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ABSTRACT

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EFFECT OF A NOVEL NUTRACEUTICAL COMBINATION ON SERUM LIPOPROTEIN FUNCTIONAL PROFILE AND CIRCULATING PCSK9

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Background. A beneficial effect on CV risk may be obtained by improving lipid-related serum lipoprotein functions such as high-density lipoproteins (HDL) cholesterol efflux capacity (CEC) (HDL-CEC) and serum cholesterol loading capacity (CLC) and by reducing Proprotein Convertase Subtilisin Kexin type 9 (PCSK9), independently of lipoprotein concentrations.
Aim. We aimed to evaluate the effect of an innovative nutraceutical (NUT) combination containing: Red yeast rice (Monacolin K 3.3 mg), Berberine 531.25 mg and leaf extract of Morus alba 200 mg (LopiGLIK® - Akademy Pharma), on HDL-CEC, serum CLC and on circulating PCSK9 levels.
Material and Methods. N=23 dyslipidemic subjects have been treated for 4 weeks with the above NUT combination. HDL-CEC was measured using specific cell-based radio isotopic assays, serum CLC and PCSK9 concentrations were measured fluorimetrically and by ELISA assay, respectively.
Results. The NUT combination significantly reduced plasma level of total cholesterol and LDL-C (-9.8% and -12.6%, respectively). Despite no changes in HDL-C, NUT combination improved total HDL-CEC in 83% of the patients, by an average of 16%, as a consequence of the increase mainly of the ABCA1-mediated CEC (+28.5%). The NUT combination significantly reduced serum CLC (-11.4%) while did not change PCSK9 plasma levels (312.9±69.4 ng/ml vs 334.8±103.5 mg/l, before and after treatment, respectively).
Conclusion. The present NUT combination improves the serum lipoprotein functional profile providing a complementary beneficial effects, without any detrimental increase of PCSK9 plasma levels.

SEVERE HYPERTRIGLICERIDEMIA IN PREGNANCY WITH HYPERCHOLESTEROLEMIA: A CASE REPORT

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Introduction. Serum triglyceride (TG) and total cholesterol (TC) become altered during pregnancy. In rare instances, sometimes associated with genetic forms of hyperlipidemia, women can develop pregnancy related hypertrigliceridemia (HTG), whose complications, namely acute pancreatitis and hyperviscosity syndrome, may be life-threatening. In particular, a very rare subgroup develops severe HTG, defined as plasma TG greater than 1000 mg/dl, showing an increased risk.
Case report. A 37-yr-old primiparous woman was admitted to Hospital to undergo caesarean section because of breech presentation at term. During the hospital stay the patient was diagnosed to have severe HTG (9950 mg/dl) with very high values of TC (1257 mg/dl). The patient also suffered from acute pancreatitis. After delivery the patient was treated by means of therapeutic plasma exchange, with an almost complete resolution of hyperlipidemia (TG 617 mg/dl; TC 275 mg/dl). After discharge the woman was followed up in our Center. A treatment with rosuvastatin and omega-3-ethyl esters was performed, determining a further reduction of plasma levels of TC (96 mg/dl) and TG (95 mg/dl). Presently the patient is healthy and still continuing therapy.
Discussion. We report this case of severe HTG in pregnancy, pointing out the dramatically high levels of serum TG and TC in a patient without obvious signs of hyperlipidemia syndrome. A careful surveillance of lipid levels in pregnancy is also proposed in women not affected by hyperlipidemia.

CHOLESTEROL SYNTHESIS AND GLUTATHIONE METABOLISM GENE PATHWAYS ARE CO-REGULATED IN CONCERT WITH GENES OF UNKNOWN FUNCTIONS. CLUSTER ANALYSIS OF PATTERNS GENERATED FROM A WEB REPOSITORY OF HEPG2 TRANSCRIPTOMES

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Background. Living cells react to external stressors by activating coordinate expression of several genes. Synergism is guaranteed by the coordinating role of transcription factors (TF). The analysis of the whole transcriptome expression by microarray analysis is a valuable tool to discover such patterns of genes co-regulation.
Objective and Methods. This paper analyzed the gene responses of HepG2 cells subjected to various stressors in different microarray transcriptome analyses in order to build patterns of co-regulation and to establish expression correlations between 20382 transcripts and 1896 TF extracted from the same experiments. Microarray transcriptome analyses were retrieved from a web repository and a powerful pipeline was developed to build regulation profiles of transcripts, to cluster them in homogeneous groups and to identify group of clusters sharing similar biological functions.
Results. This approach identified at least four clusters of genes related to: cell cycle and division (area 1), structural constituents of ribosome (area 2), cell division and mitosis (area 3), cholesterol synthesis (area 4). The area 4 contained also transcripts of genes related to glutathione metabolism, energy generation, and four genes of unknown function: TMEM97, TMEM14A, C14orf1 and C4orf27. The correlations of transcripts of cholesterol synthesis with the set of TF showed that some of them emerge as putative regulators of cholesterol metabolism, with CEBPBP, DDIT3, CDK2 and FOXM1 as most relevant candidates.
Conclusions. A novel powerful approach for the analysis or co-expressed genes under the control of a series of TF is presented. This approach revealed suggestive links between cholesterol synthesis, transcripts with other functions and TF apparently unrelated at first sight.

LIPOPROTEIN APHERESIS DISCONTINUATION IN HYPERCHOLESTEROLEMIC PATIENTS SWITCHED TO PCSK9 INHIBITORS TREATMENT

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Background and Aim. Lipoprotein Apheresis (LA) is effective in acute lowering atherogenic lipoproteins plasma concentrations, and PCSK9 inhibitors (PCSK9-i) may dampen the lipid rebound effect of LA with the possibility to decrease and in some cases even discontinue LA. The aim of our study is to evaluate if PCSK9-i therapy may be an alternative treatment to LA.
Methods. 8 patients (6M, 2F) with personal history of cardiovascular disease (CVD), average age 63,8±9,2 y, on regular LA scheduled every two weeks (Q2W) were switched to PCSK9-i therapy (6 evolocumab 140 mg Q2W; 1 Alirocumab 75 mg Q2W, 1 Alirocumab 150 mg Q2W). In 6 HeFH patients, carrying a mutation on LDLR gene, PCSK9-i was started as add-on treatment to the maximum-tolerated-dose of statins+ezetimibe combination therapy, while in 2 statin-intolerant hypercholesterolemic patients, PCSK9-i have been applied as monotherapy. Patients were examined with blood sampling before and after last LA, and at baseline, 1-3-6 and 12 months from PCSK9i administration to evaluate total-cholesterol, LDL-C, HDL-C, triglycerides.
Results. During last LA, LDL-C pre-apheresis and post-apheresis was (mean±SD) 137.4±32.3 mg/dl and 45.9±12.3 mg/dl, respectively with interapheretic values of 112,7±25,4 mg/dl, therefore far from the LDL-C goal. After 1-3-6 and 12 months of treatment with PCSK9-i, LDL-C levels were reduced to 64±21 mg/dl (-53.6±15.3%), 42±21 mg/dl (-69.5±10.1%), 39±21 mg/dl (-71.0±16.5%) and 36±9 mg/dl (-73±15.3%) respectively.
Conclusion. After switching from LA to PCSK9-I therapy all patients steadily achieved the recommended individual LDL-C levels (<70 mg/dl). Our data confirm that Alirocumab and Evolocumab treatments are effective in reducing and mantaining target-LDL-C values in patients at very high cardiovascular risk. Further investigation in larger datasets are needed.

A TREATMENT WITH ANTI-PROPROTEIN CONVERTASE SUBTILISIN-KEXIN TYPE 9 MONOCLONAL ANTIBODIES IS EFFECTIVE IN IMPROVING ARTERIAL STIFFNESS BESIDES EXPECTED LIPID PROFILE EFFECTS

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Background. Monoclonal antibodies that inhibit proprotein convertase subtilisin-kexin type 9 (PCSK9) are a new class of drugs able to lower LDL cholesterol levels. This class of drugs is indicated in the treatment of patients affected by familial hypercholesterolemia (FH) and patients with previous acute coronary syndrome.
Purpose. We investigate whether after six months of treatment with anti-PCSK9 monoclonal antibodies (alirocumab or evolocumab) any improvement of pro-atherogenic profile and arterial stiffness (AS) may be observed in patients with high cardiovascular risk already in treatment with the maximally tolerated statin therapy.
Methods. We enrolled 23 people not on LDL-C target. At enrollment and 6 months later we evaluated anthropometrics, laboratory profile, pulse wave velocity (PWV) and carotid intima-media thickness (cIMT).
Results. After 6 months of treatment we found a significant decrease of inflammatory markers (hs-CRP: -41.4%; Fibrinogen: -18.8%), LDL-C and lipoprotein(a) levels (respectively -64.2% and -36.2%). PWV (-9.8%) appeared to be improved; cIMT remained unchanged. PWV reduction appeared to be correlated with inflammatory markers and LDL-C reduction. However, ΔPWV appeared not to be dependent on ΔLDL-C or Δfibrinogen by the multiple regression analysis.
Conclusion. After 6 months of treatment the levels of CRP, Fibrinogen, LDL-C, and Lp(a), as well AS indices, are significantly improved as compared to baseline. A treatment with anti-PCSK9 monoclonal antibodies may improve significantly the arterial stiffness in patients with high cardiovascular risk and this result seems to be independent by the improving of lipid profile.

UNRAVELLING THE ROLE OF BONE MARROW ADIPOSE TISSUE IN AGING AND TYPE 2 DIABETES MELLITUS BY LIPIDOMICS: STUDY ON SAMPLES DERIVED FROM HUMAN HIPS

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By 2030, 20% of the western population will be >65-years old and develop cardiovascular diseases, mostly caused by type2 diabetes mellitus (T2DM) and its consequences. Recently, Bone Marrow Adipose Tissue (BMAT) has gained attention because its mass increases with age (from 10-15 to 70%), and because the secretion of its cytokines (IL-6; IL-8) and adipokines (leptin, adiponectin) may exert systemic effects, thus activating inflammation and adipogenesis. Based on these evidence, we want to understand the role of BMAT in aging and T2DM, by performing lipidomics on BMAT derived from 27 patients (9 with T2DM), aged 70±12, undergoing hip replacement.We first performed a qualitative analysis of their total fatty acid (FA) profile, without finding significant differences in single FA and between saturated- (SFA) and unsaturated FA (UFA) of T2DM vs. healthy controls. We found a non-significant but slight increase in triglycerides (TG) (1.5 fold) and cholesteryl esters (CE) mass (2.9 fold), together with a significant increase in C16:1 mass (in CE) and a decrease in SFA/UFA ratio in T2DM BMAT vs controls. Interestingly, the CE increase was balanced by a decrease in FC mass (4.7 fold increase of CE/FC) in T2DM patients.Altogether, since these results suggest an association between T2DM and lipid variations in BMAT, we plan to evaluate the activity of enzymes involved in lipid metabolism: while the analysis of ACAT activity and lipases urges from data documenting the unbalanced FC/CE ratio, that of SCD-1 relates to the increase in UFA

that causes bone fragility and osteoporosis in T2DM patients. To better estimate the effect of BMAT on T2DM we will correct these data for body mass index, age, sex, drug treatment and duration of T2DM, correlating them with plasma cholesterol, triglycerides, and glucose, to understand the mechanism(s) of the pathology and to identificy novel pharmacological targets for T2DM.

PCSK9 DEFICIENCY RESULTS IN INCREASED ECTOPIC FAT ACCUMULATION AND ALTERED GLUCOSE CONTROL IN EXPERIMENTAL MODELS AND IN HUMANS

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Aims. Proprotein Convertase Subtilisin Kexin Type 9 (PCSK9) targets not only the LDL receptor but also triglycerides (TGs) rich lipoproteins and fatty acids receptors (VLDLR, ApoE2R and CD36) significantly expressed in key metabolic tissues (like adipose tissue and pancreas). This raises the question whether PCSK9 reduction, via altering these interactions, might affect ectopic fat accumulation and glucose control in vivo.
Methods. PLIC Study participants were genotyped for PCSK9 R46L (Loss-of-Function, LoF) variant. fasting and postprandial (PP) lipid profiles were evaluated; insulin was measured and indexes of resistance (HOMA-IR) and secretion (HOMA-BC) were derived. Abdominal fat mass, Epicardial Fat Thickness (EFT) were quantified and presence of hepatic steatosis was evaluated.
Results. PCSK9 LoF was significantly associated with -11%, -16%, -19% and -35% in plasma LDL-C, ApoB, ApoCIII, but not different fasting and PP TGs; of note PBMCs from LoF showed increased pro-inflammatory and CD36 genes pattern mRNA expression. LoF carriers were more obese and percentage of total and abdominal fat masses were also increased vs non-carriers. Moreover, PCSK9 genetic reduction was associated with two-fold increased prevalence of hepatic steatosis and +2.7 mm EFT (p=0.022). LoF carriers showed higher glucose (vs BMI and abdominal fat-matched non-carriers), and this was reflected only by reduced HOMA-BC (8.4 (6.9-10.4)% vs 10.05 (8.1-11.6)% in non-carriers). These data are in line with findings from PCSK9 KO mice fed a high-fat diet, accumulating more visceral adipose tissue compared to PCSK9 WT (despite similar total weight and food intake). Moreover, while response to insulin was not affected, impaired glucose tolerance (+40% vs PCSK9 KO) was reflected by reduced insulin secretion by pancreatic islets.
Conclusions. Despite no clear effect of PCSK9 reduction on TGs, we question whether increased ectopic adiposity and altered glucose control is peculiar to genetic PCSK9 deficiency rather than circulating protein, target of monoclonal antibodies.

OXIDATIVE STRESS-MEDIATED ERYTHROCYTE MEMBRANE FLUIDITY ALTERATIONS IN SUDDEN SENSORINEURAL HEARING LOSS PATIENTS

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Sudden sensorineural hearing loss (SSNHL) involves an acute unexplained hearing loss, nearly always unilateral, that occurs over less than a 72-hour period. SSNHL pathogenesis is not yet fully understood. Cochlear vascular occlusion has been proposed as a potential mechanism of hearing damage and cochlear ischemia has been related to alterations of cochlear microvessels. In addition, some researchers have focused their attention on the rheological alterations and blood hyperviscosity. Erythrocyte deformability plays a key role in determining blood viscosity, and it is critical to cochlear perfusion. It has been shown that oxidative stress-induced erythrocyte membrane fluidity alterations are linked to the progression of cardiovascular diseases. To determine whether erythrocytes from SSNHL patients show signs of oxidative stress, and whether this condition can modify the hemorheologic profile in these patients, we analyzed hemorheologic profile and erythrocyte oxidative stress in 35 SSNHL patients and 35 healthy subjects, matched for age and sex. Fluorescence anisotropy was used to evaluate the fluidity of erythrocyte membranes. Our results show a significant structural and functional involvement of erythrocyte membrane alterations in SSNHL, as well as elevated levels of membrane lipid peroxidation and intracellular ROS production. In addition, erythrocyte-derived ROS and erythrocyte lipid peroxidation positively correlated with whole blood viscosity and erythrocyte deformability. Moreover, in vitro experiments demonstrated that ROS display a key role in erythrocyte membrane fluidity. These findings indicate that erythrocyte oxidative stress plays a key role in the pathogenesis of SSNHL and pave the way to new therapeutic interventions.

ECOMORPHOMETRIC EXPANSION AND EXPRESSION OF EPICARDIAL-HEPATIC-VISCERAL AND CAROTIDAL FAT IN DYSMETABOLIC PATIENTS. ADDITIONAL ROLE OF ARTERIAL HYPERTENSION AND EPICARDIAL FAT ON CARDIAC REMODELING

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Objectives. This study aims at quantitatively analyzing the expansion of corporeal visceral fat related to weight increase and sex means of standard ultrasound measurement methods. Conse-

quences of corporal visceral fat depot in hypertensive subjects has been evaluated and a quantitative score to compare patients in different groups of BMI and gender has been proposed.
Subjects and Methods. 177 patients (84 males and 93 females), aged between 27 and 66 (with average age of 49.9 and standard deviation of 0.87), were selected among 500 patients applying to the University Clinic "Murri" in a period of 6 months. They were divided in groups according to the Body Mass Index (BMI) classes, resulting in 63 Lean (L), 66 Owerweight (OW) and 48 obese (OB). Further classification was made according to hypertensive status, resulting in 111 patients (63%) previously diagnosed with hypertension and still in pharmacological treatment. Upon previously stated consensus, each patient underwent a regular medical visit in order to measure parameters such as BMI and blood pressure. Standardized ultrasound analysis was performed in order to measure epicardial fat thickness (EFAT), abdominal visceral fat thickness (VFAT), carotid wall intima-media thickness (IMT), liver steatosis grade according to semi-quantitative scale (NAFLD), indexed left ventricular mass (MSVI). Datasets were analyzed using descriptive, inferential and multivariate statistical techniques.
Results. Comparison between averaged parameters for the combinations of the three BMI categories (L, OW, OB) revealed statistically significant (ANOVA, P<0.0001) and consistent increases of BMI, VFAT and NAFLD: about 20% (OW vs Lean) and +40% (OB vs Lean) for men and about 30% (OW vs Lean) and +45% (OB versus Lean) for women. EFAT increased by 22% among OW and by 38% among OB patients (for both men and women, P<0.0001). Among women, no statistically significant difference between OW e OB patients, but a tendency to plateau, was found.IMT increased both in women OW (+12,5%) and OB (+6,2%, P=0.006) with respect to lean, while among men IMT increased only in the comparison between OB vs L (+24%) (P<0.05). MVSI expanded by 13% among OW and by 24% among OB patients (for both men and women, P<0.01). For women with hypertension, BMI growth linearly increases EFAT and EVFAT in women. For men with hypertension the same linear trend was visible only for EFAT, while VFAT does not significantly increases with BMI growth. For both sexes, the hypertensive population showed a significant IMT thickening with respect hypertensive patients, while NFLD was not significantly dependent on presence of hypertension. For both sexes, EFAT enlargement was associated with MVSI remodeling, both in hypertensive and non-hypertensive patients. Using MANOVA, EFAT was influenced by four independent variables: IMT, NAFLD, PAS and VFAT (MANOVA, P<0.0001). A new score was derived from summing up the four corrected visceral fat variables: U4SFS = (EFAT*10)+(IMT*100)+(NAFLD*100)+VFAT For such a score, cutoff values (average ±2SD) were derived for healthy (non-hypertensive, lean) patients of both sexes.Score values increase with BMI (+30-35% OW; +60% OB), with statistical significance (ANOVA, P<0.0001), when comparing all subgroups for both sexes.
Conclusions. In every different BMI class and in both sexes, there are statistically significant increments for every type of body fat, excluding EFAT in women, when comparing Lean vs OW and OB, and OW vs OB. In hypertensive patients, EFAT linearly increases with BMI, whereas both IMT and NAFLD worsen with BMI and VFAT expands similarly to other types of fat in women. The derivation of a score and relative cutoff values in both sexes allows direct comparison of Lean with OW and OB. Further studies will validate the application of the score to wider patient populations.

NOVEL COMPOUNDS TARGETING PFKFB3, A KEY GLYCOLYTIC ENZYME, AS A WAY TO INHIBIT ANGIOGENESIS

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Angiogenesis is an important contributor to atherosclerotic plaque growth and instability. Clinical evidence has linked intraplaque angiogenesis with progressive and unstable vascular disease. Proliferating endothelial cells (ECs) can switch their metabolism to being highly glycolytic enabling their growth and division in the angiogenic process. Recent studies have demonstrated the therapeutic potential of 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO), a commercially available inhibitor of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB3) one of the regulatory enzyme in glycolysis, in angiogenesis models. For this reason, PFKFB3 inhibitors seem promising compounds to be used in promoting plaque stability. We studied the in vitro effects of 3PO and of two self-synthesized inhibitors named phenoxindazole analogues (PA-1 and PA-2; based on Boyd et al., 2015) on glycolysis, cell proliferation, migration, matrix metalloproteinase (MMP) activity and gene expression in ECs. We observed that these compounds were able to significantly reduce glycolysis levels in the human endothelial cell line EA.hy926. In addition, all three compounds markedly reduced EC migration, proliferation and wound closing capacity which are essential for neovessel formation. Moreover, we demonstrated by gelation gel zymography that these inhibitors reduce the activity of proMMP-9 and MMP-2 up to 40-50% and 20-30% compared to control, respectively. Furthermore, real-time PCR results indicate that the PA compounds downregulate PFKFB3 gene expression whilst 3PO does not. As for markers of migration and angiogenesis, such as ICAM and VEGFR2, these were markedly reduced. Finally, gelatinase gene expression was downregulated by up to 80%. These findings show that PFKFB3 inhibition with PA compounds markedly reduce endothelial cell migration, proliferation and gelatinolytic activity concomitant with a significant decrease in gelatinase gene expression, EC migration and angiogenesis markers. Thus, these compounds have the potential to be tested in an animal model of angiogenesis. This project has been funded by the European Union's Horizon 2020 Marie Skłodowska-Curie grant (#67552).

EVALUATION OF CARDIOVASCULAR RISK IN THE ELDERLY AND VERY ELDERLY PATIENT, AND DETERMINANTS OF STATIN USE

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Introduction. Increasing age of the population will represent a demanding challenge in the near future, necessitating particular attention in the definition of the risk of cardiovascular (CV) diseases, the main cause of death and disability in older subjects, and

in the proper management of CV risk factors such as hypercholesterolemia. Indeed, few data are available in this regard and the indication for lipid lowering treatment is controversial, especially in the very elderly (1, 2).
Aim of this study was to analyze the determinants of cardiovascular (CV) risk and of statin use in a population of elderly outpatients, with particular focus on classical risk factors.
Methods. The study included a cohort of patients attending the Geriatric Cardiology Clinic of our Division, a significant proportion of whom was represented by very elderly (over age 80) subjects. Patients underwent anamnestic and clinical evaluation allowing for the determination of CV risk estimate according to the SCORE project algorithm (3). Information on the present use of statins was collected. The influence of the different determinants of CV risk estimate was investigated, in elderly and very elderly patients. The determinants of statin use were likewise analyzed. Standard statistical methodology, including multiple and logistic regression analyses, was performed.Results. Data from 1382 patients were collected (age 77.0±6.9 yr, 598 males, 784 females). As expected, older subjects had a significantly higher SCORE estimate (18.2±10.3 vs 10.2±6.7, p<0.001, unpaired t test) and age was significantly correlated with CV risk (r=0.560, p<0.001). Upon multiple regression analysis, age, serum cholesterol and systolic blood pressure (SBP) were significantly correlated with the SCORE value. In the very elderly subgroup the correlation with cholesterol lost statistical significance (OR 0.002-0.083, p=0.061); in this subgroup only, the comorbility index CIRS showed a significant association with the SCORE estimate (OR 0.072-0.838, p=0.021). Logistic analysis performed in the whole cohort, with SCORE ≥5 as the dependent variable, showed analogous results. Smoke was not significantly associated with a high CV risk estimate. The prevalence of statin use tended to be lower in very elderly patients (18.6% vs 22.7%, p=0.085, chi-square). At logistic analysis statin use was significantly associated with a previous history of ischemic heart disease (p<0.001) and stroke (p=0.017), but not with diabetes.
Conclusions. The result presented seem to be in line with previous evidence in younger subjects even if the original SCORE equation was not designed for older populations. In the present cohort smoke does not appear to be a relevant determinant of CV risk estimate whereas, in the very elderly, comorbility seems to associate with higher CV risk, consistently with previous reports in the literature on frail patients (4). Statin use is mainly associated with the previous CV history, but not with diabetes, whereas increasing age seems to associate with a lesser prescription rate, reflecting cautiousness towards the potential side effects of treatment, as observed in hospitalized patients (2).

References
1. Strandberg TE, et al. Evaluation and treatment of older patients with hypercholesterolemia: a clinical review. JAMA. 2014; 312: 1136-44.
2. Bertolotti M, et al. Prevalence and determinants of the use of lipid-lowering agents in a population of older hospitalized patients: the findings from the REPOSI (REgistro POliterapie Società Italiana di Medicina Interna) Study. Drugs Aging. 2017; 34: 311-9.
3. Conroy RM, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J. 2003; 24: 987-1003.
4. Verma G, et al. Trial of time: review of frailty and cardiovascular disease. Cardiol Rev. 2017; 25: 236-40.

EFFECTS OF A NUTRACETICAL COMBINATION CONTAINING MONACOLIN K AND BERBERINE ON LIPIDS, PCSK9 AND SUBCLINICAL INFLAMMATION IN HIV INFECTED PATIENTS ON HIGHLY-ACTIVE ANTIRETROVIRAL THERAPY

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Introduction. Hypercholesterolemia is a prevalent cardiovascular (CV) risk factor in human immunodeficiency virus (HIV) infected population. A chronic low-grade inflammation contribute to the pathogenesis of CV disease in HIV infection. In HIV infected patients receiving highly-active antiretroviral therapy (HAART), statin treatment is hampered by potential drug-to-drug interactions and risk of treatment intolerance. We investigated the effects of a nutraceutical combination (NC) on lipids, proprotein convertase subtilisin/kexin type 9 (PCSK9) and inflammation in hypercholesterolemic HIV infected patients receiving stable HAART.
Methods. This is a crossover interventional study in 24 patients with HIV infection on stable HAART with LDL-cholesterol (LDL-C) >100 mg/dL, not receiving any lipid-lowering treatment. After a 3-week standardized diet regimen, the effect of either a 3-month oral NC (red yeast rice-derived monacolin K 3 mg, berberine 500 mg, policosanol 10 mg, astaxanthin 0.5 mg, folic acid 0.2 mg and coenzyme Q10 2 mg) vs no active treatment (noNC) was tested on plasma total cholesterol (TC), LDL-C, HDL-C, triglycerides, lipoprotein(a), PCSK9 and high-sensitivity C-reactive protein (hsCRP) levels.
Results. At baseline a significant correlation between plasma PCSK9 levels, age (rho=0.55, p=0.005), waist circumference (rho=0.41, p=0.047) and LDL-C (rho=0.37, p=0.046) was observed. Treatment effect (NC vs noNC) was significant for the following variables: TC [-28.9 mg/dL, p<0.001), LDL-C (-27.7 mg/dL, p<0.001), plasma PCSK9 (-44.6 ng/mL, p=0.046) and hsCRP levels (-0.4 mg/L, p=0.04), while no significant effects were observed in HDL-C, tryglicerides and lipoprotein(a) levels compared to those who received noNC.
Conclusion. An oral NC containing low-dose monacolin K and berberine significantly reduced cholesterol and PCSK9 levels and attenuated subclinical inflammation in HIV infected patients on stable HAART.

ELEVATED SERUM URIC ACID LEVELS IMPAIR ENDOTHELIAL FUNCTION IN HIV PATIENTS RECEIVING HIGHLY-ACTIVE ANTIRETROVIRAL THERAPY

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Introduction. Elevated serum uric acid (SUA) levels may be associated with endothelial dysfunction. Increased rates of metabolic

syndrome (MS) and elevated SUA levels were described in human immunodeficiency virus (HIV) infected patients. We investigated whether SUA levels are associated with endothelial dysfunction in HIV positive patients receiving highly-active antiretroviral therapy (HAART) irrespective of MS.
Methods. In this cross-sectional study of 250 HIV positive patients receiving stable HAART we evaluated the relationship between MS, SUA levels and endothelial function. SUA levels and brachial artery flow-mediated dilation (bFMD) were measured. The relationship between logarithmic (LG)-transformed SUA levels and bFMD was evaluated after correction for MS.
Results. MS was detected in 28.4% of patients and elevated SUA levels (≥6 mg/dL) in 25.2%. MS was associated with higher LG-SUA levels (age-, gender- and glomerular filtration rate-adjusted beta =0.204, p=0.001). The crude linear association between LG-SUA levels and LG-bFMD (beta =-0.166, p=0.008) was abolished after correction for MS (beta =-0.089, p=0.172). When SUA levels were used as a categorical variable (≥6 mg/dL or <6 mg/dL and SUA quartiles, respectively), the association between LG-SUA levels and LG-bFMD remained significant after adjustment for MS (beta =-0.142, p=0.022 and beta =-0.163, p=0.010, respectively).
Conclusion. MS affects significantly SUA levels in HAART-treated HIV infected patients. The negative association between SUA and bFMD is independent of MS only for elevated SUA levels.

ACID LYSOSOMAL LIPASE DEFICIENCY TEST: TUSCANY REFERENCE CENTER FOR HEREDITARY DYSLIPIDEMIA EXPERIENCE

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Introduction. Acid lipase is necessary in lipoprotein hydrolysis in endosomes and lysosomes. The autosomal recessive transmission of partial or total deficiency of enzymatic activity turns into the so-called "Cholesteryl ester storage disease (CESD)", or into its most severe clinical phenotype, the Wolman disease. The most involved organs are those where LDL endocytosis is particularly active, which explains the diffuse hepatic symptoms of illness. Lipid anomalies in CESD mainly concern the cholesteryl ester storage whereas triglycerides storage is less pronounced than in Wolman syndrome. Wolman disease symptoms are evident at birth and infants generally do not survive past 1 year. In the later-onset form of acid lysosomal lipase deficiency (CESD), signs and symptoms vary and usually begin in mid-childhood, although they can appear anytime up to late adulthood. The expected lifespan of individuals with CESD depends on the severity of the associated health problems. Furthermore, it is plausible that in some cases CESD symptoms might be masked by concomitant pathologies. Unfortunately, at the moment, the poor knowledge about CESD syndrome and the limited application of its diagnostic test concur to the underestimation of this pathology.
Results. Since November 2015, according to Hamilton protocol (Clin Chim Acta. 2012; vol 413, Issues 15-16, 1207-1210) our lab started the evaluation of the acid lysosomal lipase activity in patients with suspected CESD. Data obtained were compared with those from Roma laboratories (Ospedale pediatrico Bambin Gesù), were the test is carried out routinely. Concordance was of about 80%. However, the lack of a common normal range required the use of a calibrator to which the labs involved (Pisa, Roma, Milano, Bologna, Padova, Torino, Palermo) in the acid lysosomal lipase activity evaluation should refer. Therefore, since June 2017, each lab, in turn, provides the others of a calibrator to test their performances. Results obtained so far show a 100% consistency in patients classification on acid lysosomal lipase activity.
Conclusions. In the next future, the more standardized process of analysis and the increased test availability may be determinant for earlier diagnosis of CESD and improvement of therapy.

PLASMA PHOSPHOLIPID FATTY ACID COMPOSITION, LIPID OXIDATION AND INFLAMMATORY PARAMETERS IN A POPULATION OF FISHERMEN

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Background and Aim. Dietary fish intake affects plasma phospholipid fatty acid (FA) composition. Few data are available on the role of seafood-rich diet at lower latitudes (i.e. Adriatic sea). We evaluated the potential relationships between seafood consumption and plasma phospholipid FA composition, lipid profile, lipid oxidation and inflammatory parameters in a group of fishermen living in Chioggia, in the Northern Adriatic.
Subjects and Methods. Anthropometric and biochemical parameters, plasma lipid concentrations, phospholipid FA composition (relative %), apolipoprotein B and oxidized LDL (ox-LDL) were determined in fasting plasma samples from 208 subjects (age 46.6±11.0 yrs., mean±SD). A food questionnaire was recorded.
Results. Subjects were divided into three groups according to habitual fish consumption (0-1, 2-3 seafood-based meals per week, ≥1 seafood-based meal per day). Plasma phospholipid percentage composition of n-6 polyunsaturated fatty acids (PUFAs) decreased from the lowest to the highest fish-consumption group (37.19±2.50% vs 36.05±2.62% vs 35.59±2.63%, p=0.002 ANOVA) while n-3 PUFAs increased (4.52±1.13% vs 5.24±1.52% vs 5.32±1.74%, p=0.001 ANOVA), mainly eicosapentaenoic (EPA) and docosahexaenoic (DHA) FA. Lipid profile and inflammatory parameters were similar in the three groups. Considering the whole group, total n-3 PUFAs (EPA and DHA) were significantly associated with LDL cholesterol (r=0.312, p=0.001), and DHA also with ox-LDL (r=0.206, p=0.024); there was no association between n-3 PUFAs and plasma triglycerides. N-3 PUFAs (EPA and DHA) were inversely associated with leukocytes count (r=-0.245, p=0.000), while the total n-3 PUFAs shod a direct correlation with increased interleukin-6 plasma levels (r=0.168, p=0.016).
Conclusions. Fish consumption modulates plasma phospholipid percentage composition of n-3 and n-6 PUFAs; habitual fish intake is not associated with an anti-atherogenic lipid profile while it may modulate a modest anti-inflammatory effect.

APOLIPOPROTEIN E MODULATES ADAPTIVE IMMUNE RESPONSE BY PROMOTING CELLULAR CHOLESTEROL METABOLSIM IN DENDRITIC CELLS

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Background. The role of adaptive immune response in atherosclerosis has recently gained attention owing the correlation found between T effector memory cell (TEM) expansion, T naïve cells (TN) contraction and the progression of the disease. Whether this correlation is merely the consequence of increased plasma cholesterol levels or the consequence of altered intracellular lipid metabolism is debated.
Aim. To investigate whether key players linking systemic and cellular lipid metabolism such as apolipoprotein E (ApoE) affect adaptive immune response in mouse models and humans.
Methods. Phenotypical, in vitro and in vivo functional characterization of immune cells was performed through flow cytometry in ApoE KO and WT littermates. Immunophenotyping of circulating immune cells and in vitro culture of monocyte-derived dendritic cells (MDCs) was carried out in human carriers of different ApoE isoforms.
Results. ApoE deficiency resulted in increased TEM and decreased TN levels respectively in the circulation and secondary lymphoid organs ($p<0,05$), an increased proliferation of CD4Tcells ($p<0,01$) and a faster rejection following skin graft allotransplantation (SGA, $p<0,01$) was observed. This phenotype was the consequence of myeloid-derived ApoE as WT mice transplanted with ApoEKO bone marrow presented a reduced graft survival after SGA compared to ApoEKO transplanted with WT BM ($p<0.05$), independent of plasma cholesterol levels. ApoE deficiency was also associated with an enhanced ability of dendritic cells (DCs) to trigger allogenic Tcell proliferation compared to WT DCs ($p<0,01$) but no difference was observed in Tcells proliferation induced by allogenic DCs (p=n.s.). DCs were significantly increased in the spleen of ApoE KO mice and associated with a more antigen-presenting phenotype ($p<0,01$), which was associated with an accumulation of cholesterol and oxysterols. In humans, carriers of ApoE4 isoform ($\varepsilon4/3,\varepsilon4/4$) showed increased TEM and decreased TN levels compared to ApoE2 ($\varepsilon2/2,2/3$) and ApoE3 ($\varepsilon3/3$) carriers ($p<0,01$ and $p<0,05$ respectively). This phenotype was the consequence of an enhanced ability of ApoE4 MDCs to induce Tcell polarization toward TEM compared to ApoE2 and ApoE3 carriers following mixed lymphocyte reaction. As ApoE3 and ApoE2 isoforms have similar affinity for the anti-atherogenic HDL while ApoE4 interacts preferentially to the pro-atherogenic VLDL, we tested that serum from ApoE3 but not ApoE4 carriers dampened the activated phenotype of ApoE4 MDCs and was associated with a reduction of cellular cholesterol.
Conclusion. Our data suggest that DCs-derived ApoE orchestrates the activation of DCs-Tcell axis via the control of cholesterol availability in DCs, thus connecting systemic and immune cell lipid metabolism.

REDUCTION OF VISCERAL FAT IMPROVES SERUM LIPID PROFILE IN OBESE SUBJECTS

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Background. Visceral adipose tissue (VAT) is strongly associated with metabolic abnormalities and with an increased risk of atherosclerosis and acute coronary events.In obese adults, VAT affects plasma lipoprotein composition and concentrations. Based on these observation, the study was carried out to evaluate the correlation beetwen VAT and lipidic abnormalities in obese subjects undergoing a hypocaloric-mediterranean diet.
Methods. In a 6 mo. hypocaloric mediterannean diet enrolled were 87 obese subjects (53 F:34 M, 51 yrs±1.4 SE and 56.4±1.1, respectively). At baseline and at the end of the protocol, subjects underwent the following examinations: BMI, waist circumference, bioumoral exams (i.e., insulinemia, total cholesterol, HDL-C, Triglycerides, fasting glycemia, GPT, uric acid), ultrasound examination (degree of liver steatosis, thickness of subcutaneous fat and visceral fat).
Results. At baseline males displayed a greater metabolic profile than females. While subcutaneous fat was positively correlated only with BMI, visceral fat was correlated with BMI, fasting glycemia, insulinemia, HDL, total cholesterol, triglycerides, liver steatosis. After a 6 mo. of hypocaloric mediterannean alimentary program, a significant improvement was recorded according to anthropometric, ultrasonografic and bioumoral exams. The decrease of VAT was associated with the amelioration of plasma lipid levels.
Conclusions. Visceral fat is a metabollically active tissue. Diet-induced visceral fat reduction leads to beneficial effects on lipid levels and the metabolic profile in obese subjects.

THE TWO FACES OF FENRETINIDE: GOOD FOR METABOLISM, BUT WHAT ABOUT ATHEROSCLEROSIS?

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Fenretinide is the most investigated retinoid compound for cancer prevention and treatment. Recent findings have discovered additional beneficial properties of this molecule, including the prevention of high-fat diet-induced obesity and insulin resistance. For this reason, fenretinide is undergoing clinical trials for the treatment of insulin resistance in obese subjects. Aim of this study was to evaluate if fenretinide treatment could have any beneficial effect on atherosclerosis development. To this aim, apoE-KO 9-week-old female mice were fed for 15 weeks a Western diet (control) or a Western diet added with fenretinide (0.1%) (n=20 mice/group).

Mouse body weights were monitored throughout the study. One week before the end of the study, basal glucose levels were measured and a glucose tolerance test was performed. At the end of the dietary treatment, plasma lipid profile and atherosclerosis development were evaluated. In addition, an in-depth histological analysis was performed.As expected, fenretinide administration significantly lowered glucose levels and body weight compared to controls. Additionally, plasma levels of total cholesterol, triglycerides and phospholipids were all reduced after fenretinide administration. In the liver, fenretinide remarkably lessened the storage of glycogen and lipids driven by the Western diet. Unexpectedly, this finding was accompanied by increased prevalence of extramedullary hematopoiesis and erythrophagocytosis in treated animals. Fenretinide administration was also associated to

1) a higher number of foamy macrophages in lungs and lymph nodes,
2) splenomegaly, characterized by a severe follicular atrophy and an increased extramedullary hematopoiesis.

Finally, aortic atherosclerosis development was markedly increased in fenretinide-treated mice vs controls (arch: 34.6±7.3% vs 26.1±5.8%; thoracic aorta: 14.2±4.9% vs 4.9±2.1%; abdominal aorta: 7.4±3.3% vs 3.3±1.8%; p<0.01).In conclusion, our results demonstrate for the first time, that, despite beneficial metabolic effects, fenretinide treatment could be severely detrimental for atherosclerosis development.

FACTORS ASSOCIATED WITH THE DEGREE OF LIVER AND VASCULAR DISEASE IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE: AN OBSERVATIONAL STUDY

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Background and Aims. Non-alcoholic fatty liver disease (NAFLD) is defined as accumulation of triglycerides more than 5% in the liver in the absence of other causes of liver disease and with alcohol consumption less than 30 g per day for man and 20g per day for woman. NAFLD encloses a spectrum of diseases ranging from simple steatosis to end stage liver diseases or to hepatocellular carcinoma (HCC); moreover, people with NAFLD have a higher risk of dying than general population, and the most common causes of death are cardiovascular disease, neoplastic disease and liver disease. NAFLD is becoming a relevant problem because of its high prevalence (~25% worldwide). Pathophysiology is not fully understood. The pivotal role of insulin resistance is validated from a huge number of literature, but other factors can take part to liver and cardiovascular damage progression, such as diagnosis of Diabetes Mellitus (DM), Arterial Hypetensions (AH), Dyslipidemia (D), modest alcohol consumption. The aim of this study is to analyze factors associated with liver and cardiovascular damage in patients with NAFLD.
Material and Methods. We analyzed the clinical records of 258 patients with ultrasound diagnosis of NAFLD, older than 18 years at the Outpatient clinic for liver steatosis of the University of Messina; after we obtained the informed consent we collected data on anthropometrics data, diagnosis of DM, AH, and D. Data on alcohol consumption were collected and patients declaring a consumption lower than the reported cut-off were considered as modest consumers of alcohol, whereas the other patients were "not drinkers". Data on liver damage as evaluated by liver stiffness measurement (LSM) were collected. Data on cardiovascular damage evaluated as abnormal cIMT were also collected (in 133 patients only).
Results. LSM indicative of advanced fibrosis correlates with BMI (p<0.001), CV (p<0.001), age (p<0.001), and is associated with DM (p<0.001), AH (p<0.001), and abnormal cIMT (p<0.05). The logistic regression models show that the presence of advanced fibrosis depends on age (p<0.005), BMI (p<0.001), CV (p<0.001), diagnosis of DM (p<0.001), AH (p<0.001); after the adjustment for the other factors, BMI and DM were the major risk factors for advanced fibrosis in patients with NAFLD (p<0.05, OR 1.188, CI 1.004-1.405; p<0.05 OR 3.943, CI 1.221-12.735). The logistic regression models show that abnormal cIMT depends on age (p<0.001), CV (p<0.05), DM (p<0.005), AH (p<0.005), D (p<0.05), LSM (p<0.05). After the adjustment for other factors, age resulted the major risk factor for vascular damage in patients with NAFLD (p<0.005).
Conclusion. Our data show no association between modest alcohol consumption and the degree of liver damage. In patients with NAFLD, the presence of type 2 diabetes mellitus and obesity are independently associated with advanced liver fibrosis. Since the severity of liver damage is also associated with the presence of vascular damage, it would be useful to suggest this patient to undergo carotid Doppler even to assess global CV risk and personalize the treatment.

EVOLOCUMAB REDUCED LP A IN A GROUP OF PATIENTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA.

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Background. Patients with heterozygous familial hypercholesterolemia (FH) have increased cardiovascular risk. Lp (a), a low-density lipoprotein (LDL) particle showed a consistent and independent positive association with cardiovascular disease risk in epidemiological studies. Current therapeutic options to reduce Lp(a) are limited. Aim of this study is to test the effects of Evolocumab on Lp (a) in a group of FH patients.
Methods. Twenty-five FH subjects (13 men and 12 women, mean age: 51.5±14.5 yrs) were enrolled in this study. Lp (a) and other lipids parameters were measured with a standardized methods at Visit 0 (week 0) and Visit 2 (week 12). Lp (a) was assessed by Mercodia Apo(a) ELISA, a solid phase two-site enzyme immunoassay based on the sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants of the apolipoprotein(a) molecule.
Results. After 12 week of treatment Evolocumab (140 mg) plus statin therapy, reduced Lp (a) (from 50.3±34.5 to 41.1±34.1 mg/dL, p<0.001). Mean dose-related reduction in Lp (a) was 18%. In addition Evolocumab (140 mg) reduced total cholesterol (from 269.9±60.6 to 161.7±44.5 mg/dL, p<0.001), LDL-cholesterol (from 192.9±57.3 to 86.0±37.9 mg/dL, p<0.001), and Apo B (from 1.41±0.3 to 0.7±0.2 mg/dL, p=0.001).Triglycerides and HDL were not modified.
Conclusion. Evolocumab significantly reduces Lp(a), after 12 week of treatment by up to 18%, among subjects with familial hypercholesterolemia receiving statin therapy, offering an additional, complementary benefit beyond low-density lipoprotein cholesterol reduction related to a patient's atherogenic lipid profile.

DISTINCT LIPOPROTEIN CURVES IN THE EVALUATION OF LIPID PROFILE IN OBESE CHILDREN: A NEW TOOL TO AVOID OVERTREATMENT

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Introduction. Obese children are world-wide considered at "special" risk for cardiovascular disease (CVD). As obesity may alter lipid profile, Shamir et al. (JPGN, 2017-july 6) have recently developed BMI-adjusted lipoproteins curves. Aim of our study is to evaluate lipid profile among obese children referring to our Childhood-Obesity-Center (COC), comparing traditional and BMI-adjusted curves.
Methods. In a 18-months period, 51 children (mean age 10.6 years) referred to our COC (first access). Family history for CVD and lipid disorders, anthropometric measures and clinical evaluation were assessed. Patients with BMI >97° centile for sex and age and/or positive family history for CVD and/ or for lipid disorders were addressed to lipid evaluation.R
Results. 29/51 (56%) had BMI >97° centile for age and sex, 4/51 (7%) had a positive family history for CVD and/or lipid disorders. 33/51 (64%) were addressed to further analysis, 23/51 (45%) had analysis done (others lost to follow up). Lipid values (mean; minimum-maximum; mg/dl) were: total cholesterol 159 (119-200), LDL 92.5 (64-119), HDL 40.9 (30-56), triglycerides 109 (59-320). We considered as lipid disorder the presence of either LDL or total-cholesterol or triglyceride >75° centile or HDL < 25° centile. According to traditional versus BMI-adjusted curves, patients with lipid disorder were respectively 18/23 (78%) and 12/23 (52%). Specifically, HDL was below 25° centile in 8/23 (35%) and 3/23 (13%), triglycerides above 75° centile in 10/23 (43%) and 7/23 (30%), both total cholesterol and LDL-cholesterol above 75° centile in 8/23 (35%) and 6/23 (26%).
Conclusion. Our study shows that obese children referring to COC display lipid disorders at first access, especially low HDL and elevated triglycerides. However, the percentage of patients with lipid disorders significantly decreases if evaluated by means of BMI-adjusted curves. BMI-adjusted lipoprotein curves may be a useful tool in clinical decision-making, in order to avoid overtreatment and to reduce families' and health system's costs.

EVALUATION OF LIPOPROTEIN (A) LEVELS IN HYPERCHOLESTEROLEMIC CHILDREN TO DETECT PATIENTS AT HIGH CARDIOVASCULAR RISK

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Introduction. lipoprotein(a) plays an important role in the pathogenesis of cardiovascular disease (CVD), yet its role in atherosclerosis process in children is still poorly considered. Aim of this study is to evaluate the association between lipoprotein(a) levels and family history for cardiovascular disease (CVD) in hypercholesterolemic children at their first access to our Lipid Center, in order to identify those at higher CVD risk.
Matherials and Methods. In a 18 months-period, 70 patients were referred to our Lipid Center for hypercholesterolemia. 20/70 (28%) (6 males 14 females, age 2-17, median age 11.5 years) matched clinical criteria for suspected familial hypercholesterolemia and were evaluated for detailed CVD-oriented two-generation family history (according to AAP guidelines 2008), anthropometric measures, complete blood sample lipid profile including lipoprotein(a) levels, thrombophilic screening. None was receiving pharmacological treatment or vitamin supplementation. Statistics: Mann-Whitney test for independent samples.
Results. Family history was positive in 10 (CVD+) children (first degree relatives in 2, second degree in 8) and negative in 10 (CVD). Lipoprotein(a) levels in CVD+ vs CVD- group were (median-range, mg/dl) respectively 27.6 (1.6-225) and 20.2 (3-93). Thrombophilic screening showed no abnormalities. Specific dietetic and lifestyle indications for hypercholesterolemia were given.
Conclusions. Lipoprotein(a) levels tend to be more elevated in patients CVD+, even if our limited number does not reach a statistical relevance. In our limited sample, this seems a useful tool to identify patients at higher CVD risk. Collection of a two-generation family history is an issue of utmost importance, as first degree relatives might have not had a CVD event yet, due to their young age. Families with elevated lipoprotein(a) levels are educated to avoid the acquisition of other risk factors and to lead a healthy lifestyle, in order to preserve their cardiovascular health.

IMT AND TBS: THROUGH CARDIOVASCULAR DESEASE RISK AND BONE MICOSTRUCTURAL QUALITY IN A DIABETIC POPUATION

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Literature data reported that in elderly subjects, carotid intima-media thickness (IMT) was negatively associated with bone mineral density (BMD). Paradoxically, type-2 diabetes (T2DM) patients, despite having higher BMD, present an increased risk of fragility fractures and cardiovascular complications. Some studies have reported trabecular bone score (TBS), an index of trabecular bone quality, as possibly being reduced in T2DM. This study aimed to evaluate whether in T2DM subjects TBS was better associated with IMT with respect to BMD. In 131 consecutive T2DM subjects (55 men and 76 women; mean age: 60.0±7.3 years) and 265 consecutive non-T2DM subjects (107 men and 158 women; mean age: 58.9±7.8 years) we measured carotid IMT by high-resolution ultrasonography and BMD at lumbar spine (LS-BMD), at femoral neck FN-BMD and total hip TH-BMD; TBS was calculated using TBS iNsight software. LS-BMD, FN-BMD, and TH-BMD were all significantly higher in T2DM than in non-T2DM subjects, whereas TBS was significantly lower in T2DM subjects than in controls and inversely correlated with diabetes duration. In T2DM subjects multiple regression analysis showed that IMT was positively associated with age (b=0.017; p<0.001) and inversely associated with TBS (b=-0.473; p=0.038). In non-T2DM subjects, only age was positively associated with IMT. To sum up, T2DM subjects present higher values of BMD and lower values of TBS with respect to non-diabetic controls. Moreover, in T2DM subjects TBS was found to be independently associated with carotid IMT. These findings suggest that TBS may not only capture bone fragility-related factors, but also some information associated with greater risk of developing cardiovascular diseases.

ABCA1 AND HDL3 ARE REQUIRED TO MODULATE SMOOTH MUSCLE CELLS PHENOTYPIC SWITCH AFTER CHOLESTEROL LOADING

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Cholesterol-loaded smooth muscle cells (SMCs) modify their phenotypic behavior becoming foam cells. To characterize the role of ABCA1 and HDL3 in this process, we evaluated HDL3 effects on cholesterol-induced phenotypic changes in SMCs expressing or not ABCA1.SMCs, isolated from the aortae of wild-type (WT) and Abca1 knock-out (KO) mice, were cholesterol-loaded using a "water-soluble cholesterol". Cholesterol loading downregulates the expression of Acta2 and Calponin (SMC markers), and increases the expression of Mac-2, CD11b and MHCII (inflammation-related genes and surface antigens) and of Abca1, Abcg1. HDL3 normalizes SMC marker expression and reduces the expression of inflammation-related genes/proteins in WT cells, an effect which is not observed with free apoA-I. The effect of HDL3 is almost lost in Abca1 KO cells as well as after silencing Abca1 expression in WT SMC by using ABCA1 siRNA. HDL3 does not differently affect cholesterol downloading in WT or KO cells and stimulates phospholipids removal in WT cells. Similarly, the expression of myocardin and of its modulators, such as miR-143/145, is reduced by cholesterol loading in WT and Abca1 KO SMCs; HDL3 normalizes their levels in WT cells but not in KO cells. On the contrary, cholesterol loading induces Klf4 expression while HDL3 restores Klf4 to basal levels in WT cells, but again this effect is not observed in KO cells.Our results indicate that HDL3, by interacting with ABCA1, modulates the miR143/145-myocardin axis and prevents the cholesterol-induced gene expression modification in SMCs regardless of its cholesterol unloading capacity.

CHARACTERIZATION OF PEDIATRIC PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA IN THE LIPIGEN STUDY

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Familial hypercholesterolemia (FH) is a common genetic disorder that implies exposure to high levels of LDL-cholesterol (LDL-C) from birth. This analysis was aimed at the characterization of clinical and genetic features of the FH pediatric population enrolled in the LIPIGEN (LIpid TransPort Disorders Italian GEnetic Network) study.LIPIGEN study collected clinical, biochemical and genetic data of a population of FH subjects under follow up in the Italian lipid clinic network, including results of sequencing of FH candidate genes.The LIPIGEN cohort includes 1145 pediatric patients (<18 years), of whom 48.3% are males. The mean age at diagnosis was 9.0 (SD 4.0) years; 21.4% of patients were in preschool age. The mean LDL-C level recorded at diagnosis was 219.7 (SD 104.0) mg/dL; 57.8% of patients had LDL-C >190 mg/dL, 26.6% had LDL-C >250 mg/dL and 5.9% had LDL-C >325 mg/dL. Xanthomas was observed only in 3.6% of cases. The family history of premature coronary heart disease was positive in 12.6% of cases, while the prevalence of hypercholesterolemia among first-degree family members was 91.5%. Most of the patients underwent genetic analysis; in 85.9% of them a single heterozygous pathogenic variant of the LDL receptor gene (LDLR) was detected. In these subjects, the mean LDL-c was 240.1 (SD 87.9) mg/dL and median 231.0 (IQR 192.0-272.0) mg/dL. Twenty-two subjects were found to be heterozygous carriers of two LDLR variants, with LDL-C ranging from 155.0 to 1100.2 mg/dL. In 16 subjects a homozygous LDLR variant was found; their LDL-C ranged from 460.6 to 1029.0 mg/dL.This analysis of pediatric FH patients shows a large variation of the LDL-C level and the extremely low prevalence of classic FH clinical features in mutation carriers. In most cases, FH diagnosis in pediatric age generally requires a diagnosis of FH in an adult family member.

THE DIFFERENT IMPACT OF CHRONIC AEROBIC EXERCISE ON VASCULAR AND LIVER FUNCTION IN 50 MALE ATHLETES. A 2-YEAR FOLLOW-UP

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Background. Competitive sports could accelerate atherosclerosis and damage vascular function. Aim of this study was toevaluate the progression of micro- and macro-vascular alterations associated to strenous chronic physical activity.
Materials and Methods. Ninety male subjects (50 athletes and 40 controls) underwent comprehensive vascular function evaluation, assessing ankle brachial index (ABI), augmentation index (AIX) and AIX corrected forheart rate (AIXr), intima-media thickness and pulse wave velocity assay at common carotid (c-IMT, c-PWv) and femoral arteries (f-IMT, f-PWv), non-alcoholic fatty liver disease (NAFLD), and endothelial functionassessment (LnRHI). These evaluations were performed at baseline (T0), after one year (T1) and after 2 years (T2).
Results. Athlete's f-PWv and femoral AIX at T2 were significantlyincreased in comparison to T0; LnRHI was significantly reduced in athletes at T2. PWv, IMT and AIX, LnRHI values at T2 did not significantly differ in control subjects. Athletes did not show any degree of NAFLD at T0 and T2; controls presented significantly higher values in comparison to athletes.
Conclusion. Elite sports positively affect vascular intima-mediathickness and liver steatosis, and these data were confirmed at follow-up; however, we showed a progressive worsening of f-PWv

and femoral AIX values, and LnRHI at T2 respect to T0. This study showed the progression of sport-related and district-specific vascular damage, assessed asmorphological and functional parameters.

CELIAC DISEASE MANIFESTING AS FEVER OF UNKNOWN ORIGIN; A SYSTEMIC INFLAMMATORY ACTIVATION LEADING TO CHRONIC VASCULAR DAMAGE

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Introduction. Celiac disease is an inflammatory disorder affecting subjects at any age andpresenting with a broad spectrum of symptoms; in several cases, extra-intestinal symptoms are theonly clinical manifestations or occur in conjunction with gastrointestinal symptoms.
Aim. We present the case of a 47-year old male admitted to our Internal Medicine Department, after a further episode of fever with chill, associated with headache and malaise.
Case Study. He had suffered from episodes of fever for about 20-years. At physicalexamination, gastrointestinal symptoms such as nausea, emesis, diarrheal and constipation, wereabsent.
Results. Investigations were negative for malignancy, haematological disorders, autoimmunediseases, and inflammatory bowel disease; serological and culture tests were negative for commoninfectious diseases and parasitosis. Inflammatory markers were elevated [erythrocytesedimentation rate =48 mm/hr, C-reactive protein =185 mg/L)], with the exception of normalprocalcitonin levels. Colonoscopy demonstrated villous blunting, with biopsies consistent with adiffuse chronic inflammation in the lamina propria and significant intraepithelial lymphocytosis(MARSH II). Further investigations showed an advanced atherosclerotic peripheral disease,despite the low cardiovascular risk.
Discussion. In celiac disease, the activation of gluten specific T cells in the gastrointestinalmucosa induces the activation of a proinflammatory pattern, which could contribute to the fever. The systemic inflammatory activation found in celiac disease was associated to a chronic vasculardamage, both consisting in an increased arterial stiffness and intima-media- thickness.
Conclusions. The delay in diagnosis of celiac disease could be associated to severalcomplications, such as atherosclerotic progression.

SPONTANEOUS CEREBRAL HAEMORRHAGE IN A YOUNG WEIGHT LIFTER.

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Intracranial haemorrhage is the leading cause of death relatedto a head trauma in sports, and spontaneous cerebral bleedings are a veryrare condition in young athletes.We propose the case of a young weight lifter, androgenic anabolicsteroids abuser, who developed a spontaneous massive intracranial haemorrhage during exertion. The patient was a weight lifter at acompetitive level, who had exercised on a regular basis for the past 5 years, assuming anabolic steroids and proteins for some years. Moreover, he referred a negative family history for spontaneous bleeding in thebrain. At the visit time he presented very high blood pressure values. In this case report, the side of the cerebral lesion was typical for ahypertensive brain damage. High blood pressure values, and the alterationof sodium and potassium were compatible with hyperaldosteronism; moreover, cardiac ultrasound assessment showed a hypertrophic ventriclecondition, secondary to a chronic untreated hypertension. However, incurrent literature, the effect of weight lifting and anabolic steroidsabuse on myocardial fibres is actually object of discussion.

INVERSE CORRELATION BETWEEN CIRCULATING LOW-DENSITY LIPOPROTEIN CHOLESTEROL AND N-TERMINAL PRO-B-TYPE NATRIURETIC PEPTIDE IN VERY ELDERLY HOSPITALIZED PATIENTS

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Introduction. Our studies on human adipocytes suggest that natriuretic peptides (NP) may affect plasma lipid levels also by inhibiting PCSK9 expression, a key regulator of low-density lipoprotein receptor (LDLR) activity. To test this hypothesis in a real-life clinical setting, we evaluated associations between LDL cholesterol and NT-proBNP in very elderly hospitalized patients.
Materials and Methods. A cross sectional study on 288 very elderly, admitted to our Internal Medicine and Geriatrics Department for medical conditions, a population in which increased NT-proBNP levels due to prevalent conditions such as heart failure are very common. NT-proBNP, total cholesterol (TC), HDL cholesterol (HDLc) and triglycerides (TG) were collected after stabilization of the acute illness. Patients were taking no lipid-lowering drugs. Calculated LDL-cholesterol (LDLc) was used for the analyses. NT-proBNP was analyzed both as a continuous and as a discrete variable (NT-proBNP tertiles).
Results and conclusion. Mean age was 87.7±6.2 years with female prevalence (57.3%). Median NT-proBNP: 2949 (1005-7335) pg/ml; mean TC: 145.1±40.3 mg/dl; mean HDLc: 38.4±18.6 mg/dl; median TG: 100 (75-129) mg/dl; mean LDLc: 84.0±29.5 mg/dl. We found an inverse correlation between NT-proBNP and both TC and LDLc (ρ=-0.157; p=0.008 and ρ=-0.166; p=0.005, respectively), while no correlations were found between NT-proBNP and HDLc (ρ=-0.065; p=0.275) nor TG (ρ=-0.009; p=0.874). These associations were confirmed also for NT-proBNP tertiles. The inverse association between NT-proBNP and LDLc was confirmed even after adjusting for sex, albumin, hemoglobin and cognitive impairment. We found an inverse association between NT-proBNP and LDL cholesterol levels that remained statistically significant after adjusting for covariates such as albumin, a common indicator of malnutrition or hepatic failure. The specific effect on LDLc, in our real-life clinical study, supports a possible direct role of NP on cholesterol metabolism via reduced PCSK9 expression, as suggested by in vitro studies with human adipocytes.

EVALUATION OF LYSOSOMAL ACID LIPASE LEVELS IN PATIENTS WITH LIVER DISEASE OF UNKNOWN ORIGIN

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Introduction. Lysosomal acid lipase (LAL) deficiency is a rare autosomal recessive disease characterized by progressive accumulation of cholesterol and triglycerides in the liver, spleen, lymph nodes and other organs. It is caused by deleterious mutations of the LIPA gene. Infants with LAL rarely survive beyond 6 months of age, while children and adults typically show a combination of dyslipidemia, elevated transaminases and liver damage, with progression from hepatosteatosis to cirrhosis. LAL deficiency should be particularly suspected in non-obese patients with steatosis or cryptogenic cirrhosis, especially in those presenting abnormalities in lipids metabolism.
Materials and Methods. We evaluated LAL levels in a clinical series of 40 patients with hypertransaminasemia and/or gamma-glutamyl transpeptidase increase of unknown origin since 12 months and/or evidence of ultrasound fatty liver. We excluded secondary causes of liver damage, history of alcoholic intake, use of drugs, viruses B and C, autoimmune disorders and alterations of iron and copper metabolism. Only one patient had compensated liver cirrhosis.
Results. Forty non-obese patients with cryptogenic liver disease, 20 women (50%), age 48.6±14.4 yrs (mean ±SD), were recruited; 28/40 patients (70%) had low levels of LAL with a functional reduction of 21.2±18.5% (mean ±SD) (range: 7.4-52.5%) and 40% had hypertriglyceridemia.Levels of LDL were significantly higher in patients with advanced LAL deficiency (>50° percentile) than in patient with deficit of LAL <50° percentile (155.4 mg/dL ±77.7 vs 114.2±34.8; p=0.047). Mean triglycerides and HDL levels were not significantly different between patients with LAL deficiency >50° percentile and patients with deficit of LAL <50° percentile.
Conclusion. In this group of patient LAL deficiency is significantly correlated with LDL levels; therefore could be useful to evaluate LAL levels in non-obese patients with cryptogenic liver disease. Further studies are needed in larger population samples in order to confirm our findings.

LOW ADVANCED GLYCATION END PRODUCT DIET IMPROVES THE INFLAMMATORY PROFILE AND ARTERIAL STIFFNESS OF DIABETIC SUBJECTS

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Background. Cardiovascular disease is the leading cause of mortality among individuals with type 2 diabetes, accounting for 40% to 50% of all deaths. One of the most important mechanism linking chronic hyperglycemia with vascular complication is the formation and accumulation of advanced glycation end products (AGEs). Beside endogenous AGE production, AGEs can also be found in foods and contributes significantly to the individual AGE pool. Modern diets are high in advanced glycation end products (dietary AGEs, dAGEs), derived from processing methods that exert a pivotal role in promoting atherosclerotic risk.
Objective. We studied the effect of low vs standard dAGEs diets (L-dAGEs vs S-dAGEs) on lipid profile, inflammation, and cardiovascular risk in diabetic subjects.
Methods. A 18-week randomized dietary intervention was conducted on 30 diabetic subjects. We evaluated lipid profile, high-sensitivity C-reactive protein, arterial stiffness, and intima-media thickness (IMT).
Results. After 18 weeks high-sensitivity C-reactive protein levels were significantly reduced in the L-dAGEs group compared to standard diet (0.32 [0.24-0.38] vs 0.48 [0.38-0.56] mg/dL, P<.05). We observed a non significant reduction in lipid profile in patients with L-dAGEs. With respect to baseline, L-dAGE patients showed a significant reduction in Augmentation index (27±8.6 vs 32±5.3%). A non significant reduction of Pulse Wave Velocity was observed in L-dAGE group. No difference in IMT was found from baseline to follow-up in both the groups.
Conclusions. L-dAGEs improved the inflammatory profile of diabetic subjects and seemed to reduced arterial stiffness compared with a standard diet. Further studies are needed to recommend this dietary regimen for prevention of cardiovascular risk in diabetes.

PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 (PCSK9) AND HEART METABOLISM

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Background. Proprotein convertase subtilisin kexin type 9 (PCSK9) is a 692-amino acid glycoprotein that belongs to the family of proprotein convertases. It is secreted into the circulation by the liver, interacts with several receptors of the LDL-R family but also with CD36 and favors their degradation. Viceversa increased expression of receptors of the LDL-R family was shown to favor lipid accumulation in the heart which results myocyte apoptosis, myocardial fibrosis, impaired mitochondrial function and cardiac dysfunction.
Aim. This project was aimed at testing the hypothesis that PCSK9 might regulate lipoprotein receptors expression in the heart, thus modulating mitochondria physiology, heart metabolism and cardiac function.
Methods. 2-months old WT, PCSK9 KO and Albumin CRE PCSK9 conditional KO (lacking PCSK9 production in the liver) male mice were fed for 20 weeks with SFD (Standard Fat Diet -10% Kcal fat). The heart was collected and an extensive metabolomics analysis was performed. Mitochondrial respiration was investigated under resting conditions and following maximal coupling and uncoupling conditions. Changes in the profile of mitochondrial proteins were tested by western blotting.
Results. PCSK9 deficiency resulted in the accumulation of intermediates of fatty acid oxidation, including carnitine-conjugated fatty acid (C8; 0,066±0,047 vs 0,195±0,035 and C12; 0,021±0,018

vs 0,11±0,069 pg/ug of prot, p<0,05) which was paralleled by a reduction of glucose 6-P, ribose-5P and erythrose-4P levels in the heart, suggesting a shift from fatty acid oxidation toward glycolysis. As a net effect, intermediates of Krebs cycle were reduced in PCSK9 KO hearts compared to WT samples (Citrate; 6,111±2,429 vs 3,593±1,103, α-ketoglutarate; 105,205±42,056 vs 59,657±23,939, p<0,05) suggesting an impaired activity. The WB analysis of electron transport chain subunits showed a reduced expression of key proteins of complex 1, 2 and 3 in PCSK9 KO mice compare to controls. Finally, oxygen consumption rate (OCR) was affected in PCSK9 KO mice and AlbCre-PCSK9 KO compared to controls.
Conclusion. Or data suggest that PCSK9 deficiency is associated with altered metabolic responses in the heart and support the need for an extensive characterization of this animal model.

ASSOCIATION OF PLASMA IRISIN WITH BODY COMPOSITION, PHYSICAL PERFORMANCE AND METABOLIC PROFILE IN A COHORT OF HEALTHY ELDERLY

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Physical activity is a cornerstone of primary prevention of many chronic disease, such as diabetes and metabolic syndrome. Irisin, a novel exercise-induced myokine, seems to have a key role in regulating energy metabolism, leading to weight loss and better glucose tolerance. We wondered if irisin could be a good health-status marker for the free-living elderly population so in this study we measured plasma irisin levels in a cohort of 297 healthy community-dwelling subjects enrolled in the PAN-GeA project. 297 people aged >60 years (156 women, 141 men) were enrolled. Inclusion criteria was the ability to walk for 2km without aid. A blood sample was collected for dosing plasma irisin, metabolic and inflammatory profile. Each participant underwent a BIA for body composition evaluation. VO2max was estimated with the UKK walk test. We evaluated the association between irisin and physical performance, anthropometric, body composition and metabolic profile. After adjustment for age and sex, the linear regression analysis showed that irisin (log-irisin) was significantly inversely associated with weight (β-0,002; p 0,001), free fat mass (FFM) (β-0,004; p 0,001), muscle mass (β-0,007; p<0,001), basal metabolic rate (β-0,001; p<0,01), total cholesterol (β-0,0004; p 0,03), LDL cholesterol (β-0,001; p 0,02) and triglycerides (β-0,1 p 0,02). No association was found with VO2 max (p 0,8). The multivariate regression model showed that only female sex (β -0,05; p 0,04) and FFM (β-0,004; p 0,003) were able to weakly predict irisin levels (R2 0,006). At steady state irisin is poorly predicted by anthropometric, body composition and metabolic characteristics of the subject. Maybe a dynamic evaluation of the myokine after acute exercise or acute immobilization should be more informative about musculoskeletal health status.

ARE CELLULAR LIPIDS AND OXIDATIVE STRESS INVOLVED IN ARRHYTHMOGENIC CARDIOMYOPATHY PATHOGENESIS? A LIPIDOMIC STUDY IN CARDIAC MESENCHYMAL STROMAL CELLS

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Arrhythmogenic cardiomyopathy (ACM) is an autosomal dominant rare genetic disease with low penetrance and variable expressivity, characterized by fibro-fatty substitution of ventricular myocardium, arrhythmias and sudden death, mainly in young people and athletes. Different causative genes have been identified, most of them coding for proteins of the desmosome (cell membrane component involved in cell adhesion and signaling). Mutated desmosomal proteins cause myocyte disconnection, cell death, and activate adipogenic signaling. The resulting tissue remodeling leads to progressive heart failure and arrhythmias. Several mechanisms have been proposed to explain the association between desmosome gene mutations and the origin of heart adipocytes. Among them, the inhibition of the canonical Wnt/β-catenin signaling pathway, with consequent activation of PPAR-gamma and accumulation of intracellular lipids. Cardiac Mesenchymal Stromal Cells (C-MSC) are an abundant population in the heart with structural and functional role in tissue homeostasis. Recently, it has been demonstrated that they have a pivotal role in ACM pathogenesis by differentiating into adipocytes in patients' hearts.To better characterize ACM C-MSC pathogenic processes, we conducted studies on C-MSC derived from ACM (n=5) and NON-ACM (n=4) patients' biopsies. Already at baseline ACM C-MSC showed a significant increase of oxidative stress (ROS concentrations 5.73±0.8 vs 3.73±0.4) PPAR-gamma (fold change 5±1.1 vs 1.4±0.7) compared to NON-ACM. On the same cells, grown in adipogenic medium, we also performed qualitative and quantitative lipid analysis. Using chromatographic methods (TLC, GLC) we documented a significant increase in free cholesterol (FC) (11.95±1.17 vs 8.24±1.15 ug/106 cells; p<0.05), and triglycerides (TG) (35.80±5.8 vs 19.46±3.77 ug/106 cells; p<0.05) mass in ACM- vs NON-ACM C-MSC. Increases in free fatty acids (9.37±1.7 vs 7.9±0.9 ug/106 cells) and cholesteryl esters (CE) (31.7±7.23 vs 18.84±3.9 ug/106 cells) were also present, despite not significant, together with a slight increase in FC/CE ratio. From a qualitative point of view, CE, TG and total fatty acids from C-MSC-ACM demonstrated a relative increase in monounsaturated- (MUFA; 18:1) vs saturated- (SFA 16:0, 18:0) fatty acids vs NON-ACM ones. Analyzing the total and relative mass of each phospholipid (PL) (e.g. phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin, phosphatidylserine, phosphatidic acid, phosphatidylglycerol, cardiolipin), together with their relative percentages, we documented a general mass decrease (29.32±4.6 vs 50.32±12.9 nmoles/106 cells) in C-MSC ACM (n=6) vs NON-ACM ones (n=5). We speculate that this effect could be related to the increased synthesis of TG in ACM cells, since both TG and PC (and related PLs such as PE and PS) have a common pathway up to the synthesis of diacylglycerol. The up-regulated adipogenic signaling may drive the substrate diacylglycerol towards TG biosynthesis (mediated by diglyceride acyltransferase DGAT) rather than synthesizing PC (mediated by choline/ethanolamine phosphotransferase CEPT). On the other hand, the increase in PC/PE ratio (2.11

C-MSC ACM vs 1.58 C-MSC NON-ACM) could trigger the unfolded protein response (UPR) that, in stress conditions drives cells towards apoptosis. Moreover, the decrease in PE may result from the higher oxidative stress in ACM cells vs controls, due to its elevated content in plasmalogens and polyunsaturated fatty acid (PUFA), preferential ROS substrates. To answer the question regarding the decrease in total PLs mass in C-MSC ACM and particularly that of PE (6.91±1.17 vs 14.78±5.09 nmoles/106 cells), we are planning to evaluate the mRNA, protein mass and activity of DGAT and CEPT, the key enzymes involved in the switch of PL/TG production. Morover, detailed PLs lipidomics will help to understand whether oxidative processes (e.g. PUFA and plasmalogens oxidation) may be the culprit of total PLs and particularly of PE decrease.Altogether, these data show that C-MSC from ACM patients present a perturbed lipid profile and that a more detailed analysis may contribute to understand the mechanism(s) underlying ACM pathogenesis.

DIETARY COMPOSITION MAY INFLUENCE NESFATIN-1 PLASMA CONCENTRATION IN HUMANS

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Aim. Nesfatin-1 is a energy-regulating peptide that inhibits food intake trough a leptin-independentmelanocortin pathway. It is widely expressed in different tissues with an expression level in stomach20-fold higher than in brain. It has been reported a possible influence of diet composition onNesfatin-1 production in gastrointestinal tract, in different animal models, however no data inhumans are available. We investigated the relationship between dietary intake and Nefsatin-1plasmatic levels in a cohort of healthy subjects.
Methods. 72 healthy adults, participating to the PANGeA project (Physical Activity and Nutrition for Quality Ageing), were evaluated. We collected dietary habits through a 24 h-recall interview, quantifying macronutrients intake. A blood sample was obtained from every subject for thedetermination of Nesfatin-1 plasma concentration (ELISA).
Results. Nesfatin-1 concentration positively correlated with both total lipid intake ($p<0.05$) and lipidgrams intake/body weight in kg ($p<0.05$). After dividing the population according to BMI weobserved that this correlation was significant only in subjects with a BMI <25 kg/mq. A positivecorrelation was also observed with protein intake (% protein of Kcal intake) ($p<0.01$). A linear regression analysis showed that both lipid intake (g/body weight in Kg) and protein intake (% protein of Kcal intake) were independent predictors of Nesfatin-1 concentration.
Conclusions. Lipid and protein intake seem to influence circulating levels of Nesfatin-1 in humans.It seems that there could be an impairment of this satiety pathway in patients with BMI & gt; 25, however this finding need to be confirmed in further studies.

UNRAVELLING THE GENETIC COMPLEXITY IN BICUSPID AORTIC VALVE: THE IMPORTANCE OF SEGREGATION ANALYSIS

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Background. Bicuspid aortic valve (BAV) is the most common congenital heart defect. BAV complications are: aortic regurgitation, aortic stenosis, infective endocarditis, and dilatation of the thoracic ascending aorta, thus raising concerns for the proper timing of aortic surgery especially in syndromic BAV. Familial clustering has been reported, mostly with an autosomal pattern of inheritance with reduced penetrance and variable expressivity. The 89% estimated hereditability suggests the presence of a relevant genetic contributor to the disease acting in a complex interaction with hemodynamic and environmental factors. Alongside previous data from literature suggesting the role of some genes (NOTCH1, GATA5) in BAV pathogenesis, further data concerning the association of several other genes with the disease are progressively emerging. Moreover, continuous advances in high-throughput sequencing (HTS) technologies provide unprecedented opportunities to reach a comprehensive dissection of BAV and BAV-complications heritability. However, alongside the study of vast cohorts of probands, large families with several BAV affected members currently represent the most promising opportunities for the consolidation of previously associated genes and for discovery of novel associations of genes with BAV. Aim of the study was to achieve a better comprehension of the genetic bases of BAV through segregation analysis of variants identified by HTS technology on 3 BAV index cases.
Materials and Methods. Three BAV probands admitted to the Referring Center for Marfan syndrome and Related Disorders (Tuscany, Italy) were analyzed by HTS using the 454-GS FLX Titanium platform (Roche). A targeted sequencing of 94 genes known or with plausibility to be associated with connective tissue disorders or aorthopathy or aortic wall remodelling was performed. Mutational screening was extended to the first-degree family members (n=16) who gave their informed consent.
Results. In Family 1, two not previously described mutations were identified in LRP1 gene (c.527T>A, p.Val176Asp) and PLOD1 gene (c.826A>C, p.Ser276Arg). In particular, the first variant was called potentially pathogenetic according to all applied in silico tools. Segregation analysis in this family suggests a potential contribution of the LRP1 variant to BAV phenotype. Family 2 proband showed a rare MYH11 variant (c.5073C>G, p.Arg1691Ser) called damaging for most (3/5) of the applied in silico tools and a novel ADAMTSL4 variant (c.464G>C, p.Gly155Ala) called pathogenetic according to all applied in silico tools. Despite of the characteristics of the genetic variants, in particular those of the ADAMTSL4 gene, strongly suggestive of an involvement in BAV, segregation analysis showed MYH11 mutation only to be presumably associated with BAV phenotype. In Family 3, among variants (n=4) with minor allele frequency (MAF) <0.02 identified in LTBP4 (c.1556-1567del, p.Asp519-Pro522del), COL6A3 (c.9245C>G, p.Pro3082Arg), ABCC6 (c.2171G>A, p.Arg724Lys) and LTBP1 (c.3797C>T, p.Ser1266Phe) genes, none has been shown to segregate with BAV.

Conclusions. Segregation analyses on these 3 BAV families unravelled the potential role of some genetic variants as major determinants of the BAV disease while others can be assumed to have at most a modifier role within its complex phenotype. These data suggest the multifactorial nature of BAV disorder and its complications due to the interaction of individual genetic profile, hemodynamic, and environmental factors, and highlight the crucial importance of recruiting large families with inherited predisposition to BAV in order to better define and demonstrate the specific associations between genetic variants and the phenotype.

PEDIATRIC FAMILIAL HYPERCHOLESTEROLEMIA: LIPID AND GENETIC PROFILE OF A MULTICENTRIC COHORT

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Introduction. Familial Hypercholesterolemia (FH) is a monogenic disease leading to very high levels of LDL-Cholesterol. Early identification is very important in children, since the increased risk of cardiovascular disease can be prevented by adequate therapy. The most used diagnostic criteria in children are Simon Broome's criteria. Amongst pediatric patients with a clinical suspect of FH, we aim to search for differences in lipid profile and derived parameters between patients with and without mutations in order to find additional criteria to focus on.
Material and Methods. Data from 589 pediatric patients were collected in two different Children Hospital and genetic screening was performed in 157 index cases. Inclusion criteria were the presence of LDL-Cholesterol above 3.37 mmol (130 mg/dL) and documented increase of cholesterol level on a first degree relative or premature cardiovascular disease (pCVD) on a first/second degree relative.In order to adjust lipid values for age and sex, the multiple of the 50th or 95th percentile (M50 and M95 respectively) was calculated for each patient value. LDL-C/HDL-C ratio was also calculated.
Results. 125 patients out of 157 (79.62%) carried causative variants: 70 defective variants, 51 null variants and 4 compound heterozygotes. Significant differences between mutated and not mutated were found for M95-LDL-C (p=0.039), M50-HDL-C (p=0.006), M95-NonHDL-C (p=0.03), LDL-C/HDL-C (p=0.003). Also at discriminant analysis, the best parameter to discriminate the presence of mutations was the LDL-C/HDL-C ratio (p=0.002 at Wilks' Lambda), followed by parents' hypercholesterolemia (p=0.001). LDL-C/HDL-C ratio was also statistically different amongst mutation types at multiple comparison (p=0.000025 at ANOVA with Bonferroni correction).
Conclusions. This study suggests to assess the use of LDL-C/HDL-C ratio, which revealed to be a better parameter than LDL-C, confirming the uprising role of HDL-C in FH pediatric patients.

STATIN-ASSOCIATED MYOPATHY: MYTH OR TRUTH?

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Background. In common clinical practice, the use of statin causes myopathies in 10-25% but a literature review (controlled clinical trials vs placebo) edited this data in 12,5-13%, not so different from the control group.
Aim. Evaluate the incidence of statin-related myopathy associated with significant CPK elevation in common clinical practice; evaluate their disappearance/persistence after statin suspension and/or re-challenge.
Methods. We retrospectively reviewed data from patients visited in our ambulatory during ten years (2006-2015).
Results. Data (partially incomplete) refer to 1794 different dyslipidemic patients; 1167 were in treatment with statins. During the observational period, 120 (10,3%) reported myalgia, with (21=17,5%) or without (99=82,5%) significant increase (M>450;F>400 mg/dL) of CPK levels. In 48 patients (40%) the referred pain was not confirmed as muscle-related and/or CPK increase was not significant, therefore they were not followed-up. 72 patients interrupted therapy; after a wash-out period, some of them re-challenged the same statin down-titrated, the others changed it. In 21 patients (29,2%) myalgia persisted: only 12 of them (57,1%) reached LDLc target but nobody had CPK>3xULN. Only 18 patients among all treated (1,5%) had both significant CPK increase and myalgia (statin-related myopathy?): after re-challenge 12 (66,7%) had no longer myalgia and showed a CPK level decrease; the remaining 6 patients had only mild increase of CPK but persisting myalgia. However, 15 of them (83,3%), showed a good compliance to therapy and reached LDL goal.
Conclusions. Although data of some patients were partially lacking, myalgia seems often not associated with myopathy; even when an increase of CPK-levels co-existed, re-challenge improved patient's tolerability and compliance. An isolated myalgia should never lead to a quick and unjustified suspension of therapy, because the main target is to successfully reduce LDL-levels and CVD risk. Limits: retrospective study; data acquired from medical records, not from an electronic database; many lacking data; only preliminary results.

DETECTION OF FAMILIAL CHYLOMICRONEMIA SYNDROME IN A COHORT OF PATIENTS WITH SEVERE HYPERTRIGLYCERIDEMIA THROUGH A NEXT GENERATION SEQUENCING APPROACH

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Introduction. Familial Chylomicronemia syndrome (FCS) is a rare recessive disease caused by mutations in LPL, APOC2,

APOA5, LMF1 and GPIHBP1 genes. It is characterized by very severe hypertriglyceridemia (HTG) with or without episodes of abdominal pain and recurrent acute pancreatitis. FCS diagnosis is often difficult due to its phenotypic similarity with other forms of severe hypertriglyceridemia.
Aim. The aim of our study was to detect pathogenic mutations in candidate genes in patients with suspected FCS based on specific clinical criteria and, to evaluate differences between different genotypes in terms of clinical features, lipid profile and response to pharmacological treatments.
Materials and Methods. By examining 3000 clinical records, 31 patients were classified as suspected FCS on the following criteria:
a) plasma trigliceridemia (TG) levels >1000 mg/dl in multiple determinations;
b) resistance to pharmacological therapy;
c) history of recurrent abdominal pain/acute pancreatitis.
All patients underwent fully clinical examination and information about their response to hypolipidemic therapies was collected retrospectively. Candidate genes were sequenced using Next Generation Sequencing (NGS) technique applied on Illumina platform.
Results. Twenty patients (64.5%) were identified as carriers of FCS causing mutations, of whom 66.6% were homozygotes for mutations in LPL gene. Compared to non-carriers, FCS patients showed higher prevalence of history of acute pancreatitis (P=0.04) and lower TG reduction during treatment (Padj =0.03). In addition, comparing homozygous carriers of mutations in APOC2, APOA5, LMF1 with LPL homozygotes, the latter group showed higher TG levels (P=0.002) and lower TG reduction during treatment, after adjustment for gender, BMI and therapy (-68,5% vs -13.6%, respectively, Padj =0.03).
Conclusion. Our data suggests that the proposed diagnostic criteria are highly predictive of FCS diagnosis in severe HTG patients. Moreover, mutations in LPL gene appear to be the most common cause of FCS. Notably, homozygous carriers of mutations in LPL gene show the worst lipid profile and the lowest response to therapy, suggesting a more severe clinical phenotype.

A RARE CASE OF FAMILIAL HYPERTRIGLYCERIDEMIA

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Background. Hypertriglyceridemia is a common occurrence, whose risk (cardiovascular events, pancreatitis, etc...) increases proportionally with the triglyceride values. It may be secondary to other metabolic disorders or autoimmune diseases or drugs, but the more severe forms generally have a genetic origin and some of these are very rare and difficult to diagnose.
Clinical case. Woman, 48 years, fertile, normal weight (BMI=21), with combined dyslipidemia (mainly hypertriglyceridemia) (total cholesterol 350-480; HDL <30; triglycerides 1300-2500 mg/dL) known for more than 20 years and resistant to diet and n-PUFA 1 g x 4/day (>500 mg/dL). In maternal family, 5/12 uncles had early (50-65 years) cardiovascular events. Her daughter (22 years) has a fenofibrate-resistant hypertriglyceridemia (>1350 mg/dL) and her son (18) has a hypertriglyceridemia (>1500 mg/dL) diagnosed since birth (270-320 mg/dL) but never pharmacologically treated. Both the patient and her children history (glycemia, renal, hepatic and thyroid function, screening for other endocrine and autoimmune diseases,...) were negative for secondary forms. Our patient had a hepatosteatosis (ultrasonographic diagnosis) but has never had cholelithiasis. A recent Echo-Doppler showed no signs of carotid Intima-Media Thickness. The medical examination did not reveal xanthomas/xantelasms nor discolorations in the skin folds. After 12-hour incubation at 4°C, plasma appeared milky.
Comment. Clinical and laboratory history, together with very high triglyceride levels, resistant to therapy, made us suspect a genetic form.
Diagnosis. Patient's and her childrens' blood samples were sent to the Biomedical Department of Internal Medicine, Palermo University, where they confirmed the diagnosis of hypertriglyceridemia due to heterozygous nonsense mutation (c.718 G>A) in the CREB3L3 gene both in mother and her children.
Conclusions. CREB3L3 hypertriglyceridemia is a very rare form. To date, there are not well defined clinical criteria for diagnosing. Our case, along with very few others already genetically confirmed, could help us in early identifying if there are any common clinical criteria.

HBA1C IDENTIFIES SUBJECTS WITH PREDIABETES AND SUBCLINICAL LEFT VENTRICULAR DIASTOLIC DYSFUNCTION

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Objective. Prediabetes is associated with subclinical cardiac changes associated with heart failure development. We investigated diastolic function and its association with markers of glycation and inflammation related with cardiovascular disease in patients with prediabetes. We focused on individuals with prediabetes identified only by glycated hemoglobin A1c (HbA1c 5.7-6.4%) who had normal fasting glucose (NFG) and normal glucose tolerance (NGT) after oral glucose tolerance test.
Methods. We recruited 167 subjects with NFG/NGT. All the subjects underwent HbA1c measurement; prediabetes was defined as an HbA1c value of 5.7-6.4%. Subjects were stratified into two groups according to HbA1c levels: controls (HbA1c <5.7% and NFG/NGT); and HbA1c prediabetes (HbA1c 5.7-6.4% and NFG/NGT). Doppler echocardiography, soluble receptor for advanced glycation end-products (sRAGE), endogenous secretory RAGE (esRAGE) and S100A12 were evaluated in all the participants.
Results. Patients with HbA1c prediabetes (n=106) showed a lower E/A Ratio compared with controls (n=61) (1.10±0.24 vs 1.18±0.23, P<0.05); furthermore, they showed a higher left atrium volume (28.4±5 vs 22.1±3, P<0.05) and sphericity index (SI) (0.6±0.06 vs 0.5±0.05, P<0.05). After multiple regression analysis, only HbA1c, and esRAGE were independently associated with E/A Ratio; the major determinants of left atrium volume were HbA1c and sRAGE, whereas SI was independently associated with HbA1c.
Conclusions. Subjects with HbA1c prediabetes exhibited subclinical cardiac alterations: lower E/A Ratio, higher left atrium volume and impaired SI; sRAGE, esRAGE and HbA1c were associated with these alterations. These results endorse evidence supporting the use of HbA1c as a diagnostic test for prediabetes.

A NEW PCSK9 GAIN-OF-FUNCTION VARIANT IDENTIFIED IN TWO DIFFERENT COUNTRIES

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Introduction. Familial Hypercholesterolemia (FH) is usually caused by mutation in the LDL receptor (LDLR) gene, whereas few variants are found in APOB and PCSK9 genes. Since PCSK9 acts inducing LDLR degradation after its endocytosis, an increased PCSK9 function causes decrease of LDLR on cell membrane and decreased LDL uptake. Variants in the PCSK9 gene could lead both to FH if they cause gain-of-function (GOF) or to hypobetalipoproteinemia if they cause loss-of-function. Due to this dual effect, a functional characterization is required in order to claim the variant pathogenicity in FH.Patients and methods.
Case 1. Nine years old female from Italy with untreated total cholesterol 240 mg/dL, LDL-C 167 mg/dL, HDL-C60 mg/dL, triglycerides 64 mg/dL, family history of hypercholesterolemia and premature cardiovascular disease.
Case 2. Fifty-one years female from Canary Island (Spain) with total cholesterol 375 mg/dL, LDL-C 270 mg/dL, HDL-C 62 mg/dL, triglycerides 217 mg/dL. She had 11 siblings, 8 with hypercholesterolemia, 2 of them with myocardial infarction. Exons with the flanking intronic regions of the causative genes (LDLR, PCSK9 and the exons 26 and 29 of APOB encoding the binding domain) have been amplified and directly sequenced. LDL uptake assay was performed on transfected HEK293 cells or in HEK293 and HepG2 cells incubated with purified PCSK9 variant protein. After the treatments, the cells are incubated with fluorescently labelled LDL (DiI-LDL) and analyzed by cytofluorimetry.
Results. Both cases showed the same very rare variant in PCSK9 gene, c.1496G>A p.Arg499His predicted as non-pathogenic by Polyphen-2, Mutation Taster, PMUT, SIFT and PROVEAN. In the case 2 family the variant co-segregate with the hypercholesterolemia. To test the PCSK9 total (intracellular and extracellular) activity, PCSK9 variant was transfected in HEK293 cells that are then incubated with DiI-LDL revealing a decrease of 25% in LDL uptake respect to the wt protein. To test only the extracellular activity, the uptake assay was performed in HEK293 and HepG2 cells incubated with PCSK9 variant proteins showing no differences with the wt protein.
Conclusions. Despite the predictions of non-pathogenicity, the p.Arg499His in PCSK9 gene seem to induce down-regulation of LDLR being considered causative of FH. The mechanism of pathogenicity is non canonical since the LDLR degradation is due only to the intracellular activity of PCSK9 variant. This new GOF variant was identified in 2 patients native from 2 different countries, indicating a wide distribution of the variant.

IPER-LIPOPROTEIN(A): EFFECT OF THE COMBINED TREATMENT WITH LIPOPROTEIN APHERESIS AND ALIROCUMAB IN A PATIENT WITH SEVERE CORONARY ARTERY DISEASE

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Introduction. Lp(a) is a lipoprotein particle similar to LDL-C in which apolipoprotein B100 is linked to apolipoprotein(a) through a disulfide bond. Its plasma levels are genetically determined and its concentration is continuously and independently associated with cardiovascular disease. Indeed a causal role for Lp(a) in coronary heart disease risk is suggested by mendelian randomization studies. Lp(a) is not affected by common hyopolipidemic drugs: statins and ezetimibe do not alter or, perhaps, even increase its levels; niacin somewhat decreases plasma Lp(a) but at the expense of serious adverse events. Lipoprotein apheresis, removing apoB-containing lipoproteins from plasma, is the most-effective Lp(a) lowering treatment, even if it represents a cumbersome procedure; more recently PCSK9 inhibitors showed variable Lp(a) reducing capacity.
Patients and Methods. A 63 year old male patient with severe cardiovascular disease presented to our clinic with hypertension under pharmacological control and mild hypercholesterolemia treated with rosuvastatin 20 mg. At the age of 52 he had an acute myocardial infarction and from that time onwards he was repeatedly subjected to coronary revascularization procedures; moreover he underwent surgery for abdominal aortic aneurysm. His plasma Lp(a) levels were in the range 150-200 mg/dl with minimal response to lipid-lowering agents and LDL-C was slightly above desirable concentration (72 mg/dl). Ezetimibe 10 mg was added and lipoprotein apheresis programmed. Heparin-induced Extracorporeal LDL Precipitation (H.E.L.P.) was first performed. Then apheretic procedure based on dextran-sulfate LDL absorption (LIPOSORBER) was initiated, treating initially 4,6 Lt of plasma and subsequently increasing to 6 Lt. After application to the local Ethics Committee, alirocumab 75 mg was added to the treatment regimen, alternating drug injection to lipoprotein apheresis every other week. Time-averaged concentration (Cavg) for Total Cholesterol (TC), LDL-C and Lp(a) during apheresis was estimated by Kroon equation: Cavg=Cmin+0.73 (Cmax-Cmin), where Cmax and Cmin are the immediate pre- and post-treatment levels.
Results. Table represents the number of apheretic procedures performed for each method, mean intervals between treatments and TC, LDL-C, and Lp(a) levels (mean±SD) either basally (under rosuvastatin/ezetimibe) or during different apheresis conditions. Dextrane-sulfate LDL absorption showed the highest Lp(a) removing capacity, particularly when 6 Lt of plasma were processed. The addition of alirocumab 75 mg disclosed further 57% LDL-C reduction, but without significant effect on Lp(a) concentration. Potential alirocumab up-titration was considered unsafe, due to the

particularly low post-apheresis LDL-C levels. Treatment, Apheresis (number), Mean Interval (days), Tot.-Col. (mg/dl), LDL-C (mg/dl), Lp(a) (mg/dl) Rosuvastatin+Ezetimibe (basal) 107±7** 56±6** 160±22 *HELP 21 12 96±7 46±5 139±17Liposorber (4.6 Lt) 17 10 93±7 46±5 124±17* Liposorber (6 Lt) 16 11 92±7 44±6 100±7** Liposorber (6Lt) + Alirocumab 75 mg 6 14 70±3** 19±2** 99±11** (*p<0.05 and **p<0.001 vs. HELP).

Discussion. In our patient lipoprotein apheresis proved to be a highly effective procedure in reducing plasma apoB-containing lipoproteins. In particular dextran-sulfate LDL absorption 6 Lt every 11 days was able to reduce Lp(a) by 37.5% compared to basal. The addition of biweekly administration of alirocumab 75 mg did not show any further Lp(a) reduction, despite large effect on LDL-C.

PREVALENCE OF FAMILIAL HYPERCHOLESTEROLEMIA IN A PEDIATRIC AMBULATORY POPULATION: FIRST OBSERVATION IN CALABRIA

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Background and Aims. Familial hypercholesterolemia (FH) is a genetic disorder of lipoprotein metabolism with high risk of cardiovascular (CV) events also in young individuals. FH is asymptomatic in childhood and often may be underdiagnosed. Several studies suggest that early diagnosis and treatment can prevent CV mortality in early adulthood (1). In Italy, selective screening is delegated to general practitioners or pediatricians. Suspected FH children are invited to attend lipid clinics where they undergo biochemical and genetic analysis. However, only a limited number of children complete this program, accounting for less than 10% (2). The aim was to determine the prevalence of children with possible FH in a large ambulatory care population.

Methods. A retrospective analysis was carried out among patients seen at the "Mater Domini" University Hospital and at the "Pugliese-Ciaccio" Hospital in Catanzaro (Calabria), between 2016 and 2017. Using the current Lipid guidelines (3), FH patients were identified according to age and to highest LDL-C (≥95th percentile) plus a positive familiarity for hypercholesterolemia and/or early CV events. We excluded all subjects with secondary dyslipidemia as resulting from medical records.

Results. We analysed data from 769 children and adolescents. Prevalence of suspected FH was 2.9% (n=22) (Fig. 1). The mean of the age was 9.7±4 years and LDL-cholesterol 166±50 mg/dl (Table). A positive familial history of CV events was present in 17 (77.3%) of subjects with suspected FH.

Conclusion. Suspected FH children identified will undergo genetic analysis and their families will evaluate to identify FH individuals at lipid clinic. All dyslipidemic children should be linked to a recognised specialist network managed by a pediatrician. This ensure that all children will be referred to a specialised pediatric lipid clinic and are adequately informed and treated. However, more pediatric lipid clinics should be established to identify pediatric patients with FH and other types of dyslipidemia.

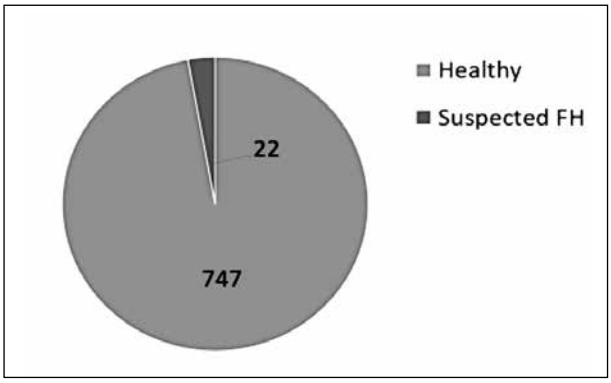

Figure 1 - Prevalence of suspected FH in a pediatric ambulatory care population.

Table - Clinical characteristics of children and adolescents with suspected Familial hypercholesterolemia.

Variables	Minimum-Maximum	Media ± SD
Age (years)	1-17	9.7±4
Weight (kg)	8-73	38±20
Height (cm)	74-166	134±25
Total-cholesterol (mg/dl)	188-354	244±53
HDL-cholesterol (mg/dl)	33-90	58±18
LDL-cholesterol (mg/dl)	105-275	166±50
Triglycerides (mg/dl)	45-200	85±44

Note. HDL = high density lipoprotein; LDL = low density lipoprotein; SD = standard. deviation.

References

1. Starr B, et al. Clin Chem Lab Med. 2008; 46: 791-803.
2. Kusters DM, et al. Arch Dis Child. 2012; 97: 272-6.
3. Stephen R, et al. Pediatrics. 2008; 122: 198-208.

A CASE REPORT OF PRIMARY CHYLOMICRONAEMIA IN A 40 YEARS OLD MAN

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Introduction. Primary Chylomicronaemia is a subgroup of severe hypertriglyceridaemia characterized by fasting high levels of chylomicrons associated with an increased risk of life-threatening pancreatits. Chy-lomicronaemia may be caused by homozygous mutations in the gene LPL, APOC2, APOA5, LMF1 and GPIHBP1 or by a cluster of several genetic variants.

Case report. A 40 years old man with primary chylomicronaemia, Hashimoto thyroiditis and a previous episode of acute

pancreatits showed hepatosplenomegaly, recurrent abdominal pain, nausea and no sign of lipaemia retinalis and eruptive xanthomas. The patient was heterozygous for a null mutation in the APOA5 gene (C883C>T, p.Gln295X), heterozygous for E2 isoform of APOE (no variants were found in LPL, APOC2, LMF1 and GPIMP1) suggesting that the co-presence of these variants to-gether with additional factors are the cause of Chylomicronaemia. Despite treatment with feno-fibrate (145 mg/die), n-3fatty acids (6 gr/die) and atorvastatin (40 mg/die), high levels of triglycer-ide (2817 mg/dl), total cholesterol (326 mg/dl), and TSH (10,93 µU/ml) were detected. After one month of both restriction of fat with diet (10-15%) and therapy with MCT oil (20 ml/die) plus levo-thyroxine (50 µgr/die), a decrease in triglyceride (655 mg/dl), total cholesterol (161 mg/dl), LDL cholesterol (33 mg/dl) and TSH (4,92 µU/ml) has been achieved. After 2 months despite of same lipid lowering therapy and normal TSH values, we detected an increase in lipid parameters (tri-glycerides 1988 mg/dl, total cholesterol 314 mg/dl), due to lack of patient's dietary compliance in the long term.
Conclusions. The presence of a mutation at heterozygous status, in association with hypothyroidism, is less se-vere than homozygous one and it may be more responsive not only to standard lipid lowering therapy, but also to lifestyle changes in order to reduce of the recurrent pancreatits. Another im-portant point is the patient's compliance with therapy, so doctor's counselling has a fundamental role in therapy management. Further studies on other pharmacological agents (antisense oligonu-cleotides against APOB, APOC3 and ANGPTL3 mRNAs; Pcsk9 inhibitors) are in progress in order to evaluate their potential benefit on lipid profile.

EFFECT OF ACUTE IMMOBILIZATION ON PLASMA IRISIN LEVELS AND ON GENE EXPRESSION OF FNDC5 AND RELATED GENES IN GLUTEAL SUBCUTANEOUS ADIPOSE TISSUE

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Irisin, a cleavage product of FNDC5 gene, is a novel exercise-induced myokine involved in muscle-adipose crosstalk with positive effects on energy metabolism. Acute inactivity poses a threat for muscle function and metabolic health of the individual. For the first time we evaluated the effect of acute inactivity on plasma irisin levels and on subcutaneous adipose tissue (SAT) gene expression of FNDC5 and a series of genes involved in its network.23 healthy male volunteers, 7 "Young" (18-25 y) and 16 "Older" (55-65 y) underwent 14-days of continuous horizontal bed rest (BR). At baseline (BR1) and after 14 days of BR (BR14) we collected a blood sample for biochemical analysis and plasma irisin dosage, and a gluteal-SAT biopsy (7 representative samples) in order to obtain RNA for subsequent gene expression analysis with microarray. We evaluated differences in gene expression of FNDC5 and a list of 56 genes, identified through a review of literature, potentially involved in irisin's pathway. At baseline plasma irisin did not differ between Young and Older (4,58 vs 5,01 mg/L respectively; p 0.74). Surprisingly, at BR14, we observed a significant increase of irisin levels in both groups (Y 4,58→6,07; O 5,01→7,05; p 0,01). Furthermore, after BR, FNDC5 was overexpressed (FC 1,8; p 0,002) in gluteal adipocyte and a different expression of 10/56 genes involved in irisin pathway (such as lipolysis and browning processes) was found (p 0,0003). Our data suggest that irisin plays a role in metabolic adaptation following acute immobilization and could be the response of healthy muscle tissue against the detrimental effects of inactivity. This underlines the importance of irisin in the muscle-adipose tissue crosstalk to maintain the global homeostasis.

USE OF PROTON PUMP INHIBITORS AND RISK OF CARDIOVASCULAR EVENTS IN LOMBARDY

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The study aimed to evaluate the risk of hospitalization for cardio/cerebrovascular events in a cohort of incident PPIs users. A nested case-control study was carried out, using the healthcare utilization databases of the Italian Lombardy Region. All residents with age >=18 and <=70 years with a first prescription of PPIs from 2003/01/01 until 2007/12/31 were selected. The observation period ranged from the index date (first-prescription date, ID) to the event, death or migration, or to the end of follow-up (2010/12/31). Patient with antithrombotic therapy or hospitalization for any cardio/cerebrovascular events during 3 years before the ID, or less than 6-month-follow-up were excluded. For each case, defined by hospitalization for non-haemorrhagic cardio/cerebrovascular event, up-to-five controls were matched by gender, age and ID. Exposure was estimated as recency (time between last prescription and event date, defining current, recent and past users) and as number of days covered (based on defined daily doses). Logistic regression was used to model the outcome risk associated with the exposure. In our cohort, 23,803 cases and 119,006 controls (males 66.2%; mean age 59.2 years) were identified. Cases had a significantly higher prevalence of diabetes, hypertension and hypercholesterolemia. In the adjusted multivariate regression analysis, risk of event was significantly higher for current (OR 1.746, 95%IC 1.639-1.860) and recent users (1.072; 1.009-1.139) compared to past users.
Results. Were confirmed by the stratified analysis for cardiovascular (current: 1.880; 1.746-2.024) and cerebrovascular events (current: 1.444; 1.281-1.626). The increased risk was also associated with a longer exposure (>60 days) and comorbidity, while there were no significant differences between PPIs.Current and persistent PPI use is associated with an increased risk of cardio/cerebrovascular events. This could call into question the beneficial benefit/risk ratio traditionally attributed to PPIs; further studies are needed to validate these evidence and to clarify the underlying mechanism of action.

GENETIC SUSCEPTIBILITY FOR ANEURYSMAL DISEASE: ROLE OF LRP1 RS1466535, ZNF335 RS3827066 AND LDLR RS6511720 GENE POLYMORPHISMS

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Introduction. A genome wide association study (GWAS) evaluated the possible correlation of 535,296 polymorphisms with the abdominal aortic aneurism (AAA); the rs1466535 polymorphism in the low density lipoprotein receptor-related protein gene (LRP1) was associated as a risk factor, both in the internal replication study, and in a replication study conducted by our group. Further analysis showed other genetic variants associated with AAA including the rs3827066 polymorphism of the ZNF335 gene (zinc finger protein 335) and rs6511720 of the LDLR gene (low density lipoprotein receptor). Previous studies carried out on murine models have shown that the LRP1 knock-out in cells of the smooth musculature leads to vascular pathologies very similar to Marfan syndrome (MFS), triggering the development of aneurysmal disease.The aim of the study was to evaluate whether these three variants rs1466535, rs3827066 and rs6511720 are specific AAA susceptibility factors or if they are related to thoracic aneurysmal disease in MFS patients.
Materials and Methods. We used GeneCatcher on Freedom Evo platform (Tecan) for genomic DNA extraction from peripheral blood and genotyping analysis was performed by real time PCR technology with TaqMan probes specific for the three polymorphisms. We enrolled 225 patients with MFS referred to the Regional Reference Center for Marfan Syndrome and Related Diseases and 536 control subjects. Statistical analyzes were performed using the SPSS v.19 software (SPSS Inc, Chicago, IL, USA).
Results. Polymorphisms are in Hardy-Weinberg equilibrium both in patients and controls. Traditional cardiovascular risk factors analysis highlights an higher prevalence of dyslipidemia, diabetes, smoking and high blood pressure in controls than in patients with MFS (34.9% vs 13.2%, p<0.0001; 5.6% vs 0%, p=0.001; 44.6% vs 17.9%, p<0.0001; 24.4% vs 17.7%, p=0.052). The genotype distribution analysis highlights a prevalence of homozygous TT for the polymorphism rs1466535 LRP1 in controls than in patients (8.1% vs 4%, p=0.042), suggesting a possible protective role. About rs3827066 ZNF335, the T allele carriers (CT+TT) had an higher prevalence in MFS patients compared to controls (28.9% vs 20.6%, p=0.025), suggesting a possible role as a risk factor. The genotype distribution of the LDLR rs6511720 polymorphism did not differ statistically between cases and controls (p=0.407). At multivariate logistic regression (adjusted for traditional cardiovascular risk factors), the rs3827066 and the rs1466535 did not remain statistically significant [rs3827066: OR=0.70 (95% CI 0.39 to 1.22), p=0.215 and rs1466535: OR=0.72 (95% CI 0.26 to 2.01), p=0.536]. Furthermore these three polymorphisms were not significant determinants of the diameters of the thoracic aortic root in MFS patients.
Conclusions. rs14665365 polymorphisms of the LRP1 gene, rs3827066 polymorphism of the ZNF335 gene and rs6511720 of the LDLR gene previously associated with AAA do not represent significant and independent determinants of thoracic aneurysmal disease in MFS patients.

UPDATE OF GENETIC SCREENING IN PATIENTS SUFFERING FROM FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction. Familial Hypercholesterolemia (FH) is a common autosomal dominant disorder characterized by high levels of total and LDL cholesterol, associated with increased cardiovascular risk. The LDLR gene is mainly involved in FH while APOB and PCSK9 genes are involved in a lower percentage of cases. Early identification and treatment of FH patients can be useful to prevent cardiovascular accidents. We aim to reanalyse data about mutation screening in the whole FH population.
Patients and Methods. We studied 585 subjects with clinically diagnosis of FH, of whom 437 were unrelated. The promoter and exon 18 of the LDLR gene, part of exons 26 and 29 of the APOB gene and all exons of the PCSK9 gene were amplified by PCR and directly sequenced. MLPA was performed to identify large rearrangements in the LDLR gene.
Results. The genetic screening revealed mutations in 334 unrelated FH patients, of whom 326 have mutation in LDLR gene, 6 in PCSK9 gene, 2 in APOB gene; in particular we found 14 patients compound heterozygotes and 4 patients homozygotes for mutations in LDLR gene. The homozygous or compound heterozygous patients show a dramatic increase of LDL cholesterol, but we also observed higher levels of LDL cholesterol in patients with a null mutation (splicing, nonsense, duplication and deletion) respect to patients with missense mutation. Finally we observed that HDL cholesterol levels gradually decreased from patients homozygous or compound heterozygous patients, to heterozygous patients with a null mutation, to heterozygous patients with missense mutations. All these difference are statistically significant with a p<0.001.
Conclusions. Our screening revealed a mutation in 76.4% of patients and LDLR is the most frequently mutated gene. In addition to the LDL, HDL emerges as a marker of FH severity. The molecular diagnosis results in an effective instrument to detect affected patients for early diagnosis.

OPENLABEL ODYSSEY APPRISE STUDY: INTERIM DATA FROM THE FIRST 843 PARTICIPANTS

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Background. PCSK9 inhibitors have been recommended as an option by the ESC/EAS Task Force in very highrisk subjects with atherosclerotic cardiovascular (CV) disease and those with HeFH with persistent high LDLC levels despite maximally tolerated statin and ezetimibe therapies.
Objective and Methods. ODYSSEY APPRISE (NCT02476006) was an open label, single arm study, conducted in a real life setting in 16 European countries and Canada. The study population included subjects with inadequately controlled lipid levels, despite maximally tolerated statins with or without additional lipidlowering therapies, excluding PCSK9 inhibitors. Based on physician's judgement, participants received either ALI 75 or 150 mg every 2 weeks (Q2W). Study endpoints included efficacy at Week (W)12 and safety.
Results. This analysis included the first 843 (out of 955) participants treated. At baseline, 57.1% of participants were receiving high intensity statin (atorvastatin 40 or 80 mg, rosuvastatin 20 or 40 mg or simvastatin 80 mg), and 55.2% reported a history of statin tolerability issues, resulting in change of statin type or lowering of statin dose. Mean baseline LDLC was 4.75±1.59 mmol/L (183.6±61.4 mg/dL). Based on clinical judgement, ALI was initially prescribed at a 75 mg or remainder received 150. LDLC levels changed from baseline to W12 by -54.1% in participants with HeFH and -58.8% in participants without HeFH. Treatment emergent adverse events were reported in 54.2% of participants, with nasopharyngitis (5.1%), myalgia (4.4%), injection site (IS) reactions (3.8%), asthenia (3.1%), diarrhoea (2.7%), IS erythema (2.4%), fatigue (2.3%), IS haematoma (2.1%) and back pain (2.0%) being the most common.
Conclusions. In a real life setting, significant LDLC reductions were observed with ALI 75/150 mg Q2W in participants at high CV risk with or without HeFH. ALI was generally well tolerated. These findings are consistent with other results reported in the ODYSSEY Phase 3 programme. Funding Acknowledgements: Sanofi and Regeneron Pharmaceuticals, Inc.

TYPE III HYPERLIPOPROTEINEMIA IN A GROUP OF OUTPATIENTS WITH MIXED HYPERLIPIDEMIA

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Background. Familial dysbetalipoproteinemia (FD) also known as type III hyperlipoproteinemia (OMIM 617347) is a genetic disorder of lipoprotein metabolism characterized by mixed hyperlipidemia, remnant accumulation and increased risk for premature cardiovascular disease. FD is an autosomal recessive disease, caused by mutations in the apolipoprotein E gene (APOE). Most familial dysbetalipoproteinemia patients are homozygous for apolipoprotein ε2, which is associated with decreased binding of apolipoprotein E to the LDL receptor. Among people carriers of an ε2ε2 genotype, 15% develops familial dysbetalipoproteinemia, which becomes evident through secondary risk factors, such as obesity and insulin resistance, that facilitate the development of FD by inhibiting remnant clearance and degradating the heparan sulfate proteoglycan receptor.
Objective and Methods. The aim of this study was to evaluate the APOE genotype in a cohort of 87 patients presenting with mixed hyperlipidemia at the Lipid Clinic in Palermo. More, among these subjects, we have analyzed those with a prevalent hypertrigliceridemia, transmitted as a dominant trait within a family, to identify the causal mutation of the candidate genes of dominant forms of primary hypertriglyceridemia by direct sequencing. Anthropometric measures, clinical and biochemical parameters, life style (smoker and/or alcohol habits) and cardiovascular outcomes were evaluated.
Results. We have identified the ε2/ε2 genotype in three probands. The phenotypic characterization of these subjects revealed a wide variety in clinical presentation. FD facilitating factors, such as insulin resistance and/or obesity and/or smoking habits conditioned the clinical presentation such as fluctuations of plasmatic triglycerides or the presence of cardiovascular events among siblings. Moreover, the sequencing of CREB3L3 gene led to the discovery of a known missense mutation in one patient with a prevalent phenotype characterized by hypertriglyceridemia.
Conclusions. We identify three patients carriers of ε2/ε2 genotype. The phenotypic expression of FD is greatly affected by clinical and genetic factors.

NEPHRO VASCULAR ATHEROSCLEROTIC HYPERTENSION: THE CHANGING CASUISTRY

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Arterial Hypertension (AH) is associated with type 2 Diabetes (D2) with a very high frequency, is considered essential in itself, and not investigated in pathogenesis. Nephro vascular hypertension (NVH) is a rare condition of secondary hypertension (<1%), pathogenetically caused by mono or bilateral artery stenosis, which causes increased secretion of Renine. Resistant arterial hypertension is a not infrequent condition (10% of essential hypertension), of which the Italian Society of Arterial Hypertension proposes the following definition: "Resistant arterial hypertension is a clinical condition characterized by a persistent failure to reach the normal clinical blood pressure values (blood pressure of 140/90 mmHg in the general population of patients with high blood pressure and heart pressure of 30/80 mmHg in patients with diabetes mellitus or nephropathy) as a result of the documented implementation of lifestyle changes and the use of the best possible therapy, including the combination of at least three classes of synergistic and optimal dosage antihypertensive drugs, one of which must be a full dosage diuretic". In personal case studies, starting from 20/09/1976, we have identified 28 cases of resistant hypertension (diagnosed with the criteria of the time), which we studied with various methods: sequential kidney scintigraphy, ECOcolor Doppler, angio RMN, arteriography. Seven of these were affected by NRV. Six of these were D2 patients, had renal artery stenosis >60%, with an average disease duration of 9.7 years, one with bilateral stenosis, 5 with ECG ischemic signs,

all with carotid stenosis >45%, LDL cholesterol calculated average 148 m/dl, 5 with newly onset IRC, 5 had aortic arterial aneurysms in various. None of the patients had received hypolypemytizer treatment before our assessment. UKPDS has shown that Insulin secretion (I) progressively declines in D2, starting from the seventh year prior to diagnosis, a situation that is inevitably associated with quantitative and qualitative alterations in the lipid structure of D2. It is known that LDL cholesterol is the main pathogenetic factor for CV diseases in primitive dyslipidemia; even in secondary forms it has a decisive role, or as an important cofactor, documented by trials with hypolypemizing drugs in primary and secondary prevention. Illuminating the CARDS study: treatment in primary prevention with statin, documenting, at the same time as the other pathogenetic factors, statistically significant decrease of the cerebri Ictus, a disease that does not recognize in the fundamental pathogenetic factor hypercholesterolemia. The re-evaluation of this case study documents a 25% prevalence of atherosclerotic NVH, which is to be suspected in the presence of RF, or aggravated by therapy with ACE-I, rapidly fattening onset of RF, unexplained hypopotassiemia. Reflecting on this case study, the lack of treatment of dyslipidemia as a severe aggravating factor of AS stenosis is highlighted. It is therefore the mandatory to aggressively treat secondary dyslipidemia of diabetes from onset, if possible also in pre-diabetic conditions.

ENDOTHELIAL BIOMARKERS IN ISCHEMIC STROKE PATIENTS TREATED WITH TPA THROMBOLYSIS: THE MAGIC STUDY

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Background. Several experimental and clinical studies have evidenced that the endothelial dysfunction is a strong predictor of ischemic stroke. However, the effect of tPA administration on endothelial biomarkers were not extensively evaluated. **Methods.** Blood was taken at baseline (B) and 24 hours after tPA from 327 patients (mean age 68, mean NIHSS 11). In the MAGIC patients the circulating antigen levels of VEGF, ICAM-1 and VCAM-1 were determined using Bio-Plex suspension array system, whereas vWF circulating levels were measured by immunoturbidimetry. B and post-tPA vWF were analyzed according to SICH, stroke OCSP subtypes and 3-month mRs.
Results. vWF levels at B were significantly higher in females than in males [F 184 (121-219)%, M 139(104-204)%, p=0.005], and correlated with age (r=0.203, p<0.001). Thrombolytic treatment was associated with a reduction of VEGF circulating levels [B: 104.4 (53.4-198.0) pg/ml; 24 h after tPA: 87.2 (37.8-150.2) pg/mL], whereas no effect on the other endothelial biomarkers was observed. B vWF, ICAM-1, VCAM-1 and VEGF levels did not differ between patients with and without SICH and death. Post-tPA vWF levels and delta ([24 hours post tPA-pre tPA]/pre tPA] VEGF values were significantly higher in patients with SICH than without [215(131-316)%, 170(98-214)%, p=0.008; delta VEGF: 0.08 (-0.003-0.38 vs -0.12 (-0.47-0.19, p<0.01]. In TACS patients we found significant higher levels B vWF levels than PACS [182(123-223)%, 149 (104-208)%, p<0.01] and higher pre-post tPA variation of VEGF levels [-0.002 (-0.30 -0.24 vs -0-016 (0.50- 0.18). Patients with mRS>2 had significantly higher B vWF than those 0-2 [185(126-223)%, 187(88-228)%, p=0.004]. At logistic regression analysis, after adjustment for major clinical determinants of outcomes, post-tPA vWF and delta VEGF did not remain a significant and independent determinant of SICH. No effect of B vWF levels on stroke subtypes or mRS>2 was also detected.
Conclusions. Our data suggest that endothelial dysfunction may contribute to the pathophysiological mechanism of ischemic stroke and poor outcomes after tPA, suggesting a detrimental role of the endothelial dysfunction in this clinical setting.

PREDICTORS OF ADVERSE CARDIOVASCULAR EVENTS IN THE START-ANTIPLATELETS REGISTER

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Background. Treatment of acute coronary syndromes (ACS) has been modified in recent years by the introduction of more potent P2Y12 inhibitors, such as prasugrel and ticagrelor. European Guidelines recommend to treat ACS patients with novel antiplatelets as first choice. In the real world, few data are available on the adherence to these recommendations, and on the follow-up of ACS in terms of thrombotic and bleeding complications with respect to antithrombotic therapy.
Methods. The Start-Antiplatelets, a multicenter, observational, prospective, non-interventional register enrolled patients who met predefined eligibility criteria (ACS). On the Register web-site, patients' data, including treatment, underlying conditions andtype of antiplatelet, are collected. A 6- and 12-month follow-up was performed.
Results. 1154 patients were registered (844 M/age: 66.4±13y). Diagnosis included: 561 STEMI (48.6%); 425 NSTEMI (36.8%) and 168 UA (14.6%). 956 (82.8%) patients underwent PCI, of whom 896 with stenting (857 DES); 185 (16%) were medically treated and 32 (2.8%) underwent coronary artery bypass graft surgery. At discharge physicians prescribed in less than 8% ACS patients a monotherapy (aspirin or clopidogrel). The dual antiplatelet treatment most prescribed was aspirin+ticagrelor (45.4%) and aspirin+clopidogrel (32.5%). Eight hundreds and thirty-four (72.3%) patients completed the follow-up (250±126 days). Sixty-two (7.4%) died; 37 (4.4%) experienced cardiovascular death (6.7% on monoterapy, 7.7% on clopidogrel+aspirin; 2.4% on prasugrel+aspirin; 2.4% on ticagrelor+aspirin). Ninenteen (2.3%) patients experienced major bleeding (8 intracranial: 3 on monoterapy, 2 on clopidogrel+aspirin; 1 on prasugrel+aspirin and 2 on ticagrelor+aspirin). The event-free survival curves for cardiovascular death and hemorrhagic complications according to the antiplatelet treatment groups and to the duration of antiplatelet therapies were performed. There was a significant difference in survival times among the treatment groups (log

rank test P<0.01) and among the different duration of antiplatelet treatment (log rank test P<0.01). By Cox regression multivariate analyses, after adjustment for several confounders, in addition to age, diabetes, ejection fraction<40%, and renal insufficiency, the duration of dual antiplatelet treatment was independently associated with total, cardiovascular mortality (HR for each SD=0.18; 95% CI, 0.12-0.27; p<0.010 and HR for each SD=0.15; 95% CI, 0.08-0.25; p<0.010) and major bleedings (HR for each SD=0.36; 95% CI, 0.19-0.67; p<0.010); (hazard ratio, 1.49; 95% CI, 1.08-2.05; P=.02) and with cardiac mortality (hazardratio, 1.81; 95% CI, 1.18-2.76; P=.006).
Conclusions. Results from the START-ANTIPLATELETS Register allow us to enhance our understanding of the risk-benefits of the different antithrombotic strategies in Italian ACS patients, by providing information on predictors of thrombotic and bleeding complications in the real world.

EFFECT OF 8-WEEK HEMPSEED OIL SUPPLEMENTATION ON LIPID PROFILE AND FATTY ACID COMPOSITION OF ERYTHROCYTES IN CHILDREN AND ADOLESCENT WITH PRIMARY HYPERLIPIDEMIA

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Children affected by primary hyperlipidemia have a high risk to develop cardiovascular diseases (CVDs) during adulthood. Dietary supplements could represent a potential strategy in the management of hyperlipidemia, particularly the positive effects of polyunsaturated fatty acids (PUFA) intake on lipid profile and CVD risk has been demonstrated. In this context, the effectiveness of hempseed oil (HSO) rich in PUFA [linoleic acid (LA) and α-linolenic acid (ALA)] in the modulation of hyperlipidemia has been poorly investigated. The aim of the study was to evaluate the effects of HSO supplementation on serum lipid profile and fatty acid (FA) composition of red blood cells (RBCs) in children and adolescents affected by primary hyperlipidemia.36 patients (6-15 ys) were enrolled in a 8-week dietary intervention study. They were randomized into 2 groups: 1- HSO group, receiving 3 g of HSO providing 1.4 g of LA and 0,7 g/die of ALA; 2- control group, receiving only the dietary guidelines. Blood samples were collected at baseline and after the intervention and serum lipid profile, RBCs FA composition and omega-3-index were analyzed. HSO supplementation significantly reduced the RBCs content of total saturated and monounsaturated FA (-5.02±7.94% and -2.12±2.23%, respectively, p>0.01), while increased the levels of total n-3 and n-6 PUFA (+1.57±1.96% and +5.39±7.18%, respectively) and omega-3 index (+1.18±1.42%) compared to control group. No significant effects were found for the serum lipid profile. In conclusion, HSO supplementation was able to improve the overall FA composition of RBC phospholipids by increasing the levels of total PUFA, n-3 and n-6 PUFA subclasses and n-3/n-6 PUFA ratio and significantly reducing RBC levels of SFA and MUFA. Further studies are necessary for a complete comprehension of the effects of HSO in the modulation of hyperlipidemia and CVD risk on this and other target population.

STUDY OF THE PATHOPHYSIOLOGY AND OUTCOMES IN ACUTE ISCHEMIC STROKE: GLOBAL TRANSCRIPTOMICS OF THROMBI OBTAINED DURING INTERVENTIONAL THROMBECTOMY

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Background. Acute ischemic stroke (AIS) is one of the leading causes of death and physical disability worldwide. AIS typically results from thrombotic or thromboembolic occlusion of a cerebral artery. Differentiation of underlying causes of AIS is highly relevant to prevent recurrent stroke. Rapid restoration of cerebral blood supply by intravenous thrombolysis and, more recently, by mechanical thrombectomy (MT) is currently the mainstay of AIS therapy. Timely and effective recanalization of occlusive thrombi or clots in such arteries may be influenced by a number of underlying factors, including clot composition and type of occluded vessel. The introduction of stent retrievers allows for a complete extraction and analysis of thrombi. The analysis (e.g histology, transcriptomics) of clots after removal may provide valuable information about underlying pathophysiology. Therefore, aim of this study was to identify specific features that might help to distinguish causes of ischemic stroke and/or determinants of clinical outcomes by investigating the global gene expression profile of RNA extracted from thrombus and from venous peripheral blood in patients with AIS undergoing interventional thrombectomy.
Method. Consecutive AIS patients undergoing endovascular treatment were collected (n=92) and followed-up for at least a 3 month. For all patients detailed clinical, imaging and biological data are available. Blood samples were collected before and 24 h after thrombectomy in tubes with anticoagulants (plasma EDTA and citrated), as well as in tubes without anticoagulant (serum) and for liquid for RNA stabilization (PAX tubes). The thrombus obtained during thrombectomy was collected in RNA later. RNA was extracted by PAX gene blood miRNA kit. The global gene expression was assessed by Affymetrix technology using GeneChip Human Trascriptome 2.0 Array allowing the analysis of 44,699 genes, >285,000 full-length transcripts coverage.
Results. In this abstract, we reported the results of the analysis of the first 10 global gene expression profiles obtained from thrombi collected during thrombectomy procedure. After data processing and application of the filtering criteria, the average of analyzable probe sets of the 10 patient thrombi numbered 2,250 out of about 245,000 coding transcripts and 40,000 non-coding transcripts. One thousand five hundred out of 2,250 probe sets identified annotated genes. Interestingly, among expressed genes in thrombi, we observed metalloproteinases 11 and 24 (MMP11 and MMP24), and interferon regulatory factor 8 and 4 (IRF8, IFR4) previously involved in activation of neural stem cells (NSCs) under physiological and regenerative conditions, synapse reorganization after traumatic brain injury and neuronal response to cerebral ischaemic-reperfusion injury. Gene onthology analysis showed that, besides the alteration of biological processes and molecular functions (related for example to regulation of apoptosis and cell death and catabolic process) - probably more transversal to various cell types in cerebral thrombi of the 10 patients investigated - the 1,500 expressed genes are involved in

biological processes and molecular function and pathways related to: cellular response to stress; neuron differentiation, development, organization, and maturation; cerebral cortex development.
Conclusion. Preliminary results of our study suggest that thrombus global gene expression profiling a promising resource to investigate causes of ischemic stroke and/or determinants of clinical outcomes.

ATHEROGENIC LIPOPROTEIN SUBFRACTIONS AND CAROTID ATHEROSCLEROSIS IN MENOPAUSAL WOMEN WITH LOW LDL-CHOLESTEROL

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Introduction. Levels of low-density lipoprotein cholesterol (LDL-c) are declining in the population with increasing adherence to healthy lifestyle and statin therapy. However, cardiovascular disease (CVD) events remain prevalent among subjects with low or normal LDL-c, a phenomenon referred to as residual risk. It is possible that residual lipid and lipoprotein risk pathways are likely to emerge as increasingly important determinants of CVD in individuals with low LDL-c.
Methods. To evaluate the association between atherogenic lipoprotein subfraction and carotid atherosclerosis, we studied 71 post-menopausal women (not taking statins) who participated to "Progetto Atena" and had low LDL-c (<130 mg/dL). They underwent anadvanced lipoprotein profiling, using the Lipoprint® system and a standardized ultrasound testing of carotid arteries.
Results. Very-low-density lipoprotein cholesterol (VLDL-c) had a statistically significant linear association with carotid intima media thickness (IMT) ($r2=0.29$; $p<0.001$), that remained significant after adjustment for age, smoking, systolic blood pressure, glucose and body mass index ($p<0.001$). A significant association was found between carotid IMT and LDL-c ($p<0.02$, after adjustment for main cardiovascular risk factors), while no association was detected between IMT and other lipid parameters. In conclusion, among post-menopausal women with low concentrations of LDL-c, the cholesterol carried in VLDL lipoproteins was strongly associated with carotid atherosclerosis. VLDL-c may represent new clinical targets for risk prediction and potential therapeutic intervention in the prevention of atherosclerotic CVD, in particular among women with low levels of LDL-c.

HELPER-DEPENDENT ADENOVIRUS-MEDIATED GENE TRANSFER OF A SECRETED LDL RECEPTOR/TRANSFERRIN CHIMERIC PROTEIN REDUCES AORTIC ATHEROSCLEROSIS IN LDL RECEPTOR-DEFICIENT MICE

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Familial hypercholesterolemia (FH) is a genetic hyperlipidemia characterized by elevated concentration of plasma LDL cholesterol. Statins are not effective in all patients whose prognosis is still quite poor. In the past, we have developed safe and effective gene therapy strategies for the expression of anti-atherogenic proteins using helper-dependent adenoviral (HD-Ad) vectors. We recently developed a HD-Ad vector for the expression of a secreted chimeric protein containing the extracellular portion of the human LDL receptor (LDLR) fused with a transferrin dimer (LDLR-TF). We evaluated the efficacy of LDLR-TF in CHOLDLA7, a cell line deficient for LDLR, in which we restored cell ability to uptake LDL; subsequently, we administered intravenously 1X10E13 vp/kg of the vector expressing LDLR-TF in LDLR-deficient mice and observed a reduction of total and LDL cholesterol and, consequently, of aortic atherosclerosis. Finally, we evaluated the biodistribution of this transgene with fluorescently-labelled LDL and observed accumulation in liver, intestine and heart after fluorescence molecular tomography analysis. We are currently evaluating the possibility of producing the LDLR-TF fusion protein using different route as the intramuscular route of administration to collect the preclinical evidences that will allow the development of a therapeutic strategy for FH patients.

CLOSE ASSOCIATION BETWEEN CAROTID AND CORONARY ATHEROSCLEROSIS ANALYZED THROUGH SYNTAX SCORE

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Background. This study analyzed the association between carotid and coronary atherosclerosis, particularly in terms of severity as well as the extent of the disease.
Methods. We conducted a study involving 478 patients (admitted to our Cardiology Unit during 2004-2014) with atherosclerotic disease, who underwent both carotid Doppler ultrasound and coronary angiography. Sex, age and conventional cardiovascular risk factors were considered. Syntax Score was used to grade the complexity of coronary disease.
Results. In our study, a clear association between carotid and coronary disease was proven: 68,2% of the population showed atherosclerosis in both carotid and coronary arteries ($p<0,0001$). The absence of carotid atherosclerosis was predominantly associated with angiographically normal coronary arteries (37,6%) rather

than a single-vessel disease (22,8%), a two-vessel disease (21,8%) or a three-vessel disease (17,8%). When carotid atherosclerosis was present a normal coronary angiography was uncommon (13,5%), while the detection of a single vessel disease was more frequent (17,2%), a two-vessel disease was even more frequent (27,6%) and the highest rate described related to a three-vessel disease (41,6%). The thickness of carotid plaque was significantly correlated with the number of diseased coronary vessels (p<0,007) and there was a significant correlation between the thickness of carotid plaque and severity of coronary disease analyzed through Syntax Score (p<0,033).
Conclusions. There is a strong correlation between carotid and coronary atherosclerosis in terms of extent and severity. It is fundamental to consider a systemic approach to atherosclerosis to obtain an adequate stratification of patients with cardiovascular risk factors, an appropriate therapeutic management and reduce the incidence of adverse events, improving the quality of life and prolonging survival.

MATRIX METALLOPROTEINASES AND CORONARY ARTERY ECTASIA

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Background. Coronary artery ectasia (CAE) is the abnormal dilatation of one or more coronary segments. Although the pathogenetic mechanisms underlying CAE are largely unknown, it seems that some of the extracellular matrix degrading enzymes such as matrix metalloproteinases (MMPs) can play an important role in the development of this condition. The aim of our study was to evaluate the relationship between MMP-9 and the development of CAE as well as the role played by the inflammatory process in this condition.
Materials and Methods. In our retrospective study we have enrolled a series of 79 patients: 47 presented, on coronary angiography, focal or diffuse ectasia of coronary arteries (cases), 32 had a free coronary tree or without hemodynamically significant stenosis (controls). All patients were subjected to blood sampling for the dosage of MMP-9 and C-reactive protein (CRP).
Results. In our study we found that the most involved coronary artery in CAE was the right coronary artery (87% in diffuse CAE and 66% in focal CAE). Serum levels of MMP-9 were higher in the patients with CAE compared with those without (5.011 mcg/ml vs. 3.93 mcg/ml, p=0,07) although statistic significance was not reached. We also found a statistically significant increase in serum levels of MMP-9 in direct proportion to the number of vessels involved in the ectatic process (p=0.025). Serum CRP levels were higher in patients with CAE (5.12 mg/dL vs 2.18 mg/dl, p=0.4) although not statistically significant and there was a direct,statistically significant, relationship between serum CRP levels and number of involved vessels (p=0.001).
Conclusions. We found a possible relationship between elevated levels of MMP-9 and CAE, as well as the relationship between levels of MMP-9 and severity of the CAE. Furthermore we found a possible relationship between elevated CRP levels and CAE and there was a directly proportional relationship between the increase of CRP and extension of the CAE.

PRECLINICAL IMPAIRMENT OF MYOCARDIAL FUNCTION AND ENDOTHELIAL VASCULAR MARKERS IN DRUG-NAÏVE PSORIATIC AND RHEUMATOID ARTHRITIS: ASSOCIATION WITH VITAMIN D LEVELS AND INFLAMMATION

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Background. Chronic inflammatory joint diseases (IJD) share increased risk of cardiovascular morbidity and mortality. Several tools have been developed to estimate the preclinical vascular and cardiac damage. Among them, arterial and ventricular stiffness indices have been extensively studied in different conditions of increased cardiovascular risk. Moreover, Vitamin D status has been postulated as potential marker or causative factor in the pathophysiology of the cardiovascular damage in IJD patients. The link between vitamin D status, inflammatory status, and cardiovascular damage development is to date not completely defined.
Aims. In the present study, we aimed to evaluate whether different degrees of inflammation and vitamin D levels may be associated to different levels of cardiovascular involvement. For this purpose, we selected 56 naïve patients suffering from IJD (27 from rheumatoid arthritis - RA - and 29 from psoriatic arthritis - PsA), analyzing several clinical, biochemical, and mechanical parameters, including lipid profile, CD34+ cells, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) pulse wave velocity (PWV) left ventricular global longitudinal and circumferential strain (GLS and GCS, respectively), vitamin D plasma levels. Patients should to not have assumed steroids, immunosuppressant drugs, DMARDs, or NSAIDs, nor Vitamin D supplementation. Forty-seven healthy subjects have been selected as control subjects.
Results. CD34+ count and vitamin D levels were lower in IJD patients as compared to controls, while fibrinogen, CRP, PWV and cIMT were higher in IJD patients. IJD patients had significantly lower GLS (mean±S.D. -16.11±2.89% vs -19.15±1.9%, respectively; p=0.05), GCS (mean±S.D. -14.21±2.7% vs -20.22±4.13%, respectively; p<0.01) versus control group. We found a correlation between GLS and/or GCS and disease-related risk factors. Vitamin D levels was found to correlate with ejection fraction, GCS, GLS, CD34+, diseases activity markers, and fibrinogen levels. Furthermore, IJD patients with higher disease activity score have showed worse GLS and GCS values as compared with patients with low diseases activity.
Conclusion. Subclinical impaired myocardial deformation and endothelial dysfunction are common in patients with IJD even in absence of clinical evidence for CVD. Higher disease activity was associated to a greater subclinical cardiovascular damage. Furthermore, Vitamin D is confirmed as novel candidate player in the endothelial homeostasis and myocardial function. Further studies on larger sample sizes could clarify whether a supplementation of Vitamin D could modify PHCs levels, inflammatory indices, myocardial function and arterial stiffness in patients affected by IJD, therefore contributing to reduce cardiovascular risk in this patients.

ADIPOSE TISSUE AND PCSK9: DO LEPTIN AND RESISTIN PLAY A ROLE?

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Background. Adipose tissue is an endocrine organ secreting active molecules, namely, adipokines. In a condition of dysfunctional visceral fat depots, adipokines may be detrimental for the cardiovascular system. The present study aimed at evaluating some of the molecular mechanisms beyond the activation of adipokine's pathway (ie, leptin and resistin) and the expression of proprotein convertase subtilisin/kexin type 9 (PCSK9) - the key regulator of low-density lipoprotein receptor.
Materials. HepG2 cells; silencing RNA (siRNA); qPCR; WB; PCSK9 promoter luciferase reporter assay and ELISA.
Results. In HepG2 cells, 48-h treatment with leptin and resistin induced PCSK9 gene expression by +195% and +250%, respectively, and PCSK9 promoter activity (+26% and +20%, respectively) via the involvement of Sterol Regulatory Element (SRE) motif. Indeed, a mutation in HNF-1 motif did not alter leptin- and resistin-driven luciferase activity. For the above described experiments, simvastatin (20 μM) was used as a positive control. Leptin raised PCSK9 secretion by 15%. In a clinical setting, a positive association between circulating leptin and PCSK9 levels (p=0.03) was found. The relationship among adipokines, PCSK9 expression and the mandatory STAT3 involvement, the latter being an important modulator in the inflammatory response, has been confirmed by knocking-down of STAT3; a phenomenon that abolished leptin- and resistin-induced expression of PCSK9 mRNA. Apolipoprotein (apo)B and microsomal triglycerides transfer protein (MTP) mRNA were increased by leptin (+57% and +60%, respectively) and resistin (+50% for both); these effects were dependent on PCSK9, as demonstrated using siRNA anti-PCSK9. As a further confirmation, a significant increment and reduction of apoB and MTP were observed in response to PCSK9 overexpression and knock-down, respectively.
Conclusions. We found a relationship between PCSK9 expression and the adipokines, leptin and resistin. The molecular basis beyond these findings requires the involvement of STAT3 pathway and the activation of SRE motif at PCSK9 promoter level.

ENVIRONMENTAL FACTORS AND SEVERE HYPERTRIGLYCERIDEMIA LEADING TO ACUTE PANCREATITIS: A CASE REPORT

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Introduction. Severe hypertriglyceridemia is due to mutations of several genes involved in enzymatic lipolytic cascade, such as LPL, ApoA5 and ApoC2. It is involved not only in cardiovascular disease, but also an other complications, especially recurrent pancreatitis.
Case report. A 47-years-old woman affected by hypertriglyceridemia and type II diabetes reported recurrent pancreatitis attacks. Two rare variants of uncertain significance were found in two different genes at double heterozygous status: LPL p.Val442Gly (c.1325T>G), APOA5 p.ALA315Val (c.944 C>T). At medical examination severe hyperlipidemia and hyperglycemia were detected (TG 2478 mg/dl, HDL 23 mg/dl, TC 420 mg/dl GLU 224 mg/dl). She was in therapy with phenofibrate 145 mg/die, N3FA 4 gr/die, simvastatin 20 mg/die, ezetimibe 10 mg/die and insulin. One year later, after further dietary counselling, and fat modified diet with MCT oil and physical aerobic activity, a significant improvement in lipid and glycemic profile (TG 491 mg/dl; HDL 27 mg/dl; TC 155 mg/dl; GLU 145 mg/dl) was detected.
Conclusion. These data demonstrate a crucial role of environmental factors in developing severe hypertriglyceridemia, leading to acute pancreatitis in patients with variants of uncertain significance.

NONALCOHOLIC FATTY LIVER DISEASE IS INDEPENDENTLY ASSOCIATED WITH AN INCREASED RISK OF CARDIAC CONDUCTION DEFECTS IN HOSPITALIZED PATIENTS WITH TYPE 2 DIABETES

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Background. Recent studies have suggested that nonalcoholic fatty liver disease (NAFLD) is associated with an increased risk of cardiac tachyarrhythmias (mainly atrial fibrillation) in patients with and without type 2 diabetes. The aim of this study was to assess whether an association also exists between NAFLD and conduction defects.
Materials and Methods. We retrospectively evaluated a hospital-based cohort of 751 patients with type 2 diabetes discharged from our Division of Endocrinology during years 2007-2014. Standard electrocardiograms were performed on all patients. NAFLD was diagnosed by ultrasonography. The fibrosis (FIB)-4 score was used to identify advanced liver fibrosis.
Results. Overall, 524 (69.8%) patients had NAFLD and 202 (26.9%) had conduction defects (defined as at least one block among first-degree atrio-ventricular block, second-degree block, third-degree block, left bundle brunch block, right bundle brunch block, left anterior hemiblock or left posterior hemiblock) on electrocardiograms. Patients with NAFLD had a greater prevalence of conduction defects than those without NAFLD (31.3% vs 16.7%, P<0.0001); this prevalence was particularly increased in NAFLD patients with high FIB-4 score. NAFLD was associated with an increased risk of conduction defects (adjusted-odds ratio 2.79, 95%CI 1.72-4.55) even after adjusting for age, sex, hypertension, hemoglobin A1c, microvascular complication status, prior ischemic heart disease, medication use and other potential confounders.

Conclusions. This is the first observational study to show that NAFLD and its severity are strongly associated with an approximately 2.8-fold higher risk of conduction defects in hospitalized patients with type 2 diabetes, independent of multiple risk factors and diabetes-related variables.

BRAIN HDL-MEDIATED CHOLESTEROL TRANSPORT IS IMPARED IN ALZHEIMER'S DISEASE

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Introduction. Brain cholesterol is transported by lipoproteins with size and density similar to plasma HDL, that have been identified in human cerebrospinal fluid (CSF), mainly contain apolipoprotein E (apoE). Brain HDL particles interact with membrane transporters such as ABCA1 and ABCG1 expressed in astrocytes and promote the efflux of cholesterol towards the neurons. The objective of the present study is to establish whether the brain HDL-mediated cholesterol transport is defective in AD.
Methods. CSF from patients with dementia related to AD (n=38), dementia not related to AD (non-AD DEM, n=16) and subjects without dementia (non-DEM, n=38) as controls, were collected by lumbar puncture. Total apoE and apoE4 levels in CSF were measured by ELISA. CSF HDL cholesterol efflux capacity was assessed by cell-based radioisotopic techniques.
Results. AD patients showed the typical neurobiomarker pattern, with decreased CSF Aβ42 and increased tau and of phospho-tau protein. CSF apoE concentrations were similar between the three groups (p=0.074), while apoE4 levels were higher in AD patients compared to controls (+4.5 fold, p<0.0001). CSF-CEC through the transporters ABCA1 and ABCG1 was markedly reduced in AD patients compared to controls (-53%, p=0.014 and -31%, p=0.009, respectively), while non-AD DEM did not differ from controls. Considering all subjects together, we detected a direct correlation between both ABCA1-and ABCG1-CEC and Aβ42 (r2=0.058; p=0.019; r2=0.064, p=0.015 for ABCA1 and ABCG1, respectively). In addition, ABCA1-CEC inversely associated with tau and phospho-tau (r2=0.115, p=0.001; r2=0.126, p=0.015).
Conclusions. Despite no change in total apoE levels, possibly reflecting HDL concentrations, an impairment of CSF capacity to promote cholesterol efflux specifically occurs in AD. These results reinforce the idea that alterations of brain cholesterol transport play an important role in AD pathogenesis and may be a potential novel pharmacological target.

STATINS FOR TREATING HETEROZIGOUS FAMILIAL HYPERCHOLESTEROLEMIA IN CHILDREN: A MONOCENTRIC EXPERIENCE

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Background. Familial Hypercholesterolemia (FH) is a genetic condition that causes lifelong elevations in LDL-Cholesterol (LDL-C). There is increasing evidence that the administration of statins early in life is beneficial in preventing or reducing atherosclerosis in subjects with FH. Nevertheless, there are still few studies in literature that evaluate the long-term efficacy of statin therapy in children and adolescents. The aim of our study is to evaluate the response to statin therapy in pediatric patients with Heterozygous Familial hypercholesterolemia (HeFH).
Materials and Methods. We retrospectively collected data from 51 patients affected by HeFH following regular follow up (every 6 months) with at least three controls after start of statins. Total Cholesterol (TC) and LDL-C levels (LDL-C) were obtained at "Bambino Gesù Children's Hospital" laboratory in the fasting state. The primary outcome was to establish how many patients are able to reach LDL-C ≤130 mg/dL.
Results. A total of 51 patients (23 male, 28 female, median age 12.5±6.53 DS, median 12.5 years old) were included in this study and followed up to 2 years. The mean values of TC and LDL-C at baseline were respectively 270.35 mg/dl ±47.93 DS and 208.6 mg/dl ±50.41 DS.Twenty-seven patients (52.9%) achieved the therapeutic goals after 6 months of treatment. Furthermore the difference of the averages of TC and LDL-C at baseline and at follow-up visits is statistically significant (p<0.0001) at 6, 12 and 24 months. No subjects discontinued therapy because of adverse events.
Conclusions. We observed that statin administration led to substantial reductions in LDL-C in more than half patients. The reductions in LDL-C reached maximal levels at months 6 and then remained at this level throughout the follow-up period.

THE DETECTION OF FAMILIAL IPERCOLESTEROLEMIA IN A CLINICAL SETTING: A SINGLE CENTER 3-YEAR EXPERIENCE IN THE LIPIGEN STUDY

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Introduction. Familial hypercholesterolemia (FH) is a genetic disorder of lipid metabolism characterized by high levels of LDL-C and increased risk of premature atherosclerosis. Despite the availability of reliable clinical criteria and laboratory methods for the identification of FH, this condition is largely undiagnosed in the routine clinical setting. Within the LIPIGEN Project, our Center since 2013 performed genetic investigation in subjects with clinical diagnosis of FH. The aim of this report is to describe the spectrum of mutations so far identified.
Materials and Methods. From October 2013 to June 2016, we enrolled 251 patients (124 males and 127 females) with clinically sus-

pected FH (DLCN score ≥3). Patients underwent complete clinical evaluation and blood sampling for searching FH-associated mutations. Next Generation Sequencing method was used to search mutations in candidate genes (LDLR, APOB, PCSK9 and LDLRAP1).
Results. Pathogenic mutations have been identified in 184 patients (73.3%) while 67 patients (27.3%) did not show any variants in candidate genes. One hundred seventy-seven (96%) were heterozygous carriers of LDLR variants, one was compound heterozygote (c.1567G>A, p.Val523Met; c.1775G>A, p.Gly592Glu). We found mutations in the LDLRAP1 gene in 3 subjects at heterozygous state and at homozygous state in one young woman (c.89-1G>C). Two heterozygous patients were identified for two rare variants in PCSK9 (c.137G>T, p.Arg46Leu) and APOB (c.10580G>A, p.Arg3500Gln) genes, respectively. In addition, we found a double heterozygous patient with a nonsense mutation on the LDLR gene (c.97C>T, p.Gln33*) and a missense mutation on the PCSK9 gene (c.137G>T, p.Arg46Leu). Within the LDLR gene we identified 30 different missense mutations (the most frequent were: c.662A>G, (p.Asp221Gly) c.2054C>T (p.Pro685Leu) and c.1775G>A (p.Gly592Glu) with frequency of 17%, 18% and 14% respectively); 5 non-sense mutations; 9 frame-shift mutations, 8 variants involving splicing sites, 4 mutations in the promoter region, 9 gene rearrangements and 2 mutations with insertion or deletion in frames. Within the LDLRAP1 gene, 2 frame shift mutations, 1 heterozygous missense mutation and 1 splicing mutation in homozygous state (c.89-1G> C) were detected. 89% of patients with DLCN score ≥8, 82% of subjects with 6-7 DLCN score and 49% of patients with 3-5 DLCN score had at least one causative mutation in one of FH-causing candidate genes.
Conclusion. This preliminary analysis demonstrated that the detection rate of FH-causing mutations our selected population is comparable to that reported in previous investigations. In addition, it confirmed that also in our cohort mutations in the LDLR was the most frequent cause of FH. Moreover, even though DLCN score >8 is highly predictive of the presence of molecularly confirmed FH, the molecular screening is of crucial importance for the confirmation of this condition also for lower DLCN score values.

ASPIRIN IN PRIMARY CARDIOVASCULAR DISEASE PREVENTION IN TYPE-2 DIABETIC PATIENTS: A LONGITUDINAL OBSERVATIONAL STUDY

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Background. Cardiovascular disease is the leading cause of death in type-2 diabetic patients. An intensive and global approach, to control all cardiovascular risk factors, reduce this kind of events and is recommended by Standards of Care. The use of aspirin (ASA) in primary prevention in type 2 diabetes remains debated.
Objective. The purpose of this observational longitudinal study was to evaluate the impact on all causes mortality of ASA, in primary prevention, in a cohort of type-2 diabetic patients.
Materials and Methods. Our study population is composed of 1917 diabetic patients attending the hospital-based Diabetes Clinic of Ferrara with a first evaluation in the period 1996-2006. Median follow up period was 10 years. Exclusion criteria: presence of overt cardiovascular disease. Cox proportional hazard analysis was performed including age, sex, satisfaction of goals for HbA1c, blood pressure, LDL-c, HDL-c and triglycerides, hypolipemic, antihypertensive and antidiabetic treatment and use of ASA (100 mg).
Results. Multivariate analysis shows that use of ASA is significantly associated with all-cause mortality (HR: 0.49; 95%CI: 0.27-0.89), together with age (HR: 1.09; 95%CI: 1.06-1.12), sex (HR: 2.40; 95%CI: 1.47-3.91) and satisfaction of HbA1c target (HR: 0.62; 95%CI: 0.39-0.97).
Conclusion. Our study show that the use of ASA in primary prevention is associated with a reduction in all-cause mortality among type-2 diabetic patients, independently from other risk factors. Our findings suggest that use of ASA in primary prevention should be recommended in type 2 diabetic patient, especially when other cardiovascular risk factors are present.

IMPACT OF LDL-R ON LYMPHOCYTES T CELL DIFFERENTIATION AND FUNCTION

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Aim. Expansion of T cells of the effector memory phenotype is known to correlate with atherosclerosis progression. Here we investigate whether LDL-receptor (LDL-R), a key player linking systemic and cellular lipid metabolism, affects the differentiation and functionality of T cells.
Methods. T cell subsets phenotyping, gene expression analysis, and functional characterization were performed by flow cytometry and qPCR following in vitro and in vivo stimulation.
Results. LDL-R KO T cells, into secondary lymphoid organs, shown no differences in T cells subsets compared to WT mice under resting conditions. In vitro, after stimulation with antiCD3/antiCD28 and MLR assay, LDL-R KO CD8+ T cells proliferation was reduced compared to WT ($p<0.05$, $p<0.01$); this finding was associated with a decreased IFNg production ($p<0.01$). *In vivo*, following antigen-specific immune cell stimulation (vaccination with Ovalbumin), LDL-R KO CD8+ T cells proliferated to a lower extent compared to WT ($p<0.01$). Furthermore LDL-R KO CD8+ T cells presented a reduction of IFNg, IL13 and Perforin ($p<0.001$, $p<0.01$, $p<0.05$). To evaluate if the reduced proliferation was the consequence of a impaired cholesterol uptake, which is foundamental for T cells expansion, we investigated the expression of keys genes involved in cholesterol metabolism in relation to the cellular lipidome. In LDL-R KO CD3+ T cells, HMGCoA-R expression was increased, while ABCA1 and ABCG1 expression was decreased compared to the WT, a profile associated with increase of cholesterol intermediate synthesis, as desmosterol ($p<0.05$). These results suggest that T cells try to compensate the reduced cholesterol uptake promoting his biosynthesis. As alterations in cholesterol metabolism might lead to a defective TCR signaling, we tested akt phosphorylation (a kinase downstream of TCR) and observed a reduction in the phosphorylation of LDL-R KO CD3+ T cells compared to WT after stimulation with antiCD3/antiCD28.
Conclusions. Our data suggest that LDL-R play an important role in immunometabolic responses in lymphocytes.

CLINICAL PREDICTORS OF A POSITIVE GENETIC TEST IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Background. There is no consensus on which are the best clinical predictors to refer patients with clinical suspicion of familial hypercholesterolemia (FH) for genetic testing. We aimed to identify clinical-laboratory parameters associated with the detection of an FH-causing mutation and to assess the performance of Dutch Lipid Clinic Network (DLCN) score and other parameters in detecting FH-causing mutations.
Patients and Methods. We enrolled 101 consecutive patients with clinical suspicion of FH, in which clinical and laboratory parameters and a conclusive result of genetic testing were available.
Results. 61 patients were mutation positive. Mutation positive patients more frequently had a familial history of LDL-hypercholesterolemia and familial cases of corneal arcus/xanthomas than mutation negative patients. There were no significant differences with respect to personal history of early cardio-cerebrovascular disease and clinically-detected corneal arcus/xanthomas between mutation positive and negative patients. Conversely, mutation positive patients showed significantly higher baseline LDL cholesterol (LDL-C) levels and DLCN score than mutation negative patients. AUROC of baseline LDL-C for predicting a positive genetic test was 0.754, IC95% 0.660-0.848. The Youden index allowed to identify LDL-C levels >225 mg/dl as the best cut-off value with sensitivity of 64%, specificity of 90%, positive predictive value (PPV) of 91% and negative predictive value (NPV) of 62%. DLCN score showed similar performance to LDL-C as predictor of FH-causing mutations (0.719, IC95% 0.619-0.819). DLCN score >5 and >8 showed sensitivity of 74% and 49%, specificity of 63% and 80%, PPV of 75% and 79%, NPV of 61% and 51%, respectively.
Conclusions. DLCN score seem to be feasible for the identification of mutation positive patients. However, LDL-C levels >225 mg/dl also show good performance in detecting FH-causing mutations, and may be used as a sole criterion to refer patients for genetic testing, when all the clinical variables required for DLCN score are unavailable.

ACHILLES TENDON ULTRASONOGRAPHY MAY PREDICT A POSITIVE GENETIC TEST IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Background. Achilles tendon (AT) xanthomas, specific for familial hypercholesterolemia (FH), may be clinically undetectable. We aimed to evaluate the usefulness of AT ultrasonography in the identification of individuals with an FH-causing mutation.
Patients and Methods. 65 consecutive patients with clinical suspicion of FH, in which clinical and laboratory parameters and a conclusive result of genetic testing were available, were submitted to bilateral AT ultrasonography with measurements of thickness and evaluation of tendon structure (abnormal structure and xanthomas).
Results. 39 patients (60%) were mutation positive. AT thickness was significantly and positively associated with age, male gender, weight, LDL cholesterol (LDL-C), Dutch Lipid Clinic Network (DLCN) score and FH severity. However, AT thickness was not significantly correlated with the result of genetic testing. 36 patients showed a pathologic finding at AT ultrasonography: 6 patients had abnormal structure, 9 patients had xanthomas and 21 patients had both abnormal structure and xanthomas. A pathologic finding at AT ultrasonography was significantly and positively associated with AT thickness, LDL-C and DLCN score, but not with gender and weight. Moreover, patients with a pathologic finding at AT ultrasonography were more frequently detected with an FH-causing mutation (37.9% vs 77.8%, p=0.001). Among pathologic findings at AT ultrasonography, only xanthomas (40% vs 83.3%, p<0.001) but not abnormal structure (52.6% vs 70.4%, p=0.150) were significantly associated with the result of genetic testing. The performance of DLCN score calculated after inclusion of ultrasonography-diagnosed AT xanthomas was significantly better than the performance of standard DLCN calculated with clinically-diagnosed AT xanthomas in detecting FH-causing mutations (AUROCs 0.782, 0.667-0.896 vs 0.686, 0.557-0.815).
Conclusions. AT ultrasonography may be a useful tool for the evaluation of patients with suspected FH. The sonographic visualization of tendon xanthomas, rather than tendon abnormal structure or tendon enlargement, may significantly improve the detection rate of FH-causing mutations.

MULTILEVEL MODELS TO ESTIMATE STANDARD INTIMA-MEDIA THICKNESS CURVES TO CHARACTERIZE THE INDIVIDUAL CARDIOVASCULAR RISK

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Objective. Taking into account the Intima-media thickness (IMT) growth patterns over time and the main cardiovascular (CV) risk factors, this study aims to estimate standard IMT curves that could contribute in defining the individual CV risk.
Design and Setting. Among subjects enrolled in the PLIC study, the growth of IMT across the study period (12 years) was assessed by use of individual growth curve modeling within multilevel models. A 2-level model was performed, with subjects at level 2 and visits at level 1. Only subjects with all the four planned visits were eligible for this study. All patients with a diagnosis of diabetes or a missing value for left or right IMT measurements were excluded from the sample. A total of 1277 participants have been included for the analysis.
Results. Equations for the left and the right IMT side, based on PLIC cohort characteristics, have been estimated. Both left and right side showed a strong linear increase in mean IMT with age, but the slope of the growth curve was bigger in the left side than

in the right side, suggesting a faster increase of the mean IMT in the left side. We observed an interaction between age and sex on the growth of IMT, suggesting that males and females did not develop IMT with similar patterns. Gender, blood pressure and LDL cholesterol levels were associated with mean IMT in both sides.
Conclusion. Our findings provide standard IMT curves that may help to define the individual cardiovascular risk. The faster increase in mean left-side IMT with age indicates that left-side IMT might be a better screen indicator of atherosclerosis. These results underscore the need for studies to consider the effect of IMT growth on clinical endpoint.

AN UNEXPLAINED CASE OF PERSISTENLY ELEVATED GGT: A LYSOSOMIAL LIPASE ACID DEFICIENCY

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Clinical signs of lysosomial lipase acid deficiency (LAL-D) in infants may arise during pregnancy with foetal ascites and the hallmarks of the disease, prominent hepatosplenomegaly, diarrhoea and vomiting, can result in malabsorption, growth and liver failure. The natural history of LAL-D in children and adults is less welldefined and detection of the disease is often incidental. Hepatomegaly is observed in most patients, but only a few number may have slight increase or, rarely, normal levels of serum transaminase and the diagnosis may be confused with more common disorders such as steatohepatitis, metabolic syndrome, non-alcoholic fatty liver disease. Elevated GGT levels are detected less commonly, with 20% of patients having values >40 U/L at baseline. Moreover, adults with LAL-D show a complex disease presentation with premature atherosclerosis, elevated LDL cholesterol and triglyceride levels plus decreased HDL cholesterol. We report a case of a 59-year-old Caucasian man affected by dyslipidaemia since he was 23. At the age of 39 a 4-fold increase of AST, ALT, GGT occurred with hepatic steatosis and cholestasis; viral and other causes of hepatitis were excluded and a liver biopsy showed hepatic fibrosis. Despite of diet, lipid lowering drugs and ursodeoxycholic acid therapy AST, ALT, ALP and GGT resulted 2-fold normal values. The maximum value of GGT and ALP was respectively 954 and 356, so that the patient had to stop every medication with a reduction, but not normalization of these serum variables. An abdomen TC scan confirmed hepatosplenomegaly. Enzyme determination showed LAL activity's reduction of 27%, which did not require replacement therapy, but supportive approach. In conclusion, the impairment of LAL activity in adult obese patients with metabolic syndrome might explain persistent hepatomegaly and elevated transaminase levels of unknown cause, not corrected by reduction in BMI or medical therapy.

A CASE OF HIGH PLASMA LP(A) ASSOCIATED WITH A MULTISITE ATHEROSCLEROTIC DISEASE AND FAILURE OF REVASCULARIZATION PROCEDURE

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The simultaneous presence of atherosclerotic lesions in at least two major vascular sites is defined "multisite" artery disease characterized by high prevalence in general population. Despite a variety of endovascular techniques to treat peripheral atherosclerotic disease, restenosis remains a common occurrence with a rate of 50%. We report a case of 58 years old Caucasian menopausal woman without metabolic syndrome presenting occlusion of right subclavian artery at the age of 48.
At the age of 52 despite of both lipid lowering diet and atorvastatin-ezetimibe association treatment, antiplatelet therapy plus smoking cessation she had stenosis of left subclavian artery treated with PTA and stenting, in the same year she presented intermittent claudication, due to a stenosis of left superficial femoral artery (SFA).
She underwent PTA and stenting of SFA in 2011 at the age of 53, thereafter she suffered from three episodes of intrastent restenosis treated with PTA and stenting, the first one in 2012 regardless antiplatelet therapy, the second one in January 2013 when she was on single antiplatelet plus anticoagulant therapy, and the last one during dual antiplatelet therapy plus oral anticoagulation. Every attempt of SFA revascularization failed and at present her maximal walking distance is less than 100 m. Based on the atypical site of atherosclerotic lesions and the frequency of the restenosis, screening tests for thrombophilic and rheumatologic disease were performed with negative findings. Considering the association between Lp(a) and atherosclerosis in menopausal women without metabolic syndrome in the area of Naples, we measured Lp(a) which resulted 69.3 mg/dl, a value near to the 90th percentile in menopausal women. In conclusion, we suggest that high concentration of Lp(a) contributes to explain premature and extensive atherosclerotic cardiovascular disease in this woman without metabolic syndrome.

A NEW CYP27A1 FRAMESHIFT VARIANT CAUSATIVE OF CEREBROTENDINOUS XANTHOMATOSIS

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Introduction. Cerebrotendinous xanthomatosis (CTX) is an autosomal recessive disease caused by mutations in the CYP27A1 gene encoding the mitochondrial sterol 27-hydroxylase enzyme. A defect of this enzyme leads to increased levels of cholestanol that accumulate in the body and cause juvenile cataracts, intellectual disability, neurological features, tendon xanthomas, and chronic diarrhoea.
Materials and Methods. The patient is a 28 years old women with a history of cognitive regression, bilateral juvenile cataracts, chronic diarrhea, but no evidence of xanthomas. The sterols were extracted from plasma by a liquid-liquid procedure and trimethylsilyl derivatives were analyzed by gas chromatography coupled to flame ionization detector (GC-FID) and by mass spectrometry (GC-MS). Genetic screening was performed by amplification and direct sequencing of the promoter and the 9 exons with intronic junctions of the CYP27A1 gene.
Results. Ataxo-spastic syndrome, facies with open mouth and protruded tongue and disinhibited behaviors were observed; Wechsler Adult Intelligence Scale - Revised Test score was <45 (rv 90-109). The magnetic resonance imaging showed brain and cerebellar atrophy with focal cerebellar white matter. Based on the suspect of CTX the plasma sterol profiles was performed showing elevated levels of cholestanol (6.75 mg/dL; r.v. <0.5) and cholesterol precursors, such as zymostenol (4.39 mg/dL; r.v. <0.05), 7-dehydrocholesterol (2.12 mg/dL; r.v. <0.19), and lathosterol (1.78 mg/dL; r.v. <0.25).Genetic screening revealed the presence of the new variant c.850_854delinsCTC in the CYP27A1 gene at homozygous status. The variant consists in the replacement of 5 nucleotide with 3 different ones leading to the removal of 2 base pair and then to a frameshift p.(Lys284Leufs*3) that produce a totally not functional enzyme. The presence of the variant at heterozygosis was ascertained in both parents which consanguinity cannot be excluded since they come from 2 nearby town. Treatment with Chenodeoxycholic acid 250 mg three times daily was started.
Conclusions. We identify a patient suffering from a very rare disease that could be treated with chenodeoxycholic acid to prevent clinical complications. The identified variant is a new null variant that could have a high prevalence in the Campania suggesting to perform a screening for cholestanol levels to early identify CTX patients.

LIPID-LOWERING THERAPY APPROACH AND ACHIEVEMENT OF TREATMENT LIPID TARGETS IN PATIENTS REFERRED TO THE PADUA LIPID CLINIC

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Aim. To evaluate clinical parameters, lipid profile, lipid-lowering therapy and the achievement of lipid treatment targets in 327 patients referred to the University of Padova Lipid Clinic.
Subjects and Methods. Anthropometric data, cardiovascular risk factors, familial dyslipidemia clinical features, carotid and cardiovascular disease, pre- and post-treatment lipid profile, CK, AST, ALT, type of statin or other lipid-lowering treatment, achievement of LDL-Cholesterol and non-HDL-Cholesterol targets were analyzed in 189 females and 138 males (171 <65 and 156 ≥65 year old).
Results. Females were older than males (61±15 vs 55±15, p<0.05), and presented higher levels of total-C (285±54 vs 267±58, p<0.05 vs males), HDL-C (67±20 vs 47±19, p<0.05) and LDL-C (190±46 vs 172 ±54, p<0.05). Subjects aged ≥65 showed higher prevalence of hypertension, diabetes, carotid stenosis and cardiovascular disease, higher levels of total-C, LDL-C, non-HDL-C, transaminases and CK. In both genders, lipid-lowering therapies reduced total-C (males 202±40 vs 265±61, p<0.05; females 210±36 vs 286±52, p<0.05; post vs pre), LDL-C (males 121±33 vs 171±58, p<0.05; females 123±31 vs 189±49, p<0.05), non-HDL-C (males 152±42 vs 221±59, p<0.05; females 145±36 vs 222±53, p<0.05) and triglycerides (males 171±111 vs 335±305, p<0.05; females 128±123 vs 171±137, p<0.05); in males we observed an increase in HDL-C (50±17 vs 46±18, p<0.05). The LDL-C target was achieved in: 41.8% of patients in primary prevention; 18.8% in secondary prevention; in patients <65 years, 33.3% in primary prevention and 20% in secondary prevention reached LDL-C target, while among patients ≥65 years 51.6% in primary prevention and 18.2% in secondary prevention were at LDL-C target. Non-HDL-C targets were reached in 16.7% of patients in secondary prevention (20% of subjects <65 years, and 15.4% ≥65 years).
Conclusions. The effectiveness of a lipid-lowering treatment was demonstrated in both genders and age groups, without safety problems; however, achievement of LDL-C and non-HDL-C targets was clinically inadequate.

CORRELATION BETWEEN BLOOD LEVELS OF S100B PROTEIN AND INCIDENCE OF HEART FAILURE OR IMA IN A CARDIOLOGY UNIT

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Background/Introduction. Since the first nineties is has been demonstrated that serum S100B levels were increased in the pathogenesis of heart disease and involved their pathogenetic mechanisms. By the years it was also noticed that after myocardial infarction both mRNA and levels of S100B protein were up-regulated. S100B by interacting with RAGE is able to start an inflammatory response with an increase of adhesion molecules and inflammatory cytokines, leading to another cardiovascular diseases, atherogenesis. On this basis, Cai et al. investigated whether or not S100B it was a associated with stable angina and acute coronary syndrome obtaining a significant correlation. In fact S100B mRNA levels were increased in large and small infarct areas. Li et Al. dosed S100B serum levels in patients affected by heart failure in order to understand if this protein could be a reliable biomarker in the studied disease. In this study S100B was increased in failing hearts but even more higher in serum of patients that had also chronic kidneys disease. Their work included also the analysis of other disease markers such as hsCRP, TNF-alpha, NT-proBNP levels together with echocardiographic assessment. What emerged was that serum S100B was an independent risk factor for chronic heart failure and for major cardiac events. Data also underlined that higher levels of this alarmin were correlated with

a worse prognosis. Differently from the already known heart disease biomarkers, S100B is a tissue-specific protein (chondrocytes, adipocytes, skeletal myofibers, cardiomyocytes, dendritic cells, etc.); it was largely demonstrated to be released after a damage and the consequent remodelling involving cardiac tissue. The obvious consequence is its increase in plasma suggesting S100B to be a more specific marker of heart disease, more than already known biomarkers Purpuse Chronic heart failure is one of the most common consequence of myocardial infarction, and is characterized by a reduction of the heart ability to face peripheral blood distribution. Chronic heart failure (HF) and myocardial infarction (IMA) are often associated to the augmentation of inflammation markers. S100B is an alarmin secreted by damaged cardiomyocites. We examined the correlation between S100B protein serum levels and the incidence of acute heart failure and IMA in symptomatic patients.
Methods. We conducted a prospective study on 90 patients aged between 50 and 72 years accepted to our Unit referring cardiac associated symptoms (thorax pain, dyspnea, arrhythmic symptoms). They were divided in three groups: healthy subjects (group A), chronic heart failure patients (group B) and IMA patients (group C). CRP, NT-proBNP, and routine exam were performed in every patient. Moreover it was made a S100B dosage was made.
Results. Results demonstrated different levels among healthy subjects. However in chronic heart failure patients the alarmin levels were higher but not significantly augmented. Instead AMI patients had mean values of S100B doubled than the other two groups and significantly augmented.
Conclusions. According to our data S100B as the potential to be, in a near future, be considered as an acute myocardial infaction marker in addiction to the ones existing. However more studies are needed to identify possible byas elements in S100B serum dosage. This together with other elements are guiding us to a better understand of micro-structural changes in damaged heart in order to consider new therapeutic targets.

PLASMA LP(A) LEVELS STRONGLY CORRELATE WITH CARDIOVASCULAR DISEASE BEYOND LDL-CHOLESTEROL IN AN ITALIAN COHORT OF HETEROZYGOUS FH

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Heterozygous familial hypercholesterolemia (HeFH) is a heritable condition characterized by severely elevated LDL cholesterol (LDL-C) and increased cardiovascular risk. Since LDL-C is not always sufficient in determining the risk of occurrence of cardiovascular events, other factors should be considered. The aim of the present study was to investigate the association of lipoprotein (a) [Lp(a)] with the FH phenotype and the role of Lp(a) in determining CV risk among patients with HeFH. One hundred twenty one Italian patients with a genetic diagnosis of HeFH (LDLR, APOB, PCSK9) were included for the analysis. Detailed biochemical profile, anthropometric variables and cardiovascular history were collected. Smoking status and the presence of concomitant diseases were also recorded. Lp(a) was measured by immunoturbidimetric assay and subjects were stratified according to the presence or absence of cardiovascular disease. Unadjusted LDL-C was higher in subject with positive history of CV event, compared to those without (364.8±107.1 vs 306.4±80.4; p=0.036), however when LDL-C was corrected for Lp(a)-cholesterol this difference was lost (p=0.075). Mean Lp(a) concentration was significantly higher in HeFH with presence of CV disease compared to those without (52.2±47.1 mg/dL vs 29.3±33.9 mg/dL; p=0.009), also when adjusted for age, sex, plasma lipids and other cardiovascular risk factors. Lp(a) levels showed a strong positive association with the presence of cardiovascular disease (R=0.250; p=0.004). No association was found instead between Lp(a) levels and the type of cardiovascular events (myocardial infarction, stroke, peripheral arterial disease). In addition HeFH carriers were tested for two LPA single-nucleotide polymorphisms (SNPs) rs10455872 and rs3798220. Our findings strongly suggest that measurement of Lp(a) could provide additional information to identify FH at the highest risk of cardiovascular events beyond LDL-C.

TREATMENT WITH FIBRATES IS ASSOCIATED WITH HIGHER LYSOSOMAL ACID LIPASE ACTIVITY IN DYSLIPIDEMIC PATIENTS

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Lysosomal acid lipase (LAL) is responsible for the hydrolysis of cholesteryl esters (CE) and triglycerides (TG) within the lysosomes; generated cholesterol and free fatty acids (FFA) are released in the cytosol where they can regulate their own synthesis and metabolism. When LAL is not active, as in case of genetic mutations, CE and TG accumulate in the lysosomal compartment, while the lack of release of cholesterol and FFA in the cytosol leads to an upregulation of their synthesis and VLDL secretion. Thus, LAL plays a central role in the intracellular homeostasis of lipids. Since there are no indications about the effect of different lipid-lowering agents on LAL activity, aim of the study was to address the relationship between LAL activity and the type of lipid-lowering therapy in a cohort of dyslipidemic patients followed at our lipid clinics.LAL activity was measured on DBS from 120 patients with hypercholesterolemia or mixed dyslipidemia. Among collected biochemical/anthropometric variables, LAL activity negatively correlated with LDL-cholesterol levels. Ninety-one patients were taking one or more lipid-lowering drugs, as statins, fibrates, ezetimibe and omega-3. When patients were divided according to the type of lipid-lowering treatment, three main treatment categories were identified on the base of similar LAL activity: patients untreated or taking only omega-3, patients taking statins with or without ezetimibe or omega-3 and patients taking fibrates with or without statins or omega-3. LAL activity was significantly different

between the three groups, with patients taking fibrates showing the highest average activity. LAL activity was significantly different between the treatment groups also when adjusted for sex, age, BMI, lipid parameters, liver function and statin use.Thus, the use of fibrates is independently associated with higher LAL activity in dyslipidemic patients, suggesting that the positive effects of PPAR-alpha activation on cellular lipid homeostasis can also include an improved LAL activity.

CARDIOVASCULAR RISK IN PATIENTS WITH TYPE 2 DIABETES MELLITUS: ROLE OF THE SPECIFIC DETERMINANTS OF DISEASE SEVERITY

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Introduction. Diabetes mellitus represents an important cardiovascular and coronary risk factor, and is currently considered a coronary disease equivalent regardless of the degree of severity and metabolic control (1). In many regards, however, this seems to represent an oversimplification (2). Several algorithms have been devised to estimate cardiovascular risk, most of which non-specific for diabetics and hardly applicable to Mediterranean countries (3, 4). Aim of this study was to investigate the variables associated with cardiovascular risk in a population of type 2 diabetes in the Emilia Romagna area.
Methods. We longitudinally analyzed the documentation of Diabetes Clinic patients, aged 35-65 without previous events, in the period 1991-2001. The occurrence of cardiovascular events in the subsequent 10 years was recorded and correlated with the main risk factors, including a number of variables specific for diabetes; standard statistical analysis was performed.
Results. 3629 patients were eligible (2071 males, 1558 females). 546 of these (15.0%) presented an event at 10 years. According to Kaplan-Meier survival curves, the variables statistically associated with the occurrence of cardiovascular events were established risk factors such as age, gender, smoking, systolic arterial pressure, HDL-cholesterol, and also specific indicators of diabetes severity like glycated hemoglobin and diabetes duration. Cox multivariate risk modeling yielded similar results.
Conclusions. In diabetic patients, disease severity and the degree of metabolic control should be considered for global risk evaluation and, in perspective, for the definition of treatment targets. Specific risk functions should be designed; the algorithm derived from these data will be utilized for a prospective evaluation of cardiovascular risk in our Regional population.

References
1. Haffner SM, et al. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med. 1998; 339: 229-34.
2. Casaccia Bertoluci M, et al. Cardiovascular risk assessment in patients with diabetes. Diabetol Metab Sy. 2017; 9: 25.
3. Stevens RJ, et al. United Kingdom Prospective Diabetes Study (UKPDS) Group. The UKPDS risk engine: a model for the risk of coronary heart disease in Type II diabetes (UKPDS 56). Clin Sci (Lond). 2001; 101: 671-9.
4. Pellegrini E, et al. Risk for cardiovascular events in an Italian population of patients with type 2 diabetes. Nutr Metab Cardiovasc Dis. 2011; 21: 885-92.
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EZETIMIBE PROTECTS MONOCYTIC CELLS FROM OXIDATIVE STRESS

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Introduction. Elevated serum cholesterol levels and risk of cardiovascular diseases is a well established association demonstrated through multiple epidemiologic studies and randomized controlled trials. The lowering of low-density lipoprotein cholesterol (LDL-C) by statin therapy is the primary target in prevention of cardiovascular morbidity and mortality. Although statin monotherapy is the backbone for LDL-C lowering, for some patients this treatment alone may not be sufficient to achieve optimal LDL-C targets or they have partial or complete intolerance to it: the use of adjuvant therapy with Ezetimibe (Eze) produces an incremental lowering of LDL-C and consequently a reduction in cardiovascular events. Eze is a drug that regulates exogenous dietary cholesterol absorption in the small intestine blocking the transmembrane protein Niemann Pick C1-like 1 (NPC1L1). Recently has been reported its potential role in protecting cells from damages due to intracellular level of reactive oxygen species (ROS) through a ROS-independent antioxidant Nuclear factor-E2-related factor (Nrf2) pathway. Uponr edox perturbation, the transcriptional factor Nrf2 normally dissociates from the Kelch-like ECH-associated protein 1 (Keap1) and translocates into the nucleus where it binds the antioxidant-response element (ARE) sequence and induces the activation of a series of antioxidant enzymes while in the presence of Eze, the activation of AMP-activated protein kinase (AMPK) and, in turn, the phosphorylation of autophagy adaptor protein p62 at Ser351 occur resulting in Keap1 sequestration on autophagy cargos by p62 and the induction of cytoprotective Nrf2 targets. Since oxidative stress (OS) may participate in the pathophysiology of cardiovascular diseases, diabetes mellitus, obesity, liver diseases and cancer, the aim of this study is to evaluate the pharmacological effect of Eze in activating antioxidant Nrf2 pathway and its safety and efficacy in protecting cells from OS.
Materials and Methods. THP-1 monocytic cell line was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin and 1% streptomycin. To evaluate antioxidant transcriptional factor activity, THP-1 cells were pretreated for 6 h and overnight (ON) with 25 µM and 50 µM of Eze. RNA was extracted and reverse transcribed, then real-time RT-PCR was conducted. Moreover, for the ON experimental condition, accumulation of nuclear Nrf2 was investigated and measured using an ELISA-based kit. To examine the effect of Eze on OS-induced ROS accumulation, THP-1 were pretreated for 1h with 50 µM of Eze and incubated for 2 h with 250 µM of palmitic acid (PA) in RPMI 1640 medium supplemented with 1% FBS to recreate a physiological ratio between bound and unbound free fatty acid. 6-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) was

used to detect ROS production and results were analyzed by flow cytometry.
Results. Data suggest the ability of Eze to activate antioxidant genes such as Nrf2 and heme oxygenase-1 (HO-1) in a dose- and time-dependent manner: after 6h Nrf2 was upregulated of 2,3 and 3,2 fold respectively for 25 µM and 50 µM treatment ($p<0,01$), while after ON incubation Nrf2 was upregulated of 3,1 and 3,5 fold respectively for 2 5 µM and 50 µM treatments ($p<0.03$). Being HO-1 a downstream gene, it was upregulated after ON condition of 4,5 and 5,6 fold respectively for 25 µM and 50 µM treatments ($p<0.001$). As a consequence of Eze Nrf2 transactivation into the nucleus, protein abundance enhances from 42,2 pg/µg for basal condition to 65,1±1,6 pg/µg and 75±1,4 pg/µg respectively for 25 µM and 50 µM conditions ($p<0.01$). Furthermore, data suggest the important role of Eze in protecting cells from PA-dependent OS with an average reduction of ROS of 22,2±11,9% ($p<0.04$).
Conclusion. Eze could be able to activate Nrf2 pathway as crucial defence mechanism against oxidative events reducing ROS and restoring intracellular homeostasis in monocytic cells.

CORRELATION BETWEEN ARTERIAL STIFFNESS AND COGNITIVE IMPAIRMENT IN AN ELDERLY POPULATION

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Background. Arterial stiffness is one of the earliest indicators of changes in vascular wall structure and function. Several recent studies have demonstrated the correlation between arterial stiffness and cardiovascular morbidity. Vascular stiffness may play an important role in the development of cognitive impairment, especially in elderly people. Arterial stiffness can be assessed using a various parameters such as intima media thickness (IMT) and pulse wave velocity (PWV).
Objective. The aim of this study was to evaluate the impact of vascular stiffness on cognitive impairment in an elderly population.
Materials and Method. 80 subjects were divided: hypertension group (n=60; mean age 75.8 yrs) and health group without hypertension (n=30, mean age 74.8 yrs). All patients underwent clinic and 24-hour BP measurement, mini mental state examination (MMSE) and carotid doppler ultrasound with Esaote MyLab 60 to measure radio frequency quality intima media thickness (RF QIMT) and PWV. We ruled out patients with diabetes mellitus, dementia, hepatic failure, renal failure, heart failure and pulmonary failure.
Results. In both groups PWV values were significantly related with age. PWV values were significantly higher in hypertensive patients ($p<0.05$). A significantly negative correlation was observed with PWV and MMSE score in both groups. In hypertensive patients PWV was independently and inversely associated with MMSE score after adjusting by age, BMI and RFQIMT ($p<0.005$). RF QIMT showed the same trend, but we found a significantly negative correlation only in hypertensive subjects.
Conclusion. This study confirms a correlation between arterial stiffness and cognitive impairment. The aging and hypertension are implicated in increasing arterial stiffness. Moreover these data support the hypothesis that arterial stiffness may be implicated in the development of cognitive impairment in hypertensive elderly subjects.

LIPID ABNORMALITIES IN PATIENTS ON STATIN THERAPY: THE TREAT STUDY

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Background. Patients receiving statins, despite achieving low plasma LDL cholesterol (LDL-c) concentration, remain exposed to a high risk of major cardiovascular events. This seems to be due to dyslipidemias related to HDL-c and triglycerides (TGs).
Objective. To assess the prevalence of anomalies in lipid profile among patients under chronic statin therapy. METHODS: The TREAT (observational retrospective sTudy on lipid abnoRmalitiEs in pAtients on sTatin therapy) is an observational, retrospective and multicenter study. Data have been collected from the clinical records of patients from 9 lipid, diabetic and cardiology clinics, visited between 2013 and 2015 and treated with statins for at least 2 years. Recorded data comprised complete lipid profile, demographic and lifestyle data, personal and family medical history. Lipid profiles were defined as abnormal following the European Society of Cardiology guidelines based on the individual cardiovascular risk score. Upon completion of data collection, a descriptive analysis has been conducted for characterizing patients enrolled. Lipid goal attainment and examined variables associated with residual dyslipidaemia have been evaluated through logistic linear regression model.
Results. In the cohort, 1216 patients treated with statins, 59.5%, 25.5% and 26.6% did not reach the LDL-c, HDL-c and TGs goals, respectively; 75.4% had an abnormality in at least one of the lipid parameters considered. Among patients at goal for LDL-c levels (40.5%), 25.8% and 75% did not reach the HDL-c and TGs goals, respectively. The logistic models showed that patients with history of cardiovascular events and at least one of the 3 lipid abnormalities had a significantly increased risk of mixed dyslipidemias.
Conclusion. The present study highlights an unmet medical need in the overall lipid profile management; it is necessary to increase the awareness on the importance of evaluating the complete lipid profile, as lipid abnormalities in addition to LDL-c increase the risk of major cardiovascular events.

ANGPTL3 FACILITATES β-ADRENERGIC-DEPENDENT LIPOLYSIS IN ADIPOCYTES: EVIDENCE FROM IN VITRO STUDIES

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Background. Detection of loss-of-function (LOF) mutations in ANGPTL3 gene in humans revealed the existence of a new lipid

phenotype, called familial combined hypolipidemia (FHBL2). ANGPLT3 protein is known to be involved in the inhibitory regulation of extracellular lipases (LPL and HL), so that its absence increases the lipolytic degradation of TG-rich lipoproteins (in particular VLDL) and HDL. Accordingly, the FHBL2 status is characterized by markedly reduced levels of all apoB-containing lipoproteins (VLDL and LDL) and HDL. Interestingly, this condition is also characterized by reduced levels of circulating free fatty acids (FFA). This indicates that ANGPTL3 also plays a role in regulating the metabolism of FFA. As plasma FFAs are mainly derived from the lipolysis of triglycerides in adipose tissue, our primary hypothesis is that ANGPTL3 regulates lipolytic pathways in adipocytes.
Methods. The main aim of present study is to investigate changes in lipolytic pathways induced by the addition of ANGPTL3 to 3T3-L1 cell line, an in vitro model of mature adipocytes. Changes in release of FFA and glycerol as well as in cell expression of genes involved in the canonical and non-canonical activation of lipolytic pathways will be investigated by a combination of biochemical measures, Western blot and RT-qPCR analyses.
Results. Compared to untreated, ANGPTL3-treated cells showed a lower release of FFA (at 30 minutes 126.6 uM vs 21.6 uM, respectively; $p<0.05$) and a markedly skewed FFA to glycerol ratio (<1.5). In contrast, when adipocytes were treated with the β-agonist isoproterenol (ISO), either before or after the addition of ANGPTL3, the release of FFA levels were markedly increased (ISO 499 uM; ANGPTL3 pre-treatment 773 uM, $p<0,03$; ANGPTL3 post-treatment 1676 uM; $p<0.01$). Treatment with ANGPTL3 increased expression of ERK1/2 while the combined treatment with ISO determined raised levels of ERK1/2 activated form (pERK1/2).
Conclusion. Our findings indicated that ANGPTL3 may act by facilitating the β-adrenergic-dependent lipolysis promoting the alternative HSL activation via-ERK1/2 phosphorylation in adipocytes. This mechanism might explain the lower FFA levels in subjects with ANGPTL3 deficiency.

NUTRACEUTICAL-POT IN PATIENTS WITH PERIPHERAL ARTERIAL OCCLUSIVE DISEASE AND STATINS INTOLERANCE: MORE THAN A CHANCE

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Aim. Peripheral Arterial Occlusive Disease is a clinical and very common manifestation of atherosclerosis in occidental countries. The main objective of these patients is the reduction of cholesterol levels (LDL-c). This objective improves claudicatio and reduce heart-associated events. We aimed evaluating the efficacy, the safety and the tolerance of a nutraceutical compound in PAOD patients which, due to an intolerance or to a volunteer refuse, weren't in treatment with any statin. We tested the hypothesis of a possible improvement of exercise performances in claudicatio patients intaking an integrator with multiple anti-atherosclerotic principles.
Methods. LDL-c level and treadmill test performance were evaluated on PAOD patients intaking this nutraceutical product were compared to patients in treatment with a placebo at T0 and at T6. The nutraceutical integrator contained: Omega-3: 651 mg; Monascus Purpureus: 417 mg; Policosanol: 10 mg; CoQ10: 10 mg; Resveratrol: 10 mg; Vitamine B6: 3 mg; Vitamine B12: 2.5 mcg, Folic Acid 300 mcg. 68 patiens were enroled and were divided in 2 groups (A group = nutraceutical, B group = placebo). The study had 3 phases: patient selection, screening part and patient evaluation under blind-treatment. Eligible patients had to be older than 40 years and had suffered from claudicatio for more than 6 months.
Results. A Group patients had a progressive and significative reduction of serum levels of total and LDL cholesterol and triglycerides than B group ($p<0,001$). A Group had also better PWV values than control group. Treadmill performance was significantly improved in patients belonging to A group than B group patients.
Conclusions. PAOD patients well tolerated nutraceutical administration and the treatment resulted being secure and effective. Whether it isn't possible using a statin in PAOD patients, a nutraceutical formulation it has to be considered as a valid alternative.

PCSK9 INDUCES MACROPHAGE POLARIZATION TO M1 PRO-INFLAMMATORY PHENOTYPE

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Intraplaque release of inflammatory cytokines from macrophages is implicated in atherogenesis by inducing the proliferation and migration of media smooth muscle cells (SMCs). PCSK9 is present and released by SMCs within the atherosclerotic plaque but its function is still unknown. In the present study, we tested the hypothesis that PCSK9 could influence macrophage polarization. THP-1-derived macrophages and human primary macrophages were exposed to different concentrations (0.250÷2.5 μg/ml) of human recombinant PCSK9 (hPCSK9). After 24 h incubation with 2.5 μg/ml hPCSK9, a significant induction of IL-1β, IL-6, TNF-α, CXCL2, and MCP1 mRNA, were observed in both cell types. hPCSK9 also significantly increased the release of both IL-6 and TNF-α from human primary macrophages (3.6±1.3 fold and 4.5±0.2 fold, respectively).
Co-culture experiments with HepG2 overexpressing hPCSK9 also showed the induction of TNF-α (2.4±0.5 fold) and IL-1β (8.6±1.8 fold) mRNA in macrophages. The effect of hPCSK9 on TNF-α mRNA in murine LDLR-/- bone marrow macrophages (BMM) was significantly impaired as compared to LDLR+/+ BMM (4.3±1.6 fold vs 31.1±6.1 fold for LDLR-/- and LDLR+/+, respectively). Of interest, a positive correlation between PCSK9 and TNF-α plasma levels of healthy adult subjects (males 533, females 537) was observed (B= 8.73, 95%CI 7.54÷9.93, $p<0.001$). Taken together, the present study provides evidence for a direct influence of PCSK9 on macrophages polarization towards the pro-inflammatory M1 phenotype, mainly dependent by the LDLR.

EFFECTS OF BARIATRIC SURGERY ON INSULIN/GLUCOSE SYSTEM AND MEAL INCRETIN RESPONSE IN OBESE PATIENTS WITH TYPE 2 DIABETES.

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Background and Aim. Bariatric surgery exerts beneficial metabolic effects in obese type 2 diabetes patients (T2DM), but its effects on the insulin/glucose system and the meal incretin response still are matter of active investigation. Aim of this study was to assess the metabolic effects of two bariatric procedures, Roux-en-Y-gastric bypass (RYGBP) and sleeve gastrectomy (SG), in obese T2DM patients.
Material and Methods. 10 (M/F=5/5) obese T2DM patients (mean±SD: age: 54.3±9.8 years, waist: 135±8,8 cm, BMI: 45.2±5.8 kg/m^2, HbA1c: 7.4±2.0%), underwent bariatric surgery (RYGB n=7; SG n=3). All patients performed a standardized 5h-mixed meal test (MMT: 186 Kcal; polenta plus parmesan cheese) at baseline (MMT0), at 1month (MMT1) and at 12 months (MMT12) after surgery to assess:
1. plasma glucose, insulin, C-peptide, total GIP and active GLP-1 concentrations;
2. beta-cell function, estimated by MMT minimal modelling;
3. Insulin-sensitivity expressed as HOMA-IR. Data were analyzed by non-parametric test at 1 and 12-month time point after surgery.

Results. After surgery:
1. BMI and waist circumference significantly decreased at 1- (ΔBMI: -5 kg/m^2 and Δwaist: -10 cm; p<0.01 for both) and 12-months (ΔBMI: -13 kg/m^2 and Δwaist: -31 cm; p<0.001 for both).
2. Both glucose and insulin curves significantly decreased (AUC-glucose by -23% and -32% during MMT1 and MMT12, p=0.007 for both; AUCinsulin by -40% and by -56% during MMT1, p=0.017, and MMT12, p=0.005, respectively);
3. The "workload" of the beta-cell expressed as the total amount of insulin (pmoles) secreted during MMT1 and MMT12 decreased by -14% (p=0.047) and by -47% (p=0.007), respectively;
4. The incretin response, expressed as AUCGLP1, improved by +79% during MMT12 (p=0.037). Finally, HOMA-IR significantly improved after surgery (p=0.005).

Conclusions. In obese T2DM patients, bariatric surgery (both SG and RYGB) exerts beneficial effects on the insulin/glucose system and the incretin response during a MMT already one month after surgery.

METABOLOMICS-IDENTIFIED METABOLITIES ASSOCIATES WITH NON SYNDROMIC AND SYNDROMIC OBESE SUBJECTS

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Introduction. Obesity is the most prevalent nutritional disorder in Western societies. More than three out of ten adults weigh at least 20% in excess of their ideal body weight. Obesity is also considered to be primarily responsible for the rising prevalence of metabolic syndrome which is defined as the coexistence in the same individual of several risk factors for atherosclerosis, including dyslipidemia, hypertension and hyperglycemia. The current study was conducted in order to identify metabolic differences in visceral adipose tissue collected from obese human subjects that were diagnosed with metabolic syndrome, obese individuals that were metabolically healthy and non-obese healthy controls.
Matherial and Methods. Extensive gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry analysis were used to obtain untargeted visceral adipose tissue metabolomics profiles.
Results. indicated a consistent increases in oxidative stress markers with the pathological obese samples as well as subtle markers of elevated glucose levels which may be consistent with metabolic syndrome. In the tissue derived from the pathological obese subjects, there were significantly elevated levels of plasmalogens which may be increased in response to the oxidative changes as well as glycerol-phosphorylcholine, glycerol-phosphorylethanolamine glycerol-phosphorylserine, ceramides and sphingolipids. We believe that these data can be helpful in recognizing new pathways that underlie metabolic-vascular complications of obesity and lead to the development of innovative targeted therapies.

EVALUATION OF RENAL RESISTIVE INDEX IN SARCOIDOSIS, A NEW MARKER OF RENAL MICROCIRCULATION DAMAGE AND ATHEROSCLEROSIS

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Background. Patients affected by sarcoidosis are to be considered at high risk, both for atherosclerosis and for renal failure and cardiovascular events. Renal resistive index (RI) is a simple to realize and important Doppler parameter that could be considered as a new marker of early atherosclerosis and renal microcirculation damage.

Aims. The aim of our study was to assess the significance of renal resistive index (RI) in patients with sarcoidosis.Methods: 15 patients (8 males, 53%, aged 45-79, mean 66 years) suffering from pulmonary sarcoidosis (PS), underwent routine laboratory tests (blood count, glycaemia, transaminases, renal function, lipid profile, serum calcium) and an Echo-color Doppler study of renal arteries and intra-parenchymal vessels. In particular, we assessed the renal resistive index (RI) and compared these values with those obtained from 15 healthy people (control group), matched by gender and age. Exclusion criteria were: presence of diabetes mellitus, anemia, renal failure, hypertension, renal artery stenosis, age >80 years, and heart rate <50 or >100 bpm.
Results. All pulmonary sarcoidosis (PS) patients had abnormal values of renal resistive index (RI), but normal serum creatinine and eGFR values and other ultrasound parameters were similar between the two groups and within normal limits; these findings may indicate the presence of early microvascular damage.
Conclusion. Echo-color Doppler ultrasound is already well-known as a useful tool for early systemic atherosclerosis diagnosis and renal resistive index (RI) is an important Doppler parameter for the evaluation of renal microcirculation. Thus, our findings show that Echo-color Doppler can be considered as the most important examination to detect and monitor early renal damage in sarcoidosis patients.

EFFICACY AND SAFETY OF A NUTRACEUTICAL COMBINATION CONTAINING THE PROBIOTIC BIFIDOBACTERIUM LONGUM BB536 IN PATIENTS WITH MODERATE HYPERCHOLESTEROLEMIA: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY

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Introduction. Specific probiotics, like the Bifidobacterium longum BB536, showing high biliary salt hydrolase activity, may contribute to lower circulating total cholesterol (TC) and LDL cholesterol (LDL-C) in subjects with hypercholesterolemia by reducing intestinal cholesterol reabsorption. Their use in association with other nutraceuticals may prove useful in the management of moderate dyslipidemia, like in subjects with borderline-high TC and LDL-C and low cardiovascular risk.
Aim. To evaluate the safety of a nutraceutical combination containing Bifidobacterium longum BB536, red yeast rice (RYR) extract (10 mg/day monacolin K), niacin and coenzyme Q10 (Lactoflorene Colesterolo®), and its efficacy on cardiovascular atherogenic risk markers (TC, HDL-cholesterol (HDL-C), non-HDL-C, LDL-C, triglycerides (TG), apolipoproteins AI (ApoAI) and B (ApoB), proprotein convertase subtilisin/kexin type 9 (PCSK9).
Methods. Study design: randomized, parallel, double-blind, placebo-controlled study; intervention duration: 12 weeks. Clinical Trials. gov n° NCT02689934. Thirtythree subjects (Males:Females =50/50%, aged 18-70 years) with moderate hypercholesterolemia (LDL-C: 130-200 mg/dL) were randomly allocated to either nutraceutical combination (n=16) or placebo (n=17).
Results. In the active-treatment group, compared to placebo group, after 12 weeks, we observed a significant reduction of LDL-C (-26% vs 0% (placebo), p<0.0001), TC (-17% vs 0%, p<0.0001), ApoB (-22% vs -1%, p=0.0003), non-HDL-C (-24% vs 0%). Similar changes were already achieved after 6 weeks. Moreover, ApoAI increased by 6% vs. placebo, HDL-C, and PCSK9 levels were unchanged and TG decreased to the same extent (-12/13%) in both arms. Lathosterol: TC was significantly reduced, whereas campesterol:TC and sitosterol:TC did not change. No adverse effects and a 97% compliance were observed.
Conclusions. A 12-week treatment with a nutraceutical combination with the probiotic Bifidobacterium longum BB536, RYR extract, niacin and coenzyme Q10 was well tolerated by subjects with borderline hypercholesterolemia and resulted in a significant improvement of the proatherogenic lipid profile. Supported by an unrestricted grant to PM from Montefarmaco OTC S.p.A. (Bollate, Milano).

HDL PARTICLES DO NOT CONTRIBUTE TO PCSK9 TRANSPORT IN PLASMA: EVIDENCE FROM GENETIC HDL DISORDERS

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Background. Proprotein convertase subtilisin/kexin type 9 (PCSK9), a protein regulating the number of cell-surface LDL receptors (LDLR), circulates bound to LDL (20-40%), Lp(a) and HDL; conversely, there is no PCSK9 associated with VLDL. Familial hypoalphalipoproteinemia (FHA) is a rare, genetically heterogeneous lipoprotein disorder, characterized in homozygotes by an almost complete deficiency of plasma HDL. Carriers of FHA represent a unique human model providing insights on the role of ApoA-I, LCAT, and ABCA1 proteins in the regulation of HDL metabolism and function. Our study was aimed at investigating whether a condition of FHA could alter PCSK9 levels and its distribution among different lipoproteins.
Methods. Carriers of mutations in LCAT, ABCA1, or APOAI genes; ELISA for human PCSK9; HepG2 cells stably over-expressing PCSK9 (HepG2PCSK9).
Results. Carriers of one or two mutations in the LCAT gene did not show significant changes in median plasma PCSK9 levels compared to controls (homozygotes, 159.4 ng/mL (124.9; 243.3); heterozygotes, 180.3 ng/mL (127.6; 251.5) and controls, 190.4 ng/mL (146.7; 264.4); P for trend =0.33). Similar results were observed in subjects carrying mutations in ABCA1 and APOAI genes. When fractionated by FPLC, PCSK9 peaked in a region between LDL and HDL in control subjects. In FHA carriers, lipoprotein profile shows a strong reduction of HDL and a prevalence of large LDL-VLDL particles. However, the distribution of PCSK9 was superimposable to those of controls and, as a further control, to the medium of HepG2PCSK9. A direct association among plasma PCSK9 concentration, age, total cholesterol, unesterified cholesterol, LDL-C, and apoB levels was observed in the entire cohort. Moreover, plasma PCSK9 levels:
1) positively correlated with unesterified cholesterol, phospholipid, and LpA-I levels;
2) negatively correlated with pre-HDL.

Conclusions. The present study demonstrates that genetic conditions of very low HDL levels do not alter either PCSK9 plasma concentrations or PCSK9 distribution among lipoproteins.

FAMILIAL HYPERCHOLESTEROLEMIA IN CALABRIA: PRELIMINARY RESULTS FROM GENETIC TESTING

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Background and Aims. In Italy, it has estimated that <1% of FH cases are actually recognized and there is a general lack of uniform public health initiatives specifically directed to close the gap of knowledge on FH.The Dutch Lipid Score (DLS) is useful tool to asses the likelihood of this diagnosis. It is a combination of criteria and according to the score "defined (points >8)", "probable (points 6-8)", or "possible (points 3-5)" indicates FH cases. In Calabria, there is not data on the FH diagnosed with both the DLS and DNA analysis. The aim was to evaluate specificity and sensitivity of DLS to identify FH in Calabrian people.
Materials and Methods. We enrolled the first 29 patients under the Lipigen project. These patients came to U.O.C of Clinical Nutrition at the "Magna Graecia" University of Catanzaro from June 2016 to February 2017. For each patients was compiled DLS and DNA analysis.
Results. Among 29 patients, 6 (21%) had a DLS more than 8. Of these 5 (17%) were positive at DNA analysis. Thus, DLS had a 94% specificity and 45% sensitivity to identify the disease. The genetic test resulted positive in 38% of patients. All FH patients had a mutation of the receptor LDLr. In particular 45% patients had the Asn370Thr mutation (Ex 8), of which one had mutation in homozygous. This mutation influences the receptor recycling. The remaining had the mutation Cys89Arg that influences the interaction with the ligand (ApoB-100).
Conclusion. Our data suggest that the DLS is a valuable and useful diagnostic tool with consistent specificity to identify patients with FH. Probably there is not the need to perform the DNA test if DLS is more than 8 and may be crucial to focus on those with a "probable" disease testing for DNA.

OBSERVATIONAL NO-PROFIT PROTOCOL TO MONITOR THE PRESCRIPTIONS AND THE COST-EFFECTIVENESS OF PCSK9 INHIBITORS IN TUSCANY REGION - CERTI (COSTO EFFICACIA REGIONE TOSCANA INIBITORI PCSK9) STUDY

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Background. Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) inhibitors were recently approved for lowering low-density lipoprotein cholesterol in heterozygous Familial Hypercholesterolemia (FH) or atherosclerotic cardiovascular disease, especially in patients where current standard care is not tolerated or ineffective. However, their long-term effect and cost-effectiveness have not yet well established.Aim of study is to evaluate, at regional level, safety, tolerability and effect on Italian health care spending of PCSK9 inhibitors.
Study design. This observational, prospective, no-profit study will be conducted in 24 Tuscany sites. It will involve 9,000 patients eligible for treatment with PCSK9 inhibitors according to the Italian Medicines Agency (AIFA) criteria and it is expected to last 10 years.This protocol will collect, through an electronic case report form, the following data:
1) PCSK9 inhibitors cost-effectiveness at 3-5 years,
2) efficiency in LDL reduction,
3) record of major cardiovascular events,
4) monitor the adherence to therapy,
5) informations about pharmaco-vigilance.
Conclusions. This study provides an opportunity to build a network for collect and integrate PCSK9 inhibitors information's about prescriptions and cost-effectiveness.

CASE REPORT: LYSOSOMAL ACID LIPASE ACTIVITY IN DRIED BLOOD SPOTS (DBS) EXTRACTS IN A HYPERCHOLESTEROLEMIC FEMALE PATIENT CARRYING MUTATIONS ON LDLR, APOB AND LIPA GENES

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Background. Lysosomal acid lipase (LAL) deficiency is a rare autosomal recessive disease caused by mutations in the gene encoding lysosomal acid lipase (LIPA) that results in reduced or absent activity of the enzyme. Wolman's disease, where LAL function is completely lost, is a severe disease that occurs during infancy with an average life expectancy of less than 4 months. Cholesteryl ester storage disease (CESD), where some residual LAL activity remains, arises later in life and is less severe, although both diseases share many common features, including dyslipidaemia and elevated serum transaminase levels.
Methods. A 70-year old lean woman presented in our Outpatient Lipid Clinic for statin-intolerance. A clinical diagnosis of Heterozygous Familial Hypercholesterolemia (HeFH) was made according to plasma LDL-C concentrations above 300 mg/dL, and a family history of hypercholesterolemia and early coronary heart disease. A patient's sister died at the age of 6 months for uncertain reasons. In her daughter, a genetic test showed the presence of a heterozygous mutation (c.126C>A, null allele) of the LDL-receptor (LDLR) gene. Both the patient and her daughter had normal serum triglyceride and transaminase levels. In the patient the genetic analysis showed the following mutations: pathogenic LDLR mutation in heterozygosis (c.126C>A, null allele), mutation in heterozygosis of APOB of uncertain clinical significance (c.2068-4T>A), and a pathogenic mutation in heterozygosis of LIPA (c.894G>A, p.Gln298=, defective). We therefore assessed LAL activity levels through DBS test.
Results. LAL activity levels were 0.51 nmol/spot/hour in the patient and 0.92 nmol/spot/hour in her daughter (normal values >0.80), respectively.

Conclusions. We report here a rare case of hypercholesterolemia characterized by three different gene mutations (LDLR, APOB and LIPA). Even if the patient had a pathogenic mutation in heterozygosis of LIPA with reduced levels of LAL activity, she did not have the "classic" CESD phenotype.

INTRAERYTHROCYTE AND SERUM MAGNESIUM CONCENTRATION IN THE METABOLIC PATHOLOGY OF ADULTS

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Introduction. Magnesium is a co-factor for many enzymes, regulates several biochemical pathways, including protein synthesis, muscle and nerve function, glucose homeostasis, blood pressure regulation, oxidative phosphorylation and glycolysis. It contributes to bone development and it is required for DNA and RNA synthesis. Impaired Mg homeostasis could play a role in several diseases. Serum magnesium reflects only 1% of the body magnesium content, since most of it is stored in bone, muscle and soft tissues. Consequently, the clinical impact of Magnesium deficiency may be underestimated measuring serum levels. Conversely, red cell magnesium measurement may indicate possible deficiency states. A Mg deficit can be the consequence of reduced intake or an increased loss. Elderly, alcoholics and patients with gastrointestinal and renal diseases have a higher risk of developing a magnesium deficiency.
Scope. The aim of this study was to measure intraerythrocytes and serum Mg concentration in a group of elderly in order to highlight possible deficiencies and evaluate the possible correlations with metabolic diseases.
Methods. A cohort of 94 patients, 38 men and 56 women (median age of 67 years, range 60-79), enrolled in the PANGeA project (Ferrara's population) underwent blood sampling for biochemical determinations, anthropometric assessment and a Bioelectrical impedance analysis (BIA), to estimate body composition. Intraerythrocyte and serum Magnesium concentration were measured by colorimetric method.
Results. Intraerythrocyte Mg concentration and serum magnesium positively correlated. Serum magnesium concentration was inversely correlated with age, systolic pressure, insulin and HOMA, and positively correlated with total cholesterol, HDL cholesterol. Intraerythrocyte magnesium levels positively correlated with BMI, waist circumference, triglycerides and insulin resistance.
Conclusions. Our data show a positive correlation between intraerythrocyte magnesium and features of the metabolic syndrome (central obesity, insulin resistance, triglyceridemia) supporting an important role of magnesium in regulating insulin sensitivity.

EFFECTS OF CARDIAC NATRIURETIC PEPTIDES AND INSULIN ON PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE-9 AND LOW-DENSITY LIPOPROTEIN RECEPTOR EXPRESSION IN HUMAN DIFFERENTIATED ADIPOCYTES

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Introduction. Proprotein convertase subtilisin/kexin type-9 (PCSK9) binds to low-density lipoprotein receptor (LDLR), leading to its degradation. PCSK9 and LDLR regulation in human adipocytes is still not determined. We investigated the presence of PCSK9 in human visceral adipose tissue (VAT) and how insulin and atrial natriuretic peptide (ANP) affect PCSK9 and LDLR regulation in human differentiated adipocytes (HDAs).
Materials and Methods. PCSK9 and LDLR gene expression and protein levels were analysed in VAT obtained from patients undergoing nephrectomy and we tested the effects of insulin (10-100 nM), ANP (100 nM) and human LDL on PCSK9/LDLR regulation in HDAs.
Results and Conclusion. PCSK9 expression was well detectable in VAT and it was positively correlated with BMI. Pre-mature form of PCSK9 was prevalent in VAT, similarly to hepatic tissue but at 5-fold lower level. Mature form was prevalent in cultured HDAs, as well as in the supernatant, suggesting active secretion. Insulin potently induced PCSK9 and LDLR expression, even at low concentration (10nM). On the contrary, ANP partially blocked insulin-induced PCSK9. A 4-hour LDL-treatment induced LDLR and the mature, but not the secreted form of PCSK9, whereas a 18-hour LDL-treatment significantly inhibited LDLR and stimulated both mature and secreted forms of PCSK9. PCSK9 is abundant in human VAT and HDAs and correlates with BMI. Insulin induces in vitro PCSK9 and LDLR expression in HDAs. ANP partially blocks insulin induction of PCSK9. Moreover, human LDL increase both mature and secreted forms of PCSK9 in HDAs and LDLR is subsequently reduced, suggesting that PCSK9 affects LDLR-mediated LDL cholesterol uptake also in HDAs.

HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA AND HIV INFECTION: THE APPROPRIATE THERAPEUTIC FLOW CHART IS STILL TO BE WRITTEN?

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Case presentation. We describe the case of a 47 year-old man with heterozygous Familial Hyperchosterolemia (heFH) and HIV infection referred to our outpatient lipid clinic, in 2014, by the infection disease consultant. We made the diagnosis of heFH with a missense mutation on LDL receptor gene. He had history of statin intolerance (previous lipid-lowering therapy was: atorvastatin 40 mg OD, rosuvastatin 20 mg OD, simvastatin/ezetimibe 40/10 mg

OD). At the cardiocascular assessment he showed subclinical atherosclerotic involvement (treadmill testing was normal, carotid artery Doppler examination revealed increased intimal medial thickness with a 30% stenosis at the bulb bilaterally). Since 2008, patient was on taking antiretroviral drugs (currently: abacavir 300 mg OD, lamivudine 300 mg OD, and dolutegravir 50 mg OD), with good immune and virological control and no AIDS-defining events. The patient (LDL 443 mg/dl, TC/HDL 13.3 at baseline), after re-challenge with atorvastatin 20 mg OD to check statin intolerance, started to Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) inhibitors evolocumab 140 mg every 2-weeks. Due to poor evolocumab efficiency (14% LDL reduction), LDL-apheresis was started (once every 2 weeks with dextran-sulphate system - Liposorber®-LA systems; Kaneka) and ezetimibe 10 mg OD has been added. To improve patient's lipid profile, Alirocumab 150 mg s.c. every 2-weeks and monacolin K 10 mg OD has been added. Because of myopathy secondary to monacolin K, this drug was substituted with Fenofibrate 145 mg OD (LDL 222 mg/dl, TC/HDL 5.0 after treatment).

Discussion. Although the HIV patient's life expectancy is increased due to antiretroviral therapy, the atherosclerotic burden is high and difficult to manage, especially when FH coexists. PCSK9 inhibitors represent a promising approach to lower LDL cholesterol in patients who experience statins intolerance, including subjects with HIV infection, in which, though, a lower PCSK9 inhibitor efficiency could occur because of higher circulating PCSK9 levels. In this particular experience, the combination of the old and new therapeutic armamentarium, has overcome the negative interaction between antiretroviral therapy and statin intolerance in a particular example personalized lipid lowering therapy.

WAIST-TO-HIP RATIO IS ASSOCIATED WITH HIGHER CORONARY AND PERIPHERAL ATHEROSCLEROTIC BURDEN IN OVERWEIGHT SUBJECTS

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Background and Aims. The association of overweight status and cardiovascular disease is not clear. Our objective was to examine the atherosclerotic burden of overweight subjects with and without abdominal obesity according to waist-to-hip ratio (WHR) using macroangiopathic imaging biomarkers.

Methods. Our population consisted in 276 overweight patients aged between 40 and 70 years, with a BMI of 25-29.9 kg/m^2 and at least 1 cardiovascular risk factor. Exclusion criteria were prior history of diabetes, cardiovascular or advanced renal disease. Abdominal obesity was defined as a WHR ≥0.85 for women and ≥0.90 for men. Coronary artery calcium (CAC) score as well as mean common carotid intima media thickness (IMT) and plaque presence were assessed.

Results. CAC score was higher in the obese group compared to non- obese subjects (9.05 [0.0-83.48] vs 0.0 [0.0-64.7] AU, p<0.01). Obese patients had higher mean IMT than non-obese (0.64 [0.56-0.72] vs 0.59 [0.55-0.67] AU, p<0.001). The prevalence of CAC in the obese group was significantly higher than the non-obese group (59.6% vs 38.5%, p<0.001. (40.4% vs 61.5%, p<0.001). Notably, 13 obese patients (8.9%) had a CAC >400 compared to 2 non-obese subjects (1.5%, p<0.01), <0.01). Moreover, carotid plaques were significantly more present in patients with abdominal obesity than in the non-obese subjects (63.7% vs 50.8%, p<0.05). In a multiple linear regression, IMT was associated with WHR levels (p<0.05). Also, logistic regression showed that high WHR was associated with CAC and carotid plaques presence (p<0.01 and <0.05, respectevely).

Conclusions. WHR increase is associated with higher coronary and peripheral atherosclerotic burden in overweight patients.

DETECTION OF COPY NUMBER VARIATION IN LDLR GENE BY NEXT GENERATION SEQUENCING IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Background. Familial Hypercholesterolemia (FH) is a dominant disorder characterized by high plasma LDL-C levels and an increased risk of premature coronary artery disease. It is caused by mutations in three genes: LDLR, APOB and PCSK9. Search for mutations is commonly performed by Sanger sequencing or Next Generation Sequencing (NGS). The majority of LDLR gene variants are point mutations (90-95%) and about 5-10% are due to copy number variations (CNVs) that require further molecular analysis to be characterized (ie Southern Blotting or Multiplex Ligation-dependent Probe Amplification (MLPA). During the last years, NGS based technology has been improved to detect CNVs as an alternative approach. A new bioinformatic tool for Ion Reporter software allow to detect CNVs using NGS data outputs obtained by Personal Genome Machine (PGM).

Objective and Methods. The aim of the study was to test if NGS data could be used to detect CNVs in LDLR gene and validate the results with MLPA. We used an Ampliseq custom panel for the analysis of FH-related genes by Ion Torrent PGM and a bioinformatic tool for the analysis of data to detect CNVs.

Results. The analysis of NGS data outputs of FH patients showed a concordance in LDLR CNVs detection between MLPA and NGS methods. CNVs discovered in FH patients by MLPA were also detected by NGS.

Conclusion. These results indicate that the detection of CNVs by NGS method represent a valid methodology that can reduce cost and time of molecular analysis in FH.

DAPAGLIFLOZIN ACUTELY RESTORES ENDOTHELIAL DYSFUNCTION, REDUCES AORTIC STIFFNESS AND RENAL RESISTIVE INDEX IN TYPE 2 DIABETIC PATIENTS: A PILOT STUDY

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Background. Sodium-glucose co-transporter-2 inhibitors reduce blood pressure (BP) and renal and cardiovascular events in patients with type 2 diabetes (T2D) through not fully elucidated mechanisms. Aim of this study was to investigate whether dapagliflozin is able to acutely modify systemic and renal vascular function.
Methods. Neuro-hormonal and vascular variables, together with 24 h-diuresis, urinary electrolytes, glucose and 8-isoprostanes were assessed before and after a 2-day treatment with dapagliflozin 10 mg QD in sixteen T2D patients; data were compared with those obtained in ten patients treated with hydrochlorothiazide 12.5 mg QD, to test whether the results could be specifically related to the mode of action of dapagliflozin or, more extensively, to its diuretic effect. Brachial artery endothelium-dependent and independent vasodilation (by flow-mediated dilation) and pulse wave velocity were assessed; renal resistive index was obtained at baseline and after glyceril trinitrate administration.
Results. Dapagliflozin decreased systolic BP and induced an increase in 24 h-diuresis to a similar extent of hydrochlorothiazide, whereas heart rate was significantly increased only by hydrochlorothiazide ($p<0.01$ for interaction time*treatment). 24 h-urinary glucose and serum magnesium were also increased by dapagliflozin, whereas 24h-urinary sodium and fasting blood glucose were unchanged in both groups. Oxidative stress was reduced in dapagliflozin group, as by a decline in urinary isoprostanes (1659±1029 to 1157±663 pg/ml, $p<0.05$). In dapagliflozin-treated patients flow-mediated dilation was significantly increased (2.8±2.2 to 4.0±2.1%, $p<0.05$), pulse-wave-velocity was reduced (10.1±1.6 to 8.9±1.6 m/s, $p<0.05$) even after correction for mean BP and renal resistive index was reduced (0.62±0.04 to 0.59±0.05, $p<0.05$); none of vascular parameters were affected by hydrochlorothiazide.
Conclusion. An acute treatment with dapagliflozin significantly improves systemic endothelial function, arterial stiffness and renal resistive index. This effect is independent of changes in BP and occurs in the presence of stable natriuresis, suggesting a fast, direct beneficial effect on the vasculature, possibly mediated by oxidative stress reduction.

METALLOPROTEINASE ACTIVITIES IN ISCHEMIC STROKE PATIENTS TREATED WITH TPA THROMBOLYSIS: PRELIMINARY RESULTS IN THE MAGIC STUDY

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Background. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that are involved in extracellular matrix degradation. After cerebral ischemia, the general neuronal response to excitotoxic injury determines the release of pro-inflammatory cytokines that stimulate the local production of MMPs and TIMPs. Circulating antigen levels of MMP9 have been proved associated with poor outcomes in stroke patients treated with tissue plasminogen activator (rtPA), but scarse data about the role of the activated form of gelatinases MMP-2 and MMP-9 are available in literature.
Methods. We evaluated the role of MMP-2 and MMP-9 activities on adverse clinical outcomes in tPA treated IS. Blood was taken at baseline (B) and 24 hours after tPA from 327 patients (mean age68, mean NIHSS 11). In 106 ischemic stroke patients the circulating antigen levels of MMP2 and MMP9 were determined using Bio-Plex suspension array system, whereas the gelatinase activity of MMP-2 and MMP-9 were assessed by zimography. vWF levels were measured by immunoturbidimetry.
Results. MMP-2 activity B did not significant correlate with the antigen levels both at B and 24 hours after tPA. On the contrary, MMP-9 activity was significantly related with MMP-9 antigen levels (B: r=0.202, p=0.049; 24 hours after tPA: r=0.342, p=0.001). No significant correlation between vWF and MMP-2/9 activities was found. The thrombolytic treatment significantly reduced the MMP-2 activity [B: 2456 Arbirary Units (AU) vs 1897 AU, p=0.001] but not the MMP-9 activity [B: 2862 (1447-6301) AU vs 2969 (1660-6291) AU, p=0.116]. MMP-2 and MMP-9 activities did not differ between females and males. MMP-9 activity significantly correlated with age both at baseline and 24 h after tPA (B: r=0.203, p=0.032; 24 h after tPA: r=0.254, p=0.014). B and delta ([24 hours post tPA-pre tPA]/pre tPA] values of MMP-2 and MMP-9 activities were analyzed across subgroups of patients undergoing symptomatic intracerebral hemorrhage, 3-month death, or 3-month modified Rankin Scale score 3 to 6. Baseline MMP-2 and 9 activity levels did not differ between patients with and without SICH, as wells between patients with mRS<=2 and mRS>2. Delta MMP-2 activity were significantly associated with death [-0.16 (-0.35- -0.13) vs -0.12 (-0.23-0.0), p=0.040], whereas delta MMP-9 activity did not differ between dead and alive patients. At logistic regression analysis, after adjustment for major clinical determinants of outcomes delta MMP-2 did not remain significantly associated with death.
Discussion. Our data provide evidence about the effect of thrombolysis on gelatinase activities and the relationship with the antigen levels of MMP 2 and 9 in ischemic stroke patients. These preliminary results did not support the use of MMP-2 and 9 activity as possible biomarkers of adverse outcomes.

MUSCULAR SAFETY OF EVOLOCUMAB, IN CLINICAL PRACTICE, IN HIGH RISK SUBJECTS INTOLERANT TO STATINS

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Evolocumab is an anti-protein convertase sublisin/kexin type 9 (PCSK9) monoclonal antibody used to reduce LDL levels in high risk hypercholesterolemic patients. In pre-marketing studies evolocumab showed a very good efficacy in lowering LDL levels with

a good safety profile. PCSK9 inibitors can cause musculoskeletal symptoms and headache as most common adverse advents, elevations in aspartate/alamine aminotransferase and creatinine kinase are reported very uncommon. Evolocumab treatment is reserved to high risk hypercholesterolemic subjects and can also be used in patients with intolerance to statins. The most common side effect of statin is myalgia/myopathy. No large date are available about musculoskeletal profile of evolocumab in patients that showed myalgia/myopathy due to statins.We wanted investigate the muscular safety profile in five high risk hypercholesterolemic patients that previously showed myalgia using statins. All of them presented a high cardiovascular risk (SCORE risk) and all of them showed myalgia with previous use of statins; two of them did not tolerate any statin therapy, the others experienced myalgia using high dosage of statins. All of patient received 140 mg i.m. of evolocumab every two weeks for six months. Therapy with evolucumab was added to previous antylipidemic therapy and not change of therapy was performed during all the six months. Nobody of six patients referred myalgia during the treatment with evolocumab. Levels of creatinine kinase (evaluated at base line, after 1.5, 3, 6 months) resulted negative for all patients. One patient referred mild injection site reaction only ones during the period of administration. The medium reduction of LDL at six month with evolocumab was 42% respect at the base line. In these five patient, that previously showed myalgia by statins, evolocumab was save in musculoskeletal profile. Nobody of them referred symptoms and no elevation of CPK was observed during the six months of evaluation.

NGS DETECTION OF NUCLEOTIDE VARIANTS IN FAMILIAL AND POLYGENIC HYPERCHOLESTEROLEMIA

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Background. Familial Hypercholesterolemia (FH) is caused by mutations in LDLR, APOB or PCSK9 genes. Mutations in LDLR gene are the most common and currently 1200 mutations have been reported. Even with exhaustive screening, in a proportion of FH patients (10%-15%) with definite clinical diagnosis no mutation can be found. This may be due to:
a) failure to detect DNA changes by current methods,
b) mutations in genetic regions usually not analyzed (e.g., introns),
c) mutations in other unknown genes.
Genome-wide association studies (GWAS) have identified 95 loci able to modulate lipid levels; more recently a 6-single nucleotide polymorphism (6-SNP) panel has been used to calculate an LDL-C genetic risk score for the characterization of patients with "polygenic FH". It has been postulated that as much as 88% of mutation-negative patients is likely to have a polygenic basis of hypercholesterolemia.
Objective and Methods. The aim of this study was to investigate monogenic and polygenic causes of FH by a targeted Next Generation Sequencing (NGS) approach to capture the coding exons and intron/exon boundaries of 19 genes affecting the main pathways of cholesterol synthesis and metabolism.
Results. The targeted resequencing of candidate genes performed in 58 FH patients led to the identification of pathogenic mutations in 20 subjects (18 carriers of mutations in LDLR gene, 2 carriers of mutations in APOB gene). In the remaining 38 mutation-negative patients we evaluated the burden of rare variants in candidate genes on the FH phenotype. ConclusionsIn this work we demonstrated that NGS approach permitted to study 19 different genes able to modulate lipid levels simultaneously. This method allowed to identified monogenic form of FH and polygenic form. Moreover, in FH patients with a low polygenic score, the presence of different rare variants in candidate gene might contribute to explain FH phenotype.

GENETIC BASES OF BICUSPID AORTIC VALVE: ROLE OF HIGH-THROUGHPUT SEQUENCING APPROACH

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Background. The progressive advancement of high-throughput sequencing (HTS) technologies significantly contributes to improve the comprehension of disorders, such as bicuspid aortic valve (BAV), whose genetic bases still remain to be finally elucidated. BAV has been described as an isolated trait or associated with syndromic conditions [e.g., Marfan syndrome (MFS) or Loeys-Dietz syndrome (LDS)]. Actually, it appears increasingly evident the contribution of multiple genes in BAV pathogenetic mechanisms, rather than a single gene. Aim of the present study is to investigate the genetic bases of BAV by using the potentiality of HTS.
Methods. Seventeen BAV patients admitted to the Referring Center for MFS and Related Disorders (Tuscany, Italy) have been analyzed by HTS approach (Illumina), based on a targeted sequencing panel including 94 genes suspected to be associated with BAV or BAV-associated conditions or known to be involved in aortic wall remodelling.
Results. Seventy-eight genetic variants with minor allele frequency (MAF) in the European population <0.05, and 43 with MAF<0.01 have been identified in 37 different genes; 34 variants have been defined damaging with at least 1 out of 4 in silico pathogenicity prediction tool used. Genes previously suspected to be associated with the disease (NOTCH1, FBN1) and genes not previously associated (TGFB1, LTBP1, LTBP4) were identified. Moreover, a computational approach aimed to evaluate significantly mutated genes with respect to background mutation rate (borrowed from cancer bioinformatics) allowed the identification of 21 candidate genes involved in BAV pathogenesis or susceptibility.
Conclusions. HTS technology and "BigData" bioinformatics approaches development provided information concerning genetic variability in candidate genes, whose further evaluation through functional studies and segregation analyses in families could contribute to improve the comprehension of BAV genetic bases.

VALUTAZIONE DEL PROFILO LIPIDICO PRIMA E DOPO INTERVENTO NUTRIZIONALE IN PAZIENTI PEDIATRICI AFFETTI DA IPERCOLESTEROLEMIA FAMILIARE

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La cardiovasculopatia aterosclerotica rappresenta ad oggi la principale causa di morte in Europa essendo responsabile del 48% dei decessi anche in giovane età. Sebbene la maggior parte degli eventi cardiovascolari si verifica nell'età adulta, è ben documentato che il processo aterosclerotico inizia già a partire dall'età pediatrica ed è correlato ai valori di colesterolemia i quali, a loro volta sono dipendenti sia da fattori genetici sia da fattori ambientali, in particolare l'intake lipidico come grassi saturi e colesterolo.
Scopo del lavoro. È stato quello di valutare se un intervento nutrizionale di tipo qualitativo potesse modificare in senso favorevole il profilo lipidico di bambini con ipercolesterolemia familiare afferenti a Centro Dislipidemie della Clinica Pediatrica dell'Ospedale San Paolo. L'intervento prevede la correzione delle abitudini alimentari al fine di migliorare gli apporti nutrizionali per garantire il fabbisogno energetico per la crescita e possibilmente modificare il profilo lipidico in senso meno aterogeno. Soggetti e metodi: la popolazione analizzata è costituita da 43 bambini affetti da ipercolesterolemia severa, 15F (43%), 28M (68%), età scolare (5-12 anni, media di 9,30±2,33), normopeso (BMI: 18±2,2 kg/m^2), prepuberi (Tanner stage: 1), in cui è stata eseguita una determinazione del profilo lipidico (CT, LDL, HDL, TG) mediante prelievo ematico a digiuno (mg/dl) al T0 (CT: 232,1; LDL: 159,6; HDL: 57,8; TG: 88,1) e al T1 (CT: 209,9; LDL: 136,1; HDL: 62,3; TG: 73,1),e sono stati calcolati i rapporti: CT/HDL (T0: 4,0±1,3; T1: 3,4±0,8) e TG/HDL (T0: 1,5±0,7; T1: 1,2±0,6). È stata quindi fatta una valutazione delle abitudini alimentari mediante somministrazione del questionario delle frequenze alimentari (FFQ) dopo 12 mesi di intervento nutrizionale.
Risultati. Dall'elaborazione dei dati si è evidenziato un miglioramento complessivo dei parametri lipidici con una riduzione del CT (-9,50%), LDL (-14,70%) e TG (-17,10%) e un aumento di HDL (+7,20%). Questa modificazione del profilo lipidico conferma l'efficacia dell'intervento dietetico nutrizionale che si evidenzia nella riduzione dei livelli di CT e di LDL ed anche la sua sicurezza in quanto i valori di HDL, seppure non statisticamente significativi, sono aumentati a conferma che l'apporto lipidico inteso come fonte energetica è stato adeguato. Dall'analisi delle abitudini alimentari abbiamo inoltre rilevato come le frequenze di consumo settimanali si siano ridotte (%) per i junk food (-56,93), per i salumi (-49,49) e per la carne (-34,09), mentre sono aumentate per i legumi (+50%), la frutta (+42,27) e la verdura (38,06). Per quanto riguarda i consumi relativi al pesce si è registrato un incremento rispetto al T0 (+27,28), ma la frequenza settimanale è risultata piuttosto bassa, attestandosi a 2,31 volte a settimana.
Conclusioni. I dati raccolti nel nostro studio evidenziano che l'intervento dietetico-nutrizionale in una popolazione di bambini con ipercolesterolemia severa su base familiare è efficace nel migliorare il profilo lipidico dei soggetti in senso meno aterogeno.

EFFECTIVENESS OF EDUCATION TO IMPROVE THE MANAGEMENT AND THE QUALIT OF LIVES OF TYPE 2 DIABETIC PATIENTS WITH A PREVIOUS MYOCARDIAL INFARCTION

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People with diabetes and cardiovascular complications need to be educated about the self-management of the disease. The aim of the study is the evaluation of nutritional education and the management of the diabetes therapy. The sample group consisted of 40 subjects (20 for the control group and 20 for the intervention group) suffering from diabetes mellitus type 2 with a previous myocardial infarction. They administered two tests to assess the degree of knowledge of diabetes, they also performed the "Six-minute walking test", the gold standard to evaluate the functional capacity of the patient. Anthropometric variables and several blood parameters were taken into consideration. Only he intervention group attended group lessons, "healthy shows cooking", physical exercise sessions and a focus on structured self-monitoring of blood glucose. Subsequently, initial assessments checked for changes and improvements. Comparisons between data collected before (0) and after (1) the study were carried out with the Wilcoxon-Mann-Whitney method. For statistical analysis SPSS 22.0 was used and the threshold of significance was set at $p<0,05$. Table 1 shows the statistical improvements in knowledge about diabetes (p=0,001), body mass index (p=0,018), waist circumference (p=0,44), meters walked (p=0,019), LDL cholesterol (p=0,033), which is the major biomarker for atherosclerosis and cardiovascular risk. This project has established a preliminary study of education for the diabetic patient and it's useful in order to verify the importance of this type of intervention, aimed at improving the living conditions of patients. Although we have not found an improvement of all the measured parametersand its functionality, informing diabetic subjects of their health conditions is essential in order to achieve patient empowerment and compliance: these are the objectives of the therapeutic education, aimed to improve the living conditions diabetes patients.

ASSOCIATION OF ALBUMIN-TO-CREATININE RATIO AND ANKLE-BRACHIAL INDEX IN NON DIABETIC DYSLIPIDEMIC OUTPATIENTS

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Introduction. Albuminuria is generally the first clinical sign of renal dysfunction in diabetic patients, and is considered a marker of general vascular dysfunction. Peripheral Arterial Disease (PAD), a widely prevalent manifestation of atherosclerosis, can be diagnosed by a low ankle-brachial index (ABI). Association of Ankle-brachial index and albuminuria are reported in diabetes and hypertension but scanty data are present in dyslipidemia.

Aim of the present study was to explore the association, if any, between ankle-brachial index and albumin-to-creatinine ratio in non diabetic dyslipidemic outpatients. Subjects and methods: oscillometric ABI (OABI) was measured in 55 consecutive non diabetic dyslipidemic outpatients attending our Division. An Omron oscillometric system (Omron Canada Inc) was used. Albuminuria was determined as Albumin-to-creatinine ratio (ACR) in morning urine sample.
Results. Descriptive statistics of the patients: 34 males, 21 females, age (mean ±SD) 50.4±13.5 years, BMI 25.±3.8 kg/m^2, SBP 124.6±13.0 mmHg, DBP 75.6±7.5 mmHg, fasting blood glucose 89.9±12.7 mg/dl, HbA1c 5.54±0.5%, total cholesterol 235.3±37.5 mg/dl, HDL cholesterol 51. ±18.1 mg/dl, triglycerides 174.4±90.8 mg/dl, LDL cholesterol 145.9±39.7 mg/dl. Mean OABI was 1.147 0.14 and mean Albumin-to-creatinine ratio was 3.22±15.72 g/mol. A significant negative correlation was found between mean OABI and ACR (Spearman's R =-0.32; p=0.041).
Conclusions. ABI is a noninvasive and reliable assay for detection of peripheral arterial disease and albuminuria a marker of early stage of nephropathy in diabetic patients. We have found an association of ABI with albuminuria in non diabetic dyslipidemic outpatients. Further studies are needed to better clarify our findings and their potential implications for clinical practice.

RESPONSE OF LIPOPROTEIN(A) LEVELS TO PCSK9 INHIBITORS: THE TUSCANY REFERENCE CENTER FOR HEREDITARY DYSLIPIDEMIA EXPERIENCE

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Introduction. Lipoprotein (a) [Lp(a)] has been classified as the single most prevalent genetic risk factor for coronary artery disease and aortic valve stenosis. Clinical trials with proprotein convertase subtilisin/kexin type 9 monoclonal antibody inhibitors (PCSK9i) have demonstrated a significant Lp(a)-lowering effect.
Methods. In our centre we enrolled 21 patients (mean age 61±8 years, male 67%) affected by hyper-Lp(a) (defined as plasma levels >50 mg/dl) and familial hypercholesterolemia assigned to PCSK9i. Concomitant coronary heart disease was present in 19/21 patients and 10/21 were chronically on LDL Apheresis (LA) treatment. No relevant comorbidity - diabetes mellitus, arterial hypertension, current smoking exposure, renal failure - were present in any patients. Blood collection were performed before the PCSK9i injection and before starting the apheresis procedure. Selective LA procedures were performed according to guidelines and manufacturer's instructions with 14 day interapheretic interval and treating 1.5 patient plasma volume for session. In subjects on LA, PCSK9i were administered at the end of apheresis section. PCSK9i (evolocumab 140 mg every 2 week in 14 subjects, alirocumab 150 mg every 2 week in 5 subjects or alirocumab 75 mg every 2 week in 2 subject) were administered, every 2 week, on top of background lipid-lowering-therapy (statins in 15 subjects, ezetimibe in 9 subjects, fibrates in 3 subjects and 3 subject not taking lipid-lowering drugs).
Results. During the 6 months study period, 1/21 patient on LA had been droped-out because of severe myalgia. Other adverse events were reported in 5/20 of patients: 3/5 showed low grade of myalgia that required a lipid-lowering down-grade, 2/5 patients presented visual impairment and were scheduled for cataract surgery. The cumulative effect of lipid lowering treatment on the all group of patients studied showed a 32% decrease in total cholesterol (p<0.001), 49% in LDL-c (p<0.001) and 15% in Lp(a) median levels (p<0.001). The subgroup of LA patients on PCSK9i (9/20) showed reduction of 34% (p<0.001) in total cholesterol, 53% (p<0.001) in LDL-c and 17% (p<0.01) in Lp(a) median levels, while subgroup on PCSK9i (11/20) showed a decrease in total cholesterol of 28% (p<0.01), 46% in LDL-c (p<0.01) and 22% (p<0.05) in Lp(a) median levels.
Conclusions. Hyper-Lp(a), especially when associated to Familial Hypercholesterolemia, is a clinical condition that requires the highest personalization in lipid-lowering therapy. While LA represent an effective treatment to lower Lp(a) and cardiovascular events, PCSK9i would be a supplementary opportunity for treatment hyper-Lp(a) patients. Our data are disappointing respect to clinical trials conducted so far on PCSK9i that report a 30% decrease in Lp(a).

COMPOUND HETEROZYGOUS MUTATION (LDLR/PCSK9) WITH A BORDER-LINE CLINICAL EXPRESSION: WHAT'S THE BEST TREATMENT OPTION?

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Background. Heterozygous mutations of LDLR, APOB or PCSK9 genes cause a severe increase in cholesterolemia ("Familial Hypercholesterolemia") and raise the risk of early cardiovascular events. Statin and ezetimibe therapy reduce LDL-cholesterolemia by up to >60%, but it is often insufficient to reach the LDL target. LDL-Apheresis can get even better results but it is not available anywhere, is very expensive and has to be frequently repeated, worsening the patient's quality of life. Today, PCSK9-inhibitors can further lower LDL-cholesterolemia and allow therapeutic targets to be reached even in the most difficult cases, but their prescription is strictly regulated.
Case report. About 2 years ago, we visited a 62-year-old female with high-cholesterolemia known for many years and resistant to traditional therapies. The latest exams, done with rosuvastatin 40/ezetimibe were: cholesterol =236 mg/dL, HDL =45, triglycerides =121, LDL =167. Her DLS was 9. We tried to enrol her in a phase III study with a PCSK9-inhibitor (alirocumab), so we repeated the lipid profile and performed a genetic study (Progenika) that revealed a compound mutation: LDLR (c.418G>T; p.Glu119*; null allele) and PCSK9 (c.60-65dupGCTGCT; p.Leu22_Leu23dup). Although "null allele" mutations are usually associated with more severe phenotypes (advanced atherosclerosis) and patients can be considered to have homozygous FH-like phenotype, our patient was in primary prevention and her clinical characteristics were border-line (repeated LDL-C was exactly =130). As inclusion criteria required LDL-C ≥130, patient could not undergo experimental treatment, and even today, that the drug is on the market and her LDL is still >100<130, she can not be treated with PCSK9-i according to the therapeutic plan.
Discussion. Our patient is undoubtedly at high-risk and has not reached the LDL-goal. Treatment with PCSK9-i would significantly reduce LDL and consequently her cardiovascular risk. Paradoxically, she would have access to LDL-Aphesis treatment, but it would be even more expensive.

Conclusions. It would be desirable that, if there is a genetic diagnosis (currently not required) and patient is not at LDL-target, therapeutic plan for PCSK9-i can be activated anyway.

STUDY OF THE POTENTIAL INFLUENCE OF OBESITY ON THE ACTIVITIES OF SERUM PARAOXONASE-1 AND LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2

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Introduction. Obesity is independently associated with disturbances in lipid and lipoprotein metabolism, oxidative stress and is a well-established independent risk factor cardiovascular diseases (CVD) (1). Human paraoxonase 1 (PON1) is a pleotropic high density lipoprotein (HDL)-associated enzyme with antioxidant and anti-inflammatory proprieties which have been suggested to contribute to the athero-protective function of the lipoprotein (2). In contrast, lipoprotein-associated phospholipase A2 (Lp-PLA2) is prevalently bound to low density lipoprotein (LDL) and might act as a pro-atherogenic player. This study aimed to explore whether and in which extent PON1 and Lp-PLA2 are associated with obesity and, thus, could act as mediators of the adverse effects of this condition on cardiovascular health (3).
Methods. The serum activities of these two enzymes were spectrophotometrically measured in 101 obese and severely obese, 101 overweight and 129 normal-weight women (controls) women (mean age: 54, intequartile range: 48-61). Markers of inflammation (high-sensitivity-C-reactive protein, hs-CRP) and oxidative stress (hydroperoxides), and lipid profile were also assessed.
Results. PON1 activity showed significant differences across the BMI groups (ANOVA, $p<0.01$), with the greatest decrease (almost 20%) observed in subjects with body mass index (BMI) >40 kg/m^2 compared to controls ($p<0.001$). PON1 and lp-PLA2 were both significantly associated to BMI but with opposite directions (negative, $r=-0.280$, $p<0.01$ and positive, $r=201$, $p<0.05$, respectively). At the multivariate analysis, PON1 emerged to be correlated with BMI in a way independent of age, inflammation and oxidative stress, thus suggesting that other factors may mediate the link between the enzyme expression/activity and obesity.
Conclusions. Our data suggest that lower PON1 and/or higher lp-PLA2 activities might contribute to increase the risk of cardiometabolic disorders in obese women.

References
1. Keaney JF, et al. Arterioscler Thromb Vasc Biol. 2003; 23: 434-9.
2. Cervellati C, et al. 2016; 64: 21-32.
3. Tellis CC, Tselepis AD. Biochim Biophys Acta. 2009; 1791: 327-38.

EVOLOCUMAB REDUCED SMALL DENSE LDL AND OTHER LIPID PARAMETERS IN A GROUP OF PATIENTS WITH HETEROZYGOUS FAMILIAL HYPERCOLESTEROLEMIA

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Backgrounds. Mall dense LDL particles have been identified as an extremely atherogenic LDL subgroup. Recently Evolocumab, the anti PCSK9 antibodies, has been successfully introduced in addiction to standard lipid lowering therapy, in particular in patient with heterozygous hypercolesterolemia. Its effectiveness is based on the inhibition of the link between PCSK9 and LDL receptor. In our study we evaluate the efficacy of Evolocumab plus statins therapy on small dense LDL reduction and other lipid parameters in 25 patients with heterozygous familial hypercolesterolemia.
Methods. We enrolled 25 patients (13 men and 12 women , mean age 51.5±14.5 yrs). Total Cholesterol, HDL-Cholesterol, LDL- Cholesterol. Triglycerides and apo B were calculated with standardized methods at Visit 0 and Visit 2 (Week 12). Lipoprotein subfractions were assessed by gel electrophoresis with Lipoprint System: 7 LDL subfractions were obtained and LDL SCORE (% sdLDL) and LDL particle diameter were calculated.
Results. After 12 week of treatment Evolocumab (140 mg every 14 days) reduced total cholesterol (from 269.9±60.6 to 161.7±44.5 mg/dl, $p<0.001$), LDL-Cholesterol (from 192.9± 57.3 to 86.0±37.9 mg/dl, $p<0.001$), HDL-Cholesterol (from 51.8±13.4 to 51.3±11.3 mg/dl, $p=0.698$), ad Apo B (from 1.41 ±0.3 to 0.7±0.2 mg/ dl, $p=0.001$), while triglycerides increased (from 106.1±50.5 to 113.7±42.7 mg/dl, $p=0.247$). LDL score was reduced (from 8.0±5.3 to 3.7±3.3 mg/dl, $p<0.001$), while particle diameter increased (from 26.8±0.3 to 27.0±0.2 nm, $p=0.007$).
Conclusion. Our study shows the efficacy of evolocumab in reducing serum lipids (Total Cholesterol, LDL-Cholesterol and APO B) and improving lipoprotein profile (decrease LDL score and increased LDL particle diameter) in a group of FH patients after 12 week of treatment. On these bases, it may be an effective tool in slowing down the progression of coronary atherosclerosis and reducing cardiovascular events in high risk patients.

CAROTID ATHEROSCLEROTIC DISEASE AND ITS CORRELATION WITH NON-ALCOHOLIC-FATTY-DISEASE (NAFLD) IN METABOLIC SYNDROME

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Objective. Relation between Non-Alcoholic-Fatty-Disease (NAFLD) and cardiovascular disease has been widely demonstrated in letterature (1-4) and the presence of carotid vascular disease in patients affected by NAFLD seems to be indipendent by common cardiovascular (CV) risk factors (5). Furthermore a higher prevalence in cardiovascular events is closely related with NASH

(6). Until now the pathogenic pathways of this relationship are not completely known. Goal of the study is to detect a relationship between hepatic steatosis severity and carotid plaque qualitative features, in patients affected by metabolic syndrome.
Methods and Results. 250 patients, 125 NAFLD subjects with a sex and age-matched non-NAFLD control group were enrolled; in both groups common CV risk factors (dyslipidaemia, smoking habits, hypertension, diabetes mellitus, glucose impaired tolerance, hyperglycemia, obesity), and morphologic vascular damage (carotid and femoral IMT and carotid plaque) were evaluated. Images of carotid plaque and hepatic segments were processed by Artery Measurement System (AMS) and characterized by Gray Scale Measurement (GSM). As result a statistically significant correlation between carotid IMT and waist circumference (r 0,07; p<0,05), glycemia (r 0,27; p<0,05), LDL cholesterol (r 0,23; p<0,05) and insulinemia (r 0,29; p<0,05) was found in both group. Within the NAFLD group, values of carotid plaque GSM were inversely related to glucose (r -0,07, p<0,05) and insulin (r-0,05, p <0,05) levels, to LDL cholesterol (r -0,37; p<0,05) and waist circumference (r-0,07; p<0,05). Hepatic GSM was strongly correlated to LDL cholesterolemia (r 0,29; p<0,05), glycemia (r 0,18; p<0,05), insulinemia (r 0,23; p <0,05) and waist circumference (r 0,06, p<0,05). At multivariate analysis the best determinants of carotid plaque GSM were waist circumference (beta -0,09; p<0,05), insulinemia (beta -0,13; p<0,05) and glycemia (beta -0,08; p<0,05), while the same for hepatic GSM were waist circumference (beta -0,06; p<0,05), insulinemia (beta 0,25; p<0,05), glycemia (beta 0,10; p<0,05) and triglyceridemia (beta 0,26; p<0,05). The most interesting result was the significant inversely relationship between hepatic and carotid plaque GSM (r0,317; p 0,004).CONCLUSIONSOur results seems to confirm a role of hepatic steatosis in predisposing in preclinical vascular damage and suggest its strong association with the common cardiovascular risk factors; furthermore our data show a higher prevalence in NAFLD patients of hypoechoic plaque (low GSM, higher lipid contents), related to higher cardiovascular risk, compared with non-NAFLD population.

References

1. Ryan MC, Wilson AM, Slavin J, et al. Associations between liver histology and severity of the metabolic syndrome in subjects with non-alcoholic fatty liver disease. Diabetes Care. 2005; 28: 1222-4.
2. Tarquini R, Lazzeri C, Boddi M, et al. Non-alcoholic fatty liver disease: a new challenge for cardiologists. G Ital Cardiol. 2010; 11: 660-9.
3. Lankarani KB, Mahmoodi M, Lotfi M, et al. Common carotid intima-media thickness in patients with non-alcoholic fatty liver disease: a population-based case-control study. Korean J Gastroenterol. 2013; 62: 344-51.
4. Ramilli S, Pretolani S, Muscari A, Pacelli B, Arienti V. Carotid lesions in outpatients with nonalcoholic fatty liver Disease. World J Gastroenterol. 2009; 15: 4770-4.
5. Targher G, Bertolini L, Padovani R, et al. Increased prevalence of cardiovascular disease in type 2 diabetic patients with nonalcoholic fatty liver disease. Diabet Med 2006; 23: 403-9.
6. Targher G, Bertolini L, Padovani R. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care. 2007; 30: 1212-8.

FAMILIAL HYPERCHOLESTEROLEMIA: FIRST RESULTS OF A 55 GENES TARGETED HIGH THROUGHPUT SEQUENCING

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Background. Familial hypercholesterolaemia (FH) is an autosomal dominant genetic disorder characterized by elevated plasma levels of low density lipoprotein-cholesterol (LDL-C), which can lead to premature cardiovascular disease. FH is caused by mutations in LDLR, APOB and PCSK9 genes found in 80%, 5%, and 1%, respectively, of FH subjects. However, many subjects with primary hypercholesterolemia did not demonstrate functional mutations in any of these genes. In a substantial proportion of FH patients without a known mutation, their high LDL-C concentrations might have a polygenic cause. Aim of this work is to sequence, through a targeted high-throughput sequencing (HTS) strategy, 55 genes, involved in FH or other alterations of lipid profile, in patients with suspected FH. We would evaluate whether this approach allows an improvement in diagnosis of genetic dyslipidemias and in research of novel associated genes, polygenic profiles impact and pharmacogenetic approaches.
Methods. Subjects with suspected FH are identified using the most common diagnostic algorithm, the Dutch Lipid Clinic Network Score (possible or probable FH). We analyzed patients in which we previously excluded, with traditional Sanger sequencing, the presence of a pathogenetic LDLR mutation. DNA libraries were prepared using Agilent Haloplex HS Enrichment System and the sequencing was performed using Illumina MiSeq Reagent Kit v3. Sequencing results were analyzed using both Agilent Sure Call software and a pipeline developed by the bioinformatics group of our Department. The possible pathogenicity of variants was evaluated using five different in silico tools (SIFT, Poly Phen, Mutation Taster, FATHMM, FATHMM-MKL, Provean).
Results. In our first HTS experiment, we sequenced 14 patients. In 3 of them we found one or two possible mutations that can explain their lipid profile. For example, one patient with possible FH have two mutations: one in APOB (S3294P) and another in LPL (N318S) gene. The second one, that has been already associated with hyperlipidaemia and hypertriglyceridaemia, could strengthen the effect of APOB variant. The remaining patients showed a burden of polymorphisms at high and low/very low allele frequency in different genes that in part represent, on the basis of some studies, known risk factors for dyslipidemia (for example variants on APOE and APOA5 genes) or atherosclerotic disease that could confirm a polygenic predisposition to the disease. It would be interesting to analyse the relatives of the index patients in order to evaluate the possible effects of the variants on the phenotype. Furthermore, our data suggested that the evaluation of genes involved in lipid-lowering drugs pharmacogenetics, such as SLCO1B1, could improve a personalized management of patients with an earlier achievement of the therapeutic target also avoiding adverse effects.
Conclusions. Our results showed the significant advantages of using a HTS approach for the diagnosis and study of FH. Indeed, with our specific 55 genes panel we can not only screen the 3 major genes involved in FH, but we can also have a larger view about the patient's genetic predisposition to the lipid disorders having a better understanding of the molecular bases of FH and other dyslipidemias and cardiovascular risk. In particular, the expanding of

the case study will allow to confirm the hypothesis that familial hypercholesterolaemia can also be caused by an accumulation of common small-effect LDL-C-raising alleles. Finally, the screening of the genes involved in lipid-lowering drugs pharmacogenetics could allow to apply a possible personalized therapy.

THE PROATHEROGENIC MICROBIOTA METABOLITE TRIMETHYLAMINE-N-OXIDE IS PRESENT IN HUMAN CEREBROSPINAL FLUID

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Trimethylamine-N-oxide (TMAO) is a small organic molecule, derived from the intestinal and hepatic metabolism of dietary choline and carnitine, that has been recently proposed as a novel prognostic marker of cardiovascular events. Indeed, its plasma levels is positively associated with indexes of atherosclerotic plaque vulnerability, major cardiac events and death. The recognized connections between gut and brain has recently raised the interest towards the impact of diet and microbiota on the functionality of the central nervous system. However, only few works revealed how dietary-derived products may exert (potentially adverse) neurological effects, and no evidence of clinical implications of TMAO in the central nervous system has been documented. The present study originates from the hypothesis that TMAO may play a direct role in the central nervous system. As a preliminary, fundamental aspect, its presence in the cerebrospinal fluid (CSF) needed to be assessed and this was the aim of the present study. CSF was collected for diagnostic purposes from 58 subjects by lumbar puncture and TMAO was quantified by using liquid chromatography-multiple-reaction monitoring mass spectrometry. The molecule was detected in all samples, at concentrations ranging between 0.11 and 6.43 µmol/L. Further analysis on CSF revealed that a total of 22 subjects were affected by Alzheimer's disease (AD), 16 were affected by non-AD related dementia and 20 were affected by other neurological disorders. However, the stratification of TMAO levels according to the neurological diagnoses revealed no difference among the three groups. Although ruling out a prognostic value of TMAO in neurological disorders, this study opens an interesting scenario about the actual role of this molecule in modulating functions of the central nervous system. Future studies in proper in vitro and in vivo models will address this issue, adding novel pieces in the gut-brain axis picture.

CHOLESTEROL EFFLUX CAPACITY DOES NOT ASSOCIATE WITH CORONARY CALCIUM, PLAQUE VULNERABILITY AND TELOMERE LENGTH IN HEALTHY OCTOGENARIANS

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Introduction. Traditional risk factors are less effective in predicting cardiovascular disease (CVD) risk in the elderly, suggesting the need to identify new biomarkers. Serum cholesterol efflux capacity (CEC) is an atheroprotective function of HDL recently identified as novel marker of CVD risk. We evaluated the association between CEC and atherosclerotic burden in a cohort of healthy octogenarians. In addition, we looked for correlations between serum CEC and telomere length (TL), as shortening of the telomeric DNA is associated to CVD risk. Finally, we compared CEC values of octogenarians with those of a cohort of younger individuals.
Methods. 59 healthy individuals ≥80 years were selected from The Brazilian Study on Healthy Aging. Cardiac computed tomography was performed to evaluate Coronary artery Calcium (CAC) and the presence or absence of plaque vulnerability features. Serum CEC was evaluated by cell-based radioisotopic techniques. CEC values were compared to those obtained from 140 sex-matched, healthy middle-aged (55±11 years) individuals.
Results. Subjects with CAC=0 displayed slightly but not significantly higher CEC values compared to subjects with CAC>0 (1.47±0.15 compared to 1.40±0.16; p=0.09). Subjects with positive or negative markers of plaques vulnerability displayed comparable CEC values. In addition, individuals with TL values under and over the median were not different in terms of CEC (1.42±0.17 compared to 1.42±0.17, ns). Interestingly, elderly subjects presented a remarkably higher CEC (+30%; p<0.0001) compared to middle-aged individuals.
Conclusion. Serum CEC is not related to atherosclerosis burden and TL in very old, free of cardiovascular events subjects, suggesting that this functional parameter may lose its protective role in elderly. However, our observation that healthy octogenarians show higher CEC compared to younger individuals possibly indicate CEC as the cause or a marker of such a healthy longevity.

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