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ABSTRACT

SOCIETA’ ITALIANA PER LO STUDIO DELL’ATEROSCLEROSI
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FH SELECTIVE SCREENING IN PEDIATRICS: A PILOT STUDY

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Introduction. Familial Hypercholesterolemia (FH) is the most common genetic disorder leading to very high cholesterol levels and premature coronary heart disease (CHD). FH is underdiagnosed and undertreated worldwide. An early detection in childhood is useful to establish an adequate management in order to prevent CHD.

Materials and Methods. We selected, in collaboration with three Coronary Care Units in the Province of Cuneo, Piedmont (Cuneo, Mondovi, Savigliano) and the Internal Medicine and Endocrinology Departments of Cuneo Hospital, families at cardiovascular risk on the basis of a positive history of CHD and/or the presence of severe hypercholesterolemia in parents and/or grandparents.

We evaluate cholesterol levels in children/adolescents belonging to these families through a capillary blood test (Afinion, Alere srl-Abbott). Children presenting TC levels ≥90° percentile were submitted to fasting venous blood sample collection to evaluate the lipid profile. FH was diagnosed according to Lipigen and Simon Broome Register Criteria and DNA analysis are in progress.

Results. Sixteen families were recruited in the study. All the families presented a positive CHD family history (43% premature CHD). We evaluated 23 subjects (M/F 14/9), aged 9.6±4.5 ys, BMI 17.5±3.8 kg/m² (4 subjects were overweight and 1 obese). Five subjects (M/F 4/1, age 7±4.5 ys) presented elevated TC levels at the capillary blood test (TC 275.4±31.0 mg/dl). The lipid profile analysis confirmed the preliminary data: TC 288±24.2 mg/dl, HDL-C 51.8±2.9 mg/dl, TG 93(42-127) mg/dl, LDL-C 221.5±32.5 mg/dl. Eighteen subjects presented normal TC levels at the capillary blood test (145.6±22 mg/dl).

Conclusion. This pilot study evidenced the relevance of an adequate screening program for FH diagnosis since childhood. Children showing a positive family history of premature CHD and/or dyslipidaemia with TC >290 mg/dl in their relatives should undergo biochemical analysis to optimize FH detection and treatment.

IN VITRO STUDIES TO IDENTIFY AND SORT EXOSOMES BASED ON THEIR SIZE AND LIPID COMPOSITION.
PRELIMINARY RESULTS

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Extracellular vesicles (EVs) are a group of membrane-derived structures released by cells, divided into two main groups: microvesicles (MV, >100 nm), and exosomes (size 50-150 nm). EVs circulate in biological fluids and transport and deliver lipids, proteins and nucleic acids to distant cells, and contribute to homeostasis, diseases development and progression. In this in vitro study, we utilized a lymph-node melanoma metastatic cell line (LM-16).

1. The most used EV isolation method is differential centrifugation (DC). By using an algorithm that takes in consideration g-force, time, viscosity of the medium and density of desired particles, to better define the properties of different EVs populations, we pelleted 4 different fractions (from 300 to 50 nm). After DC we processed the different pellets with Gaschromatography (lipid analysis) and Nanosight and TEM (distribution and dimensional characterization). Our data suggest that a decrease in EVs population with bigger diameter is counterbalanced by a decrease in relative percentage of saturated fatty acids (from 44.05% for 300nm to 71.44% for 50 nm), counterbalanced by a decrease in unsaturated ones (from 44.89% to 23.66%).

2. Since EVs are mainly made of cholesterol and phospholipids we can assume that lipid alteration can influence their functions, size and composition. We are evaluating if Simvastatin, an HMG-CoA reductase inhibitor, can alter EVs features. After 6-days incubation with 0.75 µM simvastatin and 24-hrs in serum free medium, we isolated EVs with DC. Our preliminary data shows that simvastatin increases EVs size (from 171.30 to 201.2 nm). In future we will utilize trehalose to prevent EVs aggregation and we will try to find new biomarkers to validate our method. We plan also to analyze lipid EVs composition and of their parental cells in order to understand if these alterations can lead to the biosynthesis of dysfunctional EVs to find new approaches for tumor and atherosclerosis treatment.

GUT MICROBIOTA, CAROTID VASCULAR DAMAGE AND MAJOR CARDIOVASCULAR RISK FACTORS IN THE GENERAL POPULATION: WHICH ASSOCIATION?

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Introduction. Increasing evidence indicates that alterations in Gut Microbiota (GM) composition might influence development of atherosclerosis and cardiovascular disease (CVD) (1-2), via activation of the immune system, alteration of cholesterol metabolism, and production of bacterial metabolites. The purpose of the study is to analyze GM in a large cohort, representative of the general population to relate intestinal bacterial community profiling to the presence of carotid vascular damage and to other major risk factors for CVD, and to find new diagnostic/prognostic biomarkers.

Methods. GM composition of 345 subjects from a population-based study (PLIC, “Progressione delle Lesioni Intimali Carotidi” project), was assessed through genomic DNA extraction from fecal samples and V3-V4 regions of the 16S rRNA gene sequencing by HiSeq 2500 Illumina platform, generating 250 bp paired end reads. At least 30’000 high-quality reads per sample were obtained and analyzed using QIIME; taxonomy was assigned using Greengenes.
ABSTRACT

results.

Results. We significantly highlighted a relevant separation between individuals with or without carotid vascular damage (p=0.016, unweighted UniFrac distance), implying relevant species diversity in the microbiota derived from both groups. In particular, subjects with atherosclerotic plaques, a significant increasing of pro-inflammatory genera Escherichia and a trend toward reduction of Roseburia (previously identified in subjects without chronic inflammatory conditions) was outlined. GM profiling correlated with multiple cardio-metabolic risk factors (glucose-metabolic/lipid profile, blood pressure, lifestyle habits), even in multivariate adjusted models.

Conclusions. We have provided new insights into how GM dysbiosis is related to the presence or absence of carotid vascular damage and cardio-metabolic risk factors even in the general population characterized by low CV risk. Analysis on other samples from geographical distant cohort is planned. The future challenge will be to determine:

a) whether specific bacterial species/metalobilies are the cause or consequence of the disease, through planned transcriptomic analysis;

b) determine causal biomarkers (trimethylamine N-oxide, TMAO) interaction.

GLYCOLYSIS INHIBITION WITH NOVEL COMPOUNDS SUPPRESSES ANGIOGENESIS

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Angiogenic signals induce endothelial cells (ECs) to switch their metabolism to being highly glycolytic. Clinical evidence has shown that the formation of new vessels destabilizes atherosclerotic plaques. Glycolysis modulation by inhibition of the key enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB3) is a possible therapeutic approach to reduce angiogenesis. We studied the effects of two novel self-synthesized phenoxindazole analogues inhibitors of PFKFB3 (PA-1 and PA-2) on glycolysis, cell proliferation, migration, and capillary tube formation in the human EC lines HUVEC and EA.hy926. Angiogenesis was assessed on matrigel and induces it in HUVECs. PA-1 and PA-2 markedly inhibit EC migration, proliferation and wound closing capacity. PA compounds downregulate the expression of markers of migration and angiogenesis (CCL5, VCAM-1, VEGFA and VEGFR2); 3PO suppresses the expression of ICAM-1, VCAM-1 and VEGFR2, probably explaining the differences we observed in capillary tube formation. Interestingly, 3PO suppresses several processes involved in the angiogenic process although it does not interact with PFKFB3, which implies for off-target effects. These findings suggest that glycolysis inhibition regulates EC migration, proliferation and capillary-like structure formation and this exerts a multitarget anti-angiogenic activity. Hence, PFKFB3 inhibition with PA compounds could be a promising therapeutic approach to promote plaque stability. This project has been funded by the European Union’s Horizon 2020 Marie Skłodowska-Curie grant (#67552).

GENETIC DIAGNOSIS, ACHILLES TENDON ULTRASONOGRAPHY AND DUTCH LIPID CLINIC NETWORK SCORE OF FAMILIAL HYPERCHOLESTEROLEMIA: PRELIMINARY DATA FROM THE UNIVERSITY OF PADUA OUTPATIENT LIPID CLINIC


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Aims. Ultrasonography (US) is used to detect Achilles tendon (AT) xanthomas in patients (pts) with Familial Hypercholesterolemia (FH). Our study focused on potential associations between FH genotype, clinical phenotype and ultrasonographic (US) AT findings, evaluating the contribution of AT US to identify individuals with an FH-causing mutation.

Subjects and Methods. Genetic screening, clinical and biochemical parameters were analysed in 116 pts with possible, probable or definite clinical diagnosis of FH according to the Dutch Lipid Clinic Criteria (DLCNS); 71 pts underwent bilateral AT US; ultrasonographic xanthomas (USX) were defined as the presence of AT thickness >6.15 mm in at least one of the AT and/or presence of hypoechogenic formations.

Results.

1) 85 pts were positive for LDL gene receptor mutation (35 carriers of null allele (NA) and 50 of defective (DEF) allele) and 31 with no known mutations (NM) for FH. Presence of xanthomas and gerontoxon, total and LDL-cholesterol (NA vs DEF vs NM: 326.5±97.7 mg/dl, 316.9±93.9 mg/dl, 211.1±76.3 mg/dl, p<0.000) at diagnosis were significantly higher in NA pts than other subgroups. AT thickness was significantly different among the three groups (p<0.005) and 78.2%, 72.4% and 31.6% had USX in NA, DEF and NM carriers respectively (p=0.002).

2) Among the 52 pts positive for FH-causing mutations: 36 pts had definite clinical diagnosis of FH by the DLCNS, while in 16 pts the diagnosis was either possible (5%) or probable (23%). Among these 16 pts, one had clinically evident xanthomas while 10 had USX. Thus, in nine pts the presence of USX was clinically undetected and thereby not considered for DLCNS calculation.

Conclusions.

1) Genotypic functional characterization is associated with different phenotypic clinical features, AT thickness and presence of US xanthomas.

2) AT ultrasonography may help reclassifying as definite FH, patients with DLCNS score of possible/probable FH.
ULTRASONOGRAPHIC DETECTION OF XANTHOMAS IN ACHILLES TENDONS OF SUBJECTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Aim. To analyse the diagnostic value of the Achilles tendon (AT) ultrasonography to identify xanthomas compared with clinical examination in subjects with Heterozygous Familial Hypercholesterolemia (HeFH).

Methods. We enrolled 101 subjects with HeFH between the age of 18 and 70 years old from January 2016 to October 2017. Medical history, clinical examination, complete lipid profile, and Dutch Lipid Clinic Network (DLCN) score were collected; all the subjects were submitted to bilateral AT ultrasonography to evaluate AT thickness, echo-structure and presence of hypo-echogenic formations. Subjects with previous tendon ruptures were not included.

Results. 101 subjects (63 F, and 38 M) with a mean age of 48.0±15.5 years and a mean DLCN score of 10.1±4.9. 10.2% of subjects presented xanthomas at clinical examination (XC) with an echographic pattern compatible with xanthoma (XUS), 51% presented XUS but not XC, and 38% had neither XUS or XC.

Conclusion. We have confirmed that ultrasonography is more sensitive than clinical examination to identify xanthomas, and it could represent, with medical history and clinical examination, a useful method to reach an early diagnosis of HeFH.

ENGINEERED REGULATORY T CELL ADOPTIVE THERAPY AS A NOVEL TOOL FOR THE TREATMENT OF ATHEROSCLEROSIS

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Aim. Loss of anti-inflammatory activity of regulatory Tcells (Treg) has been associated to immunoinflammatory diseases, including atherosclerosis. Therefore, the use of Treg-Adoptive Cell Therapy (ACT) is emerging as a therapeutic strategy to specifically modulate impaired immune responses. Although ACT has produced encouraging results in animal models, a main limitation remains the possibility to specifically target a selected tissue. Our aim was to develop and test a plaque-homing selective Treg-ACT in animal models.

Methods. Treg were retrovirally (IRES-EGFP vector) transfected with chemokine receptors or an empty vector and i.v. injected (2x105 GFP+ cells/mouse) in male 8-week WTD LDLR KO. Homing of transfected Treg to atherosclerotic plaque, its progression and composition was analysed by flow cytometry and histology.

Results. The chemokine CX3CL1 is selectively expressed in the aorta, but not in other tissues (lymph nodes, spleen and liver) of 8-week WTD LDLR KO, contrary to CCL2, usually associated with inflammation during atherosclerosis. Therefore, we compared homing of CCR2- and CX3CR1-transfected Treg to the aorta. While migration of CCR2-tranfected Treg was not tissue selective, CX3CR1-Treg showed a specific homing to atherosclerotic plaques (2.5% of GFP+/lived cell compared to 0.6%) with similar homing in lymph nodes and spleen. Next we investigated whether CX3CR1-Treg reduce atherosclerosis by performing plaque analysis 4 weeks after ACT. Although levels of plasma cholesterol were similar, CX3CR1- compared to control-Treg treated mice showed decreased plaque area and macrophage infiltration, and increased stability.

Conclusion. CX3CL1 is selectively expressed in atherosclerotic plaque and the CX3CL1/CX3CR1 axis may represent a pathway to target selective migration to the atherosclerotic plaque. Overexpressing CX3CR1 appears a promising ACT to promote selective homing of Treg into the plaque thus limiting atherosclerosis progression.

MOLECULAR CHARACTERIZATION OF MUTATIONS CAUSING LIPOPROTEIN LIPASE DEFICIENCY

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Background and Aims. Type I hyperlipoproteinemia is a rare autosomal recessive disorder most often due to loss of function mutations in the lipoprotein lipase gene (LPL), resulting in severe hypertriglyceridemia and recurrent pancreatitis episodes. In this work we studied the molecular mechanism beyond two already known mutations in LPL gene.

Methods. Two patients with hypertriglyceridemia were selected from the Lipid Clinic in Vienna. The first patient was compound heterozygous for c.680T>C (p.V200A) and c.1139+1G>A. The second patient compound heterozygous for c.953A>G (p.N291S) and c.1019-3C>A. LPL gene was sequenced and post-heparin plasma samples (ex vivo) were used to test LPL activity. In vitro experiments were performed in HEK293T cells transiently transfected with wild type or mutant LPL plasmids. Cell lysate and media were used to evaluate LPL production, secretion and activity by western blot analysis and LPL enzymatic assay, respectively.

Results and Conclusions. Immunoblot analysis and LPL activity measurement in plasma samples showed a faint LPL band and a strong reduction of LPL activity in both patients. In vitro experiments, showed a 10% reduction in LPL synthesis in both V200A and N291S mutant cells whereas LPL secretion was significantly reduced (80%), only in V200A. No LPL activity was detected in both
mutants. Our data show that V200A is a mutation that alter LPL production, secretion and activity whereas cells transfected with N291S showed normal levels of LPL protein but absent enzyme activity. We describe novel pathogenic LPL mutation leading to virtual absence of LPL activity and protein.

References

LP (A) AND CARDIOVASCULAR EVENTS IN A GROUP OF MEDITERRANEAN WOMEN (PROGETTO ATENA). PRELIMINARY REPORT
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Introduction. Association between Lp (a) and cardiovascular disease (CVD) was evaluated in a group of 196 women participating to Progetto Atena (N=5062), a study on the etiology of major chronic diseases in women (nested case-control study).

Methods. At the time of first visit (baseline visit) blood samples were collected and stored in liquid nitrogen (-196°C). Women at time of recruitment were free of cancer and cardiovascular diseases in women (nested case-control study). After 25 years from baseline visit Lp (a) was detected, on serum stored at time of baseline visit, by solid phase two-site enzyme immunoassay (ELISA). The women were divided into a control group (group 1, N=105) and in a group (group 2, N=91) who had cardiovascular events after the initial visit.

Results. No statistically age differences resulted between group 1 (53.6±7.8 yrs) and group 2 (56.8±7.3) (p=0.266). In the two group no statistical significance was found in the principal cardiovascular disease. After 25 years from baseline visit Lp (a) was detected, on serum stored at time of baseline visit, by solid phase two-site enzyme immunoassay (ELISA). The women were divided into a control group (group 1, N=105) and in a group (group 2, N=91) who had cardiovascular events after the initial visit.

Conclusions. These findings indicate that women who had CVD after baseline visit had elevated Lp (a) (>20 mg/dL of studied population) at baseline visit. Elevated Lp (a) was related to future cardiovascular event independently of major cardiovascular risk factor in this group of women participating to Progetto Atena. Screening and treatment of elevated Lp (a) must take into account when clinician estimated cardiovascular risk specially in women.

A CROSSTALKING BETWEEN SARCOPENIA, OSTEOPOROSIS AND ATHEROSCLEROSIS.
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Background. In recent years there has been a growing interest in evaluating the relationships between sarcopenia, bone mineral density (BMD) and fragility fracture risk.

Aim. The aim of the study was to evaluate the prevalence of sarcopenia in an older population and the correlation between sarcopenia and BMD. Methods: In 117 healthy subjects (20 males and 97 females) aged between 60 and 80 years (72.5 ± 6.1 years), we evaluated the presence of sarcopenia on the basis of EWGOP consensus: muscle mass (Skeletal Mass Index- SMI) with DXA, muscle strength with handgrip and physical performance with Short Physical Performance Battery (SPPB). We measured BMD at the lumbar spine (BMD-L), femoral neck: BMD-FN; Total: BMD-FT) and whole body (BMD-WB) with DXA (Prodigy, GE Lunar). In all subjects we were measured calcemia, phosphoremia, creatinine and 25OHD. In addition, ultrasonographic examination of the epiaortic vessels in each patient was performed by measuring IMT and presence of atheromatous plaques according to the European Carotid Surgery Trial (ECST) method and was assessed quality intima-media thickness (QMT) and pulse wave velocity (PWV) with a Mylab-70 ultrasound instrument.

Results. Men were more sarcopenic according to SMI. We found no significant differences between the genders in terms of muscle strength or SPPB. One third of the patients had sarcopenia with no significant differences between men and women, while the prevalence of osteoporosis was significantly higher in women. The values of sarcopenia showed a significant correlation with BMD (r=0.38; p<0.01) at the level of all skeletal sites. T-score values of sarcopenic patients was reduced at all levels obtaining statistical significance only at the femoral level (p<0.01). The prevalence of fractures was greater in sarcopenic patients than in non-sarcopenic patients. Muscle mass and muscle strength assessed with handgrip were found to be an independent predictor of BMD in males but not in females.Dividing patients into two groups based on presence of sarcopenia (30 patients with sarcopenia and 59 patients without) we observed that the values of IMT were higher in patients with sarcopenia (1.01±0.31 vs 0.9±0.23) with statistical significance (p<0.05).In addition the values of QMT were significantly higher in patients with sarcopenia with statistical significance (0.6±0.1 vs 0.8±0.1, p<0.05). Moreover, we found that the values of PWV were higher in patients with sarcopenia (9,1±2,5 vs 8,7±1,9) even though the difference didn’t reach statistical significance.

Conclusion. Our data confirm that an evaluation of the muscle tissue, measured by the lean appendicular mass and the muscle strength and physical performance, is important as well as the study of bone mineral density (BMD). In addition these data show an correlation of sarcopenia with atherosclerosis and consequently the higher cardiovascular risk in sarcopenic patients.
A PEDIATRIC-TAILORED APPROACH TO DETECT CHILDREN AND ADOLESCENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction. To the best of our knowledge, no validated scores to detect pediatric patients with familial hypercholesterolemia (FH) are available so far. Revised-Dutch-Lipid Clinic Network Score (rDLCSN) are worldwide used to detect adult patients with FH and are often experimentally adapted to pediatric age (PEDrDLCSN). PEDrDLCSN include premature coronary artery disease (pCAD) in a parent / first degree relative. “Premature” is meant before 55 years if male and before 65 years if female. Score higher than 8 qualify for definite FH.

Materials and Methods. The aim of this study is to evaluate the efficacy of PEDrDLCSN to select pediatric patients eligible for DNA-mutational analysis for FH in patients referring to our Lipid Center. In a 24-months period, 110 patients were referred to our Lipid Center for hypercholesterolemia. PEDrDLCSN were calculated for each patient. 28/110 (26%) showed a score of 6 or higher and underwent genetic analysis for LDL-R mutation. 4 tests are still ongoing. Parents’ median age (years) was 43 (mother) and 46 (father).

Results. 14/28 (50%) of our 28 tested patients had a mutation of LDL-R gene; 2/28 (7%) had positive pCAD in parents/first-degree relatives; 8/28 (28%) had pCAD in second-degree relatives. Out of the 14 patients with positive genetic test, 2/14 (14%) had pCAD in first-degree relatives, 7/14 (50%) had pCAD in second-degree relatives and 13/14 (93%) had positive family history for lipid disorders.

Conclusions. PEDrDLCSN is a useful tool to detect patients eligible for FH molecular diagnosis, though it should be more targeted to pediatric age. Almost all patients with positive genetic test have a positive family history for hypercholesterolemia. This item seems to be the most important one in anamnestic evaluation. As children’s parents age is often lower than the threshold proposed for pCAD, they may not have had a pCAD yet. Considering pCAD also in second-degree relatives (such as grandparents) might even improve this tool for FH detection in pediatric population.

ASSOCIATION BETWEEN GLOBAL CARDIAC CALCIFICATION (GCCS) AND OSTEOPOROSIS

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Background. Epidemiologic and clinical data have suggested the existence of a biologic linkage between bone and vascular system. Osteoporosis and atherosclerosis are two prevalent major health care concerns that frequently coexist. Several studies reported correlation between lower values of Bone Mineral Density (BMD) and cardiovascular events. Furthermore literature suggests that cardiac calcification (measured with Global Cardiac Calcium Score, GCCS) is associated with cardiovascular events and mortality.

Aim. This study aimed to evaluate if cardiac calcium deposit was correlated with BMD.

Methods. In 36 subjects assessed for bone fracture risk (mean age 72±5.7 years) we measured Bone Mineral Density (BMD) at lumbar spine (BMD-LS) at femur (Neck: BMD-FN; Total: BMD-FT) and we assessed with echocardiography a global cardiac calcium score (GCCS).GCCS is a semi-quantitative score, that was applied assigning points for calcification in the aortic root and valve, mitral annulus and valve and sub-mitral apparatus, and points for restricted leaflets mobility (1).
ABSTRACT

Can be considered a cardiovascular risk factor. Carotid ATS, and this supports the hypothesis that hepatic steatosis association between the presence and the degree of NAFLD and the burden of ATS. The results confirm the positive, but not statistically significant, relationship between the presence and the degree of hepatic steatosis (grade I, II and III) observing that increasing hepatic steatosis severity was accompanied by an increase in the IMT values, however without reaching statistical significance (p=0.1). Likewise, there was a positive, but not statistically significant, relationship between the severity of NAFLD and the burden of ATS. The results confirm the association between the presence and the degree of NAFLD and the carotid ATS, and this supports the hypothesis that hepatic steatosis can be considered a cardiovascular risk factor.

Results. The results show that there is a significant inverse correlation between BMD-FN and BMD-FT with GCCS (r=−0.285, p=0.05 and r=−0.376, p=0.05 respectively). No significant correlation was found between BMD-LS and GCCS. Dividing patients into two groups based on presence of bone fragility fractures (13 patients with fragility fractures and 23 patients without) we observed that the value of GCCS was higher in patients with bone fragility fractures (2.54±1.3 vs 2.30±1.5) but not statistically significant. Moreover, dividing patients on the basis of presence of sarcopenia (9 patients with sarcopenia and 27 patients without sarcopenia) we found that the values of GCCS was higher in patients with sarcopenia (3.0±1.4 vs 2.1±1.4) even though the difference didn’t reach statistical significance.

Conclusion. Our data suggest link between osteoporosis and cardiac calcification. The burden of cardiac calcium seems to be higher in patients with fragility fractures. These findings confirm that osteoporotic patients have an higher risk of cardiac and vascular calcification and this confirm the greater risk of cardiovascular events in osteoporotic patients.

Reference

ROLE OF NON ALCOHOLIC STEATOSIS IN CAROTID ATHEROSCLEROSIS
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There is a growing evidence in literature for a close relationship between non-alcoholic hepatic steatosis (NAFLD) and cardiovascular morbidity and mortality. However, it has not been clarified whether the increase in cardiovascular risk is due to the presence of cardiovascular risk factors associated with the metabolic syndrome, commonly found in patients with NAFLD, or if NAFLD itself is a mediator of atherosclerosis (ATS). The aim of the study was to investigate the correlation between hepatic steatosis and carotid ATS, observable in its early stages with the increase of miointimal thickness (IMT). In this study 110 patients, referred to the clinical setting of metabolic disease, were divided into two groups based on the presence or absence of fatty liver on the ultrasound examination of the abdomen (70 patients with steatosis of which 42 men and 28 women and a control group of 40 patients without steatosis). Ultrasonographic examination of the epicardial vessels in each patient was performed by measuring IMT and possible presence of atheromatous plaques according to the European Carotid Surgery Trial (ECST) method. We found an IMT of 1.32±0.68 mm in patients with steatosis and 1.14±0.76 in non-steatose subjects with a statistically significant difference (p=0.01). The presence of atheromatous carotid plaques was 40.5% in patients with steatosis and 20.5% in the control group with a statistically significant difference (p=0.01). The patients with hepatic steatosis were then ruled according to the degree of steatosis (grade I, II and III) observing that increasing hepatic steatosis severity was accompanied by an increase in the IMT values, however without reaching statistical significance (p=0.1). Likewise, there was a positive, but not statistically significant, relationship between the severity of NAFLD and the burden of ATS. The results confirm the association between the presence and the degree of NAFLD and the carotid ATS, and this supports the hypothesis that hepatic steatosis can be considered a cardiovascular risk factor.

HDL3 MODULATES THE PHENOTYPIC SWITCH INDUCED BY CIGARETTE SMOKE IN VASCULAR SMOOTH MUSCLE CELLS
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Background and Aims. Up to 50% of foam cells in human atherosclerotic plaque originates from smooth muscle cells (SMCs). Upon cholesterol loading, SMCs modify their phenotype from contractile to synthetic, with a decrease of the expression of typical SMC markers (α-actin, calponin) and an increase of pro-inflammatory markers (Mac-2). Moreover, cholesterol loading reduces the expression of myocardin, a SMC specific transcriptional factor involved in SMC differentiation, and that of its modulator, miR-143/145. On the contrary, cholesterol increases the expression of Klf4, a myocardin repressor. Data recently published by our group showed that these changes are prevented by HDL3 only in the presence of the Abca1 membrane transporter. The phenotypic behavior of SMCs is modulated also by cigarette smoke (CS), an important atherosclerotic risk factor. To characterize the role of Abca1 and HDL3 in this process, we evaluated HDL3 effects on CS-induced phenotypic changes in SMCs expressing or not Abca1.

Methods. SMCs, isolated from the aortae of wild-type (WT) and Abca1 knock-out (KO) mice, were incubated with cigarette smoke condensate (CSC). Results: CSC incubation promotes SMCs phenotypic switch by downregulating the expression of α-actin and calponin and by increasing the expression of Mac-2, Abca1, Abcg1. HDL3 prevents this effect by returning the values to baseline in WT cells, whereas the effect of HDL3 is completely lost in Abca1 KO cells. Myocardin and Klf4 expression is respectively reduced and increased by CSC treatment, indicating this pathway as a possible mechanism underlying the phenotypic modulation of SMCs.

Conclusions. Our results indicate that CSC, like cholesterol, induces the SMCs phenotypic switch from contractile to synthetic. HDL3, by interacting with Abca1, modulates the Klf4-myocardin axis and prevents the CSC-induced phenotypic switch in SMCs. In Abca1 KO cells, the preventive effect of HDL3 is completely lost, confirming that the presence of Abca1 is mandatory for the protective activity of HDL3.
EFFECTS OF PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) INHIBITORS ON CLINICAL OUTCOMES: A META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Background and Aim. Since the role of proprotein convertase subtilisin/kexin type 9 (PCSK9) in LDL receptor degradation was discovered, several pharmacological approaches to inhibit this enzyme and lower plasma LDL cholesterol (LDL-C) have been developed, such as monoclonal antibodies (mAbs), which are able to reduce plasma LDL-C up to 60% in different hypercholesterolemic patients. The aim of this meta-analysis was to investigate the effects of PCSK9 antibodies treatment on different clinical outcomes in randomized controlled trials (RCTs).

Methods. PubMed/MEDLINE and EMBASE were searched from inception to May 2018. The main inclusion criteria were: (1) English language, (2) phase 2 or 3 RCT, (3) comparing PCSK9 mAbs with placebo, (4) with effects on outcomes reported and (5) with duration longer than 8 weeks. Mantel-Haenszel method was used to estimate the pooled odds ratios (ORs) with the 95% confidence interval (CI). A continuity correction in case of rare events was also applied. Between-study heterogeneity was tested by Cochrane’s Q test and measured with the I² statistics. In cases of non-normal distribution were log-transformed. Gender differences in the population were assessed with t-test. The association between variable of interest was assessed with bivariate Pearson correlation. Multiple linear regression analysis was performed (method Stepwise) in order to identify independent predictors of IL-18 levels and insulin resistance.

Results. Twenty-six RCTs were selected, comprising more than 60,000 patients. The analysis showed that the exposure to anti-PCSK9 did not lead to a significant reduction in mortality from all causes and cardiovascular (CV) disease. An 18% reduction (OR=0.82, 95% CI: 0.77-0.87) of CV events and a 5% reduction in incidence of serious adverse events (OR=0.95, 95% CI: 0.91-0.99) were observed in subjects exposed to mAbs compared to the control group (exposed to placebo, on top of statin). A 5-years follow-up adjusted analysis confirmed the results and highlighted even more the benefit of a long-term exposure to PCSK9 mAbs.

Conclusions. This updated meta-analysis confirmed the efficacy of different PCSK9 antibodies in reducing incidence of cardiovascular events across different type of patients.

IL-18 AS A POSSIBLE LINK BETWEEN PHYSICAL INACTIVITY AND INSULIN RESISTANCE

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Introduction. According to the WHO, unhealthy dietary habits and physical inactivity, along with alcohol consumption and use of tobacco, are the pillars that support the development and maintenance of chronic diseases. Physical inactivity, and particularly sedentary lifestyle, has belatedly caught the attention of the scientific community, compared to other harmful behaviors. Physical inactivity is crucial in the development of overweight and obesity, but there is increasing evidence that physical inactivity and a sedentary lifestyle can increase the prevalence and severity of metabolic and cardiovascular diseases, independently from body weight and composition. Low grade systemic inflammation is one of the best known processes involved in pathogenesis of metabolic and cardiovascular diseases and IL-18 has recently been recognized as a potential actor in the development of atherosclerotic plaque and insulin resistance. The aim of this study was to evaluate the association between physical activity and sedentary behavior, insulin resistance and IL-18 plasma levels.

Materials and Methods. We enrolled 451 subjects (age >60 years) with preserved motor performance and good health conditions (low prevalence of chronic diseases). Subjects underwent blood sampling, bio-impedance analysis for body composition evaluation and assessment of cardiorespiratory fitness with UKK 2 km walking test and/or cycle ergometer (stress test). All subjects filled a questionnaire concerning weekly physical activity (weekly METS) and daily sedentary time (screen time). Variables with a non-normal distribution were log-transformed. Gender differences in the population were assessed with t-test. The association between variable of interest was assessed with bivariate Pearson correlation. Multiple linear regression analysis was performed (method Stepwise) in order to identify independent predictors of IL-18 levels and insulin resistance.

Results. IL-18 was significantly higher in males compared to females, (400.6 (320.8-503.0) vs 322.4 (263.1-405.5) pg/ml, p<0.001). We observed a significant association between IL-18, markers of systemic inflammation (CRP, c. Pearson 0.273, p<0.001) and insulin-resistance (HOMA-IR, c. Pearson 0.283, p<0.001), in both sexes. Moreover, only in males, we observed a significant association between IL-18 levels and markers of adiposity (BMI, WC, FM respectively with c. Pearson 0.205, 0.233, 0.230, all p<0.005). We also tested the correlation between IL-18 and lifestyle indicators in males: IL-18 values were positively associated with daily screen time (0.179, p=0.013) and inversely correlated with VO2 max and UKK fitness index (respectively c. Pearson -0.190, p=0.007 and c. Pearson -0.220, p=0.005). In order to identify the independent predictors of IL-18 in male, we built a linear regression model with IL-18 as the dependent variable. Both UKK fitness index and screen time resulted as independent predictors of IL-18 levels with opposite effect (UKK fitness index: beta coefficient -0.216, p=0.006; screen time: beta coefficient 0.216, p=0.006). Similarly, we performed a linear regression analysis entering HOMA index as the independent variable, in the whole population and age, WC, FM, VO2 max, UKK fitness index, weekly METS, daily screen time and IL-18 as dependent variables. IL-18, FM together with WC resulted as independent predictors of insulin-resistance (respectively, beta coefficient 0.210, 0.270, 0.224, all p<0.005).

Conclusions. Our data confirm the association between IL-18 and insulin-resistance. IL-18 also shows, at least in the male population, a direct association with adiposity and measures of sedentariness, and an inverse association with physical activity and cardio respiratory fitness. Interestingly, there is an independent and opposite effect of physical activity and screen time on IL-18 levels, highlighting as sedentary time has a specific role in affecting cardiovascular and metabolic risk. In conclusion, it is likely that IL-18 plays a role in the complex interaction between lifestyle, adiposity and cardiovascular risk. Further studies are needed to clarify the possible sex-specific difference in the regulation of IL-18 concentrations.
PREVALENCE OF MICRO- AND MACROVASCULAR COMPLICATIONS IN ITALIAN SUBJECTS AFFECTED BY TYPE II DIABETES MELLITUS

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Introduction. The prevalence of Diabetes Mellitus is rising year after year worldwide. About 1 in 11 adults is affected by Diabetes in the world. The persistence of elevated blood glucose levels can lead to the development of serious chronic complications: diabetic retinopathy, nephropathy, neuropathy (microvascular), coronary artery disease, peripheral arterial disease and ischemic stroke (macrovascular).

Aim of the Study. The aim of the study is evaluating the prevalence of microvascular and macrovascular complications in a Diabetic population of South Italy.

Materials and Methods. The study was conducted analyzing retrospectively a large database which contains informations related to more than 10000 patients afferent to twelve medical practitioners which work cooperatively in the same city. We evaluated: the prevalence of diabetic patients, the presence of CV risk factors (arterial pressure, smoking, lipidic profile, abdominal circumference, obesity) and the presence of chronic complications. Results: The total number of the subjects analyzed in the informatic platform was 10638. 800 of 10638 were affected by Type II Diabetes Mellitus, M=320,F=480(age 59±18.8 years). Patients affected by obesity were 68%, patients affected by hypertension were 68.75%, patients affected by hypercholesterolemia were 70%. Patients with neuropathy were 41.57%, while those who showed retinopathy were 36%, Subjects with CAD were 40 patients (5%), with Ischemic Stroke 12 (1.5%), with Peripheral obstructive chronic arteriopathy 96 (12%).

Conclusion. This study evaluated the prevalence of Type II Diabetes Mellitus and its complications in a population of South Italy showing its great increase compared to data from international and national studies. A correct life-style, a strict glycemic control, followed by a strict pressure control, lipidic control and the use of adeguated drugs is recommended in diabetic patients to prevent chronic metabolic disorders.

SERUM LIPOPROTEIN (A) PREDICTS ACUTE CORONARY SYNDROMES IN PATIENTS WITH SEVERE CAROTID STENOSIS

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Background and Aim of the Study. Lipoprotein (a) [Lp (a)] is a well established independent risk factor for the development and progression of atherosclerosis and cardiovascular disease 1,2, but the cut-off values of Lp (a) above which cardiovascular risk increases and so its pathophysiological and prognostic role remain unclear, mainly because of the published data are limited. The aim of this pilot study was to explore the role of Lp(a) in predicting the development of acute coronary syndrome (ACS) and its relationship with atherosclerotic plaque components in high-risk patients.

Materials and Methods. We included 180 patients with severe carotid artery stenosis undergoing endarterectomy. According to the most recent scientific evidences 3, we selected the Lp(a) cut-off value of 10 mg/dl to predict 24-month follow-up ACS. In addition, the relationship between the levels of Lp (a) and the different components of atherosclerotic plaque was assessed.

Results. At baseline, no significant differences were found in the two groups of patients [Lp (a) <10 mg / dl or >10 mg / dl] with regard to demographic characteristics, ongoing drug therapy and laboratory parameters, with the exception of higher levels plasma fibrinogen and D-dimer and the most frequent presence of chronic coronary artery disease in subjects with high levels of Lp (a). The main finding of our study was the association between high serum Lp(a) levels (>10 mg/dl) and the increased risk of developing an ACS at 2-year follow-up. With the histopathological analysis of carotid plaques, the only significant finding was a slight increase in the content of smooth muscle cells in the upstream regions of the plaques in subjects with Lp(a) >10 m/ml.

Conclusions. Our results suggest a possible predictive role of Lp(a) for the development of ACS in patients with advanced carotid atherosclerosis, and the cut off of 10 mg/dl could be an appropriate value to identify patients with an increased cardiovascular risk.

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SILENCING OF HISTONE DEACETYLASE 3 AT DIFFERENT STAGES OF DIFFERENTIATION: EPIGENETIC EFFECTS ON ADIPOCYTE METABOLISM

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Introduction. Obesity is a condition characterized by uncontrolled expansion of adipose tissue mass resulting in pathological weight gain. Histone deacetylases (HDACs) have emerged as crucial players in epigenetic regulation of adipocyte metabolism. Previously, our group demonstrated that selective inhibition of class I HDACs using MS-275 improves white adipocyte functionality and promotes browning of C3H/10T1/2 mesenchymal cells differentiated into adipocytes. These effects are observed in db/db and diet induced obesity mice, two models of obesity and type 2 diabetes, and in mice with adipose-selective inactivation of HDAC3.

Aim. The molecular basis of class I HDACs action in adipose tissue is not deeply characterized and it is not known whether the effects of their inhibition are exerted on adipocyte precursors or mature adipocytes. Therefore, the aim of the present work is to explore the molecular mechanism of class I HDAC action in adipocytes by evaluating the effects of HDAC3-specific silencing at different stages of differentiation.

Material and Methods. HDAC3 was silenced in C3H/10T1/2 mesenchymal stem cells at different stages of differentiation into adipocytes. shRNA targeting HDAC3 was used to generate the knock-down model.

Results. HDAC3 silencing at the beginning of differentiation enhanced adipocyte functionality by amplifying the expression of genes regulating differentiation (Pparg, Cebpa), oxidative metabolism (Acadm), browning and mitochondrial activity (Ucp1, Cox7a1). However, no differences in terms of gene expression were found in db/db and diet induced obesity mice, two models of obesity and type 2 diabetes, and in mice with adipose-selective inactivation of HDAC3.

Conclusion. HDAC3 silencing at the beginning of differentiation enhanced adipocyte functionality by amplifying the expression of genes regulating differentiation (Pparg, Cebpa), oxidative metabolism (Acadm), browning and mitochondrial activity (Ucp1, Cox7a1). However, no differences in terms of gene expression were found in db/db and diet induced obesity mice, two models of obesity and type 2 diabetes, and in mice with adipose-selective inactivation of HDAC3.

ROLE OF PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) IN THE FUNCTIONALITY OF CARDIAC MITOCHONDRIA

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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. Vice versa, increased expression of receptors of the LDL-R family was shown to favor lipid accumulation, accumulation that in the heart results in myocyte apoptosis, myocardial fibrosis, impaired mitochondrial function and cardiac dysfunction.

Aim. This project is aimed at testing the hypothesis that PCSK9 might regulate lipoprotein and fatty acid metabolism 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). MRI has been performed on these mice and the hearts have been collected. Mitochondrial respiration was investigated under resting conditions and following maximal coupling and uncoupling conditions in all mice models. A metabolomic analysis have been performed and changes in the profile of mitochondrial proteins were tested by western blotting.

Results. The lack of PCSK9 in cardiac cells is associated with a reduced ATP production as well as a reduced total energy charge (ATP: 15.07±1.49Vs9.43±0.424 and Energy charge: 15.07±1.49Vs9.43±0.424, p<0.05) indicating an altered metabolic profile that can lead to mitochondrial damage. Metabolomics data shows that PCSK9 KO and Albcre-PCSK9 KO mice have less glucose 6-P, ribose-5-P and erythrose-4-P compared to WT, alterations that are known to be associated with an increased glycolytic flow. These observations are paralleled by a reduction of the Krebs cycle intermediates in PCSK9 KO and Albcre-PCSK9 KO animals compared to WT. Western Blot analysis of protein lysates showed a reduced expression of key proteins of the electron transport chain and an increased expression of LDL receptor in both PCSK9 KO and Albcre-PCSK9 KO models. From the Oxygen Consumption data we have seen that the reduced expression of the electron transport chain complexes is associated with a reduced oxygen consumption and mitochondrial respiration. The heart of PCSK9 KO mice are also subjected to a morphological change such as an increased thickness of the left ventricular wall during systole.

Conclusion. Our data suggest that the lack of circulating PCSK9 is associated with altered lipids metabolism in the heart that associate with altered morphology and functionality. This preliminary data supports the need for an extensive characterization of this animal model.
SONOGRAPHIC EVALUATION OF ACHILLES TENDON IN FAMILIAL AND POLYGENIC HYPERCHOLESTEROLEMIA: A CASE-CONTROL STUDY

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Introduction. Familial Hypercholesterolemia (FH) is a monogenic metabolic disease in which defects in LDL binding or internalizing cause hypercholesterolemia and premature atherosclerosis. Its diagnosis relies on physical examination and biochemical, anamnestic and genetic data (some of which included into a comprehensive score, e.g. the Dutch Lipid Clinic Network Score [DLS]). Aim of this study was to evaluate the role of a simple and standardized ultrasound testing of Achilles tendon thickness, in the diagnosis of Familial Hypercholesterolemia (FH), assessing its correct classification capacity (CCC).

Patients and Methods. In a case-control study we enrolled 60 males and 60 females, 18-70 years old, with genetically confirmed heterozygous FH, who addressed a single Lipid Clinic. Each one of these patients were sex and age-matched with subjects affected by polygenic hypercholesterolemia (PH, n=120) and normolipemic controls (n=120). All these individuals underwent bilateral ultrasound measurement of Achilles tendon thickness (ATT, 2 cm from heel) and the presence of echo-structural characteristics relating to Achilles tendon xanthomatosis (ATX) was recorded.

Results. Increased ATT, by sex and aged specific cut-off values, showed a diagnostic sensibility of 72% and specificity of 79% in diagnosing FH. In the whole population the CCC of this method, examined by ROC curve analysis, was fairly good (AUC, 0.78) and that during T2DM there could be a shift of PON1 and Lp-PLA2 towards the more pro-atherogenic lipoprotein subclasses. The activity of both enzymes is conditioned by the composition of lipoprotein carriers. It’s well known that in T2DM there are significant changes in lipid profile and in the distribution of the subclasses of lipoproteins. Thus, it becomes relevant to understand the interaction of PON1 and Lp-PLA2 with subclasses of lipoproteins in T2DM. We evaluated serum levels of PON1-Arylesterase, PON1-Lactonase and Lp-PLA2 activities and lipoprotein subclasses in 202 non-diabetic subjects (Controls) and 92 T2DM outpatients. Arylesterase, but not Lactonase or Lp-PLA2 activities, was independent and inversely associated with TD2M (Odds Ratio=3.389, 95% Confidence Interval 1.069-14.756). Regarding distribution in lipoprotein subclasses, we observed that arylesterase activity was independently related to HDL-C and small intermediate density lipoprotein cholesterol (IDL-C) in Controls while, along with Lactonase, it was related with small low density lipoprotein cholesterol (LDL-C), all IDL-C subspecies and very low density lipoprotein cholesterol (VLDL-C) in T2DM (p<0.05 for all). Lp-PLA2 showed significant correlation with small LDL-C, large IDL-C and VLDL-C only in T2DM group. Our study we observed that T2DM subjects has lower levels of PON1-Arylesterase activity compared to Controls and that during T2DM there could be a shift of PON1 and Lp-PLA2 towards the more pro-atherogenic lipoprotein subclasses. The possibility of a link between the two observed phenomena required further investigations.
CURRENT IMPACT OF DIABETES ON IN-HOSPITAL SURVIVAL AND COMPLICATION OUTCOMES IN PATIENTS HOSPITALIZED FOR STROKE – THE VASD-OUTCOME STUDY

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Background. The prevalence of diabetes mellitus (DM) and accompanying cardiovascular complications is increasing worldwide. The treatment strategies for both DM and cerebrovascular accidents (CVA) have considerably improved in the recent past, thus possibly softening the detrimental effect of DM in patients with CVA. However, the availability of updated data on the current epidemiology of DM in patients hospitalized for CVA is relatively limited. AIM - To estimate the prevalence of DM in a large contemporary cohort of individuals admitted for CVA and to investigate the independent impact of DM and hyperglycemia on the in-hospital survival and complication outcomes.

Experimental Design. We assessed the prevalence of DM, previously known (known-DM) or newly-diagnosed (new-DM), in all patients (n=542, males 51.5%) with primary admission for stroke at the Stroke Unit of the Neurology Department at the Verona University Hospital from 1/1/2015 to 12/31/2016. DM was ascertained according to previous diagnosis, glucose-lowering medications at admission/discharge or random plasma glucose ≥200 mg/dL. The association of DM with in-hospital death, infections/cardiorespiratory complications, duration and costs of hospitalization was tested in all patients and separately in known-DM and new-DM. Similar analyses were applied by stratifying the whole cohort according to quartiles of plasma glucose level at hospital admittance.

Results. Prevalence of DM was 21.03% (78.1% known-DM; 21.9% new-DM 27.2%). In-hospital death rate was 10.5% (n=57 death events). Compared to non-diabetic patients, those with DM showed an increased risk of death (15.8 vs. 9.1%; crude-OR 1.87 95%CI, 1.03-3.41) and complications (48.2 vs. 33.3%; crude-OR 1.87, 1.22-2.86). DM diagnosis was associated with higher hospitalization costs (mean±SEM, 4,087±127 vs. 4,123±318 €, p=0.88) and longer hospital stay (14±1.06 vs. 8.80±0.39 days, p=0.72), although these differences did not reach statistical significance. The total number of death events occurred within the Stroke Unit. When the analyses were restricted to the subgroup of “survivors”, a significantly longer hospital stay was observed in patients with DM (2.32±1.02 vs. 0.98±0.19 days, p=0.038). After multivariable adjustment (age, sex, e-GFRMDRD, hemorrhagic stroke, lipid-lowering or anti-hypertensive therapy), DM remained an independent predictor of in-hospital complications (adj-OR 1.87, 1.22-2.80), but not mortality (adj-OR 1.79, 0.92-3.46). All study outcomes did not display significant differences between new-DM vs. known-DM. After stratification of the whole cohort according to the admittance plasma glucose levels, hyperglycemia resulted as predictor of in-hospital death and complications, independent of DM status, age, sex, e-GFRMDRD, alteplase medication and ongoing glucose-lowering, lipid-lowering or anti-hypertensive medication, with patients falling in the highest quartile (plasma glucose ≥118 mg/dL) displaying an increased risk of death (3.25, 1.40-7.54) and complications (2.90, 1.24-4.23), as compared to those comprised in the lowest tier (<86 mg/dL, reference). After stratification of the whole cohort according to age, those comprised in the highest tertile (age ≥81 years) displayed a risk of death significantly increased (adj-OR 3.25, 1.40-7.54; n=34 events), as compared to the younger subgroup falling in the lowest tier (<72 years, ref.). The opposite was observed with respect to the in-hospital complications endpoint, with younger individuals displaying a considerably higher risk, than those at older age (adj-OR 3.13, 1.42-6.9).

Conclusions. These data highlight that the occurrence of DM in patients admitted for stroke is extremely high in frequency still nowadays and it carries an excess burden of mortality and complications. Hyperglycemia per se shows an incremental risk for adverse clinical outcomes, thus underscoring the urgent need for strategies to anticipate DM diagnosis and promptly set a proper therapy in patients at high cardiovascular risk.

ASSOCIATION BETWEEN TSH AND NON-ALCOHOLIC FATTY LIVER DISEASE IN THE OVERWEIGHT-OBESE POPULATION

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Background. Non alcoholic fatty liver disease (NAFLD) is a spectrum of disorders characterized by accumulation of fat in the liver in the absence of other recognized causes of fatty. It affects up to 30% of the population in Western countries. Furthermore increased risk of incident T2DM by approximately twofold and an approximate twofold increased risk of CKD were reported. It was previously described a correlation between thyroid dysfunction, especially hypothyroidism and subclinical hypothyroidism, and Steatosis. Thyroid gland play an important role in the regulation of diverse metabolic processes, promoting hyperlipidemia and obesity, thus contributing to NAFLD. However, it is still unclear whether TSH even in the euthyroid condition is associated with Steatosis in the overweight-obese population.

Methods. In this cross-sectional study were enrolled 149 individuals (age 18-65 y) with BMI ≥25 kg/m² who underwent a liver steatosis assessment by CAP (Controlled Attenuation Parameter) measurement, referred to Clinical Nutrition Unit at the “Magna Grecia” University in the last year. Liver Transient Elastography was used to quantify liver steatosis by CAP assessment. Ten successful measurements were performed on each patient (Success rate: 60%). The ratio of the IQR of liver stiffness to the median (IQR/MLSM) was calculated as indicator of variability. The final CAP score (ranged from 100 to 400 decibels per meter (dBm)-), was the median of individual measurements. For each patient, several the laboratories test were assessed. We excluded those who had chronic hepatitis B and/or C virus infection, alcohol abuse, impaired liver function, presence of autoimmune disease. Type 1 and 2 diabetes mellitus, fasting glucose >110 mg/dL, tryglicerides >200 mg/dL, TSH >5 mIU/L or <0.3 mIU/L/L.

Results. After multivariable analysis, the CAP was associated with WHR (p<0.001), tryglicerides (p<0.006), BMI (p=0.023) and TSH tertiles (p=0.03). Participants in tertile I (low TSH values) had a lower Controlled Attenuation Parameter than those in tertile II (p=0.001) and tertile III (p<0.001).
Conclusion. We found a positive association between Controlled Attenuation Parameter and TSH values: highest levels of TSH, within the reference ranges, were associated with highest CAP values. Thyroid function, even within reference range of thyroid function tests, is significantly correlated with CAP Score in overweight-obese. Further studies on the involvement of thyroid hormone in NAFLD may help to understand its underlying mechanisms and to identify at-risk patients reducing the onset and possible progression of NAFLD and its potential complications.

IDENTIFICATION OF GENETIC VARIANTS IN METHIONINE METABOLISM GENES IN PATIENTS WITH MARFAN SYNDROME OR RELATED DISORDERS CHARACTERIZED BY NEXT GENERATION SEQUENCING ANALYSIS

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Background. Previous data from literature evidenced the role of homocysteine and MTHFR C677T polymorphism in influencing the severity of cardiovascular manifestations in patients with Marfan syndrome (MFS). In this study, we investigated the influence of genetic variants in genes related to methionine metabolism (CBS, MTHFR, MTR, MTRR, MMADHC), identified in patients admitted to the Referring Center for MFS and Related Disorders through next generation sequencing (NGS) target gene approach, on cardiovascular manifestations severity.

Methods. A NGS target gene panel including 94 genes involved in MFS and related connective tissue disorders in differential diagnosis has been used to investigate the genetic profile of 21 patients suspected to be affected by MFS, or bicuspid aortic valve with aortic dilatation [median age (range) 41 (13-70) yrs; 63.6% males].

Results. NGS target gene approach permitted the detection of 12 missense genetic variants in the 5 genes related to methionine metabolism: 4 variants in MTHFR gene (rs1801133, rs1801131, rs27552099, rs35773219), 2 in MTR gene (rs1805087, rs121913579), 5 in MTRR gene (rs1532268, rs1801394, rs10380, rs162036, rs140944718), and rs61750442 variant in MMADHC gene. Increased minor allele frequency (MAF) for MTHFR rs1801133 and rs35773219, MTRR rs10380, rs162036 and rs140944718 with respect to MAF reported from literature in the Caucasian population was observed. Statistically significant difference in Z score values of aortic diameters at the sinus of Valsalva according to DeVereux RB et al. 2012 between carriers and non-carriers of MTRR rs1801394 polymorphism (NM_000245.4:c.66A>G, NP_000244.2:p.Ile22Met) has been found [3.01 (1.77-6.20) vs 1.9 (0.1-6.0) p=0.036]. SIFT and Polyphen evaluation showed a deleterious effect of this variant.

Conclusions. Investigation of genes related to methionine metabolism by NGS approach identified genetic variants able to modulate severity of cardiovascular manifestations in MFS and related disorders. Further evaluation of these variants in a larger population could allow to confirm this datum.

LARGE GENERES PANEL TARGETED HIGH-THROUGHPUT SEQUENCING IN DETERMINING MONOGENIC AND POLYGENIC NATURE OF FAMILIAL HYPERCHOLESTEROLEMIA IN RESEARCH AND DIAGNOSIS

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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 mainly by mutations in LDLR, APOB and PCSK9 genes. However, many subjects with primary hypercholesterolaemia did not demonstrate functional mutations in any of these genes.

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. The design also allow to genotype all variants included in the Tal- mud et al. (2013) low-density lipoprotein cholesterol gene score and in statins pharmacogenomics. 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. We analyzed 30 clinically probable or definite FH patients. 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 SureCall software and a pipeline developed by the bioinformatics group of our Department. The possible pathogenicity of variants was evaluated using six different in silico tools.

Results. In 16 out of 30 patients, we found major mutations that can explain their lipid profile. The remaining patients showed a burden of polymorphisms at high and low/very low allele frequency in different genes that in part represent, based on some studies, known risk factors for or atherosclerotic disease that could confirm a polygenic predisposition to the disease. We started the segregation analysis in families of two patients. In one family the role of mutations in ABCG5 gene in lipid profile alteration was demonstrated. Furthermore, our data suggested that the simultaneous evaluation of genes involved in lipid-lowering drugs pharmacogenomics 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 an 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 define the role of other major genes (e.g. ABCG5) and have a larger view about the patient’s polygenic predisposition to the lipid disorders. The expansion 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 an early personalized therapy.

PCSK9: A ROLE IN THE PROGNOSTIC STRATIFICATION OF SEPTIC PATIENTS?

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Background. PCSK9 plays a critical role in regulating circulating cholesterol levels, through the reduction of the membrane-associated LDL receptor. In experimental models plasma PCSK9 levels were increased in sepsis. At normal levels, PCSK9 has no influence upon hepatocyte bacterial endotoxin clearance, but as levels rise, there is a progressive inhibition of clearance. It has been shown that decreased PCSK9 function increases survival of septic patients. Aim of the study was to assess prognostic value of early assessment of PCSK9 levels in septic patients.

Methods. 263 patients were enrolled in a prospective analysis aiming to find reliable biomarkers for early sepsis diagnosis. Patients admitted to High-Dependency Unit of Emergency Department with diagnosis of severe sepsis/septic shock were eligible. We evaluated vital signs and laboratory data at admission (T0), at 6 hours (T6) and after 24 hours (T24); Sequential Organ Failure Assessment (SOFA score) was calculated at T0, T6 and T24. Primary end-points were 7- and 28-day mortality.

Results. Mean age of the study population was 74±14 years, 58% male gender; mean SOFA score at admission was 5.3±2.7. The most frequent infection source was respiratory (45%). In septic patients, at every evaluation point, PCSK9 level was significantly different (T0: 696±381 vs 614±444; T6: 716±417 vs 631±421; T24: 709±413 vs 716±470 ng/mL, all p=NS). Finally we compared PCSK9 levels in patients with T0 lactate level ≤ and >2: even in this analysis we did not find any significant difference (T0: 672±485 vs 642±365; T6: 689±449 vs 664±373; T24: 711±392 vs 698±452 ng/mL, all p=NS).

Conclusions. PCSK9 levels were significantly increased in septic patients. However, the levels did not show any significant association with indexes of hypoperfusion and with the severity of organ damage, as well as with the short- and medium-term mortality rate.

STATIN-INDUCED MYOPATHY: DIFFERENT STRATEGIES FOR MANAGEMENT AND DIFFICULT CHALLENGE TO REDUCING CARDIOVASCULAR RISK

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Background. Statin-related myopathy is a common problem in clinical practice, especially due to the matter to reach LDL-C target and reduced patients’ compliance with therapy.

Aim. To evaluate patients with statin-induced myopathy characteristics and the behavior in washing-out and rechallenging therapy.

Methods. We retrospectively reviewed data from 1240 hypercholesterolemic patients visited at our Center between 2006-2015.

Results. Among 714 patients treated with statins, 113 (15.8%) presented muscle-related symptoms: 103 stopped the previous treatment; 89 of them (78.8%) agreed to do a rechallenge, 14 (12.4%) did not. After wash-out, 12 (10.6%) were still grievous for muscle symptoms, whereas 19/89 patients who did a rechallenge re-presented the same problems. CPR values among patients presenting muscle-symptoms were elevated (M=50±200 mg/dL) in 14 subjects (12.4%) but were >4xULN (myositis) only in 4 of them. Nobody had rhabdomyolysis. The most used statin in symptomatic patients was atorvastatin (43-38.0%), following by simvastatin (35-31.0%) and rosuvastatin (24-21.2%); few patients (10-8.8%) took fluvastatin, lovastatin or pravastatin but no significant relation was found and this can simply reflect the market trend. Subsequently, in the rechallenge/down-titration phase, attempting to maintaining a good efficacy/tolerability, statins more frequently used have been rosuvastatin (28-31.5%), simvastatin+ezetimibe (21-23.6%) orpravastatin (20-22.4%); 12 patients (13.5%) disagreed and continued with other Lipid-lowering drugs (monacolin K, fenofibrate). Once stopped the first statin, only 31 out of 113 patients (27.4%) reached the LDL-goal recommended by SCORE System. In particular 68 patients (60.2%) had a familial dyslipidaemia and only 14 (20,6%) had reached the LDL-C target. Moreover, patients with very-high cardiovascular risk were 17 (15.0%) but only 1 had LDL-C levels <70 mg/dL.

Conclusions. Statin-induced myopathy causes a delay and a hurdle to the reducing of LDL-C levels, especially in higher risk categories. Such patients should benefit from alternative therapeutic options.
THE WALKING ELDERLY

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Background. The hypertension is very common condition in elderly patients. Some studies demonstrate the preservation of muscle mass and physical performance improve survival in the elderly. The aim of the present study was to assess the relationship between reduced physical mobility and survival in elderly hypertensive patients.

Methods. We have observed the data stored in our hypertension treatment center for patients aged from 80 to 96 years. In our study we analyzed data from 120 elderly patients affected by hypertention; we evaluated the presence of co-morbidities that reduce walking. We divided patients into A Group and B Group. A Group: (60 patients) presence of co-morbidities or conditions that reduce walking (arthritis, peripheral neuropathy, episodes of past falls, fear of falling). B Group: (60 patients) normal walking, the patients performed the walking speed test. We analyzed the incidence at 5 years in the 2 groups of acute events (stroke, ama, acute renal fail ure), death. Dosages of standard therapies for Hypertension such as ACE-inhibitors or beta-blockers and calcium channel blockers were used. Patients affected by secondary hypertension, smoking habit, alcohol consumption, dementia, diseases of the central nervous system a clinical history of CVD, liver or kidney advanced diseases were excluded.

Results. We found a significant association between reduced ambulation and the incidence of acute events (p<0.003). We found a significant association between reduced ambulation and the incidence of death in 5 years (p<0.004). Increased walking time was related to a high incidence of adverse events and death in Group A.

Conclusions. Our results are in agreement with experimental evidences, suggesting that preserved walking, and preserved muscle mass plays an important role in the development of vascular damage and complications in elderly hypertensive patients. Reduced walking significantly increases the incidence of complications and death at 5 years in the elderly hypertensive patient.

METABOLOMIC SIGNATURE OF ANGIOPOIETIN-LIKE PROTEIN 3 DEFICIENCY IN FASTING AND POSTPRANDIAL STATE

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Objective. Angiopoietin-like 3 (ANGPTL3) is a promising therapeutic target for lowering atherogenic lipid levels and decreasing cardiovascular disease risk. We aimed to determine detailed metabolic effects of ANGPTL3 deficiency in fasting and postprandial state.

Material and Methods. The study population consisted of individuals carrying S17X loss-of-function (LOF) mutation in ANGPTL3 gene (N=6 homozygous and N=32 heterozygous carriers) and controls (N=38). We used nuclear magnetic resonance metabolomics to quantify 225 metabolic measures. We compared metabolic signatures of LOF carriers and controls in fasting state and after an energy-rich meal.

Results. In fasting, ANGPTL3 deficiency resulted in similar reductions in magnitude for low density lipoprotein cholesterol (LDL-C, p=0.74 per SD change, SE=0.16, P=2.5x10-5) and triglyceride-rich lipoproteins (cholesterol in very low density lipoprotein, b=−0.75, SE=0.16, P=7.1x10-6). Further, beta-hydroxybutyrate, a biomarker of hepatic fatty acid beta-oxidation, was elevated (b=−0.55, SE=0.17, P=0.002). Absolute levels of both cholesterol and triglycerides were substantially lowered in most lipoprotein subclasses, with the relative proportion of triglycerides being increased and cholesterol proportion reduced in triglyceride-rich lipoproteins. Homozygous ANGPTL3 LOF carriers showed blunted postprandial response in triglyceride-rich lipoproteins and several fatty acid measures, and more pronounced increases in ketone bodies when compared to controls.

Conclusions. In addition to overall triglyceride and LDL-C lowering effects, ANGPTL3 deficiency results in reduction of cholesterol content in triglyceride-rich lipoproteins. Further, ANGPTL3 LOF carriers had elevated ketone body production, suggesting enhanced fatty acid β-oxidation. The metabolic profile in individuals representing human knockout of ANGPTL3 reinforce ANGPTL3 as a promising therapeutic target for decreasing cardiometabolic risk.

A DIFFICULT CASE OF CHRONIC AND PROGRESSIVE ISCHEMIC ARTERIOPATHY IN A YOUNG PATIENT WITH HIGH LDL CHOLESTEROL AND LIPOPROTEIN (A) DESPITE MAXIMUM STANDARD LIPO-Lowering THERAPIES

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Background. Patients who didn’t reach their LDL-C target were so far forced to undergo invasive and expensive treatments such as LDL-Apheresis. PCSK9 inhibitors represent a revolution for these patients.

Aim. To evaluate efficacy and tolerability of evolocumab 140mg (E140) Q2W in a 54-yo patient in secondary prevention for multiple episodes of MI/ACS/UA again and again treated by PCI and placement of stents from the age of 49, despite maximum tolerated regimen of standard lipid lowering therapies (LDT).

Case report. The first time we visited him, patient presented BMI=27, DLS=6, LDL-C=144 and Lp(a) 104mg/dL; LDL-C score (E140) Q2W in a 54-yo patient in secondary prevention for multiple episodes of MI/ACS/UA again and again treated by PCI and placement of stents from the age of 49, despite maximum tolerated regimen of standard lipid lowering therapies (LDT).

To evaluate efficacy and tolerability of evolocumab 140mg (E140) Q2W in a 54-yo patient in secondary prevention for multiple episodes of MI/ACS/UA again and again treated by PCI and placement of stents from the age of 49, despite maximum tolerated regimen of standard lipid lowering therapies (LDT).
duced E140 mg Q2W on top of his LLT, checkin lipid and hepatic profile firstly once a month and then every three months. He quickly reached his LDL-C target (lowest LDL-C value ~5 mg/dL) and Lp(a) ~21 mg/dL. For the first time in the last five years he stabilized his coronary atherosclerosis symptoms, without needing revascularization procedures and so gaining a good quality of life (QoL) and a significant save of money for public health. Adherence and tolerability have been excellent. Laboratory data (LDL-C between 10-20 mg/dL) remained almost constant during the observation period so that we could reduce statin and suspend ezetimibe. The average reduction of LDL-C continued to be higher than 90%.

Conclusions. A long-term observation will be necessary to evaluate efficacy and tolerability of this treatment over time but, to date, E140 has shown, in this difficult case, a surprising LDL-and Lp(a) reduction, much greater than that reported in literature (2), a stabilisation of symptoms with a very good result regarding QoL and relevant money saving.

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Efficacy and Tolerability of Proprotein Convertase Subtilisin/Kexin Type 9 Inhibitors in Patients Affected by Familial Hypercholesterolemia And/ Or At High Cardiovascular Risk Not Reaching Target With Standard Therapies: A Real-Life Observational Study

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Background. PCSK9-i have recently been authorized in Italy for the treatment of HeFH, high-risk and statin intolerant patients not reaching target with standard therapies.

Aim. Evaluate efficacy and tolerability of PCSK9-i in a real-life setting in 23 patients followed at our Center.

Method. As usual, PCSK9-i were administered subcutaneously Q2W using pre-filled syringes on top of the maximum tolerated standard therapies.

Results. 8 of 23 patients received evolocumab 140, 10 of them alirocumab 75 and 5 alirocumab 150. Only three didn’t take background statin for intolerance. Two patient were treated since 2012 and 6 since 2016 because participating in phase 3 trials with alirocumab. The others started treatment between July 2017 (PCSK9-i approved in Abruzzo) and May 2018. Minimum treatment evaluation time was 3 months. Mean LDL-C reduction was -49% over the results achieved with standard therapies. One patient surprisingly increased baseline values (perhaps due to incorrect administration or to an unknown PCSK9-gene’s mutation) and two (brothers with LDL-R 2HeFH, encoded among the HoFH) were poor responders: by removing these 3 subjects from the analysis we observed a -60% LDL-C reduction, in line with literature data (1, 2). There was a significant TC reduction and HDLC increase but no significant changes of triglyceride. 17 patients out of 23 (73,9%) reached their LDL-C target; 6 patients did not, but 3 of them (the 2 brothers 2HeFH and one in secondary prevention) got a reduction (-20% for 2HeFH and -53% for high-risk pt. respectively) as expected for their categories (3, 4); 2 were poor responders (-25% perhaps because they didn’t take statins as background therapy. There were no significant difference regarding efficacy and tolerability between Evolocumab and Alirocumab.

Conclusion. Our results are similar to the few real-life available data (5, 6). Longer-term real-life studies are still needed to evaluate efficacy, tolerability and safety of these drugs.

References
ABSTRACT

Familial Hypercholesterolemia (FH) is a common autosomal co-dominant disease due to pathogenic mutations in LDLR, APOB and PCSK9 genes. FH is characterized by high LDL levels from birth and an increased risk of coronary artery disease (CAD) that can be, at least partially, reversed through lipid-lowering therapy (LLT). Prompt identification of FH among CAD patients could be extremely useful for intensification of therapy and cascade screening. Physicians should pay more attention to this lipid-linked CV risk factor in clinical practice for a correct CV prevention in hypertensive patients.

IDENTIFICATION OF PUTATIVE FH SUBJECTS AMONG PATIENTS WITH CORONARY ARTERY DISEASE

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Introduction. Familial Hypercholesterolemia (FH) is a genetic disorder that results in premature coronary artery disease (pCAD) due to lifelong exposure to elevated LDL-C levels. Heterozygous FH (1:200-250) is caused by one pathogenic variant in one of the major FH candidate genes (LDLR, APOB, PCSK9). Homozygous FH (1:200000-300000) is caused by biallelic pathogenic variants and, if untreated, can cause severe pCAD in the first decades of life.

Methods. An automatic alert was generated by clinical laboratory in case of LDL-C ≥250 mg/dl. This approach allowed to identify three patients with severe hypercholesterolemia. Patient 1 was 38 y/o male with a known hypercholesterolemia, past statin therapy suspended for muscle pain and no family history of pCAD. Total cholesterol and LDL-C were 329 and 253 mg/dl, Dutch Lipid Clinic Network Score (DLCNS) was 6. Patient 2 was a 56 y/o female with severe hypercholesterolemia since adolescence and a positive family history of pCAD. Total cholesterol and LDL-C were 331 and 261 mg/dl, Dutch Lipid Clinic Network Score (DLCNS) was 10. He recently suffered from unstable angina. Genetic testing for FH was performed in all patients.

Results. Patient 1 was a double heterozygous FH, being a carrier of missense mutation in LDLR (p.Val523Met) and APOB (p.Arg3558Cys) respectively. Patient 2 was a compound heterozygous FH having two missense mutations in two different LDLR alleles (p.Pro181Arg and p.Pro685Leu). Patient 3 was a true homozygous FH having a biallelic missense variant in LDLR (p.Gly592Glu).

Conclusions. Automatic alert by clinical laboratory is an useful tool to identify patients with a severe, possibly monogenic, hypercholesterolemia. Molecular characterization can drive the therapeutic choice and allows cascade screening in relatives.

MOLECULAR CHARACTERIZATION OF THREE PATIENTS WITH DOUBLE HETEROZYGOUS, COMPOUND HETEROZYGOUS AND HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Results and Conclusion. Mean age: 56.5±13.7 years with male prevalence (55.6%). Overweight/obese patients (OV/OB) were 70.2%. OV/OB had higher glycaemia [96 (88-107) vs 89 (83-89) mg/dl; p<0.001] and a higher risk of having diabetes mellitus (13.7% vs 5.3%; p<0.001; OR:2.8). OV/OB had an atherogenic dyslipidemia characterized by lower HDL (51.2±14.2 vs 58.5±14.9 mg/dl; p<0.001) and higher non-HDL cholesterol (148.8±40.5 vs 140.9±50.3 mg/dl; p=0.006). While 41.6% of all patients had controlled BP, only 28.5% achieved LDLc controlled according to 2016 ESC/EAS guidelines. Surprisingly, the higher the CV risk, the lower was the LDLc control (p<0.001). There was poor concordance between the guidelines analyzed. More than 30% of patients that were uncontrolled based on 2016 ESC/EAS guidelines resulted controlled based on 2013 AHA/ACC and 2014 NICE guidelines.

Conclusion. Our findings were in accordance with results from other studies and demonstrated that it is possible to give an accurate estimation of putative FH patients by combining different databases. Subjects with DLCNS≥5 should be genetically screened for FH. The application of this strategy to routine clinical setting would allow to identify FH patients and to develop cascade screening in relatives for primary prevention of CAD.
PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 AND VASCULAR CALCIFICATION UNDER UREMIC CONDITION

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Introduction. Vascular calcification represents a main risk factor of cardiovascular events in patients with chronic kidney disease (CKD). Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) levels correlates with the presence of calcific aortic valve stenosis and carriers of the PCSK9 R66L loss-of-function variant have a low calcific aortic valve stenosis.

Aim. We investigated a possible role of PCSK9 on aortic calcification by using a uremic rat model of vascular calcification and in vitro cultured human smooth muscle cells (hSMCs) overexpressing PCSK9.

Results. Sprague-Dawley rats were fed a standard diet (n=10) or uremic diet containing 0.5% adenine (n=10) for 6 weeks. The uremic condition was documented by increased urine volume (26 ml/day vs 58 ml/day), plasma creatinine (25.7 µM vs 208 µM) and phosphate levels (2.64 µM vs 6.11 µM). High phosphate concentration was associated to aortic calcification determined by measuring aorta Ca²⁺ concentrations (0.34 mg/g tissue vs 2.48 mg/g tissue) and by Von Kossa staining. This pathological condition was associated to a significant increase of total cholesterol (from 75.3 mg/dL to 107.6 mg/dL) and PCSK9 levels (from 40.1 ng/ml to 109.7 ng/ml). Higher expression of PCSK9 was also observed in kidney (+4.8 fold) and liver (+1.5 fold). The overexpression of PCSK9 in hSMCs (from 0.02 ng/ml to 11.3 ng/ml) induced a significant increase of extracellular calcification in response to 5 days exposure to 2.4 mM P0₄⁻. (-39% compared to control hSMCs), while P0₄⁻ reduced the release of PCSK9 from hSMCs (-33.6%) and the mRNA expression levels (-43%) together with significant reduction of mRNA expression of LDL receptor and HMG-CoA reductase.

Conclusions. The present study indicates a direct role of PCSK9 on vascular calcification associated to a CKD condition. Further analysis will attempt to identify the molecular mechanism of this action and to study the effect of monoclonal antibodies anti PCSK9.

SAFETY OF RED YEAST RICE SUPPLEMENTATION: A META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS


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Background. Recently, concerns regarding the safety of red yeast rice (RYR) have been raised after the publication of some case reports claiming toxicity. Since the previous meta-analyses on the effects of red RYR were mainly focused on its efficacy to improve lipid profile and other cardiovascular parameters, we carried out a

EFFECT OF BERRBERINE AND Silymarin ON SERUM LIPIDS AND FASTING PLASMA GLUCOSE: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL STUDIES

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Aim. We aimed to assess the impact of a combination of berberine and silymarin on serum lipids and fasting plasma glucose through a systematic review of literature and meta-analysis of the available randomized, double-blind, placebo-controlled clinical studies (RCTs).

Methods. A systematic literature search in SCOPUS, PubMed-Medline, ESI Web of Science and Google Scholar databases was conducted up to 2nd September 2018, in order to identify RCTs assessing changes in plasma concentrations of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and fasting plasma glucose (FPG) during treatment with berberine and silymarin in combination. Two review authors independently extracted data on study characteristics, methods and outcomes. Quantitative data synthesis was performed using a random-effects model.

Results. We identified 5 eligible RCTs, with 497 subjects overall included. Berberine and silymarin combination treatment exerted a positive effect on CT [weighted mean difference (WMD): -25.3 95%CI(-39.2, -11.4) mg/dL; p<0.001], TG [WMD: -28 95%CI(-35.3, -20.6) mg/dL; p<0.001], LDL-C [WMD: -29.1 95%CI(-39.7, -18.6) mg/dL; p<0.001] and FPG [WMD: -7.5 95%CI(-13, -1.9) mg/dL; p=0.008].

Conclusions. The present findings suggest that the coadministration of berberine and silymarin is significantly associated with an advantageous improvement in lipids and glucose profile, suggesting the possible use of this nutraceutical combination in order to promote the cardiovascular health.
meta-analysis on safety data derived from the available randomized controlled clinical trials (RCTs).

**Methods.** Several databases were searched, with no language restriction, from inception to August 20th, 2018. Primary outcomes were muscle-skeletal disorders (MuD). Secondary outcomes were non-muscle-skeletal adverse events (Non-MuD) and serious adverse events (SAE). Subgroups analyses were carried out considering the intervention (RYR alone or in association with other nutraceutical compounds), monacolin K administered daily dose (≤3, <3-5 and >5 mg/day), follow-up (>12 or ≤12 weeks), with statin therapy or statin-intolerance and type of control treatment (placebo or statin treatment). Fixed effects meta-regression analyses including some potential independent moderator variables (i.e. age at baseline and monacolin K daily dose) were planned to explore heterogeneity.

**Results.** Data were pooled from 52 RCTs comprising 110 treatment arms, which overall included 8503 subjects, with 4421 in the RYR arm and 4287 in the control one. Monacolin K administration was not associated with increased risk of MuD (OR=0.94, 95%CI 0.53,1.65). Moreover, meta-analysis showed reduced risk of Non-MuD (OR=0.59, 95%CI 0.50, 0.69) and SAE (OR=0.54, 95%CI 0.46, 0.64) versus control. Subgroups analyses confirmed the higher tolerability profile of RYR. All the results were robust in the leave-one-out sensitivity analysis. Meta-regression analyses did not suggest an increased risk for RYR-associated MuD, Non-MuD and SAE depending on age. Furthermore, increasing daily doses of monacolin K were negatively associated with increasing risk of Non-MuD (slope: -0.10; 95%CI: -0.17, -0.03; two-tailed p<0.01).

**Conclusions.** Among statin users, women showed a lower adherence level than men, regardless of age. Further studies are required to identify the most suitable interventions to increase adherence to therapy, improving health status and ensuring a more effective public resource management.

**ROLE OF MMP9 GENE POLYMORPHISM IN GENETIC SUSCEPTIBILITY FOR CAROTID ARTERY DISEASE**

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**Introduction.** It is well known that Matrix metalloproteinases are involved in atherosclerosis processes and plaque vulnerability and our previous studies showed an association between carotid artery disease (CAS) and single nucleotide polymorphisms (SNPs) of the MMP9 gene. We evaluate three single nucleotide polymorphisms (SNPs) of the metalloproteinase 9 gene (MMP9), rs1918249, rs3918256, rs3918249, according to literature evidence.

**Materials and Methods.** Polymorphisms were studied in 2814 patients with CAS and n=814 apparently healthy subjects with Real Time Technology.

**Results.** Polymorphisms are in Hardy-Weinberg equilibrium both in patients and controls. The percentage of individuals carrying the T allele of the rs3918249 in CAS patients is significantly higher than that observed in the controls (CC=40.8%, CT=43.9%, TT 15.3% vs CC=58.7%, CT=34.8%, TT=6.5%, p<0.0001). For the polymorphism rs3918256 we observed that: AA patients >58.2%, GA=36.5%, GG=5.2% vs. controls AA=45.2%, GA=42.8%, GG=11.9%, p<0.0001, showing a higher percentage of G allele carriers in the controls. The genotypic distribution of the third polymorphism, rs17576 was AA=56.9%, GA=35.7%, GG=7.4% in patients vs AA=48.0%, G=14.0%, GG 8.0% in controls, p<0.0001. At the multivariate logistic regression (adjusted for traditional cardiovascular risk factors), the carriers of the allele rs1918249 T showed a significant and independent risk for CAS [OR=2.266 (95% CI 1.64-2.50), p<0.0001]; the carriers of the allele A rs3918256 showed a protective association against the onset of CAS [OR=0.59 (95% CI 0.45-0.78), p<0.0001]; the carriers of the allele G rs17576 showed only a trend of the protective association observed in the univariate analysis, [OR=0.77 (95% CI 0.59-1.007), p=0.084]. Also, at multivariate regression analysis the condition of being carriers of a number ≥5 risk alleles is associated with an increased risk of occurrence of SC [OR=3.5 (95% CI 2.3-5.2), p<0.0001].

**Conclusion.** Our data showed a possible role of the rs3918249 MMP9 gene polymorphism in CAS onset, and a protective role of the rs rs3918256 polymorphism for as rs17576. Also, the risk conferred by the alleles Trs3918249, A rs3918256 and A rs17576 is increased in the presence of a “Burden” of risk alleles.
PREVALENCE OF STATIN-ASSOCIATED MUSCLE SYMPTOMS IN ITALY: THE PROSISA STUDY

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Introduction. Statin-associated muscle symptoms (SAMS) are one of the main reasons for statin non-adherence and/or discontinuation, contributing to adverse cardiovascular outcomes. However, a definitive diagnosis of SAMS is difficult because symptoms are subjective, influenced by comorbidities, and there is no “gold standard” diagnostic test.

Aim and Methods. The PROSISA (Project on Statin Intolerance SISA) is an observational, retrospective and multicenter study aimed at assessing the prevalence of statin intolerance due to muscular symptoms in dyslipidemic patients on statin treatment followed by Italian lipid clinics, between 1st January 2006 and 31st December 2015. Anamnestic data, biochemical values before and during statin therapy, and the potential occurrence of muscular symptoms have been collected.

Results. In the first six months, the PROSISA database accounted for 6429 patients (mean age 66.7±12 years; 53.9% males) on statins. During the statin therapy, 787 patients (12.2%), mainly treated with simvastatin (30.6%), rosuvastatin (28.3%) and atorvastatin (27.1%), reported muscular symptoms: myalgia (74.2%), cramps (25.8%), fatigue (18.8%), myositis (1.4%) and rhabdomyolysis (0.8%). Among them, 375 patients underwent dechallenge, with disappearance of muscular symptoms in 87.2% of cases, while overall 503 patients underwent rechallenge (237 with change of statin/dose reduction without interruption of therapy) mainly with rosuvastatin (30.6%), rosuvastatin (28.3%) and atorvastatin (27.1%), reported muscular symptoms in 87.2% of cases, while overall 503 patients underwent rechallenge (237 with change of statin/dose reduction without interruption of therapy) mainly with rosuvastatin (30.6%) and simvastatin (30.4%) at low dosage, with reappearance of muscular symptoms in 151 patients. Among them, the muscle symptom mainly reported was myalgia (76.2%), while no cases of myositis and rhabdomyolysis were reported.

Conclusion. This preliminary analysis offers a general outlook of SAMS. The percentage of patients in whom intolerance has been confirmed by dechallenge/rechallenge is between 26-30%, emphasizing the need to deepen the assessment beyond the simple occurrence of muscle symptoms for a definitive diagnosis of SAMS and discontinuation of treatment. The final results will be the cornerstone to obtain a complete overview of identification and management of SAMS in Italy.

EFFECTS OF ANGPTL3 IN C2C12 MUSCLE CELLS: RESULTS OF A PRELIMINARY STUDY

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Background. ANGPTL3 protein is known to be involved in the inhibitory regulation of extracellular lipases (LPL and HL). Thereby, its absence has been shown to increase the lipolytic degradation of TG-rich lipoproteins. Recently, we have reported that ANGPTL3 may also influence fatty acid (FFA), which are important substrates for energy production. In particular, we found that ANGPTL3 can influence lipolysis in 3T3-L1 adipocytes throughout a biphasic action strictly depending on the presence or absence of β-adrenergic stimulation. If this mechanism is also acting in muscles tissue is not known. Therefore, our primary purpose was to evaluate the effects of ANGPTL3 in isolated muscle cells. This would allow to improving our knowledge about the role of ANGPTL3 as also a regulator of energy substrate utilization.

Methods. Differentiated C2C12 cells will be treated by ANGPTL3 (100nM) and beta-agonist isoproterenol(100nM). Proteins and genes expression involved in intracellular lipolytic pathways will be investigated at the different time (30,60,90 minutes) by a combination of biochemical measures, Western blot, and RT-qPCR analyses.

Results. In comparison with control, the treatment of muscle cells with ANGPTL3 and beta-agonist isoproterenol (ISO) caused a reduction in the level of AMPK protein expression (after 30 minutes and 60 minutes respectively; p<0.05). ANGPTL3-treated, as well as ISO-treated cells, showed increased gene and protein expression of ERK1/2, also escalated markedly levels of ERK1/2 phosphorylation form (pERK).

Conclusions. In the present study, our data demonstrated by multiple technical approaches the effectiveness of ANGPTL3 on the intracellular signaling pathway in C2C12 muscle cells.

FAMILIAL HYPERCHOLESTEROLEMIA: 10-YEARS EXPERIENCE OF GENETIC SCREENING

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Introduction. Heterozygous Familial Hypercholesterolemia (HeFH) is the most common autosomal dominant disorder associated with high levels of LDL cholesterol (LDL-c) and increased cardiovascular risk. The genes involved in FH are LDLR, APOB and PCSK9 while LDLRAP1 is involved in a rare recessive form of FH. Homozygous FH (HoFH) is caused by mutation at homozygous status or of 2 mutations at compound heterozygous status and can lead to cardiovascular accidents since the childhood.

Patients and Methods. We enrolled 638 subjects with clinically diagnosis of FH, of whom 467 were unrelated. The LDLR, PCSK9, LDLRAP1 and part of exons 26 and 29 of the APOB genes were se-
MANAGEMENT OF HYPERCOLESTEROLEMIC SUBJECTS AT HIGH CARDIOVASCULAR RISK WITH PCSK9 INHIBITORS: EXPERIENCE IN A LIPID CLINIC

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Background. Monoclonal antibodies anti-PCS9 (Alirocumab and Evolocumab) have been recently approved by Food and Drug Administration (FDA) and European Medicines Agency (EMA) and are available in Italy for the treatment of patients with familial hypercholesterolemia (FH) and for secondary prevention in subjects with high cardiovascular (CV) risk on top of standard lipid lowering therapy (LLT) or in mono therapy in statin intolerant patients.

Objectives and Methods. The aim of this study was to evaluate the efficacy and safety of Alirocumab and Evolocumab in 50 patients (35 FH and 15 High CV Risk) in a real life setting. 33 subjects (27 FH and 6 High CV Risk) were treated with Alirocumab (75 mg or 150 mg based on LDL-C at baseline) and 17 subjects (8 FH+9 HCR) obtained Evolocumab (140 mg). Patients have self administered monoclonal antibodies subcutaneously every two weeks. Clinical and biochemical parameters, anthropometric measures, medical history, background LLT and side effects were evaluated over a period of 2 years.

Results. Patients treated with Alirocumab and Evolocumab exhibited a substantial and sustained reduction of the LDL-C plasma levels by a mean of more than 50% (LDL-C decrease variability between 46.2% and 77.3%) and LDL-C targets <100 mg/dl or <70 mg/dl were reached in all subjects. No significant change was observed in liver function tests. Injection site local reactions such skin erythema, pain, swelling and bruise were reported in 20% of patients but these adverse events progressively reduced over the subsequent drug administrations. No flu-like symptoms have been reported.

Conclusions. This study confirms the efficacy and safety of treatment with Alirocumab and Evolocumab both in FH and high CV risk patients attending a Lipid clinic.

PREVALENCE OF STATIN INTOLERANCE IN A COHORT OF OUTPATIENTS IN A LIPID CLINIC

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Background. Statins represent the gold standard in the management of dyslipidemia and are very effective in the prevention of cardiovascular diseases. They are generally safe and well tolerated however some patients experience statin intolerance issues. The prevalence of intolerance varies widely. Statin-associated muscle symptoms (SAMS) are one of the principal reasons for statin non-adherence and/or discontinuation, contributing to adverse cardiovascular outcomes.

Objective and Methods. The aim of this study was to evaluate the prevalence of statin intolerance in a group of 550 outpatients (232 males and 318 females) in primary and secondary prevention examined between 2006 and 2017 at the Lipid Clinic at the University Hospital in Palermo. Anthropometric measures, clinical and biochemical parameters, statin therapy and adverse events (AEs) were evaluated.

Results. In our cohort the prevalence of statin intolerance was of 19%. The main AEs registered were myalgia (51.7%), muscle weakness (19.5%), cramps (16.5%) and CPK increase (9%). 3 subjects suffered rhabdomyolysis. Statin intolerant subjects were mainly female (59.3%), hypothyroidism. 2 type diabetes and liver steatosis were more frequent among intolerant subjects (21.4% vs 7.2%, p value 0.02-22.3% vs 4.02%, p value 0.003 and 16.5% vs 3.8%, p value 0.002, respectively). No changes of liver and kidney function tests were registered. In most cases subjects were “switched” to other statins (76.7%) and 23.3% of intolerant patients underwent to therapy discontinuation.

Conclusions. In this study the most common statin related side effect associated with therapy non-adherence was myalgia. Statin intolerance is a significant problem worldwide and appears to be growing. A step-by-step approach, including careful examination of all other precipitating factors that may increase the risk of statin intolerance, might be useful for preventing treatment discontinuation.

EFFECT OF LEGUME CONSUMPTION ON CARDIOMETABOLIC RISK FACTORS

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Background and Aims. Numerous studies have evaluated dietary factors as conditions which can reduce cardiometabolic risk factors. In particular legumes, which are part of the Mediterran-
mean diet, have many antioxidant properties and in particular it has been shown that they can act by reducing some risk factors for metabolic syndrome. Therefore, the aim of our study was to clarify whether the intake of legumes could reduce some cardiometabolic risk factor, in particular blood triglyceride levels.

**Methods.** In this cross-sectional study, which is part of a project on the Mediterranean diet, financed by Ministry of Health and conducted between February 2013 and December 2014 at the U.O.C Nutrizione Clinica, 149 consecutive elderly individuals were enrolled with follow-up time of 11±5 months. Subjects with evidence of chronic-degenerative, endocrine, cardiovascular diseases and subjects who consumed excessive alcohol (>20 g/day) were excluded. All participants underwent an anthropometric evaluations and biochemical evaluation. In addition, all participants underwent a dietary intake assessment by a seven-day food record. For the purposes of the statistical analysis, the Δ of the triglyceride blood values between baseline and 1-year follow up was calculated. A T test was performed for independent samples to compare the averages between the groups. A ROC curve was used to identify a daily intake cut-off of legumes predicting the reduction in triglyceride levels. All statistical analyzes were performed using SPSS 20.0.

**Results.** The mean age was 69±3 aa (63% women), IMC 29±4, the mean intake of legumes was 10±12 (adjusted for 1000/Kcal). ROC curve demonstrates that intake of 7.4 g/day/1000 Kcal of legumes has a sensitivity of 70% and a specificity of 60% to predict the reduction of triglycerides. The results of this study are extremely important because they demonstrate for the first time the link between the mean diet, have many antioxidant properties and in particular it has been shown that they can act by reducing some risk factors for metabolic syndrome. Therefore, the aim of our study was to clarify whether the intake of legumes could reduce some cardiometabolic risk factor, in particular blood triglyceride levels.

**Conclusion.** The results of the present study was to evaluate prevalence and annual health care costs of patients with CAD, PAD or both in Italy.

**Background.** Coronary Artery Disease (CAD) and Peripheral Arterial Disease (PAD) have an atherosclerotic basis and their concomitant manifestation is commonly reported. The aim of the present study was to evaluate prevalence and annual health care costs of patients with CAD, PAD or both in Italy.

**Materials and Methods.** Data has been obtained reviewing the health care database of a North-Eastern Italian Region (Veneto Region) recorded between 01/01/2017 and 31/12/2017. We included adult patients (≥18 years) with diagnosis of CAD (at least one prescription of nitrates and/or CAD diagnosis at hospital discharge), and diagnosis of PAD (at least one prescription of anti-platelet drugs with concomitant prescription of anti-hypertensive agents or lipid-lowering agents and/or PAD diagnosis at hospital discharge). Patients were characterized during the period 2009-2016 for comorbidities and in year 2016 for drug use. Treatment adherence was calculated using the Medication Possession Ratio. Healthcare cost analysis included drugs and hospitalization costs.

**Results.** Over 4.9 million health-assisted subjects, 8.8% (430,254, mean age 74.2 years) received a diagnosis of CAD or PAD. Among them, 41.2% (177,157, mean age 78.8) had a PAD, 30.4% (130,833, mean age 74.2) had a CAD while 28.4% (122,244, mean age 74.7) had both diseases. Diabetes mellitus was observed in 10% of patients, heart failure in 11.8% while the 30.9% experienced a previous cardiovascular event. The cardiovascular drugs more frequently prescribed were anti-hypertensives agents (85%), anti-platelet drugs (58.7%) and lipid-lowering drugs (53%). Among patients treated with antiplatelet drugs, 36.2% were adherent to the treatment. The total mean annual healthcare cost per patient was €3,238.4 (€2,047.8, €3,390.0, €4,801.6 for patients with PAD, CAD or both, respectively). The total cost was 4.5-fold higher when compared to the mean annual healthcare of general population living in the area, 2.4-fold higher when considering comparable age and sex.

**PPAR ALPHA ACTIVATION RESCUES ACQUIRED LYSOSOMAL ACID LIPASE DEFICIENCY IN FATTY LIVER**

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**Aim.** Lysosomal acid lipase (LAL) is responsible for the hydrolysis of cholesteryl esters and triglycerides in the lysosomal compartment. Patients with NAFLD often show reduction of LAL activity in absence of LIPA gene mutations. Aim of the study was to characterize LAL deficiency in NAFLD and to investigate a possible therapeutic approach in vitro and in vivo.

**Methods.** 164 patients with biopsy-proven NAFLD were compared to 50 dyslipidemic patients with a similar incidence of metabolic syndrome but no fatty liver and to 30 controls. LAL activity was measured on dried blood spots (DBS) and liver biopsies. LAL expression and activity was tested in HepG2 cells treated with fatty acids, in the presence or absence of a PPAR-alpha agonist. A pilot study with fenofibrate on NAFLD patients was performed.

**Results.** LAL activity on DBS was reduced in NAFLD patients compared to dyslipidemic patients with a similar incidence of metabolic syndrome but no fatty liver and to 30 controls. LAL activity was inversely correlated with the degree of fibrosis, but the correlation was completely lost after correction for platelet count. LAL activities measured on DBS and liver tissues were strongly correlated. Hepatic reduction of LAL activity was not due to decreased LAL expression. HepG2 cells treated with fatty acids also displayed a reduction of LAL activity with no changes of LAL expression. The activation of PPAR-alpha before or after fatty acid loading was able to prevent and correct LAL reduction in HepG2 cells. In NAFLD patients, fenofibrate for 1 month tended to increase LAL activity.

**Conclusions.** NAFLD is associated with an acquired LAL deficiency that is likely due to a by-product inhibition of fatty acids and not to other concomitant metabolic alterations. PPAR-alpha agonists could increase LAL activity in NAFLD patients by stimulating fatty acid oxidation.
CLINICAL SIGNIFICANCE OF GENDER DIFFERENCES IN OLDER PEOPLE WITH METABOLIC SYNDROME

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Objectives. Metabolic syndrome (MetS) is defined as a cluster of risk factors that, if associated, increase the risk to develop cardiovascular disease (CVD) and type 2 diabetes mellitus (DM2). It is important to understand the impact of MetS in geriatric patients, and to assess the major gender differences, regarding both the impact of individual components and the role the syndrome plays on CV risk and comorbidities. Aims of the study are to analyse gender differences, poorly investigated in geriatric patient with MetS, and to assess whether these differences could affect the standard treatment options, often controversial in the elderly.

Methods. A total of 1382 outpatients attending our Cardiogeriatric Clinic were studied. MetS was defined in accordance with the “Harmonized definition” (2009). In all evaluated patients we used a “multidimensional geriatric assessment” including: pharmacological and medical history, clinical examination, blood tests, anthropometric parameters and the main geriatric syndromes, focusing on depression.

Results. MetS prevalence was 27,8% (27,3% in males, 28,2% in females), with a higher prevalence in women aged 85 and older compared with men in the same age group. Being a woman was found to be an independent risk factor to develop MetS (OR 2,56; p=0,001). In males, the most common component was high blood sugar levels (32,4%), while in females, was elevated waist circumference (46,9%). Interestingly 93,2% of the sample with MetS has hypertension. Even if males with MetS have a higher CV risk (39,3%) compared to women (25,3%), no differences were observed between treatment with CV drugs between genders.

Conclusions. Main differences between genders concern women >85, the prevalence of CVD, which is higher in men and dizziness (higher in women). Remarkably, the prevalence of depression is deeper associated with MetS in women. Increasing awareness in (higher in women). Remarkably, the prevalence of depression is deeper associated with MetS in women. Increasing awareness in

Lipoprotein (A) Levels Predicts Mortality and Haemorrhagic Transformation in Ischemic Stroke Patients Treated with Thrombolysis: Results from the Magic Study

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Background and Purpose. Lipoprotein (a) (Lp(a)) is endowed with both proatherosclerotic and prothrombotic properties: promotes smooth muscle cell proliferation, endothelial cell adhesion molecule expression, foam cell generation, and endothelial dysfunction. Elevated Lp(a) levels are significantly associated with higher incidence of ischemic stroke.

Aim. We aimed to investigate whether Lp(a) levels taken before recombiant plasminogen activator (rtPA) administration predict adverse clinical outcomes in rtPA treated ischemic stroke patients of the MAGIC Study.

Methods. Blood samples were taken before and 24-h after rt-PA from 327 patients (mean age 68 years, median NIHSS 11). Pre-rtPA values and delta median values [(24-h biomarker - pre-rt-PA biomarker)/(pre-rt-PA biomarker)] of Lp(a) were analyzed in relation to: 1) 3-month death, and 2) symptomatic hemorrhagic transformation.

Results. Pre-rtPA levels of Lp(a) were significantly different in patients who died with respect to survivors (141.92-258) vs 90 (44-175) mg/L, p=0.010). Similarly, Lp(a) values were significantly higher in patients with than without haemorrhagic transformation [138.72-263 vs 91.44-175 mg/L, p=0.094). Adjusting for age, sex, glycemia, baseline NIHSS, history of atrial fibrillation, orcongestive heart failure, history of inflammatory diseases or infections occurred within the last 7 days before stroke onset, pre-rt-PA Lp(a), in addition to pre-rtPA A2M and deltaMMP-9 remained significantly and independently associated with 3 month-death (OR 95% CI): pre-rtPA Lp(a): 2.63(1.26-5.48), p=0.016; A2M: 1.63(1.18-2.25), p=0.003; deltaMMP9:1.72(1.20-2.47), p=0.003). At logistic regression analysis, after adjustment for major clinical determinants of outcomes, in addition to deltaMMP9, pre-rtPA Lp(a) levels remain a significant and independent determinant ofhemorrhagic transformation (OR 95% CI) Lp(a): 1.78 (1.04-3.03), P=0.035; deltMMP9: 1.48(1.08-2.102), p=0.014).

Conclusion. Our findings suggest that in addition to A2M and deltaMMP-9 also high levels of Lp(a) are significant and independent markers of mortality and hemorrhagic transformation. Lp(a) may be used to improve prediction of unfavourable outcomes in the clinical setting of ischemic stroke patients treated with thrombolytic therapy.

PROGNOSTIC VALUE OF SEPSIS-INDUCED COAGULATION ABNORMALITIES: AN EARLY ASSESSMENT IN THE EMERGENCY DEPARTMENT

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Background. Coagulation abnormalities are frequent in patients with sepsis and coagulation activation contributes to multiple organ failure as a result of microvascular thrombosis. Several papers reported an increased mortality rate in patients with a more pronounced coagulation dysfunction in the early days of their ICU course, but few data exist about the prognostic role of abnormal coagulation parameters measured in the first 24 hours after Emergency Department admission.

Aims. To evaluate if the measurement of parameters of increased coagulation activation at ED admission could add prognostic information to the routine assessment of septic patients based on indices of hyperperfusion and parameters of organ failure.

Methods. This report utilized a portion of the data collected in a
ABSTRACT

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Results. Day-7 mortality rate was 16%. DD (T0: 4661±4562 µg/ml vs 3190±7188 µg/ml; p=0.001; T6: 4498±4931 µg/ml vs 2822±5623 µg/ml; p=0.003; T24 2905±2823 µg/ml vs 2465±988 µg/ml; p=0.008) and TAT levels (T0 29±45 vs 22±83; T6 21±22 vs 15±35; T24 16±19 vs 13±30, all p<0.05) were higher among non-survivors compared with survivors. We define an abnormal coagulation activation (COAG+) as Ddimer>1300 µg/ml and TAT>7 ng/ml (median value in our study group). At T0 and T6 COAG+ patients had higher SOFA score (T0: 5.8±2.9 vs 4.9±2.4, p=0.020; T6: 7.4±3.3 vs 5.6±2.5, p=0.001) and lactate level (T0: 3.8±4.1 vs 2.5±2.0, p=0.001; T6: 3.3±3.7 vs 1.7±1.2, p=0.002). At T0, COAG+ patients showed a higher day-7 mortality rate (HR 2.5; 95%CI 1.15-5.45, p=0.021), even after adjustment for SOFA score and lactate level.

Conclusions. Presence of abnormal coagulation activation at ED admission yielded an independent prognostic association with an increased short-term mortality rate.

TRANSCRIPTOMICS ANALYSIS OF CEREBRAL THROMBI AND PERIPHERAL VENOUS BLOOD: A PROMISING RESOURCE FOR EVALUATING PATHOPHYSIOLOGY AND OUTCOMES IN ACUTE ISCHEMIC STROKE

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Background. The thrombotic/thromboembolic occlusion of a cerebral artery leads acute ischemic stroke (AIS) which is a leading cause of morbidity and mortality. Nowadays the election therapies for AIS are represented by rapid restoration of cerebral blood supply by intravenous thrombolysis and mechanical thrombectomy. Previous studies showed that analysis of clots (e.g histological analysis of thrombus composition) can be useful in understanding AIS pathophysiology mechanisms and outcomes as clots composition and characteristics may influence the effective recanalization of occlusive thrombi in such arteries.

Aims. Of this study were 1) to investigate the global gene expression profile from RNA extracted from cerebral thrombus and peripheral blood in patients with AIS in order to identify specific genes and markers that might help to distinguish causes of ischemic stroke and/or determinants of clinical outcomes and 2) to compare the gene expression profiles obtained from clot and systemic component.

Method. Twenty-one consecutive AIS patients (7 males, 14 women) [Mean Age±SD 77.9±10.8] undergoing endovascular treatment were collected and followed-up for at least 3 months. 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 liquid for RNA stabilization. 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 Transcriptome 2.0 Array allowing the analysis of 44,689 genes, >285,000 full-length transcripts coverage.

Results. The analysis highlighted 1,712 out of 2,653 probe sets identifying annotated genes. Among expressed genes in thrombi, of greater interest, we observed metalloproteinases 11 and 24, androgen receptor, ubiquitin conjugating enzyme E2B, and interferon regulatory factor 8 and 4 previously involved in activation of neural stem cells under physiological and regenerative conditions, synapse reorganization after traumatic brain injury and neuronal response to cerebral ischaemic-reperfusion injury. Gene ontology analysis showed that, besides the expected alteration of biological processes and molecular functions related to regulation of apoptosis, cell death and catabolic processes, the expressed genes in cerebral thrombi are involved in biological processes and molecular function and pathways related to: neuronal response to stress; neuron differentiation, development, organization, and maturation; cerebral cortex development.

Conclusion. Preliminary results suggest thrombus global gene expression profiling might be a promising resource to investigate causes of ischemic stroke and/or determinants of clinical outcomes.

HELPER-DEPENDENT ADENOVIRAL VECTOR EXPRESSING MLDLR/MTF FUSION PROTEIN UNDER THE CONTROL OF A MUSCLE SPECIFIC PROMOTER FOR THE TREATMENT OF FH

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Background. Familial hypercholesterolemia (FH) is a genetic Hyperlipidemia most common due to mutations in the low-density lipoprotein receptor (LDLR) gene that result in high levels of LDL-cholesterol (LDL-C) with consequent development of xanthomas, atheromas and increased risk of premature cardiovascular complications. Several therapeutic options are available for FH patients. The conventional therapy is based on the use of high-dose statins; however, when this approach is not effective, patients often rely on more invasive interventions such as LDL apheresis. The need for additional effective therapeutic options makes gene therapy an attractive approach to reduce LDL-C in FH patients with a single vector administration. Adenoviral vectors (Ad) are highly efficient gene transfer vectors; in particular Helper dependent (HD-Ad) are characterized by long-lasting high-levels of transgene expression with limited toxicity. We have recently developed a safe and effective gene therapy strategies based on a HD-Ad vector for the expression of a circulating fusion protein composed by the extracellular portion of the murine LDL receptor (mLDLR) fused in frame with the entire murine transferrin (mTf). The chimeric protein binds and remove circulating LDLs from the bloodstream by receptor-
mediated endocytosis through the interaction with the transferin receptor (TIR). We administered this vector intravenously in LDLR-deficient mice and observed expression of the protein with a consequent improvement of lipid profile and reduction of aortic atherosclerotic lesions. In order to develop a safer strategy for clinical applications, we have recently generated a HD-Ad vector encoding the fusion protein under the control of a muscle specific promoter (HD-AdmCK-mLDLR/mTf) for intramuscular delivery. We evaluated the ability of our vector to drive the expression of the fusion protein in the C2C12 myoblast cell line and we are currently characterizing transgene expression and activity after intramuscular delivery in LDLR deficient mice.

**POSTTRANSCRIPTIONAL REGULATION OF PCSK9 EXPRESSION BY MORUS ALBA, ACTIVE COMPONENT OF LOPIGLIK®, IN HEPG2 CELL LINE: ITS ROLE IN LDL-CHOLESTEROL UPTAKE**

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**Background and Aim.** Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a pivotal regulator of low-density lipoprotein cholesterol (LDL-C) plasma levels. In a previous work, we observed that the treatment of dyslipidemic subjects with an innovative nutraceutical combination containing: Red yeast rice (Monacolin K 3.3 mg), Berberis aristata cortex extract (Berberine 531.25 mg) and Morus alba leaves extract (1-Deoxynojirimycin 4 mg) (LopiGLIK® - Akademy Pharma) did not alter the plasma levels of PCSK9. Thus, the aim of the present study was to define the effect of the LopiGLIK® nutraceutical combination on genes involved in cholesterol homeostasis, including PCSK9, and thus the molecular mechanism underlying the hypocholesterolemic effect of Morus alba leaves extract.

**Methods.** HepG2 cell line were incubated with Red yeast rice (RYR; 50 µg/ml) Berberis aristata cortex extract (BCE; 40 µg/ml) and Morus alba leaves extract (MLE; 1 mg/ml), alone or in combination, for 24 h. Their effects on PCSK9 expression (western blot), secretion (ELISA), transcription (Luciferase Promoter assay and RT-qPCR) and on LDL-uptake (flow cytometry) were then determined.

**Results.** As expected, RYR significantly induced both the 74kDa pro-PCSK9 (+3.1 fold) and the 62kDa active form (+2.1 fold), PCSK9 secretion (+9.8%±1.2%), PCSK9 mRNA (1.89±0.11 fold), and its promoter activity (+2.4±0.5 fold). In contrast, BCE reduced PCSK9 expression (-37.0%, pro-PCSK9 and -64.3%, active form), PCSK9 secretion (-44.8%±16.2%), mRNA levels (-45.0%±0.5%), and promoter activity (-63.0%±11.8%). Interestingly, MLE determined a concentration-dependent inhibition of mRNA PCSK9, with a maximal reduction at 1µg/ml (-54.8%±0.7%). The same concentration of MLE reduced PCSK9 expression levels (pro-PCSK9: -17.1%; active form: -59.3%), and its release into the cultured media (-43.8%±23.6%). MLE did not alter the PCSK9 promoter activity, suggesting a different mechanism of action compared to BCE. The same analyses were performed on additional SREBP-genes as HMG-CoAR, LDLR and FAS. At transcripts levels, RYR significantly induced both HMG-CoAR and FAS; conversely, BCE reduced them. MLE behaved as BCE did, decreasing both genes (-50%). Surprisingly, no significant variation in LDLR mRNA levels have been detected upon RYR treatment, whilst BCE partially reduced them. At the protein levels, both RYR and BCE induced LDLR expression by 2.8 and 1.5 fold, respectively. No effects on LDLR expression have been seen upon MLE treatment. The combination of BCE (40 µg/ml), RYR (50 µg/ml) and MLE (1mg/ml) reduced the PCSK9 mRNA (-77.3±0.8%), the PCSK9 expression (pro-PCSK9: -96.6%; active form: -93.5%), the extracellular protein levels (-74.8%±14.9%), and the PCSK9 promoter activity (-76.0±9.8%). In addition, the combination reduced both HMG-CoAR and FAS mRNAs, whilst a slight significant induction of LDLR was observed at protein level. MLE was able to positively modulate the LDL-DiO uptake (+3.0±0.01 fold), strengthening the RYR and BCE positive action when in combination.

**Conclusion.** BCE and MLE actively counteract the induction of PCSK9 by RYR, an effect that could explain the unchanged plasma levels of PCSK9 in patients treated with LopiGLIK®. In addition, the use of MLE, for glycemic control, together with RYR and BCE, positively increased LDL-DiO uptake by HepG2, supporting the rational of using this nutraceutical combination for controlling both hyperlipidemic and hyperglycemic conditions.

**LONG-TERM EXPOSURE TO AIR POLLUTION RAISES CIRCULATING LEVELS OF PCSK9 IN OBESE INDIVIDUALS**

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**Background.** Exposure to airborne particulate matter (PM) has been consistently associated with early death and increased morbidity, particularly raising the risk of cardiovascular disease (CVD). Obesity, one of the leading CVD risk factors, increases susceptibility to the adverse effects of PM exposure. The proprotein convertase subtilisin/kexin type 9 (PCSK9) has been related to a large number of CV risk factors, e.g. atherogenic lipoproteins, arterial stiffness, and platelet activation.

**Objective.** To evaluate, in a cohort of susceptible obese subjects, the effects of PM10 exposure on PCSK9 circulating levels and how these may be associated with the CV Framingham Risk Score (FRS).

**Methods.** In 500 obese subjects, participating in the cross-sectional SPHERE (Susceptibility to Particle Health Effects, miRNAs and Exosomes) study, we evaluated the effects of long- and short-term PM10 exposure on circulating PCSK9 levels. Results. In the studied individuals (BMI: 33.3±5.2 kg/m²) with an annual average PM10 exposure of 40.12±4.71 µg/m³, PCSK9 levels were 248.7±78.6 ng/mL. In univariate analysis, PM10 exposure (annual average) was associated to PCSK9 levels (β=1.83, SE=0.75, p=0.014) as well as to lipoproteins, i.e. LDL-C (β=0.33, SE=0.10, p=0.0008), non-HDL-C (β=0.37, SE=0.09, p<0.0001), TGs (β=0.11, SE=0.05, p=0.0165) and HDL-C (β=0.71, SE=0.24, p=0.0034). Interestingly, in a multivariable linear regression model, the association between PCSK9 and PM10 was observed only for carriers of lower concentrations of interferon (IFN)-γ, whereas it was lost in pres-
ence of higher IFN-γ levels, PCSK9 levels were positively associated with the Framingham Risk Score, which was raised by 15.8% for each 100 ng/mL rise of PCSK9.

Conclusions. Obese individuals with less inflammation, i.e. low IFN-γ appear to be hypersusceptible to PM10 exposure, as expressed by raised PCSK9 concentrations.

GENETIC CHARACTERIZATION OF A FAMILY WITH CLINICAL SUSPICION OF HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction. Homozygous familial hypercholesterolaemia (HoFH) is a rare and life-threatening disease characterized clinically by plasma cholesterol levels >500 mg/dL, extensive xanthomas, marked premature and progressive atherosclerotic cardiovascular disease. HoFH has shown to be caused by mutations in both alleles of the gene encoding the LDL receptor (LDLR).

Patients and Methods. The case report is based on a family from Campania Region; the index case was a 44-year-old woman with xanthomas, total cholesterol (TC) levels of 240 mg/dl and LDL cholesterol (LDL-c) of 186 mg/dl after maximal therapy with statins and ezetimibe, with suspicion of HoFH. Her 39-year-old brother also showed a similar lipid profile; furthermore two nephews aged 16 and 18 showed marked hypercholesterolemia. The genetic screening included the analysis of LDLR, PCSK9 and part of exons 26 and 29 of the APOB genes. MLPA was performed to identify large rearrangements in the LDLR gene.

Results. Our genetic screening revealed that index case was heterozygous for the mutation c.2312-3C>A in the intron 15 of LDLR gene. The cascade screening performed in the brother and in two nephews of the proband, revealed heterozygosity for the same mutation. We studied other 7 families with the same mutation, observing in all a very severe phenotype (baseline TC 431±112 mg/dl, LDL cholesterol 349±100 mg/dl); After genetic test the index case and her nephew, revealed heterozygosity for the same mutation. The cascade screening performed in the brother and in two nephews of the proband, revealed heterozygosity for the same mutation.

Conclusions. The clinical suspicion of HoFH is not confirmed by genetic test that revealed the presence of only one mutation in LDLR; the c.2312-3C>A is the most frequent splicing mutation causing FH in our region and is associated to a severe phenotype, while the two young nephews start early statin therapy.

ASSOCIATION BETWEEN SMALL DENSE LDL PARTICLES AND CARDIOVASCULAR EVENTS IN A GROUP OF MEDITERRANEAN WOMEN (PROGETTO ATENA). PRELIMINARY REPORT

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Association between small dense LDL (sd-LDL) and cardiovascular disease (CVD) was evaluated in a group of 193 women participating to Progetto Atena (nested case-control study). At the time of first visit blood samples were collected and stored in liquid nitrogen. After 25 years from baseline visit LDL particle separation was performed, on serum stored at time of baseline visit, by Lipoprint System: 7 LDL subfractions (LDL I to LDL 7) were obtained, mean LDL particle size and LDL score (% of sd-LDL) were calculated. Analysis was performed in 193 patients. The rest of 107 patients are currently undergoing biochemical determinations. Women were divided into a control group (group 1, N=102) and in a group (group 2, N=91) who had cardiovascular events after the initial visit. LDL score was higher in women with cardiovascular disease as compared to control women (6.5±8.6 vs 7.7±13.2 mg/dl) (p=0.386); mean LDL size was lower in group 2 than group 1 (26.8±0.5 vs 26.9±0.4 nm) (p=0.409). Cholesterol LDL resulted statistically different, group 1 (352.5±44.4 mg/dl) vs group 2 (168.5±51 mg/dl) (p=0.021). In multivariate analysis adjusted for age, LDL, Systolic blood pressure and BMI, LDL score value above the 95th percentiles (25.0 mg/dl) was found to be associated to cardiovascular disease but statistical significance was not reached (p=0.270), due to small sample size. No statistically age differences resulted between two groups. These preliminary data indicate that in women who had CVD after baseline visit, LDL score is higher and mean LDL size lower than control women. High concentrations of sdLDL are associated to future cardiovascular events in this group of Mediterranean women participating to Progetto Atena. Further studies are envisaged to investigate the specific contribution of LDL subfractions to cardiovascular risk particularly in women.

EFFECTS OF BARIATRIC SURGERY ON CARDIOVASCULAR RISK SCORE IN DIABETIC SUBJECTS

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Introduction. In obese patients with type 2 diabetes (T2DM) bariatric surgery is the most effective strategy to produce durable weight loss, diabetes remission and reduction of cardiovascular (CV) risk.

Objective. To evaluate the effect of bariatric surgery on CV risk in obese patients with T2DM using the “Progetto Cuore” (PC) risk score.

Methods. We studied 134 obese T2DM patients, M/F 50/84, age 50.4±8.6 years, BMI 45.6±7.4 kg/m², who underwent to bariatric...
ASSOCIATION BETWEEN DECREASING ESTIMATED GLOMERULAR FILTRATION RATE AND RISK OF CARDIAC CONDUCTION DEFECTS IN PATIENTS WITH TYPE 2 DIABETES

A. Mantovani, R. Rigolon, T. Turino, D. Sandri, A. Rossi, G. Zoppini, E. Bonora, G. Targher

Background. To examine the association between decreasing estimated glomerular filtration rate (eGFR) or abnormal albuminuria and risk of cardiac conduction defects in patients with type 2 diabetes mellitus (T2DM).

Methods. We examined a hospital-based sample of 923 patients with T2DM discharged from our Division of Endocrinology over the years 2007-2014. Standard electrocardiograms (ECGs) were performed in all patients; eGFR was estimated by using the chronic kidney disease epidemiology collaboration (CKD-EPI) equation, and albuminuria was measured by an immuno-nephelometric method on a morning spot urine sample.

Results. A total of 253 (27.4%) patients had some type of cardiac conduction defects on ECGs (defined as at least one heart block among first-degree atrio-ventricular block, second-degree block, third-degree block, left bundle branch block, right bundle branch block, left anterior hemi-block or left posterior hemi-block). Prevalences of patients with eGFRCKD-EPI<30, eGFRCKD-EPI 30-59.9, eGFRCKD-EPI 60-79.9 and eGFRCKD-EPI >80 ml/min/1.73 m² and abnormal albuminuria (i.e. urinary albumin-to-creatinine ratio >30 mg/g) were 7.0%, 29.4% and 41.3%, respectively. After adjustment for established cardiovascular risk factors, diabetes-related variables and potential confounders, there was a significant, graded association between decreasing eGFR values and risk of cardiac conduction defects (adjusted-odds ratios of 2.05 [95% CI 1.23-3.5], 2.85 [95% CI 1.96-5.1] and 3.62 [95% CI 1.6-8.1] for eGFRCKD-EPI <30, eGFRCKD-EPI 30-59.9 and eGFRCKD-EPI >60 ml/min/1.73 m², respectively). Conversely, abnormal albuminuria was not associated with an increased risk of conduction defects (adjusted-OR 1.09, 95% CI 0.7-1.6).

Conclusions. Decreasing eGFR is independently associated with an increased risk of persistent cardiac conduction defects in patients with T2DM.

ASSOCIATIONS BETWEEN HIGHER PLASMA CERAMIDE LEVELS AND LOWER PERCENTAGE OF POST-STRESS MYOCARDIAL PERFUSION IN PATIENTS WITH ESTABLISHED OR SUSPECTED CORONARY ARTERY DISEASE UNDERGOING MYOCARDIAL PERFUSION SCINTIGRAPHY

A. Mantovani, S. Bonapace, G. Lunardi, M. Salgarello, C. Dugo, E. Barbieri, G. Targher

Background. We recently found that distinct plasma ceramides are closely associated with stress-induced myocardial perfusion defects in patients with established or suspected coronary artery disease (CAD) undergoing myocardial perfusion scintigraphy (MPS). However, it is currently not known whether plasma ceramides are also associated with a reduction of percentage of post-stress myocardial perfusion in these patients.

Methods. We measured six previously identified high-risk plasma ceramide species [Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/20:0), Cer(d18:1/22:0), Cer(d18:1/24:0) and Cer(d18:1/24:1)] in 167 consecutive patients with established or suspected CAD, who performed a stress myocardial perfusion scintigraphy (MPS) for clinical indications. Plasma ceramides were measured by a targeted liquid chromatography-tandem mass spectrometry assay both at baseline and after MPS. Multivariable linear regression analysis was undertaken to examine the associations (standardized B coefficients) between plasma ceramides and the percentage of post-stress myocardial perfusion after adjustment for multiple cardiovascular risk factors.

Results. Seventy-eight patients had stress-induced myocardial defects on MPS (mainly located in the antero-apical wall). Of the six measured plasma ceramides, basal levels of Cer(d18:1/18:0) (B=-0.182, p<0.05), Cer(d18:1/20:0) (B=-0.184, p<0.05), Cer(d18:1/22:0) (B=-0.163, p<0.05) and Cer(d18:1/24:1) (B=-0.20, p<0.05) were inversely associated with the percentage of post-stress antero-apical wall perfusion. Notably, these significant associations persisted after adjustment for age, sex, smoking, hypertension, dyslipidemia, diabetes, previous CAD, left ventricular ejection fraction and type of stress testing. Similar results were also observed for post-stress plasma ceramides.

Conclusions. Higher circulating levels of specific ceramides, both at baseline and after stress, were independently associated with a lower percentage of post-stress antero-apical wall perfusion in patients with established or suspected CAD referred for clinically indicated MPS.
ABSTRACT

TOPIRAMATE PROTECTS APOE-DEFICIENT MICE FROM KIDNEY DAMAGE WITHOUT AFFECTING PLASMA LIPIDS

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Topiramate is an anticonvulsant drug also prescribed for migraine prophylaxis that acts through several mechanisms of action. Several studies indicate that topiramate induces weight loss and a moderate reduction of plasma lipids and glucose. Based on these favourable metabolic effects, aim of this study was to evaluate if topiramate could modulate atherosclerosis development and protect target organs of dysmetabolic conditions. Thirty apoE-deficient mice were divided into three groups and fed for 12 weeks a high fat diet (Control) or the same diet containing topiramate at 0.125% (T-low) and 0.250% (T-high). Body weight, water and food intake were monitored throughout the study. Plasma lipids and glucose levels were measured and a glucose tolerance test was performed. Atherosclerosis development was evaluated in the whole aorta and at the aortic sinus. Histological analysis of liver, kidney and adipose tissue was performed. Topiramate did not affect weight gain and food intake. Glucose tolerance and plasma lipids were not changed and, in turn, atherosclerosis development was not different among groups. Topiramate did not modify liver and adipose tissue histology. Conversely, in the kidneys, the treatment reduced the occurrence of glomerular lipidosis (on average, more than 11 glomeruli affected in Control vs 1 to 3 glomeruli affected in the treated groups) and tubular necrosis (30% in Control, 10% in T-low and 0% in T-high) and by decreasing foam cells accumulation and reducing the expression of inflammatory markers. Blood urea nitrogen levels were also reduced by treatment (-18% in T-low and -25% in T-high). Our results indicate that topiramate does not affect atherosclerosis development, but preserves kidney structure and function. The study suggests that topiramate could be investigated in drug repurposing studies for the treatment of glomerular lipidosis.

EVALUATION OF HDL FUNCTIONALITY AND DIETARY-DERIVED METABOLITES IN CALCIFIC AORTIC STENOSIS


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Aim. Calcific aortic valve disease (CAVD) is the most prevalent valvular heart disease. Its pathogenesis has not been elucidated clearly. In this study, we aimed to investigate the link between HDL function, evaluated as cholesterol efflux capacity, and the disease severity. In addition, we assessed the levels of some dietary and gut microbiota-derived metabolites and investigated their relationship with CAVD severity.

Methods. Subjects with moderate-severe calcific aortic stenosis (CAS) (n= 60), aortic sclerosis (ASC) (n= 49) and age- and sex-matched control subjects (n= 48) were included in this study. Severity of CAVD determined by echocardiographic, computed tomographic and histopathological examinations. High-density lipoprotein cholesterol efflux capacity (HDL-CEC) was assessed by a radioisotopic technique. Serum choline, betaine and gut microbiota-derived metabolite levels were measured using ultra-performance liquid chromatography-tandem mass spectroscopy.

Results. Patients with moderate-severe CAS displayed significantly lower total HDL-CEC compared to both control (p=0.011) and ASC (p=0.015) groups. Moreover, total HDL-CEC was negatively correlated with parameters suggested to have prognostic implications in AS, such as peak aortic jet velocity (AVmax) (r=-0.302, p<0.005), aortic valve calcium (AVC) score (r=-0.302, p<0.005), coronary artery calcium (CAC) score (r=-0.355, p=0.005). Total HDL-CEC was also found to be negatively correlated with plasma choline levels (r=-0.210, p=0.031). In addition, total HDL-CEC were found to be independent associates of AVmax (β=-0.419, SD: 0.139, p=0.005). Patients with moderate-severe CAS had significantly higher plasma levels of choline when compared to both control (p=0.001) and ASC (p=0.006) groups. Betaine and TMAO levels did not differ among groups (p=0.05). Higher quantities of plasma choline were also found to be associated with AVC score (p=0.001), absolute left ventricular global longitudinal strain (p=0.012) and with mitral annular calcium (MAC) score (p=0.013).

Conclusion. In this work we have demonstrated that HDL functionality, reflected with HDL-CEC, is independently associated with CAVD severity. We have also shown a relationship between presence and severity of CAVD and the plasma levels of dietary-derived metabolites. These findings suggest that HDL function and choline and betaine as well, may be a novel determinant of CAVD besides traditional risk factors.

GERANYLGERANIOL PREVENTS THE SIMVASTATIN-INDUCED PCSK9 EXPRESSION: ROLE OF THE SMALL G PROTEIN RAC1

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Statins are the gold standard therapy for the prevention and treatment of dyslipidemia, their hypolipidemic effect is related to the increased expression of the LDLR on the surface of the hepatocytes. The biological target of statin is the Hydroxymethylglutaryl-Co enzyme A reductase (HMGCR), which is the rate limiting
step of cholesterol biosynthesis; in response to their action, the intracellular pool of free sterols decreases and the transcription factors sterol responsive element binding protein (SREBP) undergoes proteolytic activation resulting in the induction of the LDL receptor (LDLR), HMGCR and of PCSK9. Interestingly the levels of PCSK9 increase as soon as 24 hours after a single dose of atorvastatin. By inhibiting the HMGCR, statins also reduce the amount of nonsteroidal isoprenoid compounds; in the early phase of treatment in fact plasma levels of mevalonate (MVA) drop up to 70% within 1-2 hours while a reduction of LDL-C starts to be detectable only after 24 h and becomes substantial after 6-7 days. Considering the correlation between the timing of PCSK9 upregulation and MVA decrease following statin treatment, we hypothesized that the prenylation of the small G-proteins, like Rac1 and RhoA, is a crucial step for the activation of PCSK9 transcription. In the CaCo-2 cell line 24 hours incubation with Simvastatin 40 μM induced both PCSK9 mRNA (10.7±3.2-fold change) and protein (2.2±0.3-fold change). The induction of PCSK9 mRNA was partially, but significantly, prevented by the co-incubation with mevalonate (MVA), farnesol (FOH) and geranylgeraniol (GGOH), while this completely prevented upregulation of secreted PCSK9, evaluated by ELISA assay. Simvastatin also reduced by −35.7±15.2% the Rac1-GTP levels, while no changes were observed on RhoA and Cdc42-GTP. This effect was also prevented by co-incubation with MVA or GGOH. We then performed RT-qPCR analysis for PCSK9 mRNA after impairing the activity of Rac1 in a number of ways. First, we took advantage of a small molecule (Raci) that is able to interfere with the interaction between Rac1 and Tiam1, and thus with the exchange between GDP to GTP. We also transfected the dominant negative mutant N17Rac1, which leads to expression of Rac1 in either a nucleotide free state or in its inactive stat. Finally, we used a siRNA against Rac1 to knock-down its expression. Each of these strategies reduced Rac1 activity led to upregulation of PCSK9, similarly to simvastatin treatment. Rac1-GTP is involved in the nuclear translocation of STAT proteins such as STAT3, which has been shown to suppress the transcription of SREBP-1 and genes involved in the de novo lipogenesis, including PCSK9. Thus, we hypothesized that STAT3 was the downstream regulator of PCSK9 transcription by simvastatin. Indeed, in the presence of simvastatin or of the Rac inhibitor, the nuclear translocation of STAT3 was significantly reduced, which in turn could activate PCSK9 transcription by SREBP. In conclusion, this study reveals a direct role of Rac1 on simvastatin-mediated PCSK9 expression via the reduction of STAT3 nuclear translocation. Those results further support the rationale for a combination therapy of statins and PCSK9 inhibitors, especially in patients with an acute myocardial infarction that receive statin in secondary prevention.

ADHERENCE TO CARDIOVASCULAR MEDICATION IN A CARDIOVASCULAR DISEASE PREVENTION CENTER

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Chronic diseases demands long term drugs administration and need constant evaluation to dedicate out-patient clinic. Adherence to the therapy is a corner stone to achieve the target to prevent cardiovascular diseases. So, treatment failure, caused by poor medication adherence, leads to frequent re-hospitalizations, poor outcome and increased health care costs. To evaluate drug adherence we can use validate tests; the Morisky medication adherence scale is a commonly used adherence screening tool. It is composed of 4 yes/no questions about past medication use patterns and is thus quick and simple to use during drug history interviews. We evaluated the adherence to therapy of 245 outpatients (age ≥18 years), consecutively visited in our cardiovascular disease prevention center unit (Lipid Center Cosenza), from 1 January to 30 July 2018. All of there were in cardiovascular treatments also in multiple drugs therapy. We used of the self reported Morisky score as a screening tool for identifying patients adherence to medications. The self report adherence in the patients begin treated was 75.9% (score 3-4) and only 39 of the 245 patients (24.1%) were categorized as non-adherent. Among non adherent patients (51% female), 38.9% were in monotherapy, while 42% took more than one drug. The 20.3% of non-adherent patients reported an adverse drug reaction. In conclusion, the data collected in our clinic show a good therapeutic adherence. Adherence can be improved with surveillance and counselling activities by doctor, nurse and pharmacist during the subsequent planned out-patient follow-up.

POTENTIAL IMPACT OF ACC/AHA GUIDELINES FOR MANAGEMENT OF ARTERIAL HYPERTENSION IN THE CHOICE OF THERAPY IN CLINICAL ASSET

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The 2017 ACC/AHA guidelines (GL) for management of arterial hypertension has changed classification and treatment of blood pressure (BP) recomanding to begin BP drug treatment at ≥130/80 mmHg for patients with an estimated 10 years ASCVD risk ≥10%. This can has a deep impact on therapeutical activity. We wanted to evaluate the percentage of patients reaching the suggested new goal and the number of drugs required to reach it. Two hundred sixty hypertensive outpatients, with an estimated 10 years ASCVD risk ≥10%, consecutively referred to our dedicated clinic, were evaluated. All of them were drug treated from more than three mounts. Of these, 41.2% were female the mean of age was 62 years. According to 2017 ACC/AHA GL, 40.7% did not reached a good BP control, instead, considering the new 2018 European GL, 27.7% did not reached BP <140/90 mmHg. The median drug prescribed was 2.15 for all patients, 2.9 for patients with BP ≥140/90 mmHg, 2.33 for patients with BP between ≥130/90 and <140/90 mmHg. In conclusion, the new ACC/AHA GL create a greater number of not at target hypertensive patients and this can cause an important effect on the use of pharmacological therapy. In our study, 43% of patients, at therapeutical goal with the 2018 european GL, became now not at target and need more antihypertensive drugs.
SAFETY OF STATINS AND MONITORING OF DRUG-RELATED MYOPATHY IN A DEDICATE CLINIC

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Statins are a widely used group of cholesterol-lowering agents with a favorable benefits for cardiovascular disease. It's known that statins can have potential adverse effects (AEs). The frequency of AEs, due to statins, is not well known. In fact, there is a wide variability between AEs reported in clinical randomized trial and the date available in post marketing settings. In recent years, pharmacovigilance has the important aim of monitoring the incidence of all adverse reactions to drugs. Unfortunately not in all countries, the reporting reaches standard levels set by the WHO. For these reasons, the Italian Agency of the Drugs (AIFA) has been promoting and financing regional projects with multidisciplinary groups to increase spontaneous reporting. We started a survey program finalized to report all adverse events from drugs observed in our Cardiovascular Prevention Clinic. We evaluated all of the 350 outpatients (≥18 years), consecutively visited in our dedicated clinic for prevention of cardiovascular diseases (Lipid Center Cosenza). All of patients were in cardiovascular treatment with drugs. A total of 30 ADRs were observed; 29 were non-serious. In particular, 4 myopathy events (3 simvastatin and 1 lovastatin) were reported (13.3%) and included in the national pharmacovigilance network (RNF). Muscular symptoms were reported by 3.4% of all statin users. Incidence of myalgia was found to be higher in the age group between 45 and 54 years old and males experienced more ADRs than females. In conclusion, we reported, regarding statins use, a lower number of AES, compared to other post-marketing study.

THE IMPACT OF THE MEDITERRANEAN DIET ON PLASMA LDL-C LEVELS IN FH CHILDREN

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Introduction. Dyslipidemia plays a major role in cardiovascular disease (CVD) particularly when Familial Hypercholesterolemia (FH) occurs. Eating habits have positive influence on the clinical outcome but the effect of the dietary counseling on FH children is not well known. The aim of the study was to investigate the Mediterranean Diet impact on lipid profile with particular focus on dietary fat quality.

Material and Methods. Fifty FH children (4-18 years), not undergoing phytosterols or statin therapy, were included in the study. Dietary intake was recorded twice, at baseline and after dietary advice, by food week diaries. The recommended dietary pattern (Mediterranean Diet) was normocaloric and characterized by wide consumption of cereals, legumes, fish, plant foods, nuts, olive oil as the main source of fat, moderate consumption of dairy products and eggs, a small amount of meat, particularly red meat. Food diaries have been analyzed to estimate nutrients intake. Plasma lipid levels were evaluated at baseline and after three month since the diet counseling.

Results. Among 50 FH children, 38 followed dietary recommendations while 12 did not. In the compliant group, after dietary advice, the mean intake of carbohydrate, protein, total fat, saturated fat (SFA), unsaturated fats was 35.7±4.5, 15.9±1.88, 30.6±4.4, 8.17±1.78, 18.8±2.8 E%, respectively, while the mean fiber intake was 12.6±2.51g. Compared to baseline, LDL-C levels in compliant FH children were reduced by 9% while in the non-compliant group LDL-C levels increased by 8.9%.

Conclusions. The lipid profile improvement here found could be correlated to the low SFAs intake and the assumption of unsaturated fatty acids, fibers as well as other bioactive compounds. Furthermore the synergy among the nutrient-rich foods in this pattern can be favorable. This study demonstrates the efficacy of the Mediterranean Diet in FH children.

DOES LIPOPROTEIN(A) PREDICT EARLY ONSET OF CARDIOVASCULAR DISEASE IN PEDIATRIC FAMILIAL HYPERCHOLESTEROLEMIA?

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Aim. Lipoprotein(a) [Lp(a)] is a marker that predicts atherosclerotic cardiovascular disease (ASCVD) in adults with familial hypercholesterolemia (FH) in combination with low-density lipoprotein cholesterol (LDL-C) level, but its role for children with FH is debated. The aim of this study is to examine Lp(a) values in a sample of pediatric patients affected by FH.

Materials and Methods. Retrospective review of pediatric patients with FH identified LDL-C, Lp(a), and family history of ASCVD.

Results. We are collecting data on our population of pediatric patients affected by FH. Twenty-four children (17 female, five males, median age at diagnosis y 8.3) from 21 families were identified right now; in 12/24 patients Lp(a) was high. Among these, 7/12 had a family history of ASCVD. In contrast, 5 patients with normal Lp(a) had a family history of ASCVD too. In a recent study, Zawacki et al. suggested that children with FH in addition to high Lp(a) may be at higher risk for ASCVD than their LDL-C alone would suggest. Follow up is in progress and data are limited. Anyway, we observed an unclear relationship.

References
PHTHALATES EXPOSURE AS DETERMINANT OF ALBUMINURIA IN TYPE 2 DIABETES SUBJECTS: A CROSS-SECTIONAL STUDY

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Background. Albuminuria is an independent risk factor for cardiovascular (CV) disease. Epidemiological studies reported an association between exposure to phthalates, a group of environmental contaminants, and states of higher CV risk like obesity and type 2 diabetes (T2D). No studies have so far addressed the presence of these compounds in urines of T2D individuals with different degrees of renal function.

Materials and Methods. Concentrations of three phthalate urinary metabolites (mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-oxoehexyl phthalate (MEOHP) and mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP)), were quantified by UHPLC-ESI-QTOF in a morning urine sample of 209 T2D subjects (75.5% normoalbuminuric, 19.7% microalbuminuric, 4.8% macroalbuminuric). Data were normalized for urinary creatinine and related to clinical parameters, adjusting for confounders by univariable and multivariable regression models.

Results. Participants were 67.8±12.4 years old; 59% males. T2D duration was 10.5±9.7 years, HbA1c was 7.18±1.31%; 29% were insulin-treated. Creatinine-adjusted urinary concentrations of MEHP; MEOHP; MEHHP (detected in 95% of the subjects) were 7.53 [4.84-12.60] µg/g, 3.04 [1.03-5.14] µg/g and 10.70 [7.02-17.40] µg/g. No difference emerged according to age, sex, BMI, T2D duration, smoking, BP, HbA1c and eGFR, even after adjusting for confounders. Exposure to MEHP (p<0.02) and MEOHP (p<0.04) was higher in high macroalbuminuric than in normoalbuminuric individuals; 4th vs 1st quartile of MEHP and MEHHP showed higher risk of albuminuria (MEHP: OR 4.83 [CI 1.45-16.06], p<0.03; MEOHP OR 3.29 [CI 1.08-10.04], p<0.04). MEHHP was higher (p=0.034) in microalbuminuric than in normoalbuminuric individuals. Smoking and BMI were associated with a 2.93 (p=0.047) and 1.34 (p=0.040) fold increase in the risk of albuminuria, respectively. In subjects with CV events, while MEHP showed a trend (p=0.061), MEHHP was associated with increased risk of albuminuria in subjects with CV events (p<0.001).

Conclusion. These findings point out for the first time an association between phthalates exposure and AER in T2D subjects independent of GFR, representing a potential early hallmark of widespread vascular damage. Though considering the limitations of a cross-sectional study, a long-term exposure to those contaminants might mark a higher CV risk, implying the need for prospective studies addressing the pathophysiologic mechanisms underlying such association.

LIVER STEATOSIS AND CAROTID INTIMA-MEDIA THICKNESS IN ADULTS. TRENDS FROM THE FOIE GRAS PROJECT IN SOUTHERN ITALY STUDYING THE ADHERENCE TO MEDITERRANEAN DIET AND PHYSICAL ACTIVITY


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Background. Nonalcoholic fatty liver disease (NAFLD) is the emerging health problem worldwide putting populations at increased risk of progressive liver damage, failure and neoplasia, and cardiovascular risk. The worrisome rise of the metabolic syndrome is also affecting subjects at a younger age but might be prevented by healthy lifestyles. AIM: To check the potential association between degree of liver steatosis, carotid intima-media thickness (CIMT), and lifestyles in adult subjects.

Methods. 60 subjects were consecutively enrolled at the Division of Internal Medicine “A. Murri”, University of Bari ‘Aldo Moro’, Southern Italy, and grouped according to the presence of ultrasonographic liver steatosis (grade 0=absent; 1=homoogeneous liver brightness; 2=proximal liver brightness+distal hypoechogenicity; 3=proximal liver brightness+distal hypoheocgenicity without visible diaphragm line). CIMT was also measured ultrasonographically (Noblus® Hitachi, 3.5, 7.5 MHz Probe, Italy). Questionnaires were used to quantitatively assess weekly physical activity levels (Metabolic Equivalent Tasks, METs; 1 MET=3.5 mL/kg/min of oxygen consumption or 1.5
Kcal/Kg/hr; Saint-Maurice et al. 2018), as markers of compliance to healthy lifestyles.

**Results.** NAFLD subjects (n=29, 16M, 13F) and healthy subjects (n=31, 14M, 17F) participated. NAFLD subjects (median steatosis score 1.5±0.12) were older (age 48.5±2.28 yrs, range 26-69 vs. 29.7±1.89 yrs, range 18-62, P<0.0001), more overweight-obese (BMI 31.3±1.19 kg/m²; 93% overweight-obese vs. 22.4±0.72 kg/m², 23% overweight-obese, P<0.0001) and with thicker CMT (0.91±0.03 mm vs. 0.59±0.02 mm, P<0.0001) than healthy subjects. The grade of steatosis increased with body size, while percent of subjects with abnormal CMT (≥1.0 mm) was 39% and 0% in NAFLD and healthy subjects, respectively (P<0.0001). Dietary adherence scored “sufficient” and was comparable in both groups (NAFLD 10.4±0.51 [median 10.5] vs. healthy 9.7±0.56 [median 9.0], P=NS). Percent of subjects scoring sufficient-high dietary adherence was 76% (NAFLD) and 84% (healthy), P=NS. Weekly physical activity levels were low and equally comparable (NAFLD 146.8±237.6 METs [median 630] vs. healthy 1906.0±237.7 METs [median 1620], P=NS) while percent of inactive subjects (i.e., <675 METs) was 52% and 26% (NAFLD and healthy subjects, respectively, P<0.001) with similar sitting times: NAFLD 253±30.5 min/day vs. healthy 311±28.3 min/day, P=NS.

**Conclusion.** In adults, liver steatosis is associated with increased CMT, pointing to increasing cardiovascular risk. This trend develops on a background of an apparent “sufficient” adherence to Mediterranean diet but poor physical activity levels. Thus, even in a typical Mediterranean area, lifestyles deserve active and urgent educational programs to counteract the universal rising trend of NAFLD.

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**LOW DENSITY LIPOPROTEIN RECEPTOR PLAYS A FUNDAMENTAL ROLE IN THE ACTIVATION OF CD8+ T LYMPHOCYTES**

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**Aim.** Cholesterol is essential for the correct function and proliferation of immune cells. The aim of this study was to evaluate whether the uptake of low-density lipoprotein by the LDL-receptor (LDLR), plays a role in the activation of T lymphocytes, that are important players in the maintenance of the chronic response associated to atherosclerosis.

**Methods.** Immunophenotypic analysis and cytokines production evaluation in T lymphocytes from WT and LDLR KO mice, after in vitro (anti-CD3/anti-CD28 stimulation, mixed lymphocytes reaction) and in vivo (vaccination, homeostatic proliferation) activation through flow cytometry, gene expression analysis and protein quantification.

**Results.** LDLR increases after in vitro activation of CD8+ T cells. By using LDLR KO mice, we found that LDLR deficiency impairs CD8+ proliferation in vitro both after anti-CD3/anti-CD28 stimulation and after allogenic stimulation (proliferated cells: 1764±38 WT, 1144±64 LDLR KO p<0.01). Furthermore, INFγ production was reduced in LDLR KO CD8+ T cells (13.9±1% WT, 8.4±0.6% LDLR KO p<0.01). Similarly, in vivo antigen-specific activation (vaccination with ovalbumin) resulted in a reduced proliferation and cytokines production (IFNγ p<0.001, IL13 p<0.01, perforin p<0.05) in CD8+ of LDLR KO mice. On the contrary, during homeostatic proliferation, a non-antigen-driven in vivo assay, WT and LDLR KO CD8+ T cell showed similar proliferation and cytokines production. Next, we investigated whether these effects were caused by an impaired T cells activation and indeed we found a reduced expression of CD69 (WT 61.6±6.1%, LDLR KO 41.8±8.5%, p<0.01), a marker of early activation, and decreased phosphorylation of Akt, a downstream molecule of the TCR that is rapidly phosphorylated after CD4+ and CD8+ T cell activation. Defects in signalling can be the consequence of altered lipid rafts formation, membrane portions enriched in cholesterol. Indeed, we observed a significant reduction of neutral lipids (MFI nile red WT 12520±2071, MFI LDLR KO 9639±272, p<0.05) in CD8+ T cells of LDLR KO mice after activation in vitro which was parallel by reduced Ctxb expression, a marker of lipid rafts.

**Conclusions.** Our results show that LDL receptor plays a critical role for immunometabolic responses in CD8+ T lymphocytes. In particular, its absence alters proliferation, activation and cytokines production in antigen-driven mechanism. LDL receptor in lymphocytes could therefore represent a checkpoint linking cellular cholesterol metabolism to adaptive immune response.

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**REVISED AMERICAN ACADEMY OF PEDIATRICS GUIDELINES ON PEDIATRIC HYPERTENSION AND CAROTID STIFFNESS IN OBESE CHILDREN**

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**Background.** A clinical practice guideline with new pediatric hypertension definitions has been recently published. These guidelines include reference tables of normative blood pressure values and new definitions of elevated BP and hypertension.

**Aim.** Of the present study was to evaluate implications of these revised AAP pediatric hypertension guidelines on the association with carotid stiffness in a group of obese children.

**Subjects and Methods.** 300 consecutive obese children from 5 to 15 years evaluated for obesity in a specialist setting care, were invited to participate in a study to investigate their cardiovascular markers of atherosclerosis. All children underwent an ultrasound carotid arterial examination. Carotid arterial stiffness was calculated using the following formula: β = (natural logarithm systolic blood pressure - natural logarithm diastolic blood pressure)/(systolic diameter - diastolic diameter)/diastolic diameter. Increased carotid stiffness was defined by a value >2.71, corresponding to the 90th percentile of lean children studied in a previous research and to the 50th percentile of obese children included in the study.

**Results.** In a logistic regression analysis stage 2 hypertension was significantly associated with increased carotid stiffness (OR 2.4, 95% CI 1.2 - 4.9; p=0.015) even after adjustment for the main cardiovascular risk factors (age, gender, BMI, LDL-cholesterol, HDL-cholesterol, triglycerides, glucose, HOMA).
Conclusion. The new pediatric clinical practice guidelines for high blood pressure have changed the clinical approach to identification of hypertension. Using these new criteria we report that in obese children with stage 2 hypertension there was an association with stiffer carotid arteries. However, in obese children with elevated blood pressure or stage 1 hypertension this association was not demonstrated. These results facilitate identification of obese children already affected by subclinical atherosclerosis and enable therapeutic interventions as early as possible to interrupt the pathological process.

EFFICACY AND SAFETY OF PCSK9 INHIBITORS: THE REAL-LIFE EXPERIENCE OF THE LIPID CLINIC IN MODENA

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Aims. PCSK9 inhibitors (PCSK9i) have proven effective and safe for reducing LDL cholesterol (LDL-C) in clinical trials. We aimed at evaluating efficacy and safety of PCSK9i in the real-life setting of the lipid clinic in Modena.

Patients and Methods. 35 consecutive patients who started treatment with PCSK9i (evolocumab 17; alirocumab 18) between Feb.2016 and Jun.2018 were followed-up for a median period of 20[6-116] weeks. Baseline and on-PCSK9i therapy clinical data and lipid profile were registered.

Results. Baseline patients characteristics were as follow: men/women 23/12; median age 60[26-76] years; 26 with familial hypercholesterolemia (FH); 25 on secondary cardiovascular prevention; 5 with total statin intolerance; 5 with type 2 diabetes (T2D); 7 on LDL-C apheresis. PCSK9i significantly reduced LDL-C by -63[-77 - -27]% (p<0.001); 83% of patients reached LDL-C therapeutic target. Lp(a) levels were also significantly reduced by -39[-73 - -21]% (p<0.001), but only 5 out of 13 patients with high baseline Lp(a) levels reached <50 mg/dl. PCSK9i LDL-C lowering efficacy was independent from baseline characteristics (FH vs. non-FH; primary vs. secondary cardiovascular prevention; presence vs. absence of T2D) and from the type of PCSK9i. LDL-C apheresis was discontinued in 88% of patients. Patients with total statin intolerance showed a less significant reduction in LDL-C and were less likely to reach LDL-C therapeutic target. No significant changes in glyceremia, CPK and aminotransferases were seen during follow-up. Three patients experienced adverse events (1 local skin reaction, 1 diffuse skin rash, 1 rhinopharyngitis) and none discontinued the treatment. Two patients experienced cardiovascular events (1 coronary and 1 carotid revascularization) during follow-up.

Conclusions. PCSK9i are effective and safe in attaining LDL-C therapeutic target in our real-life cohort of high-risk patients. Open clinical issues are: management of high-risk patients with total statin intolerance or high Lp(a) levels; PCSK9i long-term safety; PCSK9i efficacy on cardiovascular outcomes.

PREVALENCE OF FATTY LIVER AND ITS ASSOCIATION WITH CARDIOVASCULAR DISEASE IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Aims. Despite every patient with familial hypercholesterolemia (FH) is at increased lifetime risk for cardiovascular events, the risk is heterogeneous and risk stratification is challenging. Fatty liver (FL), a very common cause of chronic liver disease, is associated with an increased risk of cardiovascular disease (CVD). We aim at determining the prevalence and the predictors of FL, and at evaluating whether FL is associated with increased prevalence of CVD in FH patients.

Patients and Methods. In this pilot study, all adult patients with a clinical and/or molecular diagnosis of definite FH (Dutch Lipid Clinic Network - DLCN score >8) with available liver ultrasound data, who regularly attended our lipid clinic in Modena, were retrospectively enrolled. Main study measures were detection of FL (by liver ultrasound) and CVD (by patient history and chart review).

Results. 78 patients were enrolled in this study; men (n=39) and women (n=39) were equally represented, median age was 53[18-92] years, 44.9% (n=35) were past or current smokers, 64.1% (n=50) were overweight/obese, 6.4% (n=5) had type 2 diabetes, and 48.7% (n=38) had arterial hypertension. The prevalence of FL was 43.6% (n=34). Patients with FL were significantly more likely to have overweight/obesity (82.4% vs. 50.0%, p=0.003), type 2 diabetes (14.7% vs. 0.0%, p=0.009) and arterial hypertension (64.7% vs. 36.4%, p=0.013) than those without FL. They also had significantly higher values of BMI (p=0.001), waist circumference (p=0.001), triglycerides (p=0.007) and alanine aminotransferase (p=0.008). The prevalence of FL increased across the number of factors of the metabolic syndrome (p=0.012). Age, sex, LDL cholesterol and DLCN score were not significantly different between FH patients with and without FL. 18 (23.1%) FH patients had a history of CVD. Of these, 16 (20.5%) had coronary heart disease (CHD) (myocardial infarction, angina or coronary revascularization procedures), 5 (6.4%) had cerebrovascular disease (ischemic stroke, transient ischemic attack or carotid revascularization procedures), and 4 (5.1%) had peripheral vascular disease (symptomatic lower extremity peripheral arterial occlusive disease or lower extremity revascularization procedures); 4 patients (5.1%) had CVD in multiple sites. FH patients with FL showed a significantly higher prevalence of CVD than their counterpart without FL (35.3% vs. 13.6%, p=0.024); the association between FL and CVD was largely conveyed by CHD (CHD: 32.4% vs. 11.4%, p=0.023); cerebrovascular disease; 8.6% vs. 4.5%, p=0.444; peripheral vascular disease; 8.8% vs. 2.3%, p=0.193; multiple-site CVD: 8.8% vs. 2.3%, p=0.193. In univariate logistic regression analysis, FL was significantly associated with CVD (crude OR 3.5, 95%CI 1.1-10.5; p=0.029) and CHD (crude OR 3.7, 95%CI 1.2-12.1; p=0.028). Together with FL, age (p=0.007), smoking status (p=0.003), arterial hypertension (p=0.008), fasting glucose (p=0.012), HDL cholesterol (p=0.041) and LDL cholesterol (p=0.001) were significantly associated with CVD, whereas sex, BMI, waist circumference, metabolic syndrome, Lp(a) and DLCN score were not. The association between FL and CVD remained significant even after adjustment for age, sex and smoking status (adjusted OR 4.3, 95%CI 1.1-16.6; p=0.037), but it was weakened.
Efficacy and Safety of PCSK9 Inhibitors in a Cohort of Dyslipidemic Patients

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Introduction. The use of PCSK9-inhibitors represents the new frontier in the management of hypercholesterolemia. Studies of efficacy have shown a reduction in LDL-C levels up to 60%. The aim of the work was to evaluate the reproducibility in real-life of the efficacy, durability and safety data of anti-PCSK9.

Methodology. Treatment with Alirocumab (75-150 mg sc every 14 days) or Evolocumab (140 mg sc every 14 days) was initiated in 27 subjects with not on target LDL-C levels according to ESC guidelines/EAS 2016. Heterozygous FH was present in 15 subjects (55%); among these, 5 were in primary prevention, 7 in secondary prevention and 3 were intolerant to statins. 12 subjects (45%) had a form of polygenic hypercholesterolemia and were at very high CV risk or in secondary prevention; among these, 7 were intolerants to statins. All subjects were treated with ezetimibe 10 mg/day. The serum levels of total cholesterol, HDL cholesterol and triglycerides were measured before and at 2 (n=27), 6 (n=19) and 12 (n=14) months since start of treatment. Data are expressed as m ± SD. Data were analyzed with Student’s T distribution for simple data or for paired data as appropriate.

Results. After two months of therapy, LDL-C levels were reduced by an average of 53.9% compared to baseline (from 195±78 mg/dL pre-treatment to 87±58 mg/dL, p<0.005), while there were no statistically significant differences among LDL-C at 2 months and LDL-C at 6 and 12 months of therapy (87.4±73 and 98±74 mg/dL, respectively) (p=ns). Similar results were obtained for non-HDL cholesterol: 224±82 mg/dL pre-treatment vs 107±71 mg/dL at 2 months (p=0.005 vs baseline) and 113±73 mg/dL after 12 months (p=0.005 vs baseline). A significant reduction of triglycerides was observed after two months of treatment (131±48 mg/dL at baseline vs 99±25 mg/dL at 2 months, p=0.005), at 6 months (99.7±34 mg/dL, p=0.023), but not at 12 months (109±39 mg/dL, p=ns). No significant changes in HDL-C levels were observed before and after the introduction of anti-PCSK9 therapy: 48±10 mg/dL at baseline vs 47±9 mg/dL at 2 months (p=ns), 48±9.5 mg/dL at 6 months (p=ns) and 51±8 mg/dL at 12 months (p=ns). The baseline LDL-C in the group of subjects intolerant to statins (n=10) was 187±174 mg/dL and 199±81 mg/dL in subjects receiving statins (n=17) (p=ns). After 2 months of treatment the LDL-C values were reduced to 100.6±54 mg/dL in the subjects intolerant to statins (46.2%) and to 79±60 mg/dL (60.4%) in the subjects taking statins, without statistically significant differences between the two groups (p=ns). No significant side effects were found. Compliance to treatment was 100%.

Discussion. These data confirm the efficacy of PCSK9 inhibitors in the reduction of LDL and non-HDL cholesterol values. The reduction of LDL-C levels is similar in subjects treated with statins and ezetimibe and in subjects intolerant to statins who take only ezetimibe. The maximal effect is achieved after two months of treatment and is maintained over time. 85% of subjects achieved LDL-C target values, a difficult result to reach until few months ago. The absence of significant side effects argues in favor of the safety of treatment with the anti-PCSK9 antibodies.

Performance Evaluation of the Dutch Lipid Clinic Network Score Using an Italian Database of Familial Hypercholesterolemia Patients

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Background. Familial hypercholesterolemia (FH) is a common genetic condition characterised by high plasma levels of low-density lipoprotein cholesterol (LDL-C) from birth and increased risk of premature coronary heart disease. Although it is a severe disorder, FH is currently under-diagnosed. The Dutch Lipid Clinic Network (DLCN) score is commonly used by healthcare professionals as a tool that guides FH diagnosis in the setting of general medicine. Aims: To evaluate the performance of the DLCN score, understanding whether missing information may affect the identification of FH subjects.

Methods. Patients are enrolled in the LIPIGEN study, an Italian integrated network aimed at improving the identification of patients with genetic dyslipidaemias. The analysis was carried out in all mutation-positive patients, aged 18 years and older, who underwent clinical evaluation and had available information on LDL-C levels.

Results. The DLCN score was applied on 1377 adults (mean age 42.9±14.2 years) with genetic diagnosis of FH, resulting in 28.5% of the sample classified as probable FH and 37.9% as definite FH. In total, 43.4% had at least one missing data out of 8, and about 10.0% had at least 4 missing data. When analyzed based on the type of missing data, a higher percentage of subjects with at least 1 missing data in the clinical history (54.6%) or physical examination (40.4%) was classified as possible FH (DLCN score 3-5). Moreover, it has been proven that using real or estimated pre-treatment LDL-C levels may significantly modify the DLCN score, highlighting an overestimation of the number of patients with definite FH diagnosis when pre-treatment LDL-C levels are calculated.
**ABSTRACT**

**Introduction.** Atherosclerosis is a leading cause of cardiovascular morbidity and mortality worldwide. Inflammation drives the formation, progression, and complication of atherosclerotic plaques. The plaque complications lead to plaque erosion, plaque rupture and thrombus formation. We aimed to investigate the accuracy of circulating Gal-3 in predicting advanced atherosclerotic plaque presence and to evaluate the relationship of circulating Gal-3 both with plaque complications leading to plaque erosion, plaque rupture and thrombus formation. We aimed to investigate the accuracy of circulating Gal-3 in predicting advanced atherosclerotic plaque presence and to evaluate the relationship of circulating Gal-3 both with plaque complications leading to plaque erosion, plaque rupture and thrombus formation.

**Materials and Methods.** Advanced human carotid plaques from 99 patients undergoing carotid endarterectomy were histologically classified as type Va (fibroatheroma), type Vb (mainly calcific) and type VI (complicated lesion) according to American Heart Association guidelines. Additional characteristics such as percentage of fibrosis, calcifications, necrosis and number of vessels were also recorded for each plaque. Gal-3 was measured in serum samples from patients and 78 healthy controls by immunometric assays. In the subgroup of 40 patients Gal-3 was directly detected in the plaques by immunohistochemistry.

**Results.** Gal-3 serum levels were significantly higher in patients than in controls (19.8±5.8 vs 14.0±3.6 ng/mL, p<0.0001). Analysis of ROC curves confirmed the discriminating power of the marker with an AUC of 0.806 (p<0.0001). At multivariate logistic regression Gal-3 serum levels remained associated with plaque presence independently from age, sex and LDL-c with an odd ratio of 1.26 (1.13-1.41, p<0.0001). No differences were found between Gal-3 serum levels among the different plaque types. No correlation was observed between serum and intra-plaque Gal-3 levels. A significant association between Gal-3 plaque levels and the number of vessels present in the atherosclerotic plaque was observed (p=0.002).

**Conclusion.** Our data show that Gal-3 is a good marker discriminating between subjects with and without advanced atherosclerotic plaque independently from age, sex and LDL-c. The association between Gal-3 and number of vessels suggests that Gal-3 could act as a pro-angiogenic factor.

**Conclusions.** Although the DLCN score is a useful tool for the FH diagnosis, it may be limited by the complexity to retrieve all the information, suggesting a crucial role of the clinical judgement in the identification of FH subjects.

**GALACTIN-3 SERUM LEVELS AND ADVANCED ATHEROSCLEROTIC PLAQUES: CORRELATION WITH PLAQUE PRESENCE AND NUMBER OF VESSELS**

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**A CASE OF PREMATURE Atherosclerosis AND Ipercholesterolemia Low RESPONSIVE TO THERAPY: WHEN SUSPECT SITOSTEROLEMIA**

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**Introduction.** Sitosterolemia is a rare disorder of lipid metabolism that is inherited in an autosomal recessive pattern, characterized by several plant sterols in blood. Signs and symptoms consist in large xanthomas can cause pain, difficulty with movement, joint stiffness and blood abnormalities that occasionally are the only signs of the disorder, resulting in hemolytic anemia, stomatocytosis and macrothrombocytopenia.

**Aim.** Report a case of sitosterolemia characterized by ipercholesterolemia, tendom xanthomas, premature atherosclerosis and minor thrombocytopenia.

**Results.** We report the case of 57-year-old man diagnosed with hypercholesterolemia and macrothrombocytopenia. He presented with lipid profile suggestive for familial hypercholesterolemia (FH), with total cholesterol 320 mg/dl e LDL 260, tendinous xanthomas, xanthelasmamas and early onset atherosclerotic cardiovascular disease (DLCNS 12); hemorrhagic diathesis (epistaxis in childhood, hematuria and post-traumatic ecchymosis during anticoagulant/antiplatelet therapy for mechanical valve); light thrombocytopenia (PTS 116 x10.9/L), increase of LAD 332 U/L with low level of haptoglobin, normal bilirubin, negative direct and indirect Coombs test, normal platelets aggregate and immunophenotype test. Furthermore the genetic screening for FH resulted negative. He presented increased plasma sitosterol levels 123,7 μg/ml (v.n. 0,7-7,0) and the hypothesis of sitosterolemia was confirmed by genetical and molecular analysis that shown the presence of a homozygous mutation of the gene ABCG8, exon, c.320C/G (p.S107X). The sterol absorption inhibitor ezetimibe was associated to rosuvastatin therapy and diet low in shellfish sterols and plant sterols (vegetable oils, margarine, nuts, seeds, avocados, and chocolate), with reduction of total cholesterol (-30,6%) and LDL-C (-42,4%) with reduction of xanthomas, xanthelasmas and early onset atherosclerotic cardiovas-
MEASURING EFFEROCYTOSIS IN ATHEROSCLEROSIS: NEW INSIGHTS FROM TRANSGENIC MOUSE MODELS

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Background and Aims. Macrophage apoptosis may play opposite roles in atherosclerosis, limiting plaque progression in early lesions, while worsening the disease at late stages. Late apoptotic cells produce proatherogenic inflammatory responses and eff erocytosis, the adequate clearance of apoptotic bodies and cells by macrophages, is fundamental to hamper the atherosclerotic plaque progression. Indeed, an impaired eff erocytic process leads to wide necrotic cores and extensive pro-inflammatory response, thus increasing plaque vulnerability. Studying eff erocytosis’ efficacy in atherosclerotic lesions could help to evaluate plaque progression and eventually develop proeff erocytic therapies. To address this issue, we applied a peculiar method for evaluating apoptosis and eff erocytosis in vivo in athero-prone transgenic mouse models.

Materials and Methods. LDLR-/- mice on a cholesterol-rich diet were irradiated and transplanted with bone marrow isolated from transgenic mice overexpressing sphingosine 1-phosphate receptor 1 (S1P1) in myeloid lineage (S1P1-Lyz and S1P1-F4/80) or control mice. After sacrifice, aortic root cryosections were analyzed to compare mice expressing S1P1-F4/80 or S1P1-Lyz with control mice. After staining with a specific marker (MOMA-2) to stain macrophage positive area, TUNEL assay was performed to detect apoptotic signals. Apoptotic signals associated with macrophage cytosol were classified as eff erocytosis, while free TUNEL signals were classified as apoptosis. Efferocytic signals were normalized to the respective lesion area (mean±SD weighted on mm²).

Results and Conclusions. Apoptosis rate was significantly higher in controls compared to S1P1-F4/80 and S1P1-Lyz mice (16.1±13.98 vs 7.1±3.98 and 5.1±1.64, respectively). Conversely, eff erocytosis significantly increased in S1P1-F4/80 and S1P1-Lyz compared to control group (6.26±3.85 and 11.17±2.36 vs 2.46±2.20, respectively). These data confirm previous in vitro studies pointing to an antiapoptotic role for S1P-S1P1 axis. Accordingly, in vivo evaluation of both apoptosis and eff erocytosis is an efficient method that could be useful in analysing atherosclerotic lesion progression and plaque stability.

EFFECT OF PERIODONTAL DISEASE ON GLUCOREGULATORY HORMONES IN INDIVIDUALS WITH MORBID OBESITY

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The link between periodontitis (PD) and obesity is well known; however, the influence of PD on the glucoregulatory hormone pattern has never been explored. We measured clinical periodontal parameters (probing pocket depth, PPD, bleeding on probing, BOP, clinical attachment level, CAL) in 110 severely obese, non-diabetic individuals. Insulin, glucagon, GLP-1 and GIP were assessed after three days of standardized diet. 47 subjects had periodontitis (PD+) and 63 did not (PD-). PD+ showed a mean number of teeth of 26, 30.3% of gingival sites with PPD>4 mm, 35.2% of BOP sites and a mean CAL loss of 4.1 mm, suggestive of a generalised tissue loss. hsCRP was above the normal range in both groups (4.31±3.80 vs 3.67±2.62 mg/l, p=0.29). PD+ showed higher IL-6 levels (3.4±3.9 vs 5.2±4.1 pg/ml, p=0.02); IL-1α did not differ. No relationship emerged between cytokines and hormones. Compared with PD-, PD+ had higher glucagon (26.60[10.07] vs 23.34[16.80] pmol/l, p<0.0001) and GIP levels (10.56[13.30] vs 6.43[8.43] pmol/l, p<0.001), and lower GLP-1 (11.78[10.07] vs 23.34[16.80] pmol/l, p<0.0001). Insulin did not differ. In PD+, after adjustment for confounders, PPD was directly related to glucagon (β=0.424, p=0.002) and inversely to GLP-1 (β=−0.159, p=0.044). In PD+, CAL and PPD were related with glucagon (β=0.404, p=0.003 and r=0.456, p=0.001 respectively). In a multiple regression analysis, such relationship held for PPD (β=0.431, p=0.002); when adding incretins in the model, glucagon and GLP-1 were both associated (full model r²=0.30). In comparing different stages of PD, glucagon was related with the severity of periodontitis (16.17±9.85, 25.25±4.34, 39.24±4.45 ng/l from stage I to III, p=0.03). We describe here for the first time an impaired incretin axis combined with a relative hyperglucagonemia in obese non-diabetic subjects with PD and subclinical inflammation; this hormonal pattern might contribute to deteriorate their glucose tolerance, partially explaining the higher risk of diabetes observed in these patients.

MICROARRAY ANALYSIS OF LIVER GENE EXPRESSION IN APOA-I MILANO KNOCK-IN MOUSE MODELS

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Atherosclerosis, the dominant cause of CVD, is a vascular site-specific chronic inflammation initiated in response to retained and modified lipids within arterial wall that can lead to clinically sig-
significant endpoints. Several epidemiological studies have shown that there is an independent and inverse relationship between circulating HDL-C levels and CVD. However, it has been proven difficult to successful reduce CVD risk with drugs increasing HDL-cholesterol. Moreover, it was reported that upon acute-phase response, HDL of both patients and animals not only lose some of normal functionality, but also gain atypical functions. The natural variant of human apoA-I, i.e. the apoA-I Milano is the result of a point mutation, with an arginine to cysteine substitution at position 173. Carriers exhibit hypertriglyceridemia with markedly reduced HDL and apoA-I plasma levels, a condition generally associated with a high risk of premature coronary disease. Evaluation of the cardiovascular status in apoA-I carriers, compared with control subjects from the same kindred, did not reveal any evidence of increased CVD. 

Aim of this study was the identification of pivotal molecules that to a large extent differentiate two mouse models expressing this natural variant. In our study two different mouse models have been generated and used, i.e. the hA-IM k-in (hA-IM), and the hA-IM k-in overexpressing human apoA-II (hA-IM/AII) mice. This aim has been accomplished by using the Affimetrix GeneChip Mouse Gene ST system. The results obtained from the DAVID platform showed 1965 differentially regulated genes, 955 downregulated (from -1 to -3.3 fold change) and 1014 upregulated (from 1 to 4.6 fold change), in hA-IM compared to hA-IM/AII mice. Among them, we found genes related to metabolic and inflammatory pathways, for example: the coagulation cascade (alpha-2-macroglobulin) and the melanogenesis pathway (elongation enzymes). These preliminary data, requiring validation, indicate that hA-IM could be involved in several pro and anti-atherogenic pathways.

MYOCARDIAL OVEREXPRESSION OF ANKRD1 RESULTS IN SINUS VENOSUS DEFECTS AND LEADS TO ADULT DIASTOLIC DYSFUNCTION

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Increased Ankrd1 levels linked to gain of function mutations have been correlated to selective congenital heart disease onset and adult cardiomyopathy occurrence in humans. Recently, we have identified Ankrd1 as a candidate gene for TAPVR in isolated patients. Our reported TAPVR patients presented either increased Ankrd1 transcript levels or a missense T116M mutation, resulting in greater protein stability. The link between increased ankrd1 level and cardiac structural and functional disease onset is not understood. To get insight into this problem, two “gain of function” mouse models by overexpressing both ankrd1 WT and ankrd1 T116M, under the control of the c-mHIC promoter, have been previously generated. Embryos from control and transgenic mice have been harvested at 10.5 and 14.5 days post coitum and processed to perform morphological and expression analyses. Both transgenic mice were characterized by impaired cardiac remodeling, which strongly affects the developing sinoatrial region and leads to sinus venous defects. Transgenic mice survived to adulthood but developed left atrial enlargement accompanied by severe diastolic dysfunction. Isolated myofibils from embryonic to adult hearts presented progressive and differential alterations in contractile parameters, indicating a functional shift towards stiffer and hyper-contractile phenotype. At the molecular level, these changes are accompanied by dynamic alterations in titin isoforms ratio. Embryonic and neonatal transgenic cardiomyocytes present irregular shape and sarcomeric disorganization, which progresses into sarcomeric loss and mitocondrial damage in adult ventricular but not atrial cardiomyocytes. Our data indicate that genetic mutations leading to increased ankrd1 levels can lead both to congenital heart disease and adult cardiomyopathy via a common cellular mechanisms, i.e. the progressive alteration of cardiomyocyte contractile and functional properties. Ankrd1 is a critical sensor-signaling molecule, which finely modulates cardiomyocyte stiffness/function/properties during development and postnatal life.

A CASE OF HYPOBETALIPOPROTEINEMIA DUE TO A NOVEL APOB GENE PATHOGENIC VARIANT AFFECTING SPLICING

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Background. Familial hypobetalipoproteinemia (FHBL) is a dominant monogenic disorder characterized by a significant reduction of plasma LDL-C. The main candidate genes involved in FHBL are APOB, ANGPTL3, and PCSK9. The APOB-linked FHBL may be associated with liver steatosis and chronic diarrhea, depending on the specific genotype involved.

Aim of the Study. Genetic characterization by NGS of a patient thought to have FHBL.

Results. A 17-year-old obese boy was referred to our Lipid Clinic for a long-standing history of very low plasma cholesterol levels associated with episodes of abdominal pain and diarrhea. At the age of 11y, during a hospitalization, several clinical and instrumental examinations were performed: abdominal echography documented hepatomegaly with diffuse steatosis without focal lesions, while hematological parameters were normal with the exception of plasma lipids (total cholesterol 72, LDL-C 15.5, HDL-C 30, triglycerides 35 mg/dL). At the age of 17y after admission to our Lipid Clinic blood samples were collected for DNA analysis and the evaluation of other biochemical parameters. The only reported symptoms were intermittent episodes of diarrhea especially following the consumption of fatty meals. Genetic analysis showed that the proband was heterozygous for a splice site variant in intron 3 of APOB gene (c.237 +1G>A) predicted to be pathogenic in silico. An APOB minigene construct harboring the mutation, generated in vitro two abnormal transcripts producing an identical truncated apoB protein (p.Val800Leufs*10). In addition to the APOB pathogenic variant, the NGS analysis revealed variants in other genes which might contribute to hypobetalipoproteinemia.

Conclusion. This report highlights the importance of genetic diagnosis to explain a rare phenotype of hypobetalipoproteinemia and further confirms that a broader gene analysis performed through NGS might help in defining more accurately the link between each single genotype and a specific clinic phenotype.
GENETIC BACKGROUND OF HDL DEFICIENCY DIFFERENTLY AFFECTS MONOCYTE PHENOTYPE AND FUNCTION


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Aim. In epidemiological studies HDL-cholesterol showed a robust inverse relationship with coronary heart disease. Besides their role in promoting cholesterol efflux from macrophages, HDLs can contribute to atheroprotection limiting the inflammatory responses during atherogenesis. Since monocytes can contribute in creating a proinflammatory milieu, in this study we aim to evaluate the effect of genetic HDL deficiency on pure monocyte phenotype and functionality.

Methods and Results. Monocytes were isolated from fresh blood in carriers of genetic hypoalphalipoproteinemia due to mutations in LCAT (n=12), ABCA1 (n=10) and APOA1 (n=9) and from controls (n=9) matched for age, gender and BMI. Exclusion criteria were known cardiovascular disease, renal disease and diabetes. While no difference in the monocyte subset distribution was found between carriers and controls, expression of the integrin CD11c, as assessed by flow cytometry, was nearly half in mutant LCAT (ΔMFI 220±56 vs 415±38 p=0.032) and consistent in all monocyte subset. According to the decrease in CD11c, only monocyte in LCAT deficiency displayed a reduction in transendothelial migratory capacity ex vivo compared to control monocyte (39.7±1.92 vs 48.0±1.83 percentage of migrated monocytes p=0.006). Monocytes were 24-hour challenged with LPS and Pam3Cys as toll-like receptor ligands and showed comparable cytokine production in ABCA1 and APOA1 genotype. On the contrary, monocyte isolated from LCAT deficient subjects proved to be less efficient in producing IL-8 and IL1-β upon stimulation compared to matched controls. A sub-analysis in LCAT mutations carriers was performed, revealing a gene-dose dependent effect, for all the parameters considered. No difference in circulating levels of hsCRP, MCP-1, TNF-α and IL-6 was appreciated.

Conclusions. Genetic HDL deficiency due to ABCA1 or APOA1 mutations does not affect monocyte phenotype and functionality. Conversely, LCAT deficiency seems to reduce the pro-inflammatory potential, contributing to increase the atheroprotective role of HDL often seen in this disease.

MICE WITH HUMANIZED LIVER FOR STUDYING EPIGENETIC MARKS INVOLVED IN REGULATION IN CARDIOMETABOLIC DISEASES

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Introduction. The HUMAN project aims to identify the genetic basis of cardiometabolic diseases using mouse models with humanized liver bearing allelic variants associated to disease state. To overcome the differences between human and mouse metabolism, the HUMAN consortium developed mouse models that closely resembles human metabolism. This study aims to characterize the epigenome landscape of histone marks in humanized mouse livers and correlate it to the metabolic phenotypes. The focus is on the different distribution of the histone 3 lysine 27 acetylation (H3K27ac) mark of active enhancers and histone 3 lysine 4 trimethylation (H3K4me3) mark of active promoters in livers from donors with allelic variants for TCFT7L2 and, moreover, on the comparison with murine hepatocytes.

Methods. Chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) of H3K27ac and H3K4me3 histone marks.

Results. The histone mark H3K27ac showed higher enrichment on regulatory region of PPARα ( Peroxisome proliferator-activated receptor alpha), even though not statistically significant (p=0,4285), in mice with protective allele (30%) vs. mice with risk allele (22%). Histone mark H3K27ac was more enriched in human PPARα of mice humanized with TCF7L2 protective alleles (30%) compared to the homologous PPARα region in murine hepatocytes (18%, p=0,0406). These data correlate with the increased expression of FGF21, target of PPARα, in humanized livers compared to mouse livers. The enhanced expression of FGF21 may explain the higher oxidative metabolism observed in mice with humanized liver.

Conclusion. ChIP-seq protocol allowed to investigate changes in epigenetic markers in mice with humanized livers bearing a protective allelic variant of TCF7L2 compared to murine hepatocytes. Our results indicate that mice with humanized livers have an enhanced oxidative metabolism. These data suggest a possible role of the PPARα-FGF21 axis in the higher oxidative capacity observed in mice with humanized livers. [Supported by grant EU FP7-602757 HUMAN].
INTERLEUKIN-11 EXERTS ANTIATHEROGENIC EFFECTS IN VITRO AND IN VIVO

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Background and Aims. Interleukin-11 (IL-11) is a stromal cell derived cytokine, highly expressed by smooth muscle cells (SMCs), heart, skin and testis. It belongs to the IL-6-type cytokine family, which shares the gp130 subunit in signaling cascade activation. Specifically, IL-11 signals via IL-11RA, leading to gp130 homodimerization and STAT3 activation. Beyond its known role in stimulating the maturation of megakaryocytes, it also exerts anti-inflammatory effects in T-cells and macrophages. Our preliminary observations indicated that both high-density lipoproteins (HDL) and sphingosine 1-phosphate (S1P) dose dependently induce IL-11 production in SMCs, via S1P2 stimulation. Since HDL-S1P showed antiatherogenic potential, we investigated the effects of exogenous administration of IL-11 to athero-prone mouse models and in different in vitro settings.

Materials and Methods. LDLR-/- mice on cholesterol-rich diet received human recombinant IL-11 (oprelvekin) at 50 μg/kg or vehicle i.p. thrice a week for 12 weeks. At sacrifice hearts and aortae were dissected for morphometric and immunostaining analysis of lesions. For in vitro analysis, cultured macrophages were exposed to IL-11, lipid charge or other stimuli and analyzed accordingly.

Results and Conclusions. IL-11 treatment significantly reduced apoptosis and inflammation in aortic lesions of LDLR-/- mice. For in vitro analysis, cultured macrophages were exposed to IL-11, lipid charge or other stimuli and analyzed accordingly. IL-11 showed a significant increase in efferocytosis rate compared to controls. Macrophages exposed to IL-11 showed increased IL-11 production in SMCs, via S1P2 stimulation. Since HDL-S1P showed antiatherogenic potential, we investigated the effects of exogenous administration of IL-11 to athero-prone mouse models and in different in vitro settings.

NOVEL VARIANTS OF SAR1B GENE IN CHILDREN WITH CHYLOMICRON RETENTION DISEASE

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Background. Chylomicron Retention Disease (CMRD) is an exceedingly rare recessive disorder characterized by severe intestinal lipid malabsorption, due to impaired chylomicron secretion, associated with reduced plasma cholesterol and low density lipoprotein cholesterol (hypobetalipoproteinemia). CMRD is caused by mutations in SAR1B gene encoding Sar1b protein, a guanosine triphosphatase, involved in chylomicron transport in enterocytes. Here we describe three variants of SAR1B gene found in three unrelated children with recessive hypobetalipoproteinemia born from consanguineous parents.

Methods. Full clinical investigation including intestinal biopsy and sequencing of recessive hypobetalipoproteinemia candidate genes (MTTP and SAR1B).

Results. Case 1 was a 4 months old male with steatorrhea, fat soluble vitamins deficiency, failure to thrive and severe hypobetalipoproteinemia. Intestinal biopsy revealed lipid accumulation in enterocytes. He resulted to be homozygous for a novel SAR1B nonsense variant (c.49 C>T, p.Gln17*) predicted to encode a short truncated Sar1b protein. Case 2 was a 4 years old male with intestinal fat malabsorption, lipid accumulation in enterocytes and severe hypobetalipoproteinemia. He resulted to be homozygous for a novel SAR1B missense variant (c.409 G>C, p.Asp137His) affecting the guanine recognition site of Sar1b protein.

IMPACT OF DIFFERENT OBESITY AND METABOLIC PHENOTYPES ON CARDIOVASCULAR OUTCOME IN PATIENTS AFTER CORONARY REVASULARIZATION

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Methods. A sample of 674 consecutive acute coronary syndrome (ACS) patients were recruited from January 2008 to January 2010 during their hospitalization in our Department of Cardiology. Clinical blood pressure (BP) readings were obtained in the supine position, after 5 minutes of quiet rest, with an aneroid sphygmomanometer. He (racing weight) was measured to the nearest centimeter, and weight was measured to the nearest one-hal kilogram. BMI was calculated as body weight (kilograms) divided by the squared height (meters) and further sub-divided into 2 categories: <25 kg/m² (normal weight) and >30 kg/m² (obese). Examinations were performed by a single investigator who was unaware of the patients’ BP and other clinical data. Metabolic status, waist and hip circumferences were measured to the nearest centimeter with the patient standing and arms hanging relaxed. Then we categorized all participants in four group according to absence or presence of MetS and obesity as following: metabolically healthy and normal weight (MHNW), metabolically unhealthy but normal weight (MUNW), metabolically healthy but obese (MHO) and metabolically unhealthy and obese (MUO).

Study outcomes. The patients were followed from coronary revascularization until they experienced one of the primary endpoints. Clinical outcome was evaluated by monitoring major adverse cardiovascular events (death, fatal or non-fatal re-infarction with or without PCI and stroke), seen as cumulative cardiovascular (cCV) events for all statistical analyses. Procedure-related AMI within 24 hours was not included in the end-point. Statistical Analysis. Data are presented as means, standard deviations, and frequency of occurrence (%). To summarize, all unhealthy phenotypes had higher CV risk of recurrences compared with the reference group (MHNW); this finding did not change after further adjustments for age, sex, smoking and cholesterol levels. Our findings showed that metabolically unhealthy phenotypes were associated with increased CV risk despite ongoing therapy. This data emphasizes the importance of metabolic health also in patients with coronary revascularization.
Case 3 was a 6 years old malnourished and dysmorphic male with steatorrhea, fat-soluble vitamin deficiency, lipid accumulation in enterocytes and liver steatosis. He resulted to be homoygous for a ~6 kb deletion in SAR1B gene eliminating exon 2. exon 2 deletion eliminates the first ATG translation initiation codon, a condition predicted to generate a Sar1b protein devoid of the normal aminoterminal domain.

**Conclusions.** Intestinal fat malabsorption in a child with recessive hypobetalipoproteinemia should raise the suspicion of a monogenic disorder of chylomicron formation/secretion such as ABL or CMRD. The analysis of the corresponding candidate genes allows a rapid diagnosis and avoids delays in the diagnostic process and in the implementation of appropriate treatment.

**L-ARGININE PREVENTS OSTEOGENIC DIFFERENTIATION OF AORTIC INTERSTITIAL VALVE CELLS**

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**Introduction.** Oxidative stress and reduced bioavailability of nitric oxide (NO) are considered pathological mechanisms potentially involved in the calcific degeneration of aortic valve.

**Aim.** To study the effects of L-Arginine (L-Arg), the main precursor of NO, on the osteogenic differentiation of aortic interstitial valve cells (VIC).

**Methods.** We isolated a clonal population of bovine VIC that expresses markers of osteogenic differentiation (such as alkaline phosphatase, ALP) and induces the calcification of collagen matrix after stimulation with endotoxin (LPS 500 ng/ml). VICs were cultured in vitro with different combinations of LPS±L-Arg (50 or 100 nM) and cell extracts were collected to perform proteomic analysis (iTRAQ) and gene expression experiments (RT-PCR).

**Results.** L-Arg treatment prevents the over-expression of ALP in the VIC treated with LPS (p <0.001) and reduces the matrix calcification (p<0.05). The proteomic analysis allowed to identify 49 proteins with an altered expression profile after stimulation with LPS and significantly modified by the treatment with L-Arg. These include proteins involved in the redox homeostasis of the cells (such as Xanthine Dehydrogenase, Catalase, Aldehyde Oxidase), remodeling of the extracellular matrix (ADAMTS14, Basigin, COL3A1) and cellular signaling (Fibrillin-1, Legumain, S100A13).

**Conclusions.** L-Arg treatment prevents osteogenic differentiation of VIC and reduces matrix calcification. This effect is achieved through the modulation of proteins involved in the cellular redox system, the remodeling of the extracellular matrix and the inflammatory activation of the VIC.

**PYROPHOSPHATE IS A POTENT INHIBITOR OF VASCULAR CALCIFICATION AND CAN BE USED TO DEVELOP BIOLOGICAL MATRIX RESISTANT TO ECTOPIC MINERALIZATION**

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**Background.** Previous studies showed that the pro-calcific differentiation of interstitial aortic valve cells (VIC) is accompanied by reduced production of pyrophosphate (PPi), a potent inhibitor of calcification. Aim: To define the anti-calcification properties of various polyphosphate compounds and to develop calcification-resistant vascular/valve matrices.

**Methods.** An in vitro model of calcification induced by high serum concentrations (FSB 50%) was developed. Type I collagen matrices were treated for 12 days with FSB 50% added with different concentrations of PPi, nucleotides (ATP, ADP, AMP), alendronate, mevalonic acid-Pi and farnesyl-PPi. Calcification of the matrix was quantified by colorimetric assay and histological staining (Von Kossa). The same approach was used to evaluate the anti-calcification properties of the different compounds on decellularized porcine aortic valves, decellularized bovine pericardium and commercial aortic valve bioprosthesis. A tissue engineering technique was also developed to covalently bind alendronate and mevalonol-5-Pi acid to the collagen matrix.

**Results.** PPi is able to inhibit, in a dose-dependent manner, the serum-induced calcification of type I collagen matrices (p<0.001). The same inhibition capacity was observed for ATP, ADP, 5-Pi mevalonic acid, farnesyl-PPi and alendronate (p<0.001 for all compounds). No effect was observed for the AMP (PPi-free compound). The same inhibitory effects were obtained by using the previous compounds on decellularized porcine valvar leaflets, decellularized bovine pericardium and commercial bioprosthesis. Subsequently, Alendronate and acid-mevalonol-5-Pi were covalently anchored to a collagen type I matrix. Both matrix treatments were effective in inhibiting calcium deposition (p<0.05).

**Conclusions.** The PPi isolated or contained within different polyphosphate compounds is able to inhibit the mineralization of the extracellular matrix. This property can be exploited for the development of biological matrices, both valvular and vascular able to withstand the calcification processes.

**COMORBIDITIES PREDICT DISABILITY IN OBESE PATIENT. PRELIMINARY DATA BASED ON THE ICF-OB QUESTIONNAIRE**

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Obesity is a chronic condition resulting in decreased physical function and disability. Recently a questionnaire (Q) based on the International Classification of Function, Disability and Health was developed to address disability in obese-patients (ICF-OB).
Aim. The aim of study was:
1) to assess disability in obese-patients by means of ICF-OB-Q;
2) to compare disability-score with other phenotypic scores such as the Edmonton-Obesity-Staging System (EOSS) and the comorbidities-index;
3) to identify predictors of disability.

Methods. Observational study on 48 adult patients, M/F 12/36, age 47.5 years (range 18-69) BMI 38.9±5.8 kg/m². In all patients demographic, anamnestic, anthropometric, and biochemical data were collected. The EOSS stage and the comorbidities index has been defined. The ICF-OB-Q, consisting of 4 parts: Medical-Area (QAM), psychological-area (QAP), physical-function-area (QAF), dietitians-area (QAD), was administered by a team of specialists from four disciplines (medicine, psychology, physical-therapy, diet and nutrition). According to disability-scores, we defined 4 different conditions: Negligible-disability (score <0.5), Mild-disability (score between ≥0.5 and <1.0), Moderate-disability (score between ≥1.0 and <1.5), and Severe-disability (score ≥1.5).

Results. Most patients had a mild/moderate-disability. According to EOSS-system, 6.3% of subjects were within stage-0; 12.5% in Stage-1, 39.6% in Stage-2, 39.6% in Stage-3 and 1 patient only in stage-4. 75% of patients had multiple comorbidities. The disability-scores recorded by QAM and QAF correlated with EOSS-staging-scores and the comorbidities-index (p<0.05). Schooling was significantly correlated with QAM-score (p=0.004) while employment correlated with QAF-score (p=0.019), EOSS-stage (p=0.007) and comorbidities-index (p=0.018). Moreover, in a multivariate-analysis, comorbidities-index was an independent predictor of disability in medical-area (β stand. 0.37 p<0.01; IC 0.27-1.88) and the disability of physical-function-area (β stand. 0.56 p<0.05; IC 0.55-3.9).

Conclusions. The ICF-OB-Q is a validated tool for detecting disability as a first step for a multidisciplinary approach to taking care of obese-patients. Our preliminary data can be reinforced in the much broader case studies.

THE CALABRIAN NETWORK ATEROSTOP 2 FOR DIAGNOSIS AND CURE OF HYPERLIPIDEMIA IN ADULT AND CHILDREN

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Background and Aims. To date, a reliable estimate of the number of people with Familial Hypercholesterolemia (FH) is available only for 23 countries. Considering an estimated theoretical prevalence of heterozygous FH in the population of 1/500, less than 1% of FH cases are diagnosed in many countries including Italy, demonstrating the low priority attributed to FH worldwide (1). About 4000 subjects may be affected by this fearsome pathology in Calabria (2), but of these currently less than 200 have been identified. Thus, there is the urgent need to implement strategies to identify FH in Calabria.

Materials and Methods. In 1998, a network for the diagnosis and treatment of hyperlipidemia was conceived and approved by Calabria Region. It involved Internists working in the local hospitals which were coordinated by University. All participants in this Network were well trained in lipidology. In 2017 a pediatric network was conceived for the diagnosis also in children. After training courses, now the General Practitioners and outpatients pediatricians collaborate to send those with hypercholesterolemia to the Lipid Center of the Network. Recently, the Network performed a screening to find new FH cases.

Results. In the last 2 years a total of 1300 subjects with hypercholesterolemia were screened from different cities. 132 blood samples were collected and sent for genetic testing (by Lipigen Project). To date, 18 were FH positive with the following genetic variants: 8 patients were heterozygosis for the LDLR gene (c.418G>A:p.G14L and c.1775G>A:p.G592G); 2 patients heterozygosis for the APOB gene (c.2938G>A:p.A98Tand c.5856T>C:p.H1952), 1 patient was homozygosis for a variant of the LDLR gene (c.1109 A>C:p.N370T), 2 double heterozygosis for the variants LDLR/APOB (c.119A>C:p.A37T and c.268-4T>A) and finally 2 patients with triple heterozygosis of the LDLR gene (c.2657C>T:p.C889A), APOB gene (c.12541C>T:p.P425L) and LDLRAF1 gene (c.672C>T:p.S224).

Conclusion. The hospital-based network is an operational reference for all practitioners in the region and helps to integrate efforts aimed at bridging the gap between scientific evidence and clinical practice. This organisational model takes advantage of hospital units, which devote a fraction of their time to the prevention of cardiovascular disease (3).

Efficacy and Safety of PCSK9 Inhibitors in Clinical Practice: A Monocentric Experience

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The recent approval of novel anti-PCSK9 therapy opened a new scenario for the treatment of hypercholesterolemic patients.

Aim. To evaluate the efficacy and safety of PCSK9-inhibitors (PCSK9) in clinical practice. Methods: observational study on 59 patients (31M/28F) that from December 2015 to August 2018 started PCSK9 therapy on the top of previous lipid-lowering-therapy (16 with alirocumab 75 mg, 14 with alirocumab 150 mg and 29 with evolocumab 140 mg). Lipid profile and glucose were evaluated at 3, 6 and 12 months.

Results. 22 patients had mutations of LDLR gene. 50% were statin-intolerant, 68% were in secondary prevention, 70% had peripheral vasculopathy, 15% were affected by diabetes and 14% by pre-diabetes. At baseline (N=59), LDL-c (median, interquartile range) was 140 [123-180] mg/dl at 3 months (N=50) 55 [29.2-81.2] mg/dl at 6 months (N=37) 56 [31-71.5] mg/dl at 12 months (N=23) 49 [37-90] mg/dl. At 3 months 76% of the patients were on target for LDL-c, 70% at 6 months and 74% at 12 months. At 6 months the median reduction of LDL-c from basal was 86 mg and at 12 months 90 mg, independently from the PCSK9 used, from age and sex, from previous histo-
ry of CHD, from gene mutations and from diabetes. Statin-intolerant patients had a significative reduction in LDL-c values, but remained at higher LDL-c values if compared with normotolerant patients. Therapy with PCSK9i was well tolerated. One patient reported a reduction of cognitive function. One died for heart attack. One patient was a non-responder. We observed an increasing trend in glucose levels during the follow-up, mainly between 6 and 12 months.

**Conclusion.** Our experience confirmed efficacy and safety of PCSK9i. Further studies are needed to explore their long-term effect on glucose levels and to explore why rare patients are non-responders.

**AN ALTERED LIPID OXIDATION PREDICTS METABOLIC SYNDROME AND TYPE 2 DIABETES**

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**Background.** Metabolic Syndrome (MetS) and Type 2 diabetes (T2D) are clinical conditions involving the impaired uptake and utilization of glucose, altered lipid metabolism and the disruption of the metabolic signaling pathways that regulate insulin secretion from pancreas. Diabetes has been linked to an impaired ability to oxidize fatty acids. Fat oxidation can be assessed clinically by a respiratory quotient measurement during fasting. We hypothesised that a respiratory quotient might predict metabolic syndrome and T2D onset.

**Methods.** In this longitudinal study we used an existing database of 233 individuals who had complete nutritional and biochemical data at baseline and after 12-month follow-up. All participants underwent an indirect calorimetry to measure the respiratory quotient. We excluded participants with diabetes, chronic diseases and those who had changed food habits in the previous three months. Only 88 subjects met the inclusion criteria.

**Results.** Two individuals developed type 2 diabetes and 10 metabolic syndrome after one year. Mean basal respiratory quotient was 0.91±0.09 in those who developed metabolic syndrome/diabetes. Participants in the high respiratory quotient group (>0.91) had a higher incidence of metabolic syndrome/diabetes than those in the low quotient group (25% vs 8% p=0.04). In the high respiratory quotient group, Kaplan-Meier curves showed a greater probability of having metabolic syndrome/diabetes than those in the low respiratory quotient group (log Rank χ2 test=8.44; p=0.004). A multivariable Cox proportional hazards model demonstrated that energy expenditure and weight increase did not predict metabolic syndrome/diabetes [HR (95% CI) = 1 (0.996-1.005), p=0.86 and 3.9 (0.407-38.061), p=0.23 respectively].

**Conclusions.** A greater probability of metabolic syndrome/diabetes was found in individuals with a basal respiratory quotient of >0.91 than in those with a respiratory quotient of ≤0.91 after 1 year. In the short term anthropometric measurements and their variation overtime were not correlated with metabolic syndrome/diabetes.
ASSESSMENT OF ARTERIAL STIFFNESS IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA BEFORE AND AFTER TREATMENT WITH IPCSK9

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Background. Familial hypercholesterolemia (FH) is a genetic disease characterized by elevated levels of serum LDL cholesterol (LDL-C) and high cardiovascular risk (CVR). The prognosis of FH patient correlates with lifelong LDL-C levels, and therapy is aimed to achieve and maintain LDL-C target over years, for high (primary prevention, LDL-C <100 mg/dl) and highest (secondary prevention, LDL-C <70 mg/dl) CVR patients. Endothelial and vascular dysfunction occur years before clinical manifestations of atherosclerosis (in the early stages of atherogenesis), even before the instrumental recognition of atherosclerotic lesions. PWV is considered a reliable and early marker of arterial stiffness and an independent prognostic predictor for cardiovascular mortality.

Aim. To evaluate the effectiveness of six months of treatment with anti-PCSK9 monoclonal antibody on lipid profile and vascular arterial function, in a setting of patients with heterozygous familial hypercholesterolemia (HeFH) in primary prevention, who did not achieve the expected LDL-C levels despite the best conventional therapy (at least 6 months) with high efficacy statin at the maximum tolerated dose in association with ezetimibe.

Materials and Methods. We enrolled 19 patients (12 male and 7 female, aged between 35 and 69 years) with HeFH in primary prevention, for which it was considered useful to start treatment with PCSK9 inhibitor drugs; 12 patients received treatment with Evolocumab 140 mg every 2 weeks; 7 patients were treated with Alirocumab at a dose of 75 (1) or 150 (6) mg every 2 weeks. At the time of enrollment (T0) and 6 months after the treatment with monoclonal antibodies (T1) we performed blood chemistry, arterial stiffness and anthropometric parameters. Complete lipid profile, fibrinogen, ApoB, Lp(a), COT, GPT, CPK, fasting glucose, insulin and many other parameters were determined by routine methods. High sensitivity CRP was determined using the ELISA method. Both the Lipid Centre involved in the study performed PWV measurement by SphygmoCor. Carotid artery echo-Doppler scan was performed in accordance with the ESC/ESH guidelines.

Results. After 6 months of treatment (T1) with IPCSK9 compared to baseline values (T0) we found a significant reduction in levels of inflammation indices (CRP), lipid profile (LDL-C, TC, triglycerides, HDL-C, Lp(a), ApoB). Furthermore, PWV showed a small but significant reduction a T1 (9.33±2.2 vs 10.19±2.0, Δ=8.9%, p<0.001). Due to the very small sample size, to verify whether arterial properties improvement, as measured by PWV, associate with Δ changes of other study variables is not statistically plausible.

Conclusions. After 6 months of treatment with monoclonal anti-PCSK9 antibodies, the levels of CRP, LDL-C, Lp(a), TC, TG, were significantly reduced in patients with heterozygous familial hypercholesterolemia; moreover, the artery properties significantly improved after therapy. This did not allow to speculate on a pathophysiological model in which LDL-C or CRP lowering allow PWV improvement in a direct and/or indirect way; therefore, larger observation should address this focus, also given the biological plausibility of the association.

PERSONALIZED REGIMEN FOR PCSK9 INHIBITORS: A THERAPEUTIC OPTION WHICH MAINTAINS EFFICACY AND REDUCES COSTS

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Background. PCSK9 inhibitor (PCSK9i) represent a breakthrough in the treatment of hypercholesterolemia, however PCSK9 therapy have significant limitation in term of sustainability of economic public healthcare. The aim of the study was to verify the hypothesis that a longer interval between PCSK9i administration maintains LDL cholesterol-lowering efficacy.

Methods. We enrolled 14 FH patients (mean age 60±9 years, 11 male) with known cardiovascular disease and LDL-C level below 70 mg/dl after at least 3 months of PCSK9 therapy. Therapy was administered every 2 weeks (11/14 evolocumab 140 mg, 3/14 alirocumab 150 mg) on tot maximally tolerated lipid lowering therapy (statins 5/14, ezetimibe 5/14, statins+ezetimibe 2/14, statins+fibrate 2/14). After 3 months, the interval between PCSK9i administration was extended to 3 weeks for at least 3 months.

Results. The PCSK9i, administered with a 3-week interval, maintained the desired LDL-C target (40±18 at 2-week vs. 59±25 at 3-week intervals). Only 2/14 patients had to discontinue the 3-week interval regimen because LDL-C was above 70 mg/dl.

Conclusions. There are two ways to improve the cost-effectiveness of PCSK9 inhibitors: lower drug-cost and limit the use to high-risk patients. Inevitably, these two options are incompatible with the low negotiation margin of pharmaceutical companies and with the ethical issues related to the patient’s right to receive the best available care. In our study, we sought a third alternative: longer dosing intervals. Certainly, we cannot demonstrate that prevention of cardiovascular events is maintained with the longer dosing interval by which the LDL cholesterol-lowering efficacy is preserved. These data deserved be confirmed in a larger population.
SPHINGOSINE-1-PHOSPHATE (S1P) RECEPTOR TYPE 1 EXERTS Atheroprotective Effects in Murine Macrophages

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ABSTRACT

Introduction. Atherosclerosis is a chronic inflammatory disease leading to the formation of vascular lesions caused by both an excessive accumulation of cholesterol and cell death in arterial walls. High Density Lipoproteins (HDLs) are thought to exert multipotent protective effects against atherosclerosis. Indeed, HDLs mediate cholesterol transport from the atherosclerotic plaque through the interaction with cholesterol transporters expressed in macrophages. The protective effects of HDLs have been partially attributed to specific molecules, to which they serve as carriers, such as sphingosine-1-phosphate (S1P) (1, 2). The aim of this study was to investigate molecular mechanisms underlying the protective atheroprotective effects of S1P exerted by interaction with macrophages. Specifically, we were interested in the role of sphingosine-1-phosphate (S1P)/S1P receptor 1 (S1PRI) axis in modulating macrophage functions relevant to the pathogenesis of atherosclerosis.

Methods. We used a transgenic mouse model overexpressing the S1PRI in macrophages. The functional macrophage phenotype has been evaluated by examining surface markers of macrophage polarization and activation and differential gene expression by means of flow cytometry and qPCR technique, respectively. To investigate the receptor-mediated lipoprotein uptake, we assessed the cholesterol accumulation in peritoneal macrophages by determining the uptake of fluorescently labelled acetylated and oxidized low density lipoproteins (LDL). The cholesterol transport from macrophages was evaluated with a radiotracer technique using apolipoprotein A1 (apoA1) and HDLs as extracellular acceptors. The expression of proteins regulating cholesterol handling, inflammatory response and the expression of cell surface receptors involved in the foam cells formation were determined by qPCR. The propensity of S1P to prevent innate immunity signalling in macrophages and relative effect on M2 polarization, was examined in vitro by evaluating the liberation of anti-inflammatory cytokines/chemokines such as IL-1RA, IL-10, IL-4 and CCL20 from peritoneal macrophages.

Results. Compared to WT cells, peritoneal macrophages overexpressing S1PRI showed an increased expression of cholesterol transporters ABCA1 and ABCG1. Accordingly, an improved cholesterol transport to acceptors apoA1 and HDLs was observed in S1PRI-overexpressing macrophages. The favourable effects exerted by S1PRI on cholesterol uptake and transport were likely related to the increased expression and activity of a transcription factor liver X receptor (LXR). Moreover, LXRs regulates the expression of several other important factors modulating macrophage biology in lesions undergoing regression, such as Arg-1, IRF8, Lgm1. In particular, Arg-1 expression is enhanced through binding of hematopoietic transcription factors IRF8 and PU.1 to its promoter (3). These results have been recapitulated in the present study. Our preliminary results suggest that S1PRI overexpression induces M2a alternative macrophages phenotype and inhibits inflammatory response.

Conclusions. Current results suggest that SIP exerts beneficial atheroprotective effects in macrophages by modulating cellular cholesterol metabolism and inflammatory response mainly through interaction with the macrophage S1PRI. Hence, S1PRI may be considered as a potential target for future therapies against atherosclerotic disease.

References
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Background and Aims. Inflammation is a key regulatory process that links hypercholesterolemia and immune mechanisms promoting atherosclerosis. Inflammatory biomarkers may be helpful to better define the atherosclerotic burden in patients with high cholesterol levels such as familial hypercholesterolemia (FH). Our aim was to evaluate the concentration of S100A12 protein in FH patients and its association with pulse wave velocity (PWV) and carotid intima-media thickness (IMT).

Methods and Results. We measured lipid profile, S100A12, PWV and IMT in 94 hypercholesterolemic participants. FH diagnosis was attributed to patients with a Dutch score ≥4 and confirmed with the presence of a genetic mutation. FH patients had a greater amount of low-density lipoprotein and apolipoprotein B and a lower amount of high-density lipoprotein and apolipoprotein A1 than non-FH subjects (4.37±1.78 vs 3.8±0.55 mmol/L, p<0.05; 1.15±0.35 vs 1.04±0.15 g/L, p<0.05; 1.44±0.34 mmol/L, p<0.05; 1.35±0.2 vs 1.49±0.28 g/L, p<0.01). PWV and IMT were higher in FH patients than non-FH subjects (8.63±2.02 vs 6.98±0.73 m/s, p<0.05; 0.77 [0.63-0.97] vs 0.66 [0.58-0.77] mm, p<0.01). S100A12 was independently correlated with genetic mutation (p<0.01), PWV and IMT (p for both <0.001).

Conclusion. FH patients exhibited higher S100A12 levels than non-FH subjects.
DISEASE ACTIVITY SCORE PREDICTS CARDIAC AND VASCULAR IMPAIRMENT IN VERY EARLY PSORIATIC AND RHEUMATOID ARTHRITIS PATIENTS

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ABSTRACT

Background. Patients with inflammatory joint disease (IJD), including rheumatoid (RA) and psoriatic arthritis (PsA), exhibit increased rates of cardiovascular disease (CVD) morbidity and mortality. Chronic inflammation and immune dysregulation are thought to play a substantial role. Aim: To evaluate if disease activity score (DAS) could predict CV risk in terms of cardiac and vascular impairment, before clinical signs of atherosclerosis are manifested. The prevalence of subclinical atherosclerosis has been already assessed in RA and PsA, but how precociously also myocardial dysfunction is impaired remains unknown. In order to address this question, we assessed myocardial and arterial impairment, circulating CD34+ cell count, vitamin D levels, additionally to DA scores in IJD patients of recent onset.

Methods. DA scores were estimated in patients with RA (n=41) and PsA (n=35) without traditional CV risk factors, and 58 matched healthy controls (HC). Besides CRP-DAS28, BASDAI, PASI, VAS, HAQ were also calculated. Furthermore, global longitudinal and circumferential strain (GLS and GCS) were estimated to assess the myocardial function, Pulse Wave Velocity (PWV) and carotid intima-media thickness (cIMT) were measured to assess artery vascular involvement; circulating CD34+ counts were evaluated by flow cytometry, and vitamin D levels by HPLC. CRP, ESR, Fibrinogen were also determined by routine methods as inflammatory markers.

Results. We verified whether DA scores, and particularly CRP-DAS28, are able to predict cardiac and vascular involvement; consistently, we performed multivariate regression models considering GLS, GCS, PWV, cIMT and CD34+ as response variable(s), respectively. DAS28, CRP, ESR, Fibrinogen, age, gender, disease duration, BMI, lipid profile, systolic and diastolic blood pressure values, and Vitamin D levels. We found that DAS28 predict all the cardiovascular indices taken into account, and also CD34+ cell counts. As further analysis, we repeated the statistics in RA and PsA patients, separately, and also in two different subgroups according to DAS28 score, with a cut-off level of 2.9 (low activity disease vs high activity diseased subjects). In RA we found a linear association between DAS28 and GLS, GCS, PWV, cIMT and CD34+ cells; these results were maintained also when patients were subdivided basing on disease activity grade. In PsA we found DAS28 as predictor of GLS, GCS, PWV, and CD34+ cells; however, this association was confirmed only for GLS in lower disease activity patients. In all patients, the predictive power of DAS28 was lacking in presence of an IMT ≥1.3.

Conclusions. DAS28 was found as a strong predictor of cardiac and vascular involvement in early stage IJD; if confirmed on larger scale, this observation could suggest the clinical evaluation by scores for patients routine CV risk stratification, at least when the early stage of IJD is confirmed by routine carotid US IMT examination (cIMT<1.3 mm), in presence of additional CV risk factors.

IDENTIFICATION OF P.LEU167DEL APOE GENE MUTATION BY NEXT GENERATION SEQUENCING IN A LARGE HYPERCHOLESTEROLEMIC FAMILY

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ABSTRACT

Introduction. Familial Hypercholesterolemia is a genetic disorder caused by mutations in LDLR, APOB or PCSK9 genes. Recently other genes and genetic loci (16q22.1 and 8q24.22) have been associated with hypercholesterolemia. APOE variants are well known determinants of lipid variability and a peculiar form of hyperlipidemia known as type III hyperlipoproteinemia.

Materials and Methods. We have studied a large family with definite clinical diagnosis of hypercholesterolemia. Sanger sequencing analysis did not reveal any pathogenic mutations in LDLR, APOB and PCSK9 genes. More, we performed MLPA analysis to evaluate CNVs in LDLR gene and no mutations were identified. During the last years the NGS technology has been applied to analyze a large number of genes related to lipid metabolism. We performed NGS analysis in the proband by using a custom panel of 17 high-cholesterol-related genes by Ion Torrent PGM. This approach allowed the resequencing of candidate genes in addition to other recently discovered genes associated to hypercholesterol-olemia.

Results and Conclusion. The data output of NGS analysis confirmed the absence of mutations in LDLR, APOB and PCSK9 genes and allowed to identify in the proband an APOE mutation in heterozygosity (p.Leu167del). The family cascade screening revealed the presence of the same mutation in the affected subjects while no carriers of mutation were found among the normolipidemic ones. As already known, p.Leu167del derange ApoE interaction with lipids and the affinity of apoE to its receptors and causes hypercholesterolemia. In conclusion we report the third kindred with FH due to an APOE gene mutation.

BIGLYCAN-INDUCED INFLAMMATORY RESPONSE IN HUMAN MONOCYTE: EX VIVO EXPERIMENTAL MODEL

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ABSTRACT

Background. Monocytes are critically involved in the pathogenesis of atherosclerosis, capable of secreting many factors such as chemokines, cytokines, growth factors, and reactive oxygen species, thus contributing to wall lesion development. Biglycan (BGN), a small leucine rich proteoglycan, plays a pivotal role in initiating lipid deposition in the arterial subintimal space, by its ability to bind and retain apoB-containing lipoprotein, including

ABSTRACT

ABSTRACT
A COMPLEX GENOTYPE IDENTIFIED IN A PROBAND AFFECTED BY SEVERE HYPERTRIGLYCERIDEMIA

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Primary forms of HTG include mild-to-moderate and severe forms; the rare severe hypertriglyceridemias are thought to be monogenic, autosomal recessive and caused by homozygous or compound heterozygous loss of function mutations of few known genes pathophysiologically involved in the intravascular lipolysis of the TG-rich lipoproteins namely lipoprotein lipase (LPL), apolipoprotein CII (APOCII), apolipoprotein AV (APOAV), glycophosphatidylinositol (GPI)-anchored high-density lipoprotein-binding protein 1 (GPIHB1), Lpase maturation factor 1 (LMF1) and GPIFD1. The proband, a 19 years old boy, showed a severe hypertriglyceridemia (up to 1400 mg/dl). No hepatosplenomegaly was observed and secondary forms of hypertriglyceridemia were excluded. Family clinical history was lacking because he was an adopted child, so we could not determine the mode of inheritance of the HTG trait. We decided to use a Next Generation Sequencing approach to study the coding exons and intron/exon boundaries of genes affecting the main pathways of triglyceride metabolism. The targeted resequencing of candidate genes led to the discovery of a complex genotype characterized by three different rare variants in three candidate genes: p.Asn318Ser in LPL gene, p.Ser107Ala in GPIHBP1 gene and p.Lys77Gln in APOC2 gene. All variants were confirmed by Sanger sequencing; LPL variant was found in homozygosity, while GPIHBP1 and APOC2 variants were found in heterozygosity. The variant p.Asn318Ser in LPL gene has been already identified in heterozygosity in different subjects and classified as “non deleterious”, “dis- ease causing” or “tolerated” based on SNP’s3D, Mutation Testing and SIFT. GPIHPB1 and APOC2 variants were classified as “probably damaging” and “possibly damaging” respectively by using bioinformatic tool Polyphen-2. More studies are needed to understand the impact of these variants in the development of the hypertriglyceridemia in this proband. We report a case of HTG likely caused by a high polygenic burden determined by complex heterozygosity for variants in LPL, APOC2 and GPIHBP1 genes.

EVALUATION OF Atherosclerotic CORONARY ARTERY DISEASE BURDEN BY CT SCAN IN PATIENTS WITH MOLECULARLY DEFINED HETEROZYGOUS FAMILIAL HYPERCOLESTEROLEMIA (HeFH) AS COMPARED TO THOSE WITH POLYGENIC FAMILIAL HYPERCOLESTEROLEMIA (PFH)

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Aim. Heterozygous Familial Hypercholesterolemia (HeFH) is the most common genetic disorder characterized by high levels of LDL cholesterol (LDL-C) and associated with an increased risk of coronary heart disease (CHD). In addition to the monogenic variant, it has been reported that the HeFH phenotype may also have polygenic molecular bases (PFH). To date few studies have compared HeFH and PFH in terms of atherosclerotic burden. The aim of this study is the evaluation of atherosclerotic coronary artery disease by CT scan in subject with monogenic HeFH as compared to individuals with PFH.

Methods. Ninety one subjects, aging 18-75 years, clinically diagnosed as FH based on a DLCN score >3, were screened for mutation in the major FH-causing genes, LDLR, APOB, PCSK9 and LDLRAP1 by next generation sequencing. In those who were mutations negative, a 6-SNP genetic risk score (6-GRS) was calculated to determine the presence of a polygenic background, as previously reported. Seventy three patients were found to carry monogenic HeFH and 18 were classified as PFH based on an elevated 6-GRS. All patients were asked to undergo coronary CTA imaging. Coronary atherosclerosis burden was evaluated based upon the presence and severity of coronary stenosis and coronary calcium (Agaston score).

Results. In comparison with PFH patients, those with HeFH were younger (51±8 yrs vs. 46±13 yrs, respectively, P=0.103), had higher levels of LDL-C (167±53 mg/dl vs. 185±85 mg/dl, respectively; P=0.709) and reported higher use of statins (38.9% vs. 76.4%, respectively; P<0.005). The prevalence of other cardiovascular risk factors was comparable between groups. We found that a coronary involvement was almost absent in patients under the age of 50 years. Among HeFH, 24 patients (32.9%) had non-significant coronary artery lesions (stenosis <50%) and 16 patients (21.9%) had coronary lesions of which at least one significant. Overall, 54.8% of HeFH and 55.6% of PFH showed any grade of coronary atherosclerosis and this difference was not statistically significant. Two patients
ROLE OF LRP1 LOCUS AS POSSIBLE GENETIC DETERMINANT OF BICUSPID AORTIC VALVE

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Background. Bicuspid aortic valve (BAV) represents the most common congenital heart defect, as it affects 0.5-2% of the general population. An increased risk of aortic valve stenosis, as well as aortopathy, has been documented in BAV patients. Moreover, presence and severity of calcification contributes to worsen the clinical outcome. Actually, disease progression may also involve processes sharing risk factors with the atherosclerotic process, among which lipid profile alteration. Genetic variants in LRP1 gene, encoding for the Low Density Lipoprotein Receptor-Related Protein 1, a receptor belonging to low density lipoprotein receptor family and involved in multiple cellular processes, have also been associated with the aneurysmal disease.

Aim. Of the present study was to investigate the role of LRP1 as possible genetic determinant of BAV.

Methods. Thirty-eight BAV patients admitted to the Referring Center for MPS and Related Disorders (Tuscan, Italy) have been analyzed by Next Generation Sequencing (NGS) approach (Illumina), based on a targeted sequencing panel of 97 genes suspected to be associated with BAV or BAV-associated conditions or known to be involved in aortic wall remodeling including LRP1.

Results. Data from NGS evidenced the presence of LRP1 rare genetic variants in 6 out of 38 BAV patients [c.527T>A (p.Val176Asp); c.1458G>A (p.Arg2613Gln); c.1458G>A (p.Arg2292Cys); c.2323G>C (p.Ala775Pro); c.7838G>A (p.Pro2613Gln)]. For one of these 6 patients, relatives were available for performing genetic analysis. In this family, p.Val176Asp (p.Arg2613Gln) was also classified as potentially pathogenetic by all in silico prediction tools used.

Conclusions. Data from the present study allow to support the role of LRP1 locus as potential candidate gene for BAV, thus contributing to evidence its possible involvement in modulating the pathophysiological processes underlying disease development.
ALIROCUMAB IN DYSLIPIDEMIC PATIENTS: THE SANTA CROCE E CARLE HOSPITAL OF CUNEO EXPERIENCE

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Introduction. Dyslipidemic patients are at increased cardiovascular risk and often do not achieve LDL goals with statins/ezetimibe. Moreover drug intolerance could challenge the clinician. Alirocumab is a monoclonal antibody targeted to PCSK-9 utilized to reduce LDL-Cholesterol levels that could be utilized also in patients with statin intolerance. Aim of the Study: was to retrospectively evaluate the effect of a 6 months treatment with alirocumab of 8 dyslipidemic patients according to Italian Medicines Agency (AIFA) prescription rules.

Subjects and Methods. 8 dyslipidemic patients were evaluated before and 6 months after alirocumab treatment (75 or 150 mg s.c. every two weeks). 4 patients fulfilled a diagnosis of Heterozygous Familial Hypercholesterolemia, the other 4 were diagnostic of Familial combined Dyslipidemia. Four patients were statin intolerant. Data after six month treatment are available of only 7 patients. Student’s T-test was performed to compare means of the variables studied before and after the treatment.

Results. (means±S.D.) age 54.3±14.4 years, Male/Female ~7/1, Body Mass Index 28.04±3.02 kg/m2, Systolic Blood Pressure 130.2±12.9 mmHg, Diastolic Blood Pressure 80.0±9.6 mmHg, total cholesterol 312.7±52.3 mg/dl, HDL-Cholesterol 47.9±12.1 mg/dl, Triglycerides 197.3±100.1 mg/dl, calculated LDL-Cholesterol 225.4±56.4 mg/dl, Non-HDL-Cholesterol 264.9±56.6 mg/dl. After treatment lipid values were: total cholesterol 187.6±39.5 mg/dl (p=0.0001 vs baseline), HDL-Cholesterol 51.7±13.1 mg/dl (p<0.05), Triglycerides 147.8±65.6 mg/dl (p=0.064), calculated LDL-cholesterol 108.7±52.5 mg/dl (p<0.001 vs baseline), Non-HDL-Cholesterol 135.9±40.6 mg/dl (p<0.001 vs baseline). In particular absolute LDL-Cholesterol reduction was 118.7±30.7 mg/dl corresponding to 53.6±10.6 percent reduction. No adverse events were reported among patients (in particular no injection site reactions were reported).

Conclusions. Alirocumab 75 mg or 150 mg every two weeks is a very effective and safe treatment to reduce significantly LDL-C and Non-HDL-Cholesterol levels in patients with familial dyslipidemias with or without statin intolerance. A trend toward an improvement also of HDL-cholesterol and Triglycerides was also observed although not significant.

EVALUATION OF SUBCLINICAL MYOCARDIAL DAMAGE IN PATIENTS WITH CHRONIC INTESTINAL INFLAMMATORY DISEASE BEFORE AND DURING TREATMENT WITH BIOLOGICAL DRUGS

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Background. The idiopathic inflammatory bowel diseases comprise two types of chronic intestinal disorders: Crohn’s disease and ulcerative colitis. The prevalence of IBD in Western countries is estimated to be up to 0.5% of the general population, with growing incidence. The current treatment of IBD includes mesalazine (oral and rectal formulations), glucocorticoids (CCS, conventional and other forms like budesonide or beclometasone), antibiotics (typically ciprofloxacin and metronidazole), immunosuppressants (mostly azathioprine/6-mercaptopurine or methotrexate) and anti-TNF agents (infliximab, adalimumab, certolizumab pegol and golimumab). Recently, the anti-integrin monoclonal antibody vedolizumab and the antibody against IL-12/23 ustekinumab have been approved for IBD.

Aim. To evaluate the subclinical cardiac and vascular damage in patients with refractory (as considered after six months of conventional therapy with CCS plus mesalazine) chronic intestinal inflammatory disease before and after treatment with biological drugs (infliximab, adalimumab, vedolizumab). Furthermore, circulating CD34+ cell count was also assessed.

Methods. Pulse Wave Velocity (PWV), global longitudinal strain (GLS) and circulating CD34+ cells were evaluated to estimate subclinical cardiovascular involvement in 16 patients with IBD, before (T0) and after (T1) a six-months treatment with biological drugs. Carotid-femoral PWV was measured by routine methods. GLS was measured by speckle tracking echocardiography. Circulating CD34+ were counted by flow cytometry. In addition, inflammatory indices (ESR, GRF, fibrinogen) and EF% were also evaluated.

Results. At T1, no statistically significant differences were detected as regards ESR, PWV, EF with respect to T0; in contrast, some parameters appeared statistically improved as compared to baseline, including: CRP (p<0.013), GLS (p<0.001) and CD34+ (p<0.001). The interdependence analysis performed on the mean percent changes showed a significant correlation between ΔPWV and ΔGLS: as ΔPWV decreases ΔGLS increases, improving ventricular performance.

Conclusions. Patients with IBD have a greater risk of developing CV disease, especially when IBD is biological uncontrolled; in our study we have shown that in refractory IBD biological drugs may have a favorable effect on inflammatory status and symptoms/biological compensation, but also as regards CV risk as suggested by favorable change in plasma levels of CRP, circulating levels of CD34+ and GLS values. This study needs to be enhanced and reproduced on larger patients cohort to confirm this preliminary data and to address the question of whether therapy with these drugs may have a role also in favorably modulating CV risk.
THE ACTIVITY OF LYSOsomAL ACID LIPESE IN DRIED BLOOD SPOT IN NON-ALCOHOLIC FATTY LIVER DISEASE AND IN CRYPTOgenic CIRRHOsis: 5 YEArs OF LABORATORY EXPERIENCE

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Introduction. Lysosomal acid lipase (LAL) plays a key role in lipid metabolism through the hydrolysis of cholesteryl esters and triglycerides in lysosome. In this study, lysosomal acid lipase activity was evaluated in dried blood spot (DBS) of adult patients with non-alcoholic fatty liver disease (NAFLD), cryptogenic cirrhosis (CC) or cirrhosis of different aetiology.

Methods. Dried blood spot (DBS) samples were prepared spotting 75 ul of blood. LAL activity is measured using the fluorimetric substrate 4-methylumbelliferone palmitate in the presence of cardiolipin and Lalstat 2, respectively activator and inhibitor of LAL.

Results. In the first study, LAL activity was measured in 240 NAFLD patients. Median LAL activity was significantly reduced in NAFLD [0.78 vs 1.15 p<0.001]. Our findings suggest a strong association between impaired LAL activity and NAFLD. The second study was about a multicentre cohort including 274 patients with liver cirrhosis of different aetiology from 19 centres of Internal Medicine, Gastroenterology and Hepatology distributed throughout Italy. In the whole cohort, median LAL activity value was 0.58 nmol/spot/h. 0.49 and 0.65 in the groups of CC and known-aetiology cirrhosis, respectively (p<0.002). We found a marked reduction of LAL activity in patients with cryogenic cirrhosis compared to the other known aetiologies. In another paper, we investigated the association between spleen dimensions and LAL activity in NAFLD patients. We included 425 consecutive patients who underwent abdominal ultrasound to evaluate hepatic steatosis and spleen dimensions. LAL activity was lower in 56 patients with splenomegaly, as compared to those without. At multivariable logistic regression analysis, age, LAL activity and platelets were significantly associated with splenomegaly. These results suggest that LAL may contribute to spleen enlargement in this setting. Further studies will clarify the role of LAL in chronic liver diseases.

POST-PRANDIAL LIPEMIA AND CD36

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Aim. CD36 (Cluster of Differentiation CD36) is a membrane glycoprotein found at the surface of many cells types which binds with high affinity oxidized lipoproteins and free fatty acids (FFA). While the role of CD36 in the arterial wall during atherosclerosis has been extensively studied, on the other hand its expression at relatively high levels also in circulating cells such as leukocytes (PBMCs) is intriguing. We therefore asked a) whether CD36 expression in these cells type associates with increased fatty acids flux during post-prandial lipemia and b) if this is related to altered inflammatory pattern in these cells, modulating cellular responses.

Methods. Subjects underwent a postprandial phase study and were asked to undergo an oral lipid load. The concentration of FFA in plasma and PBMCs and changes in the expression of gene and protein expression in PBMCs during post-prandial lipemia (PP) were evaluated. In the attempt to study whether modulating CD36 expression in vivo associates with different postprandial response, the lipid load was given to carriers of an intronic variant of CD36 gene, the rs1761667, associated with reduced transcript and production of the protein.
Results. After three hours from the OFL, we confirmed significant increases in plasma triglycerides (TG), which was paralleled to the increase in TG content in triglyceride-rich lipoproteins (TGRLs) and to an increased IL-6, IL-1β, MCP-1 and TNF-α genes expression, supporting a correlation between cellular inflammation and the PP phase. An increase of FFA in plasma and PBMCs was also observed, suggesting that the elevation of plasma lipemia is associated with an intracellular accumulation of FFA in circulating cells. Elevations in CD36 gene expression (+14±11 fold of induction) were observed in the same cells. Moreover cells isolated from the carriers of mutant allele during the PP phase, showed both a reduction in the transcript (0.44±0.29 fold of induction), in CD36 protein levels (21%), and reduced concentration of FFA, compared to subjects with the common allele.

Conclusions. The post-prandial response is associated with changes in CD36 in circulating cells which appears to be related with changes in the inflammatory response. The physiopathological relevance of this observation and the molecular mechanisms involved remain to be explored.

ALCOHOL PATTERN CONSUMPTION DIFFERENTLY AFFECTS THE EFFICIENCY OF MACROPHAGE REVERSE CHOLESTEROL TRANSPORT IN VIVO

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Moderate alcohol consumption is inversely correlated with cardiovascular morbidity and mortality, whereas binge consumption has the opposite effect. Cardiovascular disease can be prevented or retarded by the reverse cholesterol transport (RCT), in which excess cholesterol is removed from the macrophages of the artery wall. We aimed to evaluate whether moderate and binge consumption of alcohol differently impact RCT in vivo. RCT was measured with a standardized, radioisotope-based technique in apolipoprotein E knock out mice. Group I (n=10), received placebo, mimicking the abstainers; group II (n=10) received 0.8 g/kg alcohol/day for 28 days, mimicking a moderate intake; group III (n=10), received 0.8 g/kg alcohol/day for 5 days/week and 2.8 g/kg alcohol/day for 2 days/week, mimicking a moderate/binge intake. On day 26, mice were injected with 3H-cholesterol loaded macrophages and the radioactivity was quantified in plasma, liver and feces. Alcohol intake caused a dose-dependent increase in plasma total cholesterol (272 mg/dl+59, 283 mg/dl+53, 374 mg/dl+86 in group I, II and III respectively). Binge consumption significantly increased plasma non-HDL cholesterol (126 mg/dl+40, 113 mg/dl+25, 165 mg/dl+65; in group I, II and II and III respectively) and triglycerides (110 mg/dl+36, 88 mg/dl+16, 136 mg/dl+30; in group I, II and III). Radioactivity in plasma (1.7%±0.6, 2.6%±1.9, 2.0%±0.5; in group I, II and III) and liver (2.4%±0.7, 4.2%±0.8, 3.4%±1.0 in group I, II and III) was higher in the moderate alcohol group. The elimination of radioactivity in the feces was similar in all groups: 5.3%±2.3, 4.9%±2.3, 4.1%±0.9; in group I, II and III. Overall, the removal of radioactivity from macrophages along the RCT pathway was higher in the moderate group: 12.2%±3.1, 15.1%±3.7, 13.3%±2.4; in group I, II and III respectively. Moderate alcohol consumption in mice was associated with limited impact on macrophage RCT, whereas binge consumption exerted pro-atherosclerotic effects, including the increase of total and non-HDL cholesterol and the impairment of cholesterol excretion from the body.

MALE HYPOGONADISM NEGATIVELY IMPACT ON SERUM LIPOPROTEIN FUNCTIONS RELATED TO CHOLESTEROL HOMEOSTASIS

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Background and Aim. Low testosterone levels are associated with an increased incidence of cardiovascular (CV) events, but the underlying biochemical mechanisms are not yet fully understood. The clinical condition of hypogonadism, characterised by low levels of testosterone, offers a unique model to unravel the possible role of lipoprotein-associated abnormalities in CV risk. In particular, the assessment of the functional capacities of high-density lipoproteins (HDL) may provide novel insights besides traditional risk factors. Thus, to determine whether reduced testosterone levels correlate with lipoprotein functionality, HDL cholesterol efflux capacity (CEC) and serum cholesterol loading capacity (CLC) were evaluated in a series of hypogonadal patients and control subjects.

Methods. Hypogonadal men (n=19) and healthy aged matched men selected as controls (n=12) were enrolled for the study and characterized for plasma lipids with standard methods. Testosterone concentrations were measured by electrochemiluminescence immunoassay. HDL CEC was evaluated by cell-based radioisotopic techniques allowing the measurements of the single cholesterol efflux pathways. Serum CLC was assessed by fluorometric techniques.

Results. Hypogonadism significantly reduced HDL ATP-binding cassette transporter A1 (ABCA1)- and ATP binding cassette transporter G1 (ABCG1)-mediated efflux (21% and 45.6%, respectively) with a 14.3% decrement of total CEC. In the whole series, a positive correlation between testosterone levels and both total HDL CEC (r²=0.345, p=0.0008) and ABCG1 HDL CEC (r²=0.360, p=0.0006) was observed. Conversely, serum CLC, which was markedly raised (+44.5%) in hypogonadal patients, inversely correlated with testosterone levels (r²=0.233, p=0.008). HDL-C concentrations did not correlate with either testosterone levels or HDL CECs.

Conclusion. Altogether, these findings show that in hypogonadal patients pro-atherogenic lipoprotein-associated changes leads to reduced cholesterol efflux and increased influx. These results also offer for the first time a functional biochemical explanation for the increased CV risk in patients affected by low levels of testosterone.
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