infants. The other SOD2 polymorphism (rs5746136) was also associated with significant lower gestational age (p=0.038).
Moreover, carriers of the -844C/T CAT polymorphism had a higher gestational age (p=0.041) compared to homozygous wild-type infants. After haplotypes reconstruction, at multivariable analysis adjusted for gestational age and birth weight, gg in SOD1 and gt in SOD2 rare haplotypes resulted significantly associated with different clinical complications in premature infants. CAT atcc haplotype was a risk factor for BPD and SOD3 tgc haplotype was a protective factor for BPD and IVH. Further study are needed to confirm our findings and to elucidate the mechanisms which induce these correlations. These results could be useful in identifying subjects at high risk of developing FRDs to whom prevention strategies should be particularly addressed.

PRO-INFLAMMATORY ENVIRONMENT AND EXPOSURE TO OX-LDL PROMOTES DIFFERENTIATION OF EARLY ENDOTHELIAL PROGENITOR CELLS INTO ANTIGEN-PRESENTING CELLS

M.C. Vinci[1], L. Zampetti[1], F. Prandi[1], G.L. Polvani[2], M. Pesce[1]
[1]Laboratorio di Ingegneria Tissutale Cardiovascolare, Centro Cardiologico Monzino IRCCS, Milan, Italy; [2]Dipartimento di Scienze Cardiovascolari, Università di Milano, Italy
E-mail: cristina.vinci@ccfm.it

Introduction. Culture of peripheral blood mononuclear cells (PBMCs) in endothelial cell (ECs)-specific media reveals two phenotypically and morphologically distinct cell types with pro-angiogenic abilities, 1) "early" EPCs and 2) late-outgrowth EPCs (OECs) (1, 2). Differently from OECs, early EPCs express CD14 and CD45 markers, sharing common features with circulating monocytes. The pro-angiogenic function of early EPCs is well-established (3-5) and the number and the clonogenic activity of these cells inversely correlates with coronary artery disease (CAD) risk factors and Framingham score (6). However we and others have found that eEPCs transplantation promotes atherogenesis (7) and causes a chronic inflammatory condition into ischemic tissues (8), thus raising the possibility that eEPCs may also act as pro-inflammatory cells. Given the recent demonstration of an unexpected plasticity and phenotypic adaptation to local conditions (9) of CD14+ cells, we hypothesized that exposure of eEPCs to a pro-inflammatory environment and/or to oxidized LDL (oxLDL) turns them into professional immune-modulatory cells such as antigen presenting cells (APCs).

Materials and Methods. PBMCs were isolated as previously described (8, 10) from healthy human peripheral blood. PBMCs were then seeded on fibronectin-coated tissue culture dishes (BD Biosciences, Bedford, MA) in endothelial basal medium supplemented with growth factors (EBM-2 and EGM-2 Bullet-Kit, Cambrex, Milan, Italy) plus 10% fetal bovine serum (FBS). After 1 week of culture the cells were further cultured for additional 5 days in presence of IL4, GM-CSF, and TNFalpha, IL1beta or ox-LDL. Early EPC and APC phenotype was evaluated by cytofluorimetric analysis for CD80, CD86, CD83, CD3, CD31, CD146, CD144, KDR, and CD14 (BD Bioscience) expression before and after pro-inflammatory cytokine/oxLDL treatment.
Results. After 1 week of culture on fibronectin-coated plates in EGM-2 complete media, PBMCs were identified as eEPCs for their morphological and phenotypic features (1). These cells were spindle-shaped and expressed typical endothelial markers such as CD31, DiI-labeled acetylated low-density lipoprotein uptake and Ulex Europaeus agglutinin binding. Further culture in pro-inflammatory environment (IL4, GM-CSF, TNF alpha and IL1 beta) or medium containing ox-LDL, determined a dendritic cell like morphology and expression of fully mature antigen presenting cells markers such as CD80 and CD83 (CD80, 74.66%±4.01; CD83, 22.085%±4.31 n=4; P<0.001 Vs CTR, t test). Further experiments are ongoing to evaluate the chemo/cytokine profile, the ability to stimulate allogenic lymphocytes by mixed-lymphocyte reaction (MLR) and the global gene expression profile in EPCs-derived APCs by pro-inflammatory/oxLDL exposure vs. conventionally derived APCs.
Conclusions. Our results indicate that PBMNC commitment into early EPCs is not a definitive event. In fact, exposure of these cells to pro-inflammatory stimuli or risk conditions promotes differentiation into an APC-like phenotype which may have a pro-atherogenic function. Here we suggest that pro-inflammatory-risk-factor-related environment may induce not only EPC dysfunction but also the acquisition of immune-modulatory cell phenotype.
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A RARE DELETION OF ABCA1 GENE IN A PATIENT WITH TANGIER DISEASE

P. Zanoni[1], E. Favari[2], M.P. Adorni[2], A. Sechi[3], F. Bernini[2], S. Calandra[1]
[1]Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy; [2]Department of Pharmacological and Biological Sciences and Applied Chemistry, University of Parma, Italy; [3]Center for Rare Diseases, Azienda Ospedaliero-Universitaria, Udine, Italy
E-mail: lormetazepam2@alice.it

Object of the Study. Analysis of ABCA1 Gene and cell cholesterol efflux pathways in a patient with severe hypoalphalipoproteinemia and some clinical manifestations of Tangier Disease (TD).
Methods and Results. We investigated a 60 years-old diabetic woman with splenomegaly, thrombocytopenia and anemia associated with peripheral neuropathy. In view of the presence of foams cells in the bone marrow she was thought to have Gaucher disease or Niemann-Pick type B disease. However, the normal activity of beta-glucosidase and acid sphingomyelinase and the presence of extremely low plasma levels HDL-C (2 mg/dl) and apoA-I (6 mg/dl) suggested the diagnosis of TD. The sequence of ABCA1 gene did not reveal point/minute mutations. However, the inability to amplify three exons (ex. 32, 33 and 34) suggested the presence of a deletion involving these exons. The amplification of the genomic region encompassing exons 31-35 by long range PCR, generated a fragment of 6Kb in control DNA vs a 2.5 Kb fragment in patient's DNA, thus indicating that the patient was homozygous for a 3.5 Kb deletion. This deletion results from a recombination between Alu sequences located in intron 31 and intron 34 respectively. The mutant allele generates an abnormal mRNA encoding a truncated protein of 1502 amino acids expected to be devoid of function. ABCA-1 mediated cholesterol efflux was abolished in patient's fibroblasts incubated under basal conditions or in the presence of probucol, a specific ABCA1 inhibitor. Under basal conditions cholesterol content in the plasma membrane of TD fibroblasts was similar to that found in control fibroblasts. However, following the stimulation of ABCA1 expression by LXR-RXR agonists, plasma membrane cholesterol increased in control fibroblasts but did not change in TD fibroblasts, as we have previously documented in several TD cell lines.
Conclusion. We report a novel mutation in ABCA1 gene which is one of the few large deletions in this gene observed so far.

EFFETTI ACUTI DELLA LDL-AFERESI SUI LIVELLI PLASMATICI DI PENTRAXINA3 IN PAZIENTI AFFETTI DA IPERCOLESTEROLEMIA E MALATTIA CARDIOVASCOLARE

F. Zardi[1], M.G. Zenti[1], G. Fainelli[1], M. Messa[2], A. Lupo[2], R.C. Bonadonna[1], E. Bonora[1]
[1]Divisione di Endocrinologia e Malattie del Metabolismo; [2]Divisione di Nefrologia
E-mail: mariagrazia.zenti@univr.it

Background. L'infiammazione gioca un ruolo critico nello sviluppo e nella progressione dell'aterosclerosi. Fra i marcatori di flogosi la Proteina C reattiva (PRC) e la Pentraxina3 (PTX3), due proteine della superfamiglia delle pentraxine, sono predittori indipendenti di eventi cardiovascolari. La PCR viene principalmente prodotta dal fegato mentre PTX3 viene prodotta da molti tipi cellulari presenti nelle lesioni aterosclerotiche. Questa proteina è coinvolta nel reclutamento leucocitario rappresentando un possibile meccanismo di amplificazione del danno tissutale. Non vi sono al momento dati sull'effetto acuto della LDL-aferesi sui livelli plasmatici di PTX3.
Obiettivo. Valutare gli effetti sulla PTX3 di un singolo trattamento di LDL aferesi in pazienti ad elevato rischio cardiovascolare.
Metodi. Sono stati studiati 9 pazienti (6M, 3F; età media 58 anni, range 41-70) affetti da ipercolesterolemia familiare non controllata dal trattamento ipolipemizzante alla dose massimale e con cardiopatia ischemica, sottoposti a trattamento cronico con LDL-aferesi (sistema HELP) a cadenza quindicinale. Prima e dopo un singolo trattamento aferetico è stato eseguito prelievo ematico per la determinazione di PTX3, PCR, profilo lipidico, fibrinogeno, emocro-

mo, glicemia, insulina. PTX3 è stata misurata con metodo ELISA (Quantikine, R&D Systems, Inc., Minneapolis, USA).
Risultati. Prima della seduta aferetica il colesterolo LDL era (media±DS) di 159±45.7 mg/dl. Dopo la seduta aferetica la riduzione del Col-LDL era del 63±12,7%; parallelamente PTX3 ha presentato una riduzione del 22.6±24%, (PTX3 basale 1.62±0.7 ng/ml, PTX3 dopo aferesi 1.23±0.5 ng/ml, $p<0.05$) e PCR ha presentato una riduzione del 56.4±10.9% (PCR basale 1.12±0.74 mg/L, PCR dopo aferesi 0.45±0.28 mg/L, $p<0.003$). I valori basali di PTX3 e le variazioni di questo parametro dopo aferesi non mostravano significative correlazioni con i corrispondenti valori di PCR.
Conclusioni. Una singola seduta di LDL-aferesi determina una significativa riduzione dei livelli plasmatici di PTX3. Poiché PTX3 sembra giocare un ruolo cruciale nell'amplificazione del danno vascolare, si può ipotizzare che la riduzione dei livelli di questa molecola possa contribuire ai noti benefici cardiovascolari della terapia con LDL-aferesi nei soggetti con ipercolesterolemia familiare. Studi specifici al riguardo sono necessari per confermare questa ipotesi.

THE NATURAL COMPOUND BERBERINE AFFECTS MACROPHAGE ABCA1 EXPRESSION AND FUNCTION TROUGH A TRANSCRIPTIONAL EFFECT

F. Zimetti, M.P. Adorni, N. Ronda, F. Bernini, E. Favari
Dept. of Pharmacological and Biological Sciences, and Applied Chemistry, University of Parma, Italy
E-mail: francesca.zimetti@unipr.it

The ATP binding cassette transporter A1 (ABCA1) plays a major role in macrophage cholesterol efflux, the first step of the atheroprotective mechanism known as reverse cholesterol transport (RCT). Berberine (BBR) is an alkaloid used in traditional Chinese medicine that recently has been object of investigation for its cardiovascular protective effects. BBR reduces the serum level of cholesterol by increasing LDL receptor mRNA in the liver cells trough a post-trancriptional mechanism that stabilizes the LDLr mRNA. BBR in vitro showed other antiatherogenic properties including the ability to reduce the expression of matrix metalloproteinase 9 (MMP-9), the LPS-induced expression of proinflammatory cytokines and the secretion of tumor necrosis factor-alpha (TNF-alpha) in macrophages. However, BBR was also shown to promote foam cell formation. Whether BBR treatment reduces the risk of atherosclerosis requires further investigation. The objective of this study was to evaluate the effect of BBR on ABCA1 expression and function in mouse peritoneal macrophages (MPM).
Methods. For efflux studies MPM were labeled with 3H-free cholesterol (FC) for 24 h and successively incubated overnight with 50 µg/ml of acetylated LDL (acLDL) or LXR-RXR agonists (22-OH cholesterol 5 µg/ml 9 cis-retinoic acid 10 µM), in the absence or presence of BBR 1 µM. Efflux was promoted to Apo A-I 10 µg/ml for 4 h and it was calculated as a percentage of radioactivity released in the medium over the total radioactivity incorporated by cells. Changes in ABCA1 mRNA in response to BBR treatment were assessed by quantitative real-time reverse transcription-PCR; total ABCA1 protein expression was evaluated by western blot.
Results. In acLDL-loaded and in LXR-RXR treated MPM, BBR 1µM reduced ABCA1-cholesterol efflux to apoA-I by 51%±9.2 (n=3) and 25%±6.9 (n=3) respectively. Incubation with acLDL and LXR-RXR agonists induced ABCA1 mRNA levels by 4 and 3 folds respectively; in both conditions, BBR decreased ABCA1 mRNA levels by about 50%; consistent with the effect on mRNA levels, in acLDL-enriched or LXR-RXR treated MPM, incubation with BBR was able to reduce ABCA1 protein expression. BBR did not affect either cholesterol uptake (88.31±7.64 µg cholesterol/mg protein versus 91.41±3.25 µg cholesterol/mg protein without or with BBR respectively) and percentage of esterification (47.89%±3.41 versus 45.9%±2.53 without or with BBR respectively).
Conclusions. BBR reduced ABCA1-efflux in acLDL-loaded and LXR-RXR treated MPM; the effect observed on ABCA1 function was a result of an impact of BBR at either mRNA and protein expression levels. No interference of BBR was observed in cholesterol uptake or esterification.
Our results suggested that BBR reduces ABCA1-mediated efflux through a transcriptional effect and that, despite its cholesterol-lowering effect, it may have a potential pro-atherosclerotic activity. The molecular mechanism by which BBR decreases ABCA1 is now object of our studies and it could shed more light into the ABCA1 modulation pathways in macrophages.

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