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STEATOEPATITE NON ALCOLICA E FIBROSI EPATICA IN UNA CASISTICA DI PAZIENTI OBESI SOTTOPOSTI A CHIRURGIA BARIATRICA

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Obiettivi dello studio. Valutare la prevalenza di steatoepatite non alcolica (NASH) e fibrosi epatica in pazienti con obesità grave candidati ad intervento di chirurgia bariatrica. È stato inoltre valutato l'andamento delle transaminasi plasmatiche, adiponectina e di alcuni scores non invasivi di fibrosi epatica avanzata a distanza di 6 e 12 mesi dall'intervento chirurgico.
Popolazione e Metodi. Abbiamo studiato un campione di 28 pazienti affetti da obesità grave (75% donne, età mediana 41 anni, BMI mediano 45 kg/m^2), che sono stati sottoposti ad intervento in elezione di sleeve gastrectomy (19 pazienti) o bypass gastrico (9 pazienti). In tutti i pazienti sono stati eseguiti accertamenti metabolici (incluso 2-h clamp euglicemico iperinsulinemico) al baseline ed è stata eseguita una biopsia epatica durante l'intervento chirurgico.
Risultati. Dei 28 pazienti inclusi nello studio, 16 (57%) hanno soddisfatto i criteri istologici per una diagnosi di NASH, mentre i restanti 12 (43%) pazienti non avevano NASH al baseline. Di questi 12 pazienti privi di NASH alla biopsia, 8 pazienti avevano steatosi macrovescicolare di grado lieve o severo (NAFL), mentre solo 4 pazienti (pari al 14.3% del campione totale) erano esenti da NAFLD alla biopsia epatica. Per quanto riguarda il grado di fibrosi epatica, 4 pazienti (14.3%) non avevano fibrosi (stadio F0), 14 (50%) pazienti avevano fibrosi moderata (F2) e 10 (35.7%) avevano "bridging fibrosis" (F3). Nessuno dei pazienti aveva cirrosi epatica (F4). Quando i pazienti venivano suddivisi sulla base della presenza/assenza di NASH e/o della severità di fibrosi epatica (F3 vs. F0-2), i due gruppi di pazienti erano comparabili per età, sesso e le principali variabili biochimiche esaminate, incluso transaminasi, APRI index, FIB-4 score e sensibilità insulinica (M-clamp). L'intervento chirurgico induceva, sia dopo 6 che 12 mesi, un marcato calo ponderale ed una significativa riduzione dei livelli circolanti di adiponectina in entrambi i gruppi. Al contrario, i valori di transaminasi e gli scores non invasivi di fibrosi epatica avanzata non hanno mostrato alcuna significativa variazione dopo 6 e 12 mesi dall'intervento chirurgico in nessuno dei gruppi di pazienti considerati (NASH vs. no-NASH e F3 vs. F0-2).
Conclusioni. Nei pazienti con obesità grave candidati a chururgia bariatrica la NAFLD è una patologia assai comune (essendo presente in circa 85% del campione) ed è già presente anche nelle sue forme istologiche più severe (NASH nel 57% dei casi e fibrosi avanzata nel 35.7% dei casi), pur rimanendo queste forme spesso clinicamente silenti (o paucisintomatiche) e senza accompagnarsi a significative alterazioni delle transaminasi circolanti. Ciò suggerisce la necessità di una diagnosi precoce e tempestiva delle forme più severe della NAFLD (che sono quelle associate ad elevato rischio di progressione verso la cirrosi ed HCC) in tutti i soggetti obesi che vengono sottoposti a chirurgia bariatrica (da eseguirsi almeno in fase intra-operatoria).

NEED TO BRIDGE THE GAP BETWEEN RESEARCH AND CLINICAL PRACTICE: THE UNMEASURED ADDED VALUE OF LP(A)

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Elevated plasma levels of lipoprotein(a) [Lp (a)] (≥50 mg/dL, observed in approximately 20% of the general population) represent an independent risk factor for cardiovascular (CV) disease. Lp(a) levels are mostly genetically determined, therefore they are relatively stable throughout life. The most recent European guidelines for the management of dyslipidaemias recommend assessing Lp (a) levels at least once in life, to identify individuals who, being exposed to high levels of this atherogenic lipoprotein since birth, suffer from a CV risk higher than that determined by elevated LDL-C levels alone. The SANTORINI study is an observational study that aims to evaluate how, in clinical practice, patients with high and very high CV risk are treated, collecting data at enrolment and after 12 months. Italy participates in this study with 1531 patients at very high risk and 446 at high risk. At enrolment, in the overall population, mean LDL-C and Lp(a) levels were 98.4 mg/dL and 52.5 mg/dL. When stratified by risk, very high-risk patients had mean LDL-C and Lp (a) values of 94.6 mg/dL and 48.7 mg/dL, respectively, while high-risk patients had values of 111.4 mg/dL and 77.9 mg/dL, respectively. In addition to the low percentage of subjects receiving an appropriate lipid-lowering therapy, which is reflected in the observed LDL-C values, it is also known that the levels of Lp (a), especially in the high-risk group, confer an increase individual risk, regardless of LDL-C levels. The assessment of Lp (a) levels, therefore, allows the identification of subjects at greater risk of CV events and avoids an underestimation of the risk, inappropriate therapies, and reduced goal achievement.

LIVER STIFFNESS RELATES TO AN INCREASED RISK FOR ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Non alcoholic fatty liver disease (NAFLD) is a common and emerging liver disease in adults and represents a large and growing public health problem. NAFLD is closely associated with obesity, type 2 diabetes mellitus, metabolic syndrome, and cardiovascular events. The diagnosis of NAFLD is based on the following three criteria: non- alcoholic, detection of steatosis either by imaging or by histology, and appropriate exclusion of other liver diseases. The prevalence is estimated around 25% worldwide, it is around 50% in type 2 diabetes and around 50% in patients with dyslipidemia. NAFLD patients are usually asymptomatic until the condition progresses to liver cirrhosis. Patients affected by NAFLD show a lower-than-expected survival because of increases in the risk of cardiac arrhythmias such as atrial fibrillation and ventricular arrhythmia. NAFLD is often detected based on the presence of hepatic steatosis on abdominal ultrasound (US) during routine health checkups. US is gold standard examination for NAFLD diagnosis, as it is a non- invasive method, relatively cheap and widely available. Liver Stiffness Measurement (LSM) through FibroScanTM (transitory elastography) is a reliable index to assess liver librosis. Similar diagnostic accuracy is shown by Shear Wave Elastography (SWE), a technique US devices have been improved with. Purpose of the study is to evaluate ASCVD risk in NAFLD where the liver fibrosis could represent, alone, a non-lipid marker of cardiovascular risk.

Methods. We evaluate 41 patients (15 F, aged 58.71±13.56) who presented NAFLD according to the recent international guidelines. We excluded from the study patients in steroid therapy. Using the liver ultrasound we evaluate the liver stiffness as fibrosis marker as Stiffness measured by share wave technology. As controls, we compared 88 patients who were NAFLD negative (33F, aged 57.39±9.91). To evaluate the cardiovascular risk we calculate for each patient Atherosclerosis Cardiovascular disease score (ASCVD-10-yr).

Results. In NAFLD group, arterial essential hypertension was in 58.53% (24) and diabetes in 34.15% (14). Similar percentages of comorbidities were found in Controls. Patients with NAFLD has an increased liver fibrosis (median fibrosis class 1 [1QR 1-3]), compared to controls (F 0) ($p<0.05$) and an increased cardiovascular risk compared to controls (ASCVD-10-yr 17.21±17.99% vs 7.93±7.68; $p<0.05$). Shear wave kPa range (Spearman r 0.37, $p<0.01$) Fib4 and NFS score were directly correlated to ASCVD-10-yr. The same direct correlation was found between ASCVD-10-yr and absolute liver stiffness values evaluated in kPa (Pearson r 0.39, $p<0.05$). In particular higher fibrosis (F2-F3) was related to a nearly 50% increased ASCVD in 10 years.

Conclusion. Our data suggest that NAFLD may relate to a reduced life-expectancy mostly due to cardiovascular disease. Dyslipidemia and dysregulation of glucose homeostasis are underlying risk factors in NAFLD that contribute to the increased ASCVD risk, but the predilection for ectopic fat deposition in the liver and other tissues, in particular epicardial fat, seems to be associated with heightened risk of ASCVD beyond the risk attributable to traditional risk factors.These data may be useful in evaluation of both cardiovascular disease and liver steatosis due the connection we found between the two parameters. Thus, a comprehensive evaluation may be useful in order to optimize the tailored therapy for these patients. It would also be interesting to follow the decrease in visceral fat storage in patients with new antidiabetic medication like GLP-1 receptor antagonists. Further studies are surely needed.

A NEW METHOD OF EXTRACELLULAR VESICLES SEPARATION AND CHARACTERIZATION HIGHLIGHTS THEIR IN VITRO HETEROGENEITY-RELATED BIOLOGICAL PROPERTIES

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Extracellular vesicles (EVs) partecipate, by transferring their cargo from cell to cell, in pathophysiological processes. Unfortunately, unproper separation and characterization methods impair the full comprehension of their functions. To overcome this problem, we set up an ultracentrifugation method to size-separate different EV populations derived from a melanoma cell line, following an algorithm developed by Livshits. We characterized EVs by transmission electron microscopy (TEM), atomic force microscopy and dynamic light scattering (Zetasizer). Fatty acid (FA) profile and protein content were evaluated by gas-chromatography and mass spectrometry. Purity from external proteins was assayed by CONAN method. Size analysis not only confirmed the existence of different EV populations, but also the theoretical sizes calculated by the algorithm. Gas-chromatography analysis revealed a continuous percentage increase in saturated FA ranging from parental cells to smaller EVs (33.61%-64.79%), suggesting different membrane properties among populations. Mass spectrometry analysis identified 2003 proteins with qualitative and quantitative differential distribution among the separated populations. As expected, the MetaCore pathway analysis performed on individual cargos evidenced, besides common signaling pathways, molecular properties that specifically characterize each fraction. This suggests distinctive behaviors and biological functions for different EVs. Finally, the interactomic analysis, performed on identified proteins from vesicles with smallest diameter, evidenced a complex and highly integrated network of involved in a fine regulation of target-cell and environmental plasticity. Our separation method may be applied to any cell line, being helpful in defining the role of specific EV populations in cardiovascular diseases and in finding new pharmacological treatments able to modulate EV functions.Supported by EXTRALIPO Bando SEED 2019.

EFFECT OF SEMAGLUTIDE ON GLOBAL LONGITUDINAL STRAIN AND GLOBAL MYOCARDIAL WORK EFFICIENCY IN PATIENTS WITH UNCONTROLLED TYPE 2 DIABETES MELLITUS AND OBESITY

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Background. Glucagon-like peptide-1 receptor agonists (GLP1-RAs) are peptide molecules that exert their action through potentiation of insulin secretion, suppression of glucagon release, delayed gastric emptying, and weight loss. Cardiovascular outcome trials (CVOTs) demonstrated that GLP1-RAs have effectively reduced cardiovascular (CV) risk in type 2 diabetes mellitus (T2DM) patients, so their use is recommended by guidelines. The objective of our work was to evaluate the effect, in a cohort of uncontrolled diabetic patients, of Semaglutide, on oxidative stress markers (8-isoprostane and NOX-2), platelet activation indicator (Sp-Selectin) and subclinical myocardial damage evaluated by measurement of deformation and efficiency parameters, obtained by speckle tracking echocardiography (STE).

Materials and Methods. We performed a retrospective analysis enrolling 70 Caucasian patients (mean age 65.5±8.2, 54 men and 16 women) who met the following inclusion criteria: diagnosis of T2DM within 5 years, uncontrolled T2DM, obesity. Exclusion criteria were previous CV events, atrial fibrillation, heart failure. All clinical evaluation and laboratory tests were performed at baseline and after six months of treatment. The serum values of oxidative stress markers (8-isoprostane, NOX-2) and platelets activation (Sp-selectin) were assessed with ELISA sandwich. Echocardiographic recordings were performed by a single blind operator. Continuous variables were expressed as mean±standard deviation. For all continuous variables, comparisons between baseline (T0) and post-treatment values (T6) were performed using paired Student's t test. A linear regression analysis was performed to assess the relationship between variation in Global Longitudinal Strain (GLS), GLS endo-epi ratio, and global myocardial work efficiency (GWE), expressed as Δ of variation between baseline and follow-up (ΔT0–6) and the variation of metabolic, inflammatory, oxidative stress and platelets activation covariates that significantly improved after the treatment (expressed as ΔT0-6).

Results. Among enrolled patients, 85.7% presented hypertension, 18.5% had chronic kidney disease and 41.4% had dyslipidaemia. All patients were in treatment with statins and metformin, 42.8% with insulin, 24.2% with diuretics, 37.1% wit ACE inhibitors, and 48.5% with angiotensin receptor antagonists. The mean dose of Semaglutide was 0.59±0.29 mg/week without serious adverse events. At six months, data showed significant improvement in hemodynamic and clinical parameters such as systolic and diastolic blood pressure (SBP, DBP), heart rate ($p<0.004$), NT-ProBNP ($p<0.0001$); and metabolic parameters: fasting plasma glucose, insulinemia, HOMA, IGF-1, HbA1c and BMI ($p<0.0001$). Lipid profile and renal function also showed an improvement ($p<0.0001$). In addition, there was a significant reduction in biomarkers of oxidative stress such as 8-isoprostane, Nox-2 ($p<0.0001$), biomarkers of platelet activity such as Sp-Selectin ($p<0.0001$) and high-sensitivity C-reactive protein (hs-CRP) ($p<0.0001$). In addition, we observed a significant improvement in left ventricular myocardial deformation parameters such as GLS ($p<0.0001$), GLS endo/epi ($p<0.0001$) and GWE ($p<0.0001$). The linear correlation analysis showed that ΔGLS endo-epi was inversely correlated with ΔHOMA ($p=0.001$), Δuric acid ($p=0.012$), ΔNox-2 ($p=0.016$), ΔSp-selectin ($p=0.010$); ΔGLS was inversely correlated with ΔHOMA ($p=0.011$), Δuric acid ($p=0.025$) and ΔSp-selectin ($p<0.0001$); ΔGWE was inversely correlated with ΔHOMA ($p=0.011$), Δuric acid ($p=0.025$) and ΔSp-selectin ($p<0.0001$). From stepwise multivariate linear regression model, ΔSp-selectin, ΔHOMA, Δuric acid and Δ NOX-2 justifying respectively 18.1%, 7.2%, 5.6% and 5.3% of ΔGLS endo-epi; ΔSp-selectin, Δuric acid and ΔHOMA justifying 27.8%, 4.4% and 5.6% of ΔGLS respectively. Instead ΔNOX-2, Δhs-CRP and ΔBMI justifying 22.3%, 15.6% and 4.0 % of ΔGWE respectively. Results of our study demonstrated that six months treatment with Semaglutide, in patients with uncontrolled T2DM and obesity, improved GLS, GLS endo-epi ratio and GWE. It's plausible that this improvement may be justified by the reduction of inflammatory, oxidative stress and platelet activation parameters together with favourable metabolic changes; thus promoting the protection of the cardiac microcirculation with improving in myocardial contractility.

GENETIC CHARACTERIZATION OF LIPOPROTEIN(A) KRINGLE IV TYPE 2 REPEAT POLYMORPHISM: COMPARISON BETWEEN DIGITAL DROPLET AND REAL-TIME PCR

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Background. Evidence to support the role of lipoprotein(a) [Lp(a)] as a risk factor for atherosclerosis and thrombosis continue to increase. Lp(a) levels strongly differ among individuals, and many studies have shown the relationship between variants in the LPA gene, encoding apolipoprotein(a), and the increase in Lp(a) levels, closely related to the increase in cardiovascular risk. The major genetic determinant of these levels is a copy number variation (CNV) polymorphism, consisting of a variable number of repeats of a 5 kb region that includes exons 4 and 5 of the gene, encoding the protein domain Kringle IV type 2 (KIV-2). A lot of studies were able to support causality between Lp(a) and clinical outcomes through the analysis of KIV-2 repeat polymorphism. However, the peculiar structural characteristics of this variant constitute a significant challenge to the development of accurate methods for its detection. In this study, we compared quantitative real-time PCR (qPCR) and digital droplet PCR (ddPCR) in KIV-2 repeats determination.
Methods. 100 patients with possible/probable/certain diagnosis of Familial Hypercholesterolemia according to the Dutch Lipid Clinic Network score were analysed. Demographic and laboratory/clinical characteristics of the study population were collected. CNV values were obtained with qPCR using the 7900HT Sequence Detection System and with ddPCR using the QX200 Droplet Generator and reader system. Telomerase reverse transcriptase gene (TERT) was used as a single-copy reference gene in both techniques. To make CNV values comparable within and between different plates, three internal controls (CNV: C1=41-47, C2=50, C3=63-68) were used.
Results. In the whole cohort, qPCR analysis showed median values of repeats of 29.45 [IQR: 20.89-41.49], while ddPCR of 10.24 [IQR: 8.92-12.26]. Correlation analysis between the two methods was slightly significant, because of the greater dispersion of data obtained by qPCR compared with ddPCR. In fact, we found a very huge discrepancy in results using qPCR; really, C1, C2, C3 internal controls measurement throughout different experimental sessions reported lower data dispersion and greater stability in ddPCR with respect to qPCR: control sample C2, used as a reference sample for qPCR and estimated to have around 50 repeats, was confirmed to have a mean value of 54.85±1.11 with ddPCR analysis; control C1 and C3 showed a mean±SD of 37.59±9.05 in qPCR vs 44.28±2.74 in ddPCR and 101.18±45.12 in qPCR vs 65.02±3.53 in ddPCR, respectively. Spearman's rho test showed an inverse proportional correlation between Lp(a) levels of each patient and the CNV polymorphism, as expected, but higher and significant when evaluated with ddPCR despite qPCR (R=-0.393, p<0.001 vs R=-0,220, p=0,028, respectively). Dividing patients in two groups based on Lp(a) concentration (300 mg/L as cut off), a significant lower number of repeats of the KIV2 domain emerged among patients with greater levels of Lp(a) compared with the other group in both methods but with strongly evidence with ddPCR than in qPCR (P<0.001 and P=0.003, respectively).
Conclusion. Data obtained support the contribution of this molecular characterization approach in CNVs measurement, so strengthening the need of confirming results in larger cohorts in the effort of identifying a suitable method for the evaluation of a complex polymorphic variant representing the main genetic determinant of Lp(a) levels. The achievement of this goal might pave the way to improve genetic characterization of Lp(a) trait, in particular in the dyslipidaemic population, in order to better frame the risk profile of these patients.

TRIGLYCERIDES-GLYCAEMIA INDEX PREDICTS CARDIOVASCULAR EVENTS IN NON-ALCOHOLIC FATTY LIVER DISEASE

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Introduction. Non-alcoholic fatty liver disease (NAFLD) prevalence is steadily growing. The NAFLD epidemic is strongly associated with that of obesity and type 2 diabetes. NAFLD is considered the hepatic manifestation of metabolic syndrome and most patients affected by NAFLD develop cardiometabolic complications rather than liver ones. Aim of the study was to investigate which of Triglycerides and glucose index (TyG) and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) indexes better predicts cardiovascular events in NAFLD patients.
Methods. This is a post-hoc analysis performed in 830 patients enrolled in the Plinio Study (Progression of LIver Damage and Cardiometabolic Disorders in Non-alcoholic Fatty Liver Disease: an Observational Cohort Study. ClinicalTrials.gov Identifier: NCT04036357). The study includes dysmetabolic patients investigated for the presence of NAFLD. TyG index (ln[Fasting triglycerides (mg/dl) x Fasting glucose (mg/dl)] / 2) and HOMA-IR (Fasting insulin (mg/dl) x Fasting glucose (mg/dl)/405) were calculated as insulin resistance scores. Data on incident Cardiovascular Events (CVEs) were collected during the follow-up.
Results. NAFLD was present in 82.8% of patients. Both TyG index (4.8±0.3 vs. 4.6±0.2; p<0.001) and HOMA-IR (3.4 [2.4-5.6] vs. 1.7 [0.9-2.5], p<0.001) were higher in patients with NAFLD. Higher tertile of both scores independently associated with NAFLD diagnosis (TyG index III tertile aOR: 4.02, p<0.001; Homa-IR III tertile aOR: 7.31, p<0.001) after correction for age, sex, obesity, diabetes, arterial hypertension, prior CVEs and low-Fib4 score. Median follow-up duration was 47.6 [23.9-75.7] corresponding to 3629.6 person-years in NAFLD subgroup. The incidence rate of CVEs in NAFLD patients was 1.5%/year. Multivariable regression analyses showed that TyG Index III tertile (aHR: 1.86; p<0.01) but not Homa-IR III tertile predicted CVEs incidence after correction for age, sex, obesity, diabetes, arterial hypertension, prior CVEs, low Fib-4 score.
Conclusion. Unlike the HOMA-IR, TyG index predicts CVEs in NAFLD patients, and its use could help identify patients in need of more careful cardiovascular prevention.

IN VITRO AND EX VIVO STUDIES TO EVALUATE THE EFFECTS OF EDOXABAN ON PLATELET FUNCTION

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Background. All anticoagulants are expected to have an indirect effect on platelet function since they interfere with the generation or activity of thrombin, but the impact of Direct Oral Anticoagulants (DOACs) is largely unknown. Previous studies conducted with Dabigatran, Apixaban and Rivaroxaban showed a reduction in endogenous thrombin potential (ETP) and in platelet aggregation induced by thrombin and tissue-factor (TF) in a dose dependent manner. Aim of this study was to evaluate the effects of Edoxaban on platelet function by in vitro and ex vivo studies.
Methods. We evaluated platelet aggregation (PA), thrombin generation (TG) and thromboxane B2 (TXB2) levels in 20 healthy donors: samples were incubated in vitro with increasing concentrations of Edoxaban [E50, E150, E250 (ng/mL)] or vehicle as control. We also investigated the same parameters in 12 patients with Atrial fibrillation (AF) treated with Edoxaban (ex vivo study). A PAP-8 aggregometer was used to assess PA on PRP samples, induced by ADP (5 µM), TRAP-6 (10 µM), Human Thrombin (THR, 0.182 mU/µL) and TF. TG was measured using the Calibrated Automated Thrombogram System (CAT). Serum TXB2 was measured by using the TXB2 EIA kit, according to the manufacturer's instructions.
Results. The incubation of PRP with different Edoxaban concentrations significantly reduced TF-induced PA with respect to vehicle [E50 by 21% ($p=0.033$), E150 by 33% ($p=0.004$), and E250 by 52% ($p<0.001$)]. TF-induced PA was significantly lower in patients treated with Edoxaban than in controls ($p<0.001$). ADP and TRAP-6-induced PA was not inhibited by any Edoxaban concentrations in the in vitro study, and also ex vivo experiments failed to demonstrate any difference between ADP and TRAP-6-induced PA from AF patients treated with Edoxaban and controls. THR-induced aggregation in E150 group showed a trend towards a reduction, though did not reach the statistical significance. Among the parameters related to TG, Lag Time was significantly ($p<0.001$) and positively related to Edoxaban concentrations. Patients showed more prolonged Lag Time values ($p=0.031$) with respect to those observed in controls. ETP and Peak were significantly reduced in vitro ($p<0.001$) by the incubation of Edoxaban in a dose dependent manner. In in vitro study, ETP ratio values were significantly reduced according to increasing Edoxaban concentrations. AF patients showed reduced levels of ETP ratio with respect to controls ($p<0.001$). We found a 24% decrease in serum TXB2 concentration in the E250 group vs control ($p<0.01$), while the reduction is not significant in the other Edoxaban concentrations.
Conclusions. Our data show that Edoxaban is able to interfere with platelet function. In particular, it significantly reduces TF-induced platelet aggregation in a dose-dependent manner and also TG, although thrombin-induced aggregation is not affected by the drug, supporting Edoxaban's indirect effect on thrombin by inhibiting FXa. In addition, the reduction of TXB2 levels by Edoxaban suggests that the drug is endowed with an antiplatelet effect, which may, in turn, lead to the delayed/reduced formation of coagulation complexes reinforcing its antithrombotic potential.

ROLE OF DENDRITIC CELL IMMUNORECEPTOR 2 (DCIR2) IN EXPERIMENTAL ATHEROSCLEROSIS

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Background and Aim. DCIR2 (Dendritic cell immunoreceptor 2) is a C-type lectin receptor mainly expressed by dendritic cells, responsible for modulating the adaptive response towards pathogens. The peculiarity of this receptor is the inhibitory intracellular domain able to dampen the response driven by other immune cell receptors. Thus, given the strong impact of the immune system in atherogenesis, the aim of this project was to study the contribution of DCIR2 in atherosclerosis.
Materials and Methods. Male Dcir2-/- Ldlr-/- (DKO) and Ldlr-/- mice were fed a cholesterol-enriched diet for 12 weeks to encourage atherogenesis. Subsequently, paralleled to the analysis of circulating lipid and immune cells, the characterization of atheromatous plaque within ascending aorta was performed.
Results. Compared with the control counterpart, DKO mice showed decreased plasma cholesterol and triglycerides levels (-42%, $p<0.05$; -25%, $p<0.01$ respectively), which resulted in a better phenotype of the atherosclerotic plaque - characterized by the reduced plaque formation both at aortic sinus level (-58%, $p<0.01$) and along the first tract of the aorta (-64%, $p<0.05$) for 300µm -. Although atheromatous plaques were less necrotic (-91%, $p<0.05$), they did not differ either in the fibrotic component or in the content of smooth muscle cells and macrophages. Of note, while a significant decrease in circulating neutrophils and monocytes (-47%, $p<0.05$; -38%, $p<0.01$) was observed in the DKO mice compared to Ldlr-/-, the number of bone marrow myeloid precursors increased (+42% $p<0.05$).
Conclusions. DCIR2 is a receptor mainly expressed by dendritic cells and is involved in the modulation of the immune response; our data suggest that it is also implicated in the development of atherosclerosis. We are currently investigating whether this is related to a different regulation of the immune-inflammatory response.

THE DUAL ACTIVITY OF FUROXANS MAY AMELIORATE DIFFERENT ASPECTS OF ATHEROSCLEROSIS: AN IN VITRO STUDY ON SMOOTH MUSCLE CELLS

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Atherosclerosis is a multifactorial disease in which, beyond lipid accumulation and inflammation, nitric oxide (NO) unbalance and smooth muscle cell (SMC) proliferation play pivotal roles. To find a new pharmacological approach, we synthesized furoxans, which demonstrated to release NO in a controlled fashion and we tested their ability in inhibiting SMC proliferation, together with the comprehension of their mechanism of action. We measured SMC proliferation by cell counting (Coulter Counter) after 72 hours of incubation or by thymidine incorporation (20 hours). Proteomics was assessed by SILAC followed by MetaCore analysis or by western blot techniques.We demonstrated that all the tested furoxans inhibit SMC growth and cause rat aorta rings vasodilation, albeit with different potency. To comprehend their antiproliferative mechanism, after blocking the position 4 of their ring by a phenyl group, we found that their inhibitory potency paralleled with the electron-attractor capacity of the group in 3. Extending the study to related furoxans (in which groups in 3 and 4 are interchanged) and furazans (analogues without ring-opening capacity and therefore unable to release NO), we found that 4-Ph-3-R furoxans were the most potent inhibitors of SMC proliferation, followed by 3-Ph-4-R furoxans. Furazans were not effective, documenting that the opening of the ring is essential for growth inhibition. To understand the molecular basis of this effect, we demonstrated that the mechanism is neither cGMP- nor polyamine-dependent, the two main NO-mediated pathways involved in SMC proliferation. Finally, proteomic experiments assessed that specific proteins (12) and specific networks involved in cell homeostasis (e.g. SUMO1, BANF1) are modulated by furoxans, probably by interaction with adducts generated by their ring opening, rather than NO release. Altogether, thanks to their pharmacological flexibility compared with classical NO donors, furoxans may be tested in animal models of atherosclerosis to assess their efficacy as antiatherosclerotic molecules.

PCSK9 AND LEPTIN PLASMA LEVELS IN ANOREXIA NERVOSA

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Aim. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a regulator of low-density-lipoprotein cholesterol (LDL-C), a major risk factor of cardiovascular (CV) disease. Since the hormone leptin has been suggested to have a role in CV risk regulation, possibly by modulating LDL receptor expression through the PCSK9 pathway, nutritional status may represent a potential regulator. Thus, evaluation of PCSK9 levels in human eating disorders appears of interest. In this report we evaluated the lipoprotein profile, PCSK9 and leptin levels in subjects affected by Anorexia Nervosa (AN), to improve the understanding of the metabolic alterations in this disease.
Methods and Results. We have set up a case-control observational study, enrolling 20 anorexic adolescent females and 20 normal adolescent females as control group, age and sex matched. AN subjects showed a lower BMI, total cholesterol and LDL-C respect to control group, with lipoprotein levels in the normal range. Further, adolescent AN girls show significantly higher PCSK9 (+24%, $p<0.005$) and lower leptin levels (-43%, $p<0.01$), compared to the control group.
Conclusions. Raised levels of PCSK9 and reduced leptin, stimulate further research unravelling the role of liver and adipose tissue in the handling of PCSK9/LDL metabolism in adolescent anorexia nervosa.

ANGPTL3 AND PCSK9 DIRECTLY INTERACT AND COORDINATE THE REGULATION OF CELLULAR METABOLISM IN VITRO

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Background and Aims. ANGPTL3 and PCSK9 are known regulators of lipoprotein metabolism. Patients harboring homozygous loss of function mutations in the ANGPTL3 gene, show reduced levels of circulating PCSK9, indicating a possible coordinate regulation of these two proteins. This study aimed to establish whether the two proteins can cross-regulate in different conditions of nutritional availability.
Material and Methods. ANGPTL3, PCSK9, or both genes were overexpressed in HepG2 cells grown in glucose-rich (Feeding) and glucose-poor (Fasting) conditions. We performed Real-time PCR to study ANGPTL3 and PCSK9 mRNA levels, Co-immunoprecipitations (Co-Ip) to verify protein-protein interaction, and western-blotting to quantify the produced proteins and apoB secretion both intracellularly and extracellularly in the culture medium.
Results. Glucose determines a 5-fold increase in the ANGPTL3 mRNA levels and a 1.5-fold increase in the PCSK9 mRNA levels in HepG2 cells. The Co-immunoprecipitation in baseline growth conditions highlighted a direct protein-protein interaction of PCSK9 and ANGPTL3 intracellularly. The western blot analysis showed that the two proteins have a similar secretion pattern dependent on glucose availability in the culture medium. ANGPTL3 overexpression determines PCSK9 intracellular accumulation in fasting conditions, the opposite is observed in the overexpression of PCSK9. The double overexpression determines a consensual secretion increase of both proteins, more evident in feeding conditions. In addition, also apoB secretion appears to be tightly dependent on glucose levels showing a substantial increase in the case of ANGPTL3 and PCSK9 overexpression (Fold-change x2,8).
Conclusion. ANGPTL3 and PCSK9 are transcriptionally cross-regulated, they respond to changes in glucose availability and show co-secretion patterns in vitro. The two proteins are in close intracellular interaction, they are finely regulated in the same direction in response to metabolic stimuli and both promote apoB secretion and accumulation in culture media.

THE LOW-DENSITY LIPOPROTEIN RECEPTOR-MTORC1 AXIS COORDINATES CD8 T CELL ACTIVATION

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Background. Activation of T lymphocytes combines functional to metabolic rewiring of cell machinery, including cholesterol homeostasis. Here we evaluated the role of LDLR, as a key regulator of cholesterol cellular uptake, on T cell biology.
Methods. Immunophenotypic characterization of T cells from WT and LDLR KO mice was performed in vitro (anti-CD3/CD28) and in vivo (ovalbumin vaccination) coupled to proteomics and WB analysis on isolated T cells. T cells from FH (familial hypercholesterolemia) patients, carrying mutations in the LDLR gene, were tested.
Results. LDLR mRNA expression increased after in vitro activation of CD8, but not CD4 T cells, suggesting a different regulation of cholesterol homeostasis between T cell subsets. Functionally, deficiency of LDLR mainly dampened CD8 vs CD4 activation as demonstrated by in vitro proliferation (-35%, $p<0.01$) and INFγ production (-39.6%,$p<0.01$), and in vivo proliferation and cytokine production (↓IFNγ $p<0.001$,↓IL13 $p<0.01$,↓perforin $p<0.05$) after ovalbumin vaccination. Addition of LDL to serum free media increased by roughly 15%($p<0.01$) CD8 proliferation in WT but not in KO and in CD4 cells. By proteomic and WB analysis we associated this phenotype to a reduced activation of mTORC1 (pmTOR -40%,$p<0.01$) and impaired lysosomal organization (reduced lysotracker and LAMP-1 expression). CD8 T cells from FH patients proliferated less (-36%,$p>0.05$) compared to sex- and age-matched controls; in addition, CD8 from FH vaccinated for seasonal influenza were tested in vitro with virus-derived peptides, showing a decreased granzyme production (-60.3%,$p<0.01$) compared to CD8 from vaccinated controls.
Conclusions. LDLR plays a critical role in regulating the immunometabolic responses in CD8 Tcells by fuelling the cholesterol-lysosome-mTORC1 axis.

EFFECT OF PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 INHIBITORS ON PULSE WAVE VELOCITY AND MONOCYTE-TO-HIGH-DENSITY-LIPOPROTEIN-CHOLESTEROL RATIO IN FAMILIAL HYPERCHOLESTEROLEMIA SUBJECTS: RESULTS FROM A SINGLE-LIPID-UNIT REAL-LIFE SETTING

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Introduction. Subjects with familial hypercholesterolemia (FH) are characterized by an increased amount of low-density lipoprotein cholesterol (LDL-C) that promotes a continuous inflammatory stimulus. Our aim was to evaluate the effect of proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9-i) on inflammatory biomarkers, neutrophil-to-lymphocyte ratio (NLR), monocyte-to-high-density lipoprotein ratio (MHR), and on early atherosclerosis damage analyzed by pulse wave velocity (PWV) in a cohort of FH subjects.
Methods. In this prospective observational study, we evaluated 56 FH subjects on high-intensity statins plus ezetimibe and with an off-target LDL-C. All subjects were placed on PCSK9-i therapy and obtained biochemical analysis as well as PWV evaluation at baseline and after six months of PCSK9-i therapy.
Results. After six months of add-on PCSK9-i therapy, only 42.9% of FH subjects attained LDL-C targets. As expected, a significant reduction of LDL-C (-49.61%, $p<0.001$) was observed after PCSK9-i therapy. Neutrophil count (NC) and MHR were reduced by PCSK9-i (-13.82% and -10.47%, respectively, p value for both<0.05) and PWV significantly decreased after PCSK9-i therapy (-20.4%, $p<0.05$). Finally, simple regression analyses showed that Δ PWV was significantly associated with Δ LDL-C ($p<0.01$), Δ NC and Δ MHR (p value for both <0.05).
Conclusions. In conclusion, PCSK9-i therapy significantly improved lipid and inflammatory profiles and PWV values in FH subjects; our results support the positive effect of PCSK9-i in clinical practice.

EFFICACY AND SAFETY OF LOMITAPIDE IN PEDIATRIC PATIENTS WITH HOMOZYGOUS FAMILIAL HYPERCOLESTEROLEMIA: IS DISCONTINUATION OF LIPOPROTEIN AFHERESIS POSSIBLE?

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Introduction. Lomitapide is a microsomal transfer protein inhibitor approved for the treatment of adults with homozygous familial hypercholesterolemia (HoFH). The use of lomitapide in HoFH pediatric subjects is described only by few case-reports.AIM OF THE STUDY: In this study, as part of the first multicenter trial in the word, we evaluated the efficacy and the safety of lomitapide on top of conventional therapy with statin, ezetimibe and lipoprotein apheresis (LA) in pediatric subjects with HoFH.
Subjects and Methods. 2 males and 2 females, aged between 6 and 10 years, with HoFH (3 out of 4 with genetic diagnosis) were included in the study. Lomitapide was initiated at a starting dose of 2 mg/day and progressively escalated up to 20 mg/day. The efficacy of lomitapide was defined by the LDL cholesterol change, hepatic function and liver ultrasound were assessed.RESULTS: Untreated LDL cholesterol (LDL-C) was 794±53 mg/dl (mean±SD). With rosuvastatin 5-20 mg plus ezetimibe 10 mg plus weekly LA, LDL-C was 324±27 mg/dl. The addition of lomitapide 20 mg/day, resulted in a robust LDL-C decrease (221±52 mg/dl after 28-40 weeks of treatment), allowing the reduction of LA frequency in 3 children (every two weeks) and the LA suspension in 1. Furthermore, lomitapide induced reduction and/or resolution of cutaneous xanthomas present at the beginning of the study. Three out of 4 patients showed a transient mild elevation of AST and ALT (<2xULN), and the mild hepatic steatosis evaluated by liver ultrasound at the beginning of the study remained stable during the therapy. At present, none of the children has discontinued lomitapide treatment.
Conclusions. Our findings suggest that lomitapide is effective and safe in children with HoFH, leading to a significant reduction in LDL-C and allowing a reduction/suspension of LA with a considerable impact on the quality of life of children and their families.

IDENTIFICATION OF A NOVEL NONSENSE MUTATION IN THE APOB GENE BY NEXT GENERATION SEQUENCING

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Introduction. Familial hypobetalipoproteinemia (FHBL) is an autosomal codominant disorder of lipoprotein metabolism characterized by low plasma levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and apolipoprotein B (apoB) below the 5th percentile of the distribution in the population. It may be due to loss-of-function mutations in APOB or, less frequently, in PCSK9 genes. The most frequent dominant monogenic form of HBL is the Familial Hypobetalipoproteinemia type-1 (FHBL-1, OMIM # 615558). The 50% of FHBL-1 cases is caused by mutations in APOB gene which result in assembly defects and secretion of lipoproteins containing apoB. Most of the FHBL-1 subjects are heterozygous carriers of nonsense pathogenetic variants and frameshift of the APOB gene which interfere with the complete translation of the mRNA coding the apoB protein, determining the formation of truncated forms of apoB. FHBL heterozygotes are generally asymptomatic but often develop fatty liver.
Materials and Methods. We designed a custom panel for Next Generation Sequencing (NGS) in order to analyze known genes involved in FHBL by Ion Torrent GeneStudio S5 Plus. We sequenced the FHBL candidate genes in 10 patients presenting LDL-C and ApoB levels below the 5th percentile.
Results and Conclusion. In the majority of subjects no functionally relevant mutations in candidate genes were detected. Two unrelated patients was found to be carrier of a novel heterozygous nonsense mutation in the exon 26 of the APOB gene (c.10324C>T, p.Gln3442Ter). The mutation lead to the formation of a premature stop codon and an apoB truncated protein of an expected size of 75.8% of wild type apoB (apoB-75.8).In this work we describe a novel nonsense mutation of the APOB gene responsible for FHBL identified by a Next generation sequencing approach.

SUBOPTIMAL ADHERENCE TO STATIN THERAPY IN CHILDREN AND ADOLESCENTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA DESPITE A HIGH THERAPEUTIC EFFICACY: IS THE CARDIOVASCULAR RISK UNDERESTIMATED?

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Background. European guidelines currently support the initiation of statin by age 8-10 years in patients with heterozygous familial hypercholesterolemia (HeFH) to slow the progression of endothelial disfunction and to reduce the risk of cardiovascular disease in adulthood. However, to date, there is lack of data on adherence to statins in the paediatric population. Therefore, we describe our real-life paediatric experience about efficacy and adherence of statin therapy.
Methods. This is a monocentric, observational study recruiting children and adolescents with genetically confirmed HeFH. Anthropometric measures, fasting lipid profile and therapeutic data were collected at diagnosis of HeFH [T0], on lipid-lowering diet [T1], four weeks after starting statin therapy [T2] and yearly during the first two years on statin [T3 and T4].
Results. 24 HeFH children and adolescents (17/24 female) were started on statin at a mean age of 13.77±3.09 years (12 on atorvastatin, 10 on pravastatin and 2 on simvastatin). On lipid-lowering diet, lipid metabolism did not change significantly [LDL-C 237.61±47.18 vs. 218.22±50.15 mg/dl, p 0.11], while on statin the improvement was quick and persistent [LDL-C T1 218.22±50.15, T2 163.85±27.64, T3 153.12±34.90, T4 156.37±34.11 mg/dl, $p<0.05$]. The mean reduction of LDL-C in comparison to baseline levels was: -34.68±12.99% at T2 and -30.42±20.78% at T4. Despite this efficacy and excluding one case of statin-intolerance, 9/23 patients (about 39%) dropped out after one year of statin therapy with a higher prevalence among families without an history of precocious cardio-vascular events ($p<0.05$).
Conclusions. We report an overall scarce adherence to statins in our paediatric HeFH population despite an efficacy in line with international data. GP involvement, a more effective communication with patients and their families to emphasize the high HeFH-related cardiovascular risk, and a periodic follow-up including telemedicine may be tools to achieve a better adherence.

"IN MEDIO STAT VIRTUS": EFFICACY AND SAFETY OF STATINS ON ALTERNATE DAYS

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Background. Statins are the main treatment for hypercholesterolemia but patients taking statins sometimes refer muscle-related symptoms, a frequent cause of treatment discontinuation.As an alternative to high-intensity statin monotherapy, moderate-intensity statin with ezetimibe combination therapy can lower LDL cholesterol concentrations effectively and reduce adverse effects.AIM The aim of the study was to evaluate the efficacy of therapy with statin/ezetimibe combination on alternate days and only ezetimibe every other days, both in terms of safety of this treatment and in terms of overall reduction of the lipid profile, in a population of patients, predominantly in primary prevention, with myalgia statin-related, previously treated with statin/ezetimibe combination every day.
Methods. In this study were involved 49 subjects (19 male; 30 female. Median age: 61 years) in primary (46 subjects) and secondary (3 subjects) prevention with statin/ezetimibe combination treatment every day. The lipid profile (cholesterol tot; HDL; triglycerides) and CPK values were analyzed at baseline and also 3 months after the introduction of therapy with rosuvastatin/ezetimibe (5/10 mg) combination on alternate days and only ezetimibe (10 mg) every other days. LDL was calculated using Friedewald formula.
Results. In the analyzed population median baseline values were: total cholesterol 250 mg/dL, LDL-C 172,3 mg/dL, HDL-C 52,3 mg/dL; triglycerides 163 mg/dL and CPK 261 U/l.3 months after the introduction of alternative treatment median values were: total cholesterol 185.4 mg/dL, LDL-C 100 mg/dL, HDL-C 53 mg/dL; triglycerides 118 mg/dL and CPK 194 U/l.It has been showed a reduction of nearly 40 % LDL values and 33 % CPK values, with improvement of safety and compliance to therapy.
Conclusion. Our data suggest good efficacy of treatment with statin/ezetimibe combination on alternate days and only ezetimibe every other days thanks to the improvement of compliance. Moreover it could represent an advantageous therapy in terms of pharmacoeconomy.

CAROTID AND AORTIC INTIMA-MEDIA THICKNESS IN PEDIATRIC AGE: COMPARISON BETWEEN FAMILIAL HYPERCHOLESTEROLEMIA AND POLIGENIC DISLIPIDEMIAS

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Objectives. Carotid and aortic intima-media thickness (cIMT and aIMT) are surrogate markers of subclinical atherosclerosis. Patients with primary hyperlipidemias have higher cIMT and aIMT values than controls, even in pediatric age. AIM: The aim of the study was to evaluate cIMT and aIMT in pediatric subjects with monogenic (familiar hypercholesterolemia- FH) and polygenic dyslipidemias (PD) and to investigate any lipid profile or clinical data correlations.
Patients and Methods. The study included 390 hyperlipidemic subjects (135 FH, 255 PD; age 5-18 years). Clinical data (age, sex, weight, BMI) and pre-therapy lipid profile were analyzed by standard methods; cIMT and aIMT were tested by B-mode ultrasound with a 7.5-10 MHz linear array transducer.
Results. Significantly higher TC, LDL-C, non-HDL-C levels and lower triglycerides were observed in the FH group compared to the PD group. Mean cIMT values were higher in FH vs PC (0.462±0.079 vs 0.445±0.058 mm, p=0.014) as were left cIMT values (0.462±0.080 vs 0.443±0.059 mm, p=0.006). Stratifying by age, these differences were just confirmed since 10 years of age. No significant results were obtained for right cIMT or aIMT. In the FH group, mean and left cIMT values positively correlated with TC, non-HDL-C, aIMT, age, weight, BMI and male sex. In the PD group correlations were confirmed with aIMT, age, weight, BMI and male sex, but not with TC and non-HDL-C.
Conclusion. Present results confirm the early onset of atherosclerosis, particularly in FH subjects. The increased cardiovascular risk, even compared to other forms of primary dyslipidemia, stresses the importance of an early diagnosis and treatment.

TELEMEDICINE FOR THE CARE OF PEDIATRIC PATIENTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA IN THE COVID-19 PANDEMIC ERA AND BEYOND

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Telemedicine is the use of electronic methods to deliver health care and/or health education from a distance. It is a useful tool in providing a modality for continued care as expected during pandemic era.
Methods. We conducted a single-centre prospective pilot study to evaluate the feasibility and applicability of telemedicine services in the management of children with Familial Hypercholesterolemia. The objective was to provide consultation for drugs and select children who needed inpatient care. The study was conducted during the period between March 2020 and June 2020. The first 30 parents of children affected by FH and routinely followed in our center and asked for consultation were informed about the availability of teleconsultation services. A digital platform for a teleconference (Zoom or Jetsi) was used to connect pediatricians and families. The medical data received was entered in the internal electronic medical records system (OBG clinico) and a summary was e-mailed to the family. At the end of the e-consult, the parents/caregivers were asked to complete a brief survey and rate the teleconsultation experience on a scale of 0 (not satisfied) to 5 (fully satisfied).
Results. During the study period, a total of 22 e-consults were done; 8/30 parents refused teleconsultation because of little confidence in the technology. At least 4 fathers and 18 mothers personally completed the survey. The study group comprised 22 patients - 8 boys and 14 girls. The mean age of the children in this study was 10,7 years (range 5-17 years). The patient/family satisfaction score for e-consults was "5" in 54,5% and "4" in 45,4% of the parents. Based on our experience, we conclude that telemedicine may be an effective modality in triaging children with Familial Hypercholesterolemia for follow-up and in providing individualized tailored advice. This results in enhanced satisfaction due to a stronger doctor-patient-family relationship and may improve clinical outcomes also outside the pandemic period.
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PROPROTEIN CONVERTASE SUBTILISIN KEXIN TYPE 9 INHIBITORS REDUCE PLATELET NETS RELEASE THAT DRIVE THROMBOSIS IN FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction. Elevated low-density lipoprotein-cholesterol levels (LDL-C) contribute to chronic systemic inflammation in heterozygous familial hypercholesterolemia (HeFH). it is known that the treatment of HeFH patients with PCSK9 inhibitors (PCSK9i) significantly reduces LDL-C levels. Moreover, neutrophil extracellular traps (NETs) release has been shown to induce activation of endothelial cells and platelets, resulting in a proinflammatory response. Thus, NETs may play a role in triggering atherosclerotic plaque formation and thrombosis. This study aims to describe the inflammatory profile of HeFH patients and explore the effect of PCSK9 inhibitor (PCSK9i) on NETs release, and thrombus formation, and finally, investigate the molecular mechanisms governing its occurrence.
Materials and Methods. We studied 40 patients with heterozygous familial hypercholesterolemia (HeFH) on treatment with the maximum tolerated statin dose ± ezetimibe before and after six months of PCSK9i therapy. We analyzed NETs release and thrombus formation by measuring in plasma citrullination of histone H3 (CitH3) and thrombus-formation analysis system (T-TAS), respectively. Furthermore, we investigated by in vitro study if plasma post-PCSK9i reduced NETs release and thrombus formation.
Results. In vivo studies showed decreased circulating levels of CitH3 and under laminar flow platelet-dependent thrombus growth in HeFH patients after PCSK9i treatment compared to patients before treatment. In vitro study showed that plasma from HeFH patients after PCSK9i treatment decreased NETs release by neutrophils and thrombus formation.
Conclusion. This study provides evidence that HeFH patients showed an increased NETs release and thrombus growth that were inhibited by the treatment with PCSK9i which may represent a novel approach to counteract inflammation in these patients.

GENETIC SUSCEPTIBILITY IN VACCINE INDUCED THROMBOTIC THROMBOCYTOPENIA (VITT)

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Background. In late February 2021, a prothrombotic syndrome was observed in a small number of individuals who received the adenoviral vector-based vaccine Vaxzevria (AstraZeneca). Similar pictures were observed in some individuals who received the Janssen (Johnson & Johnson) vaccine. This syndrome has been named vaccine-induced thrombotic thrombocytopenia (VITT) and is characterized by multiple venous/arterial thrombosis, with atypical venous thrombosis of cerebral and splanchnic districts, associated with thrombocytopenia. Hemorrhagic manifestations and sometimes disseminated intravascular coagulation (DIC) are present. Evidences suggest that this rare syndrome is caused by platelet-activating antibodies directed against platelet factor 4 (PF4), a chemokine stored in the platelets' alpha granules necessary for platelet aggregation, also known to be involved in the atherosclerotic plaque formation. Aim of this work is to apply a Whole Exome Sequencing (WES) analysis approach, for the identification of possible genetic predisposition profiles underlying VITT.
Methods. Due to the referral role of the Center for Atherothrombotic Diseases (University of Florence/AOU Careggi) for the management of COVID-19 patients and diagnosis of VITT, fifty patients were examined. Ten out of fifty patients were diagnosed with VITT. In our analysis we used Next Generation Sequencing techniques, by a WES approach, with Illumina NextSeq500 platform and SureSelect XT HS enrichment kit (Agilent Technologies), to analyse six out ten VITT patients.
Results. VITT patients analyzed were 6 females (mean age 64,2±13,8), who received an adenoviral vector-based vaccine. WES analysis revealed a total of 140,563 variants. Rare variants (MAF <1%) identified range from 1,619 to 1,774 and their distribution by type (frameshift, missense, splicing, nonsense, UTR, samesense, intronic) is similar in the six patients.In this work we decided to focus on rare variants involved in different biological processes underlying VITT.We found a total of 89 rare variants in genes involved in integrin signalling pathways (ITGA2B, ITGAD, ITGB4, GP6, FGA, FGB), in thrombocytopenia (MASTL, PDIA6, FYB, MYH9), and other genes inducing/inhibiting platelet aggregation/activation processes. Interestingly, the two patients (VITT05 and VITT18), with most severe clinical complications, showed a higher number of rare variants identified in such pathways (21 and 27 variants, respectively). Among the abovementioned pathways, 15 variants with putative functional effect have been identified in genes encoding for molecules of the integrin pathway, which display an additional role in the atherosclerotic mechanism/process. Interestingly, both VITT05 and VITT18, patients with a more severe phenotype, carried variants in GP6 gene, encoding a collagen receptor involved in collagen-induced platelet adhesion and activation.
Conclusions. WES analysis exhibits a considerable number of variants in molecular pathways involved in integrin signalling, thrombocytopenia, platelet aggregation/activation and atherosclerotic processes. The two patients with the worst clinical outcome presented a significantly higher number of suggestive rare variants with respect to other patients investigated; consequently, it is not possible to exclude the potential contribution of a greater number of rare suggestive variants in the modulation of the phenotype of patients with worse clinical course. Further investigation on other mechanisms (inflammation, immunity, viral response) and functional assays are needed for more clarity with respect to the impact of genetic background on VITT susceptibility.

A SMALL LIPOPROTEIN THAT CAN MAKE THE DIFFERENCE: THE ROLE OF LIPOPROTEIN(A) IN CARDIOVASCULAR RISK STRATIFICATION AND REVERSE SCREENING IN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLAEMIA

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Background and Aims. Familial hypercholesterolemia (FH) is a genetic disease involving 1:250 subjects in the general population. FH detection and treatment, starting from childhood, helps gaining decades of life. Cardiovascular-disease (CVD) risk stratification is fundamental in FH children. Lipoprotein(a) [Lp(a)] is universally recognized as an independent CVD risk factor. Lp(a) values above 30 mg/dl are considered a threshold for increased CVD. Moreover, Lp(a) determination in FH children can improve CVD family risk stratification.
Matherials and Methods. In a 30 months period, 70 patients were referred for the first time to the Centre for Paediatric Dyslipidaemias of Guglielmo da Saliceto Hospital in Piacenza for hypercholesterolemia; 33/70 (20 male, age mean±sd 11.2±4.3 years) were assessed for suspect FH. Pediatric evaluation, anthropometric parameters, complete lipid profile including Lp(a) and genetic analysis for FH were performed. Nutritional intervention and follow up were started. If patient's Lp(a) level was ≥30 mg/dl, Lp(a) assessment in first degree family members was recommended.
Results. Lipid profile of the study population was (mean±sd, mg/dl): total cholesterol 266.3±46.7, LDL-cholesterol 187.8±36.8, HDL-cholesterol 56.3±12.3, triglycerides 108.6±62.4. Mean Lp(a) level was 27.9 mg/dl. 7/33 patients had Lp(a) ≥30 mg/dl, so Lp(a) assessment was recommended in their parents and 4 out of 7 patients had at least one parent with Lp(a)≥30 mg/dl. 4 out of 7 patients with elevated Lp(a) and 12/26 patients with normal Lp(a), after a 6 month nutritional treatment, were put on lipid lowering pharmacological therapy.
Conclusions. Lp(a) evaluation in FH pediatric patients can improve CVD risk stratification, so as to start early drug therapy after nutritional and lifestyle intervention. Elevated Lp(a) in children is an alarm sign that should lead clinicians to perform reverse screening in adult components of the child's family. Pediatric Lipidologists have a fundamental role in CVD prevention in the whole family.

TRANSCRIPTOMICS: AN ENTICING APPROACH TO UNDERSTANDING THE PATHOPHYSIOLOGY AND CLINICAL OUTCOMES OF ACUTE ISCHEMIC STROKE

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Background. Acute ischemic stroke (AIS) represents one of the principal causes of neurological morbidity and mortality worldwide and is characterized by a multifactorial etiology, caused by interactions among blood vessel and environmental and genetic factors. For a prompt and efficient cerebral blood restoration, intravenous thrombolysis with rt-PA is often combined with mechanical thrombectomy (MT). MT represents a golden ticket from a research perspective, providing cerebral thrombi (CT) as new study material, enabling in-depth studies concerning their cellular composition and etiology correlation. In addition, it represents a key pillar for the creation of virtual predictive models of different gene expression based on annotated histopathological evidence. The trend towards a medicine based on personalized and individualized prevention and treatment strategies has led to the need to investigate the genetic aspect of the disease, due to its significant contribution to the genesis of the ischemic event. For that, focus of the research is the highlighting and exploration of profiles in which peripheral blood (PB) mirrors CT through the analysis of global gene expression profiles, and the identification of promising markers that can serve as sentinels for different pathophysiological mechanisms and/or determinants of clinical outcomes, such as haemorrhagic transformation, 24h edema, modified 3 months Rankin scale-mRS, death. This approach could allow to gain deeper insights into the pathogenesis of the disease through investigation of the relationship between gene expression and phenotypic differences.
Methods. We performed gene expression profiles of RNA samples obtained from 40 CT and 37 PB of 52 patients. The CT obtained during MT were stored in RNA later, while PB, collected before and 24 hours after MT, in tubes containing a reagent that protects RNA from degradation and minimizes ex vivo changes in gene expression. RNA was extracted by PAX gene blood miRNA kit; the global gene expression profile was assessed by Affymetrix technology using GeneChip Human Transcriptome Array 2.0, allowing the analysis of 44,699 genes, with more than 285,000 full-length transcripts coverage. Data analysis was performed in R environment with dedicated pipelines.
Results. Data processing and the application of appropriate filtering criteria showed an average of analyzable probe sets of 440,085 in CT and 602,874 in PB. In the two different type of specimens 20,341 were found to be common features, whereas 3 and 562 symbols were unique in CT and PB, respectively. The Gene Ontology (GO) enrichment analysis allowed the identification of the biological processes, common and peculiar, in CT and PB, indicating that peripheral and local mechanisms of damage and response to damage are present in both. The significance analysis of microarrays, according to different outcomes and GO analysis, brought into focus 221 significant biological processes associated with poor outcome according to mRS in CT, and 27 terms associated with 24h edema in PB. Among significant terms in CT, those associated with regulation of neutrophil mediated immunity and activation play a crucial role. Concerning PB, particularly significant enriched terms were associated with regulation and activation of transcriptomes of cells.
Conclusions. Our results provided interesting insights into the mechanisms underlying the AIS and the response to treatments. In particular, the analysis of CT and PB gene expression profiles, differentially expressed probe sets and their biological processes alterations according to stroke outcomes, has not only confirmed and extended several known pathophysiological mechanisms, but also suggested novel pathways to be explored that may provide an important starting point for expanding knowledge on this cryptic disease.

SPHINGOSINE-1-PHOSPHATE (S1P) RECEPTORS AND CHOLESTEROL TRANSPORTERS EXPRESSION IN MACROPHAGES

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Background and Goals. Atherosclerosis is a chronic multifactorial disease characterized by an accumulation of cholesterol in arterial macrophages. The bioactive lipid sphingosine-1-phosphate (S1P) acts as an extracellular and intracellular signaling molecule regulating the immune, cardiovascular and nervous system. Several lines of evidence point to a crucial role for the S1P/S1P-receptor (S1PR) axis in cancer and in chronic inflammatory diseases, such as atherosclerosis. In vivo studies have shown that pharmacological stimulation of S1PR can exert atheroprotective effects. Fingolimod (FTY720, Gilenya®), an unselective S1PR modulator, was the first oral therapy approved for relapsing–remitting multiple sclerosis. In animal models, fingolimod can reduce the progression of atherosclerosis. We explored the effects of S1P/S1PR stimulation on the expression of cholesterol transporters in macrophages in vitro.
Materials and Methods. Murine macrophages were cultivated under cholesterol normal or loading (acetylated LDL, AcLDL) conditions and exposed or not to different concentrations of S1PR modulators. The expression of target genes and proteins, such as ABCA1, ABCG1 and SR-BI, in macrophages, was evaluated by real-time qPCR and Western blot.
Results. Treatment with S1PR modulators, particularly fingolimod, affected cholesterol transporters expression, regardless of AcLDL stimulation. Treatment specifically increased the expression of SR-BI, which is normally downregulated under cholesterol loading conditions.
Conclusions. Our preliminary observations suggest that the modulation of S1PRs may affect the expression of ABCA1, ABCG1 and SR-BI in macrophages. This effect may partially account for the atheroprotective role attributed to S1P/S1PR axis stimulation in the context of atherosclerosis.

FOUR VARIANTS IN CREB3L3 GENE ARE ASSOCIATED WITH DIFFERENT PHENOTYPES

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Aim. Dyslipidemia are characterized by an heterogeneous genetic background with several genes influencing different lipid fractions. The CREB3L3 gene was recently suggested as a non-canonical gene causative of hypertriglyceridemia (HTG). We aim to report our variants in CREB3L3 identified in patients with suspect of different diseases.
Patients and Methods. Four patients with different clinical suspects (2 with HTG) were screened by NGS using a large panel of 94 genes associated with dyslipidaemia, cholestasis and/or liver diseases. Variant presence was confirmed by Sanger sequencing.
Patient 1: a 9 years old girl with TG 8280 mg/dL, total cholesterol 834 mg/dL, HDL-cholesterol 26 mg/dL, acute lymphoblastic leukemia treated with PEG-arginase.
Patient 2: a 25 years old woman hospitalized urgently for acute pancreatitis with TG> 1500mg/dL.
Patient 3: a 5 years old girl analyzed to confirm the status of carrier for a variant causing Wilson's disease.
Patient 4: a 58 years old man with clinical suspect of FH.
Results. All patients resulted heterozygotes for rare variants in CREB3L3 gene: patient 1 for the nonsense variant c.724C>T - p.(Arg242*), previously reported as causative of HTG; patient 2 for the new missense variant c.742C>T - p.(Arg248Cys); patient 3 for the new splicing variant c.577-1G>A predicted to cause the loss of only 2 aminoacids p.(Gln193_Gln194del); Patient 4 for the missense variant c.700C>G - p.(Leu234Val) and LDLR gene (c.1135T>C - p.(Cys379Arg). Only the first variant was classified as likely pathogenic, whereas the last 3 variants were classified as USV. The last 2 variants were identified in patients without HTG.
Conclusions. Among 4 patients carrying different variants in CREB3L3 gene, only 2 patients showed HTG. Only one of these patients carried a variant classified as likely pathogenic. This report highlights that variants in CREB3L3 gene should be carefully evaluated for the association with HTG.

THE IMPACT OF LIPOPROTEIN(A) GENOTYPE ON THE PHENOTYPE OF INDIVIDUALS WITH CLINICAL FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction. Evidence suggests that LPA genotypes, associated with elevated lipoprotein(a) [Lp(a)] levels, can result in a phenotype suggestive of clinical familial hypercholesterolemia (FH). This study aimed at determining the prevalence of LPA risk variants in FH individuals enrolled in the Italian LIPIGEN study, with (FH/M+) or without (FH/M-) a causative variant.
Methods. We selected adults (aged ≥18 years) with a clinical diagnosis of FH and with the genetic test performed in a centralized laboratory searching for possible causative variants in candidate genes and evaluating the two common Lp(a)-raising single nucleotide polymorphisms rs3798220 and rs10455872. A Lp(a) genetic score was calculated for each participant by summing the number risk-increasing alleles. We compared baseline lipid levels and clinical variables by presence of FH causative mutations and LPA genotype.
Results. A total of 930 FH/M+ and 765 FH/M– patients were identified. Among them, 10.2% and 21.0% were characterized by one or two copies of either rs10455872 or rs3798220, respectively. FH/M- subjects had higher levels of Lp(a) than FH/M+ patients (median values 41 mg/dL [9-103] vs 19 mg/dL [8-41], p<.0001), with increasing Lp(a) concentrations among subjects with the same FH genetic background based on increasing value of Lp(a) genetic score. The adjustment of LDL-C levels based on lipoprotein(a) concentration reduced from 68% to 42% the proportion of subjects with LDL-C level ≥190 mg/dL. Overall, in the 4.6% of clinically diagnosed FH patients, the phenotype was not explained by a monogenic or polygenic aetiology, but genotype associated with high Lp(a) levels emerged as the sole culprit.
Conclusion. Our study supports the importance of measuring lipoprotein(a) in patients with familial hypercholesterolemia to improve the diagnosis and the prediction of cardiovascular risk. Subjects in whom hypercholesterolaemia is driven by high Lp(a) values, would benefit more from therapies targeting this protein.

THE SPECTRUM OF FATTY LIVER DISEASE AND THE ROLE OF GENETIC, METABOLIC AND LIFESTYLE FACTORS ASSOCIATED WITH ITS SEVERITY IN HETEROZYGOUS APOLIPOPROTEIN B-RELATED FAMILIAL HYPOBETALIPOPROTEINEMIA

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Introduction and Aims. Heterozygous apolipoprotein B (APOB)-related familial hypobetalipoproteinemia (FHBL) is a genetic disorder characterized by low levels of LDL cholesterol and apoB owing to disrupted secretion of apoB-containing lipoproteins. Patients with heterozygous APOB-related FHBL are prone to develop fatty liver disease (FLD) that has been anecdotally associated with the risk of progression toward cirrhosis. However, the actual prevalence of and the factors associated with severe FLD in APOB-related FHBL patients still remain to be elucidated. Our study aims to characterize the spectrum of FLD and to determine the association of genetic, metabolic and lifestyle factors with the severity of FLD in a single cohort of heterozygous APOB-related FHBL patients.
Methods. 21 adults with genetically-proven heterozygous APOB-related FHBL (men 57.1%, age 51[23-74] years) were consecutively enrolled in the Lipid Clinic in Modena. Genetic and clinical data were retrieved. The presence and severity of liver steatosis was evaluated by liver ultrasonography; measurement of liver stiffness with ultrasound elastography techniques (Fibroscan® and 2-dimensional shear wave elastography (2D-SWE)) was performed. A subgroup of patients underwent liver biopsy; histological presence of nonalcoholic steatohepatitis (NASH) and staging of fibrosis were defined according to Kleiner's criteria. Patients were classified as carriers of significant fibrosis if Fibroscan® and/or 2D-SWE yielded liver stiffness results ≥8Kpa and/or histological fibrosis stage was ≥F2. Lifestyle habits were evaluated by self-administration of three-day food diary, Sofi's Mediterranean diet adherence score and international physical activity questionnaires.
Results. 4 patients had APOB missense or intronic splice-site variants and 17 patients were carriers of APOB truncating mutations, of whom 5 determine the synthesis of protein longer than apoB-48 and 12 shorter than apoB-48. The prevalence of abdominal obesity, arterial hypertension, type 2 diabetes mellitus (T2DM) and metabolic syndrome was 57.1%, 28.6%, 14.3% and 14.3%, respectively. 16 patients (76.2%) had liver steatosis at ultrasound, whose grade was mild-to-moderate in 10 patients and severe in 6 patients. The presence of any grade of liver steatosis was significantly associated with apoB length: only 1 out of 5 patients (20%) carrying truncated apoB longer than apoB-48 vs. 11 out of 12 subjects (91.7%) carrying truncated apoB shorter than apoB-48 showed liver steatosis (p=0.010). However, the severity of liver steatosis was not associated with apoB length. The main factors significantly associated with the presence of severe steatosis were indices of adiposity (BMI, p=0.036; waist circumference, p=0.006), glucose homeostasis (fasting glucose, p=0.008; HbA1c, p=0.045) and insulin resistance (HOMA-IR, p=0.018), and markers of liver injury (GOT, p=0.036; GPT, p=0.011). Of note, the presence of severe steatosis was significantly associated with significant fibrosis (p=0.031).4 patients had liver stiffness ≥8Kpa, 1 of which with overt cirrhosis. 5 out of 6 patients submitted to liver biopsy showed NASH with significant liver fibrosis (4 with F2 and 1 with F3 stage). In total, significant fibrosis according to liver stiffness and/or histology was present in 6 patients (28.6%). Significant fibrosis was not associated with APOB length, but was significantly associated with metabolic comorbidities (abdominal obesity, p=0.019; insulin resistance, p=0.004; T2DM, p=0.015), platelet count (p=0.003) and markers of liver injury (GPT, p=0.029; GGT, p=0.045).With regards to lifestyle, few patients were adherent to Mediterranean diet (16.7%) and most of them reported unhealthy dietary habits with excessive fat intake (80%). Moreover, 23.8% of patients were inactive.
Conclusions. FLD affect the majority of patients with heterozygous APOB-related FHBL, especially when apoB length is shorter than apoB-48. However, the severity of liver steatosis and fibrosis seems to be influenced by metabolic comorbidities, rather than by APOB mutations per se. Efforts to promote healthy lifestyles and prevent obesity and diabetes should be made in order to avoid FLD progression.

HDAC INHIBITION PROMOTES OSTEOGENIC DIFFERENTIATION IN VALVE INTERSTITIAL CELLS

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Background. Calcific aortic valve disease (CAVD) is the most common valvulopathy in the Western world, but the pathophysiological processes involved in the disease are still poorly understood. The progression of CAVD depends mainly on the differentiation of valve interstitial cells (VICs) towards an osteogenic phenotype. Valproic acid (VPA), an inhibitor of histone deacetylases (HDAC), has been shown to promote the osteogenic differentiation of different cell types, but evidence is lacking on its potential involvement in CAVD.
Aim. This research project aims to determine the potential effects of VPA and HDAC inhibition in isolated VICs.
Methods. Isolated human VICs were treated with 5 mM VPA alone or in an osteogenic medium (OM) for 12 days. At the end of the treatment, proteins and RNA were extracted for western blotting analysis and gene expression studies (RT-PCR). The activity of alkaline phosphatase (ALP) and calcium deposition were quantified through colorimetric assays.
Results. Isolated VICs treated with VPA showed an increase in ALP activity compared to the control group, which became statistically significant ($p<0.05$) when the cells were in OM. A significant overexpression of osteogenic markers (such as ALP and BMP2) and IL-6 was detected when VICs were treated with OM and/or VPA. The supplementation of VPA to the OM also increased calcium deposition in isolated VICs. Moreover, the protein expression of acetylated histone H3 was significantly increased in an OM supplemented with VPA, due to VPA-mediated HDAC inhibition.
Conclusions. These preliminary data suggests that VPA induces the osteogenic differentiation of VICs, probably through HDAC inhibition. Further studies are needed to confirm these findings and understand whether the pharmacological modulation of HDAC may represent a therapeutic strategy for CAVD.

METABOLIC AND CARDIAC MORPHO-FUNCTIONAL IMPROVEMENTS AFTER PCSK9 INHIBITORS ADMINISTRATION

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Background. Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) play a key role in cholesterol's metabolism regulation through the degradation of low-density lipoprotein receptor (LDL-R). Recent studies suggest its possible role in cardiovascular (CV) diseases, promoting vascular inflammation, reactive oxygen species generation and atherosclerotic plaque formation. Furthermore, higher TG/HDL ratio has recently emerged as a marker of increased atherosclerotic extension and it can identify subjects with higher CV risk profile. Global longitudinal strain (GLS), global myocardial work efficiency (GWE), reservoir (PALS) and pump (PACS) atrial function evaluated by speckle-tracking echocardiography are able to identify early subclinical ventricular and atrial cardiomyopathy. In the last years, PCSK9 inhibitors have been introduced as innovative therapies for LDL plasma levels' reduction, showing strong CV protection. The aim of our study was to investigate, at baseline and after 6-months follow-up, PCSK9 inhibitors effects in patients with established coronary disease who were statin-intolerant and/or not reaching the target of LDL-C <55 mg/dl using the maximum tolerated drug dosage.
Materials and Methods. We enrolled 30 patients (24 males and 6 females, mean age 66±8 years), 97% showed arterial hypertension, 70% chronic kidney disease, 67% polidistrectual atherosclerosis, 50% type 2 diabetes mellitus (T2DM) and 20% chronic heart failure NYHA class II-III. All patients underwent main anthropometric and hemodynamic parameters evaluation, biochemical analysis, oxidative stress markers assessment, and advanced echocardiogram at baseline and after six months of therapy. The serum values of oxidative stress marker (NOX-2) and platelets activation (Sp-selectin) were assessed with ELISA sandwich. Echocardiographic recordings were performed using an E-95 Pro ultrasound system (GE Technologies, Milwaukee, Wisconsin, USA) using a 2.5 MHz transducer.
Results. There were no significant differences regarding systolic blood pressure (SBP), heart rate and glycaemia after six month of therapy. As expected, lipid profile was greatly improved in all the subjects, reaching the target of LDL-C <55 mg/dl. We obtained a statistically significant reduction of total-cholesterol (Δ=-32%, p<0.0001), LDL-C (Δ= -60%, p<0.0001), TG (Δ=-26%, p<0.0001), TG/HDL ratio (Δ= -27%, p<0.0001); an increase of HDL-C (Δ=+9%, p=0.001) and an improvement of glomerular filtrate evaluated by CKD-EPI (Δ=+6%, p=0.007). We observed a statistically significant reduction of NOX-2 (Δ=-27%, p<0.0001) and Sp-selectin (Δ=-36%, p<0.0001). Concerning echocardiographic parameters, we obtained a statistically significant increase of PALS (Δ= +16.9%, p<0.0001), PACS (Δ=+21%, p<0.0001), GWE (Δ=+9.7%, p<0.0001), GLS (Δ=+24%, p<0.0001) and a statistically significant reduction of global wasted work (GWW) (Δ=-15%, p<0.0001), left atrial volume index (LAVI) (Δ= - 8%, p<0.0001) and E/e' ratio (Δ= -19%, p<0.0001) respectively. The linear correlation analysis showed that ΔPACS was significantly and inversely correlated with ΔTG/HDL (r=-0.406, p=0.013) and ΔNOX-2 (r=-0.416, p=0.011); ΔPALS was significantly and inversely correlated with TG/HDL (r=-0.473, p=0.004) and ΔNOX-2 (r= 0.435, p=0.008); ΔE/e' was significantly and directly correlated with ΔTG/HDL (r=0.654, p<0.0001) and ΔNOX-2 (r=0.438, p=0.008); ΔGWE was inversely correlated with ΔNOX-2 (r=-0.422, p=0.01).Our study demonstrated for the first time that PCSK9 inhibitors are able to reduce left ventricular filling pressure, to increase atrial function (reservoir and pump) and global cardiac performance. Furthermore, PCSK9 inhibitors are able to increase CKD-EPI after six months of treatment in high CV risk population. Our results could be partially explained with a reduction of oxidative stress markers, inflammation and cardio-lipotoxicity, probably linked to a modulation of PCSK9's heart expression and its toxic effect on CV and renal function. In addition, we observed a TG/HDL ratio's reduction related to cardio metabolic and lipid profile improvement. Further studies are necessary to better investigate systemic benefit in a larger population with longer follow-up.

BLACK GARLIC AND POMEGRANATE STANDARDIZED EXTRACTS FOR BLOOD PRESSURE IMPROVEMENT: A NON-RANDOMIZED DIET-CONTROLLED STUDY

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Recently released position papers by the European Society of Hypertension (ESH) and the Italian Society of Hypertension (SIIA) provide therapeutic recommendations for the use of nutraceuticals in the management of high blood pressure (BP) and hypertension, opening up new perspectives in the field. This not-randomized diet-controlled clinical study aimed to evaluate if daily dietary supplementation with back garlic and pomegranate (namely SelectSIEVE® SlowBeat) could advantageously affect BP in individuals with high-normal BP or stage I hypertension. Enrolled subjects were adhering to a Mediterranean DASH (Dietary Approaches to Stop Hypertension) diet for two weeks before deciding whether to continue following Mediterranean DASH diet alone or in association with SelectSIEVE® SlowBeat. At the end of the study, dietary supplementation with SelectSIEVE® SlowBeat was associated with significant improvement in systolic blood pressure (SBP) and diastolic blood pressure DBP compared to baseline. SBP improved also in comparison with control. In conclusion, the study shows that dietary supplementation with extracts from black garlic and pomegranate safely exert significant improvements in BP in healthy individuals adhering to a Mediterranean DASH diet.

EFFICACY AND SAFETY OF LOMITAPIDE IN FAMILIAL CHYLOMICRONAEMIA SYNDROME

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Background and aims. Familial chylomicronaemia syndrome (FCS) is a rare autosomal recessive disorder, resulting in elevated triglycerides (TGs), abdominal pain and pancreatitis. Treatment options are limited. Lomitapide, a microsomal triglyceride transfer protein inhibitor, is approved for the treatment of homozygous familial hypercholesterolaemia. Whether its therapeutic use may be extended to FCS remains unknown. The aim of this study was to evaluate the efficacy and safety of lomitapide in adult patients with FCS.
Methods. The open-label, single-arm 'LOCHNES' study of lomitapide in FCS enrolled patients >18 years with genetically confirmed FCS, elevated fasting TG ≥750 mg/dL and history of pancreatitis. Patients were administered lomitapide to maximum tolerated dose for 26 weeks. The primary endpoint was the percent change in TGs from baseline to Week 26.
Results. Eighteen patients enrolled with median baseline TG levels 1803.5mg/dL (97.5% CI, 1452-2391 mg/dL). At Week 26, median fasting TGs reduced to 305mg/dL (97.5% CI 219-801mg/dL; 70.5% reduction); median lomitapide dose was 35 mg/day; 13 patients achieved TGs ≤750 mg/dL. Adverse events were mild-to-moderate and mainly related to gastrointestinal tolerability. Liver imaging at baseline and Week 26 revealed hepatic fat increases from median 12.0% to 32.5 %, while median hepatic stiffness remained normal. No patient experienced acute pancreatitis or severe abdominal pain during lomitapide treatment.
Conclusions. Lomitapide is effective and well tolerated in reducing TGs in FCS patients with a history of pancreatitis. Larger studies are warranted to determine lomitapide effectiveness in FCS.

DIETARY CHOLINE SUPPLEMENTATION WORSENS ATHEROSCLEROSIS DEVELOPMENT AND MODULATES MULTIPLE METABOLIC PATHWAYS IN EKO MICE

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Background & Aim. Scientific evidence revealed that there is a positive correlation between increased risk of cardiovascular events and plasma levels of TMAO, a molecule originating from the degradation of dietary choline by the gut microbiota. This study was aimed at investigating whether dietary choline affects additional metabolic pathways besides that leading to TMAO production.
Methods. Ten-week-old EKO female mice were fed for 16 weeks two standard rodent diets differing for a low (0.09%) or high (1.2%) choline content. Atherosclerosis development was quantified at the aortic sinus and targeted plasma metabolomic was performed. Furthermore, hepatic gene expression of selected target genes was analyzed by qPCR.RESULTS. Confirming previous observations, high choline intake was associated with greater atherosclerosis development and increased plasma levels of TMAO. In contrast, despite the different dietary content, plasma choline concentration did not differ between the two groups. Likewise, cholesterolemia and triglyceridemia were not significantly affected by choline intake. Interestingly, high choline feeding was associated with lower plasma levels of the thiol group-containing amino acid homocysteine and a concomitant increase of its related metabolites, methionine, sarcosine and carnitine. In agreement with the observed metabolomic changes, EKO mice fed high-choline diet displayed a significant increase in the expression of Aldh7a1, Slc44a1, Sardh and Gnmt in liver, together with a trend towards a higher expression of Chdh, Bhmt and Dmgdh.
Conclusions. Taken together, our data confirm that an increased dietary intake of choline worsens atherosclerosis burden and leads to increased plasma levels of TMAO. Interestingly, choline intake not only influences the choline-TMAO pathway, but also modulates metabolic processes affecting methionine, sarcosine, glycine levels, as well as homocysteine plasma concentrations. These observations shed a new light on the scenarios through which dietary choline might influence the development of atherosclerosis and modify cardiovascular risk.

IMPACT OF OPA1 AND MITOCHONDRIAL DYNAMICS ON SYSTEMIC LIPID METABOLISM AND ATHEROSCLEROSIS

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Aims. Mitochondria, in eukaryotic cells, are one of the principal organelles involved in cellular metabolism, energy generation, calcium homeostasis, hormones, sterol synthesis and bile acids (BAs) production. Mitochondria continuously undergo biogenesis, fusion, fission and mitophagy, maintaining a continuous balance between all forms. On these premises, we test the impact of OPA1, an essential protein of the inner mitochondria membrane fusion, on mitochondrial interaction with other organelles and the following impact on lipid metabolism and the development of metabolic dysfunction and atherosclerosis.
Methods. OPA1 liver KO (Opa1LKO) on HFD and Opa1LKO, OPA1 He male mice, OPA1 Tg on LDLR Ko background were fed with Western type diet (WTD) respectively for 12 weeks and 20 weeks. Inverse calorimetry, ITT, GTT, Lipid Tolerance Test (LTT) were performed, and paraffin-embedded tissues were used for histological analysis. Frozen tissues were further used for integrated OMICs analysis.
Results. Opa1LKO mice display altered systemic metabolism and reduced body weight due to altered lipoprotein circulation without alteration on muscle functionality. Opa1LKO mice altered bile acid production, as showed by RNAseq and proteomics data, leading to the manifestation of cholestasis and the consequent hepatic fibrosis. Opa1LKO mice show an improved glycemic profile, reduced liver steatosis and a reduction in circulating triglycerides levels following LTT. In line on LDLR KO background, Opa1 hepatocyte deficiency is associated with reduced lipoprotein amount and OPa1 overexpression is therefore associated with increased lipoprotein metabolism. Despite this increased lipoprotein metabolism, no differences were observed in atherosclerotic plaque development.
Conclusion. Hepatic Opa1 deficiency protects mice from HFD-induced metabolic dysfunction resulting in a reduction of lipid metabolism as a consequence of an alteration in bile acids production. OPA1 systemic modulation despite major alteration on lipoprotein metabolism is not strongly affecting atherosclerotic plaque development.

EFFICACY OF LONG TERM TREATMENT WITH EVINACUMAB IN FAMILIAL HOMOZYGOUS HYPERCHOLESTEROLEMIA (HOFH): FROM TRIAL TO REAL WORLD EXPERIENCE

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Background. Homozygous Familial Hypercholesterolemia (HoFH) is a genetic disorder, characterized by absent (null-null) or impaired (non-null) LDL-receptor activity, resulting in a rimarkable increase of low-density lipoprotein cholesterol (LDL-C), early onset coronary atherosclerosis, and premature death; thus requires aggressive LDL-C-lowering to prevent complications. Traditional therapeutic strategies, such as lifestyle modifications, invasive treatment, as apheresis and drugs to reduce LDL cholesterol, are insufficient to achieve the objectives of ESC 2019 guidelines in these patients. Evinacumab, a human monoclonal antibody inhibitor of angiopoietin-like protein 3 (ANGPTL3), is the latest lipid-lowering drug, which received FDA approval in February 2021(exclusively in patients with HoFH) in addition to other lipid-lowering therapies, after a phase III trial, ELIPSE HoFH.
Material and Methods. Seven of our patients (3 males and 4 women) with HoFH, who participated in the ELIPSE trial, continued intravenous infusion of evinacumab (with compassionate use) at 15 mg / kg of body weight over at 60 minutes once monthly from October 2021 to date.
Results. In line with the data of the trial, after 12 months of treatment, our real word experience confirms a stable reduction of LDL-C (from 277 mg/dl to 92 mg/dl , p 0.002), triglycerides((from 80 mg/dl to 34 mg/dl , p 0.18) and HDL (from 43.2 mg/dl to 34.1 mg/dl , p 0.001) and at the same time the absence of adverse events.
Conclusions. The significant reduction of LDL-C and the need for new treatment options for HoFH, in the absence of side effects, make evinacumab an important step in the therapy of HoFH, although high costs and intravenous administration still limit its use and approval by AIFA.

INVESTIGATING THE EFFECT OF DIABETIC CONDITIONS ON THE PROGRESSION OF CALCIFIC AORTIC VALVE DISEASE

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Background. Calcific aortic valve disease (CAVD) is the most common heart valve disease. To date, no effective pharmacological therapy has proven to halt or delay its progression. Diabetic subjects are at higher risk of developing cardiovascular complications, including CAVD. Moreover, diabetes contributes to the progression of CAVD, but the pathophysiological mechanisms are still not completely understood.
Aim. This research project aims to assess the molecular mechanisms leading to diabetic CAVD. In particular, we investigated the effects of a high glucose treatment in isolated VICs and of hyperglycemic conditions on animal models of CAVD.
Methods. LDLr-/- and LDLr-/-:ApoB100/100 mice were fed with either a diabetogenic or control diet for 6, 12 and 26 weeks. The aortic valves were collected for RNA sequencing followed by gene expression analysis. For the in vitro system, non-human primate VICs were treated with low- or high-glucose culture media (5.5 mM or 25 mM, respectively) and inorganic phosphate (Pi; final concentration 2.6 mM) to induce their osteogenic differentiation. After 5 days of treatment, the total RNA was extracted for gene expression analysis (RT-qPCR) and calcium deposition was determined through a colorimetric assay.
Results. Based on our findings, hyperglycemic conditions downregulate cardiogenic pathways and cardioprotective genes (including GATA4, TBX5, NPPA, and NKX2-5) in the aortic valves of LDLr-/- and LDLr-/-: ApoB100/100 mice. Moreover, the diabetogenic diet promotes the expression of genes involved in inflammatory and immune processes, accelerating the progression of CAVD.In our in vitro model, a high glucose treatment alone does not directly affect calcium deposition or the osteogenic differentiation of isolated VICs. In addition, elevated glucose levels dysregulate cardiogenic genes (such as NKX2-5 and TBX5) in our in vitro model. Taken together, these findings suggest that further mechanistic studies are warranted and may lead to the discovery of novel potential targets for diabetic CAVD.

AN UNTARGETED LIPIDOMIC ANALYSIS REVEALS DEPLETION OF SEVERAL PHOSPHOLIPID CLASSES IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA ON TREATMENT WITH EVOLOCUMAB

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Background. Familial hypercholesterolemia (FH) is caused by mutations in genes involved in low-density lipoprotein cholesterol (LDL-C) metabolism, including those for pro-protein convertase subtilisin/kexin type 9 (PCSK-9). The effect of PCSK-9 inhibition on the plasma lipidome has been poorly explored.
Methods. Using an ultra-high-performance liquid chromatography-electrospray ionization-quadrupole-time of flight-mass spectrometry method, the plasma lipidome of FH subjects before and at different time intervals during treatment with the PCSK-9 inhibitor Evolocumab was explored.
Results. In 25 FH subjects, heterozygotes or compound heterozygotes for different LDL receptor mutations, untargeted lipidomic revealed significant reductions in 26 lipid classes belonging to phosphatidylcholine (PC), sphingomyelin (SM), ceramide (CER), cholesteryl ester (CE), triacylglycerol (TG) and phosphatidylinositol (PI). Lipid changes were graded between baseline and 4- and 12-week treatment. At 12-week treatment, five polyunsaturated diacyl PC, accounting for 38.6 to 49.2% of total PC at baseline; two ether/vinyl ether forms; seven SM; five CER and glucosyl/galactosyl-ceramide (HEX-CER) were reduced, as was the unsaturation index of HEX-CER and lactosyl-CER (LAC-CER). Although non quantitative modifications were observed in phosphatidylethanolamine (PE) during treatment with Evolocumab, shorter and more saturated fatty acyl chains were documented.
Conclusions. Depletion of several phospholipid classes occurs in plasma of FH patients during treatment with the PCSK-9 inhibitor Evolocumab. The mechanism underlying these changes likely involves the de novo synthesis of SM and CER through the activation of the key enzyme sphingomyelin synthase by oxidized LDL and argues for a multifaceted system leading to vascular improvement in users of PCSK-9 inhibitors.

A CASE OF SEVERE MULTIFACTORIAL CHYLOMICRONEMIA SYNDROME TREATED WITH VOLANESORSEN, ARE THESE PECULIAR CASES NEGLECTED AND UNDERTREATED?

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Background. Chylomicronemia can be either monogenic (Familial Chylomicronemia Syndrome or FCS) or multifactorial (Multifactorial Chylomicronemia Syndrome or MCS). Unlike FCS, MCS is usually asymptomatic and more responsive to pharmacological treatment, although there are cases that are in a grey zone between the two conditions. Our goal was to detect new cases of FCS and patients with MCS with a clinical phenotype similar to FCS among a wide cohort of patients.
Materials and Methods. For our single centre retrospective study based in Turin (Italy) we extracted data from a wide laboratory database, ranging from 2016 to 2020. Data from patients with triglycerides>885 mg/dl in more than one detection were collected in order to rule out secondary non-metabolic conditions causing hypertriglyceridemia, such as renal failure and neoplasms. Remaining subjects were subjected to a phone interview and a visit to fill out Moulin and Lipigen scores. Willing patients were then sent to a salivary genetic test for known genes for FCS.
Results. Starting from a database of 563,765 blood tests for triglycerides, 10 patients were selected for the phone interview, clinical evaluation and score assessment. 4 of them were subjected to the genetic test. One patient resulted double heterozygous for variants of LPL and APOA5 genes. This subject had a high Moulin and Lipigen score and a history of hardly manageable hypertriglyceridemia and recurrent pancreatitis. Since the subsequent start of a treatment with volanesorsen in February 2022, triglycerides have been in range and pancreatitis events have not occurred yet.
Conclusions. Under the definition of MCS a broad spectrum of conditions are included, and some are "clinically FCS" but not "genetically FCS". Probably It is now time for a stratification of clinically significant MCS patients and for gathering data in order to possibly expand the indications for volanesorsen to these subjects.

ROLE OF OPTIC ATROPHY 1-MEDIATED MITOCHONDRIAL DYNAMICS IN KUPFFER CELLS ON SYSTEMIC METABOLISM

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Aim. Kupffer cells are liver tissue-resident macrophages essential to liver pathophysiology as they play a critical role in the innate immune response. They are able to influence immune activity but also systemic metabolism through the synthesis of proteins with metabolic activity (i.e., CETP). OPA1 (Optic Atrophy 1) is a dynamin-related protein located in the inner mitochondrial membrane, which has a pro-fusion activity and thus is one of the main leaders involved in mitochondrial dynamics, since it regulates cristae morphology and consequently also oxidative phosphorylation. This project aims to study the role of mitochondrial dynamics within Kupffer cells and its possible effects on systemic metabolism by exploiting mice selectively lacking OPA1 in Kupffer Cells.
Methods. OPA1 flox/flox Clec4F-Cre+ and control mice were fed a standard diet for 22 weeks. The metabolic phenotype was assessed by indirect calorimetry through metabolic cages. Blood and liver were collected for immunophenotyping by flow cytometry analysis. Plasma total cholesterol and triglycerides dosages were performed. Liver histology was assessed with specific tissue stainings.
Results. OPA1 flox/flox Clec4F-Cre+ showed less energy expenditure (-9.44%; $p<0.05$), less oxygen consumption (-9.44%; $p<0.05$) and less carbon dioxide production (-9,48%; $p<0.01$), despite an increase in movement (+26.6%) flox/flox Clec4F-Cre- control mice. Systemic immune profile was similar, while the percentage of Kupffer Cells in the liver was reduced in OPA1 flox/flox Clec4F-Cre + compared to OPA1 flox/flox Clec4F- Cre - (-25% $p<0.05$). No significant differences in cholesterol and triglyceride levels were observed between the two groups as well as in liver histology.
Conclusions. Our data suggest that OPA1-mediated alteration of mitochondrial dynamics affects Kupffer cells and impacts systemic energy phenotype. To unveil whether this pathway plays a role in liver diseases, ongoing studies are investigating the role of OPA1 deficiency in KC in models of diet-induced obesity.

DIAGNOSIS AND MANAGEMENT OF FAMILIAL CHYLOMICRONEMIA SYNDROME IN A NEWBORN GIRL

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Introduction. Familial chylomicronaemia syndrome (FCS) is a rare, severe, monogenic, recessive disorder caused by loss-of-function mutations in both alleles of one or more of the genes that control the intravascular lipolytic cascade of triglyceride (TG)-rich lipoproteins. FCS is characterized by severe hypertriglyceridemia (TGs >10 mmol/L - 886 mg/dL) due to the accumulation of chylomicrons during fasting. FCS patients also develop eruptive xanthomas, lipemia retinalis, recurrent abdominal pain, acute and/or recurrent pancreatitis, hepato-splenomegaly and memory loss. The standard of care of FCS is based on a strict dietary regimen with <10% of energy from fat and supplementation with medium-chain TGs. Long-term adherence to this diet is poor.
Material and Methods. Here we describe a female neonate, born at term of uneventful pregnancy from healthy non-consanguineous parents. At 6th day of life a capillary blood micro-sample showed lactescent serum. TG levels were 3632 mg/dl and the clinical suspicion of FCS was made. A Next Generation Sequencing (NGS) custom panel was used to analyze candidate genes involved in the pathways of triglyceride synthesis and metabolism. Implementation of a nutritional management plan was started.
Results and Conclusions. NGS analysis allowed to identify a previously described homozygous pathogenetic mutation in LPL gene (c.829G>A p.Asp277Asn). Genetic molecular cascade screening allowed to identify the mutation in heterozygosity in both parents. Milk formula supplemented with medium chain triglycerides (MCT) oil, vitamins and oligoelements ensured an adequate intake of nutrients and TGs were stably <500 mg/dl over the weeks. At 6 months complementary feeding was introduced with a specific low-fat diet. Feeding has been well tolerated and TG levels have been as low as 339 mg/dl. In conclusion, early diagnosis and nutritional management of FCS in newborn are crucial to guarantee adequate growth and neuro-psycho-motor development and prevention severe complication.

PATHOGENICITY EVALUATION OF VARIANTS ASSOCIATED WITH FAMILIAL HYPERCHOLESTEROLEMIA: COMPARISON BETWEEN GUIDELINES

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Aim. Familial Hypercholesterolemia (FH) is the most frequent genetic disease caused by pathogenic variants in three main causative genes (LDLR, APOB and PCSK9) and characterized by high level of LDL-cholesterol and higher cardiovascular risk. There are two forms of this disease: the heterozygous (HeFH) and the homozygous one (HoFH), characterized by the presence of one and two pathogenic variants, respectively. We aim to perform the pathogenicity evaluation of a large number of variants identified in our laboratory according to different guidelines.
Materials and Methods. The pathogenicity evaluation of 193 variants, identified in the laboratory from 2008 to 2022, was made comparing the ACMG's guidelines (Richards et al. 2015) with the most recent FH-specific suggestions (Chora et al. 2018 for APOB and PCSK9 and ClinGen - Chora et al. 2022 - for LDLR).
Results. Using the ACMG's guidelines for the classification of the 137 variants identified in LDLR, 18 are USV and 113 are pathogenic/likely pathogenic, while following the most recent suggestion 38 are USV and 92 are pathogenic/likely pathogenic. The different weight given to several functional assays, widely used to test the protein function, is the major determinant of classification changes for 13 variants. Some variants identified in patients with a clear HoFH phenotype and just in a single HeFH relative were reclassified as USV. No differences were observed between the different guidelines in the evaluation of the 45 variants identified in APOB and the 11 ones identified in PCSK9.
Conclusions. Despite new guidelines suggested criteria specific for the FH genetic features that are very useful for a standardization of pathogenicity evaluation, their application resulted in many variants classified as USV. These guidelines should be improved allowing to consider with more strength the available data, very few in case of the rarest variants.

THE IMPACT OF STATIN THERAPY ON IN-HOSPITAL PROGNOSIS OF PATIENTS AT HIGH-TO-VERY HIGH CARDIOVASCULAR RISK ADMITTED WITH COVID-19

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Background. Compelling evidence suggests that statins may reduce the risk of COVID-19-related complications through their multiple pleiotropic effects. This study aimed to assess the impact of preadmission statin therapy and its continuation upon hospitalization on clinical outcomes of patients at high-to-very high cardiovascular (CV) risk with COVID-19, as well as to assess the possible influence of preadmission statin therapy on endothelial function at hospitalization.
Methods. A cohort of hospitalized COVID-19 patients at high-to-very high CV risk was retrospectively enrolled. The association between statin therapy and either endothelial function, as assessed by brachial artery flow-mediated dilation (bFMD) at hospital admission, or the composite endpoint of intensive care unit (ICU) admission/in-hospital death was assessed through univariable and multivariable analyses.
Results. Among 342 enrolled patients (mean age 79 ± 11 years, males 60%), 119 (35%) were treated with statins prior to hospital admission whereas 223 (65%) were not. Upon hospitalization, 91 patients continued statin therapy, 28 patients discontinued it, and 3 patients introduced it de novo. Also, 25 (7%) patients were admitted to ICU, 75 (22%) patients died, and 92 patients (27%) met the composite endpoint of ICU admission/in-hospital death. At multi-adjusted Cox regression preadmission statin therapy was associated with up to a 70% reduced risk of ICU admission/in-hospital death (HR 0.296, 95% CI 0.161-0.541, p<0.001). Also, at different multi-adjusted linear regressions it was positively associated with bFMD at hospital admission. However, no longer freedom from ICU admission/in-hospital death emerged according to statin continuation versus discontinuation upon hospitalization (p= 0.674).
Conclusions. Statins may positively affect the prognosis of hospitalized COVID-19 patients at high-to-very high CV risk, possibly due to their endothelium-protective effects, at least in the early phases of infection. In the COVID-19 era, statins may have an adjunctive role against COVID-19 adverse outcomes in patients at high-to-very high CV risk.

DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMIZED CLINICAL TRIAL COMPARING THE EFFECT OF A COMBINED NUTRACEUTICAL CONTAINING RED YEAST RICE ASSOCIATED TO ARTICHOKE EXTRACT OR BERBERINE ON LIPID PROFILE AND LIVER PARAMETERS IN PATIENTS AFFECTED BY POLYGENIC HYPERCHOLESTEROLEMIA

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Background. Increasing evidence suggests that the combination of low-dose lipid-lowering nutraceutical compounds is an effective and safe tool to improve total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) in subjects affected by mild-to-moderate hypercholesterolemia.
Aim. To test comparatively and versus placebo the lipid-lowering efficacy of berberine and artichoke extract in combination with low dose of monacolins from red yeast rice.
Methods. A 3-arms, randomized, double-blind, placebo-controlled, parallel-groups clinical trial was carried out on 60 healthy adult volunteers with a diagnosis of polygenic hypercholesterolemia. Enrolled subjects were randomized to be treated with monacolins 2.8 mg + artichoke 200 mg [ATC group], monacolins 2.8 mg + berberis aristata 588 mg [BBR group] or placebo.
Results. After 8 weeks of treatment, all patients experienced a significant improvement in baseline TC, LDL-C, apolipoprotein B (Apo-B) (P<0.01 always) (ATC group: TC= -18.9%, LDL-C= -26.7%, Apo-B = -19.6%; BBR group: TC= -18.4%, LDL-C= -25.8%, apoB= -23.2%; placebo: TC= -6.2%, LDL-C= -8%, Apo-B= -8.4%). Observed LDL-C variations in actively treated subjects were statistically significant not only compared to baseline but also compared to placebo. Subjects in ATC and BBR group respectively reached significantly lower body mass index and improved baseline high-density lipoprotein cholesterol (HDL-C) and triglycerides levels. Finally, baseline waist circumference and the hepatic steatosis index significantly decreased in both ATC and BBR.
Conclusion. In our short-term trial, subjects affected by polygenic hypercholesterolemia experienced a significant improvement in several cardiovascular risk factors in both monacolin-berberine and monacolin-artichoke treated patients.

EFFECT OF DIETARY SUPPLEMENTATION WITH DIURIPRES® ON BLOOD PRESSURE, VASCULAR HEALTH AND METABOLIC PARAMETERS IN INDIVIDUALS WITH HIGH-NORMAL BLOOD PRESSURE OR STAGE I HYPERTENSION: THE CONDOR RANDOMIZED STUDY

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Our aim was to evaluate if a nutritional intervention with a dietary supplement (Diuripres®) containing magnesium, standardized extract of orthosiphon, hawthorn and hibiscus could positively affect blood pressure (BP), vascular health and metabolic parameters in 60 individuals with high-normal BP or stage I hypertension. Participants followed a low-fat low-sodium Medi-terranean diet for 4 weeks before being randomly allocated to 8-week treatment with 2 pills each day of either Diuripres® or placebo. Diuripres® significantly decreased systolic BP compared to placebo after 4 weeks (3.1±0.8 mmHg; p< 0.05) and more consistently after 8 weeks (3.4±0.9 mmHg; p<0.05). At 8-week follow-up, after correction for multiple testing, dietary supplementation with Diuripres® was associated with significant improvements in diastolic BP (-3.1±0.6 mmHg; p<0.05), aortic BP (-4.3±0.4 mmHg; p<0.05), and high-sensitivity C-reactive protein (hs-CRP; 0.04±0.01 mg/dL; p<0.05) in comparison with baseline. The reductions in diastolic BP (–3.8±0.7 mmHg; p< 0.05), aortic BP (-5.2±1.0 mmHg; p<0.05) and hs-CRP (-0.03±0.01 mg/dL; p< 0.05) were also significant compared to placebo. Therefore, our study show that dietary supplementation with Diuripres® may be useful in individuals with high-normal BP or stage I hypertension.

IMPACT OF ANTI-OESTROGEN THERAPY ON SERUM LEVELS OF LIPOPROTEIN(A) IN WOMEN: A SYSTEMATIC REVIEW AND META-ANALYSIS OF DOUBLE-BLIND PLACEBO-CONTROLLED CLINICAL STUDIES

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Endocrine-therapy for breast cancer is associated with an increased risk of developing atherothrombotic events, of which high serum levels of lipoprotein(a) (Lp(a)) are a well-recognized risk factor. The aim of our study was to systematically evaluate the effect on Lp(a) of different endocrine therapies for breast cancer in high-risk women. A systematic literature search was conducted in multiple electronic databases to identify the randomized, double-blind, placebo-controlled clinical studies on this topic. Effect size for changes in Lp(a) was expressed as mean difference (MD) and 95% confidence intervals (CI). Data were pooled from 10 clinical trials comprising 24 treatment arms, which included 2049 women (1128 women in the active-treated arms and 921 women in the control arms). Unexpectedly, meta-analysis of data suggested that anti-oestrogen therapy in women significantly reduced Lp(a) [MD= -5.92% (95%CI: -9.05%,-2.8%)]. Then, the increased risk of thromboembolic events in women undergoing endocrine-therapy for breast cancer does not appear to be mediated by an increase in Lp(a) serum levels.

A CASE OF SITOSTEROLEMIA: HEMATOLOGICAL DIAGNOSIS WITHOUT ABNORMAL LIPID PROFILE

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Background. Sitosterolemia is a rare genetic disorder, characterized by phytosterol accumulation in the blood due to mutations of ATP-binding cassette (ABC) transporters ABCG5 and ABCG8. The latter promote the secretion of phytosterol into the bile and the intestinal lumen. Subjects affected by sitosterolemia are almost asymptomatic, while elevated plasma low-density lipoprotein cholesterol (LDL-C) and accelerated atherosclerosis are often found. Other manifestations include hematologic problems such as hemolytic anemia with stomatocytosis and splenomegaly. The aim of this study is to describe a case of a 30-year-old Italian male with a diagnosis of sitosterolemia by hematological phenotype.
Material and Methods. The patient came to the attention of the Hematologists for microcytic anemia and splenomegaly. Hematologists, excluding the main causes of microcytic anemia, and performed tests that revealed the possible diagnosis of sitosterolemia. Then, he came to the Lipid Clinic of San Martino Hospital in Genoa without atherosclerotic process, as documented by carotid ultrasound, and with the following lipid profile: total cholesterol 140 mg/dL, high-density lipoprotein cholesterol 46 mg/dL, calculated LDL-C 73 mg/dL, triglicerides 104 mg/dL. Subsequently, genetic analysis and the sitosterols dosage were performed.
Results. Genetic tests of the patient found the following mutations: c.293 C>G, p.Ala98Gly and c.80 G>C, p.Gly27Ala of ABCG5. He also presented high plasma levels of Colestanol, Campesterol and β-sitosterol (1,71 mg/dL, 0,45 mg/dL and 0,40 mg/dL respectively). Therefore, patient was diagnosed with sitosterolemia, and Ezetimibe 10 mg therapy and low phytosterol diet was administered.After 4 months of therapy, we did not observe changes in the lipid profile while a reduction in plasma phytosterols was reported (Colestanol 1 mg/dL, Campesterol 0.34 mg/dL and β-sitosterol 0.35 mg/dL).
Conclusion. This case report confirms that sitosterolemia may not show elevated plasma LDL-C. The identified mutations, although described as benign in the literature, may be responsible for the proband's haematological findings.

EFFICACY OF DIFFERENT NUTRACEUTICAL COMBINATIONS CONTAINING MONACOLIN K AND BERBERINE ON LIPID PROFILE: THE EXPERIENCE OF THE OUTPATIENT LIPID CLINIC OF GENOA

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Background. The 2019 ESC/EAS guidelines for the management of dyslipidemia suggest nutraceutical treatment as adjuvant of lifestyle intervention to reach the LDL cholesterol (LDL-C) target in low cardiovascular risk. On June 2022, the European Union established a daily limit for all monacolins in supplements of less than 3 mg. The main focus of this study was to evaluate the efficacy in improving lipid profile of the new formulation of Normolip 5 Forte® ESI (NUT 1), composed of 2,2 mg of monacolin K and 500 mg of berberine; secondary, we aim to compare the efficacy of NUT1 with two other nutraceuticals with different composition produced by the same pharmaceutical company.
Material and Methods. This single-center open-label clinical trial included 90 patients with polygenic hypercholesterolemia not controlled by diet alone, referring to the Lipid Clinic of San Martino Hospital in Genoa. Patients were equally divided to receive NUT1 or a nutraceutical containing 2.2 mg of monacolin K without berberine (NUT2) or a supplement composed of 10 mg of monacolin K without berberine (NUT3). At baseline, anthropometric characteristics, lipid profile and risk score were evaluated. After 8-weeks intervention, all considered parameters were revaluated.
Results. After 8 weeks of treatment with NUT1, a significant reduction in total cholesterol (-13%, p<0.0001) and LDL-C (-18%, p<0.0001) was reported. Comparing the three different nutraceuticals, we observed that NUT1 effect on lipid profile was similar to the NUT2, with a LDL-C reduction of -17% (p<0,0001); instead, patients administered with NUT3 reported a greater reduction of LDL-C (-24%, p<0,0001) compared to NUT1.
Conclusion. This study confirms that the association of monacolin and berberine is effective in improve lipid profile; however, patients who received NUT2 achieved a similar LDL-C reduction compared to patients who received NUT1. Furthermore, a greater reduction of LDL-C was observed in subjects who received NUT3.

HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA IN CHILDREN: CASE REPORT

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B.L is a young boy born in January 2008, who underwent a dermatological examination in 2016 after the appearance of skin lesions in the popliteal fossa with no other symptoms associated. The dermatologist diagnosed cutaneous xanthomas and indicated the execution of a lipid profile. Baseline exams (October 2016) showed TC 539 mg/dl and LDL-C 452 mg/dl (HDL-C and TG in range), confirmed in a second lipid profile a few days later (LDL-C 511 mg/dl). He was therefore sent for a specialist consultation. The family history revealed a hypercholesterolemia in both the parents (mother, LDL 152 mg/dl, treated with atorvastatin, father, LDL 136 mg/dl, not treated) and in maternal grandfather and grandmother. No history of early cardiovascular disease. Remote physiological and pathological anamnesis was negative. Clinical examination revealed 3 little skin xanthomas (2-3 mm diameter) in the popliteal fossa, cardiovascular and other system examination was normal; his weight was 33,4 kg (75° pct), height 131 cm (50-75° pct) and BMI 18,5 kg/m^2 (75° pct, overweight). Other laboratory exams (thyroid profile, renal profile and fasting plasma glucose) were normal, as well as baseline AST, ALT and CK. Genetic examination (NGS) was performed and two different mutations were found (c.1048C>T: premature stop codon in exon 7 of LDL-R, c.1775G>A encoding for a defective LDL-R). The family segregation study revealed that the first one was inherited from the father and second one from the mother. A first step of therapy was started with physical activity and dietary recommendations (CHILD-2 and school menu changes), and statin therapy (starting with Simvastatin 20 mg/day). The other in-depth investigations were negative (echo Doppler and carotid intima-media-thickness, echocardiogram and ECG, stress test and computed tomography of coronary arteries). The therapy was gradually increased in the next months until the dosage of 40 mg of Rosuvastatin and 20 mg of Ezetimibe, with the achievement of an LDL-C of 210 mg/dl. In June 2020 Evolocumab 140 mg x2/month was started, later increased to 420 mg once a month and then 420 mg twice a month, reaching a LDL-C of 120 mg/dl. Drugs were always well tolerated, with normal follow-up exams. Familial hypercholesterolemia (FH) is often underdiagnosed and undertreated, particularly in children, since physical signs are rarely observed. Lipid profile screening would guarantee the early identification of these patients and genetic analysis should be performed on childhood patients with family history or clinical data very suggestive for FH. Risk factors can be identified in children and adolescents and early treatment can reduce LDL-C burden, improve endothelial function and attenuate the development of atherosclerosis, adding decades of healthy living.

THE GENETIC MANIPULATION OF HDL/APOA-I LEVELS IN MICE AFFECTS THE LIPID DEPOSITION IN PERIPHERAL ORGANS

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The reverse cholesterol transport is a multistep process whereby excess cholesterol is conveyed by HDL from the peripheral tissues to the liver for excretion. In this study, the impact of the genetic manipulation of HDL/apoA-I levels on lipid deposition in intestine, liver, kidney and adrenals was investigated. Mice with extremely low plasma HDL levels, deficient for both murine apoA-I and apoE (DKO), were compared with mice characterized by elevated HDL, deficient for both apoA-I/apoE, but overexpressing human apoA-I (DKO/hA-I). Mice, both female and male, were fed a standard rodent diet until one year of age. Plasma lipids were quantified by enzymatic methods. Intestine, liver, kidney and adrenal morphology was evaluated by light microscopy on frozen sections. Plasma total cholesterol concentration in DKO mice was comparable with that of wild-type mice and 3-fold lower than that observed in DKO/hA-I mice. Plasma HDL-C was almost absent in DKO mice and strongly elevated in DKO/hA-I mice. The H&E-stained sections did not reveal the presence of morphological alterations in the tissues analyzed: intestinal villi and crypts were regular, steatosis in liver parenchyma, as well as foam cells in renal glomeruli were absent and adrenal size was comparable in both genotypes. The neutral lipid-specific staining with Oil Red O showed instead interesting differences. The intestine did not exhibit HDL-mediated effects on lipid deposition. On the contrary, in the hepatic parenchyma, an increased accumulation of lipids around the centrilobular vein was observed in DKO/hA-I mice only. In addition, within the glomeruli and the adrenal cortex of DKO/hA-I mice, lipid accumulation was significantly higher than in DKO. On summary, although DKO mice are almost completely devoid of HDL and prone to atherosclerosis development, they do not exhibit signs of abnormal lipid accumulation in liver, kidney and adrenals, as in DKO/hA-I mice, characterized by elevated HDL levels.

STATIN-INDUCED AUTOIMMUNE MYOSITIS: A PROPOSAL OF AN "EXPERIENCE-BASED" DIAGNOSTIC ALGORITHM FROM THE ANALYSIS OF 69 PATIENTS

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Background. Statin-induced autoimmune Myositis (SIAM) represents a rare clinical entity that can be triggered by a prolonged statin treatment. Its pathogenetic substrate consists of an autoimmune-mediated mechanism, evidenced by the detection of antibodies directed against the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (anti-HMGCR Ab), target enzyme of statin therapies.
Objective. To evaluate the different features of SIAM cases described in literature and to extrapolate a diagnostic algorithm. Methods: We have analyzed the clinical data of 69 patients diagnosed with SIAM. Sixty-seven patients have been collected from the fifty-five available and complete case records regarding SIAM in the literature; the other 2 patients represent our direct clinical experience and they are described in detail as case records. From the analysis of the clinical features of 69 patients, we have constructed a proposal of an "experience-based" diagnostic algorithm for SIAM, in order to facilitate the diagnosis of nuanced SIAM clinical cases. The algorithm involves Anti-HMGCR antibody testing, musculoskeletal MR, EMG/EEG of upper-lower limbs and, where possible, the muscle biopsy.
Conclusion. We advise a close monitoring of symptoms during statin treatment and the employment of this algorithm to early recognize and treat adverse events such as SIAM, avoiding no longer reversible and sometimes fatal complications.

THE LIFETIME RISK OF ATHEROSCLEROTIC CARDIOVASCULAR DISEASE DOES NOT VARY BASED ON GENETICALLY DETERMINED OR CLINICALLY MEASURED LIPOPROTEIN(A) LEVELS

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Lipoprotein(a) (Lp(a)) concentration has been causally associated with increased risk of atherosclerotic cardiovascular disease (ASCVD). Whether ASCVD risk varies according to genotype determined or measured Lp(a) levels is still unknown.A total of 445,744 participants (mean age: 57.3 years; female sex: 54.3%) enrolled in the UK Biobank were included in this Mendelian randomization analysis. For each participant, we calculated the LPA genetic risk score by summing the number of risk-increasing alleles inherited at rs3798220 and rs10455872 variants. The primary outcome was the incidence of major coronary events (MCE), a composite of fatal or non-fatal myocardial infarction, or coronary revascularization. Using adjusted Cox proportional hazards models and Kaplan-Meier curves, we compared the cumulative lifetime risk of MCE among subjects with different LPA genotype and measured Lp(a) levels, expressed as hazard ratio (HR) and 95% confidence interval (95%CI).Participants with one copy of either rs10455872 or rs3798220 had a HR for MCE of 1.47 (95%CI, 1.42-1.51) compared with wild-type subjects (median Lp(a): 146.3 nmol/L and 13.6 nmol/L, respectively). Stratifying the population according to measured Lp(a) concentrations comparable to those observed for the genetic score, we found similar increased MCE risk for the same Lp(a) change (HR 1.47; 95%CI, 1.41-1.53). Moreover, even among subjects with the same LPA genotype, increasing quintiles of measured Lp(a) concentration were associated with a step-wise increase in MCE risk. Conversely, among subjects with different LPA genotype, but similar median Lp(a) concentrations, the lifetime risk of incident MCE was comparable.Our findings demonstrated that LPA genetic risk score and measured Lp(a) concentration provide comparable risk for incident MCE. However, since measured Lp(a) showed a wide range of values among individuals with the same genotype, with the risk changing accordingly, we emphasize the importance of measuring Lp(a) level in clinical practice to better identify patients at risk, regardless of genotype.

EVIDENCE FOR THE REFINEMENT OF THE DIAGNOSTIC APPROACH IN CHILDREN AND ADOLESCENTS AFFECTED BY FAMILIAL HYPERCHOLESTEROLEMIA: DATA FROM THE LIPIGEN STUDY

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Background. The high cardiovascular risk associated with familial hypercholesterolemia (FH) makes early identification crucial. However, the detection of FH subjects in childhood poses several issues. We aimed to highlight and quantify limits of a diagnosis of FH in childhood based on the presence of the typical features of FH, included in one of the most applied diagnostic tool, the Dutch Lipid Clinic Network (DLCN) score, and to suggest additional parameters to refine the FH diagnosis at young age.
Methods. From the LIPIGEN study, we selected 1188 (≥18years) and 708 (<18years) FH patients with a positive genetic test for heterozygous FH, with no missing DLCN parameters about physical examination and personal clinical history, and untreated LDL-C value available. The prevalence of the main FH features was compared between the two groups, and data about premature CHD also in second-degree family members were integrated in a paediatric sub-group (N=374).
Results. The lower prevalence of typical FH characteristics in children/adolescents vs adults was confirmed: tendon xanthoma 2.1% vs 13.1, arcus cornealis 1.6% vs 11.2%, respectively. No children presented clinical history of premature CHD or cerebral/peripheral vascular disease (in adults 8.8% and 5.6%, respectively). The presence of tendon xanthoma and/or corneal arcus as well as hypercholesterolemia in first-degree relatives were comparable among adults and children (18.7% vs 20.0% and 92.9% vs 93.5%, respectively). The prevalence of premature CHD in first degree relatives was significantly higher in adults compared to subjects under 18 years (38.9% vs 19.7%). Within the paediatric sub-group with data about second degree relatives (representative of the whole paediatric cohort), a premature CHD events in parents was reported in 63 of 374 subjects (16.8%), but the percentage increased to 54.0% extending the evaluation also in second degree relatives.
Conclusion. In children, the DLCN parameters are clearly less informative than in adults. A refinement of the diagnostic approach with a tailored data collection is needed for the diagnosis of FH at young age.

CORONARY ARTERY CALCIUM IS STRONGLY ASSOCIATED WITH PULSE WAVE VELOCITY AND LDL-CHOLESTEROL BURDEN IN SUBJECTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Background and Aims. Familial hypercholesterolemia (FH) is a genetic disorder characterized by high plasma levels of low-density lipoprotein cholesterol (LDL-C) and premature cardiovascular (CV) diseases. Coronary artery calcification (CAC) assessment and arterial stiffness measured as pulse wave velocity (PWV) are accurate in CV risk assessment, but so far data on HeFH are missing. Evaluation of aortic stenosis by Doppler echocardiography with markers of severity, aortic valve area (AVA) and mean gradient (MG) are still unresolved. In this study we evaluated CAC, PWV and the relationship between aortic valve calcium/stenosis severity in a HeFH cohort in order to improve risk stratification and therapy timing.
Methods. One-hundred genetically characterized HeFH subjects were recruited at our lipid clinic and CAC, PWV measurement and LDL-C burden calculation were assessed. Physiologic/structural determinants of aortic valve area (AVA)/mean gradient (MG) relationship associated with aortic stenosis were also analyzed.
Results. Mean age was 45±16 years. 25% of patients had hypertension;15% were in secondary prevention. On univariate analysis, we found strong positive correlations between CAC and both PWV ($r=0.52$ $p>0.0001$) and total LDL-C burden ($r=0.52$ $p<0.0001$). No other associations with lipid parameters were found. Multivariate analysis showed that CAC was independently associated with PWV adjusted for sex, total LDL-C burden, systolic blood pressure, smoking, LDL-C, HDL-C and statin treatment.
Conclusions. Arterial stiffness is independently correlated with CAC in HeFH subjects with similar total LDL-C burden and CV risk profile. The assessment of PWV in HeFH patients could represent a valuable tool to refine the CV risk and therapeutic management.

HE DIES YOUNG WHO IS DEAR TO THE GODS

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Since the discovery and commercialisation of antibiotics, cardiovascular diseases have become the most significant cause of death in western economies. The identification of risk factors (family history, LDL-hypercholesterolaemia, hypertension, diabetes, smoking, sedentariness...) prompted basic research to look for drugs reducing these factors. It is to the credit of the Padua school that it intuited the multiplicative nature of the risk of the association of some of these factors, later named the Metabolic Syndrome: includes, in addition to diabetes and conditions preceding it (IFG; IGT), hypertension, hypertriglyceridemia, low LDL-cholesterol values, and racially determined values of abdominal circumference. This datum, apparently purely anthropometric, is strongly pathogenetically connected with the elements of the syndrome, explaining various alterations, due to the flow of NEFAs from the abdominal district to the liver: hypertriglyceridemia due to the 'mass effect' of the NEFAs themselves; alteration of the post-synthetic metabolism of lipoproteins, with the formation of small, dense HDL. Much has been debated about the existence of this morbid form ('Requiescat in pace' wrote one of its early detractors). A controlled study demonstrated an increased CV risk in the presence of 3, 4 or 5 elements of this syndrome. Another metabolically deleterious effect of NEFAs, exerted in this case in the systemic circulation, is the one described by Randle, christened by his name, or 'glucose-fatty-acid cycle': describes the competition for cellular use of glucose by fatty acids, resulting in the problematic cellular internalisation of sugar, with persistence of sugar in the circulation. At the level of endothelial cells, it exerts an atherogenic effect summative to that due to the increased synthesis of triglyceride-rich lipoproteins (TRLP). In gynoid obesity, the centripetal flow of NEFAs occurs through the inferior vena cava: this pathogenetic aspect, explains the lower impact of this condition on CV disease, as it lacks the increased synthesis of TRLP. In 'pear' obesity, CV morbidity is still higher than in subjects with normal BMI. A Caucasian male (P.P.) with no history of family history of CV disease, with a personal history of significant sporting activity in his youth, who was a personal friend during his summer stay at our ASL, was entrusted to us as a patient in July 2002 by his wife, who was concerned about his persistent weight gain, also in relation to his strong diabetic family history. The patient reported nocturia in recent times. The patient's anthropometric data were: height cm 181; weight kg 107.000; BMI 32; abdomen circumference cm 104. Objective examination documented: pure MV over all range; cardiac action rhythmic, normo frequent, distal arterial pulses pulsating, symmetrical, BP 145/95 (he had not checked blood pressure habitually for years); abdomen globular, treatable, organs within limits. The patient was a smoker of 30 cigarettes a day; drank one bottle of wine a day, occasionally super alcohol. . Informed of the definite diagnosis of diabetes, and of the high cardiovascular risk due to the concomitant metabolic syndrome and cigarette smoking, he was prescribed Metformin in graduated doses up to 2,550 mg, Aspirin 100 mg, Atorvastatin 20 mg, as well as an invitation to take aerobic physical activity, to assiduously monitor his blood pressure, to stop smoking, and to lose weight. He also received a prescription to undergo ECG and funduscopy. The patient was puzzled by our instructions, objecting that he was in good health and 'felt great'; he would discuss our prescription with his doctor. He claimed to be able to normalise all altered parameters with his lifestyle. Back at home, he discussed our prescriptions with the doctor: both considered them 'excessive'. He informed us that he would follow up with behavioural therapy alone. The following spring, we were informed by his wife that the patient, seized one evening, first by thoracic discomfort, then by frank precordial pain radiating to the left arm, was quickly transported to the local hospital, where he was diagnosed with an extensive anterior infarction and taken to the haemodynamic room, where he unfortunately did not arrive alive. The case is emblematic in several respects: death occurs in most cases within 90 minutes of the onset of symptoms; the high morbidity and mortality from IMA in the diabetic, a patient whose mortality is as high as the second heart attack in the non-diabetic; the patients' guilty nihilism, sometimes shared by the treating physicians, in not aggressively treating diabetes and all the other factors of the syndrome, when present. The fatal event in the case described, could have benefited from the pleiotropic effects of Metformin on cardiovascular disease, at a dosage of 2,550 mg; of Aspirin in primary prevention; of the reduction of cholesterolaemia, also due to the pleiotropic effect of these molecules, on plaque stabilisation and nitric oxide production. The therapeutic inertia of physicians in applying the guidelines of the Scientific Societies is known as a cause of morbidity and mortality. Similarly, non-acceptance of treatment, or discontinuation of treatment by patients, is another cause of treatment failure. P.P. (29/02/1956-22/03/2003) joking about his own date of birth, predicted that he would die young anyway, claiming that he would turn one year every four, having been born on the last day of February of a leap year. His prediction was unfortunately true: in addition to unchangeable constitutional factors, there were environmental factors that were not adequately dealt with.

SCREENING OF POLYMORPHISMS F2 RS1799963, F5 RS6025 AND IDENTIFICATION OF RARE COAGULATION VARIANTS IN COVID-19 PATIENTS

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Background and Aims. Severe SARS-CoV-2 infections are characterized by perturbation of physiological coagulation mechanisms. COVID-19 is associated with a high incidence of thrombotic complications, such as venous thromboembolism or arterial thrombotic events (myocardial infarction and cerebral events). An association between thrombophilia and the most severe clinical course of COVID-19 has been suggested. Thrombophilia is a condition of altered haemostasis, characterized by increased blood clotting, which predisposes to adverse thrombotic events. This condition may be due to hereditary factors, acquired changes or, in the prevalence of cases, an association of genetic and acquired factors. Among the hereditary thrombophilia factors, the most frequent causes are factor V Leiden polymorphism (rs6025) in F5 gene, and the G20210A polymorphism (rs1799963) in F2 gene. Aim of the study was to identify genetic variants and/or genetic profiles associated with severity of the disease and thrombotic events susceptibility.
Methods. Starting from these considerations, an NGS analysis was conducted on a cohort of n=40 patients with COVID-19; genetic analysis included a sequencing panel of 11 genes (PROC, PROS1, FGA, FGB, FGG, SERPINC1, F2, F5, F10, PLAT, PLG) known to be involved in the coagulation process. Moreover, a genotyping analysis of rs6025 and rs1799963 polymorphisms has been conducted through Real Time PCR on the whole cohort of n=994 patients hospitalized at the AOU Careggi with COVID-19.
Results. As regards NGS analysis, 29 rare variants (MAF≤1%) have been identified at the heterozygous state in 24 of the 40 patients studied: 7 missense variants (on the F10, F2, PLAT, SERPINC1, F5 and FGB genes), 13 synonymous variants (on FGB, F2, PLAT, PLG, PROC and F5 genes), 4 variants concerning zone 3'-5' UTR/downstream (on F10, PLAT, PROC and F5 genes) and 5 non-deep intronic variants (on F5, PROC, F10 and PLG genes). In particular, there are five rare variants which were identified in two different patients each. A higher prevalence of rare missense variants with potential pathogenic prediction in ICU or death patients (26.7%) was observed than in ordinary ward patients (8%). Concerning common genetic thrombophilia, in the whole n=994 patients cohort, n=45 were heterozygous for the rs1799963 polymorphism and n=31 were heterozygous for rs6025 polymorphism. MAF for F2 G20210A was 0.023, higher than that reported in the literature for the population of Tuscany (0.016), while for the FV Leiden was 0.016, comparable to that observed in the tuscan population (0.020). Among a subgroup of n=324 patients, for which information concerning the clinical outcome was available, emerged that in patients who developed a thromboembolic event (5.9%) there was a higher allelic frequency, but not statistically significant, of FVL polymorphism, compared with patients who did not develop such an event (0.026 vs. 0.018, p=0.519); no differences were observed for F2 G20210A polymorphism.
Conclusions. The presence of common genetic factors of hereditary thrombophilia does not seem to indicate a significant contribution in modulating the risk of developing thromboembolic complications in SARS-CoV-2 patients; on the other hand, NGS results show that genetic variability, due to rare variants, might modulate clinical severity of COVID-19 disease in patients.

THE OPTIMIZED INTAKE OF PROTEIN-RICH FOODS TO REDUCE CARDIOVASCULAR DISEASE RISK CAN HELP MITIGATE CLIMATE CHANGE

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Ischemic heart disease is the leading cause of mortality in Europe, contributing to half of total cardiovascular disease (CVD) deaths. Food choices account for 50% of all CVD deaths and ~37% of total greenhouse gas (GHG) emissions, with red meat at the top of the list of harmful foods for planetary health.To identify the most suitable intakes of different protein-rich foods to reduce CVD risk, we performed a systematic evaluation of the relationship between their habitual consumption and CVD incidence or mortality in meta-analyses of prospective studies. Thereafter, we calculated the impact on climate of the transition from the current intake of protein-rich foods in Europe towards the optimal intake for CVD prevention, using the Carbon Footprint indicator. We identified 100g and 25g weekly of red and processed meat as the most appropriate intakes for the optimization of CVD prevention, corresponding to a reduction of 87.6% and 91.7% respectively from current consumption. Among dairy products, cheese and full-fat milk should be reduced (-52.9% and -69% respectively) while yogurt consumption should markedly increase (up to 200g/day, +528.8%). A much higher intake would be required for legumes (+716.6%), while fish and eggs should be increased to a lesser extent. Overall, these variations would avoid the emission of 18.7 kgCO2eq./capita/week (-56.6%). The increase in GHG emissions linked to higher consumption of protein-rich foods like legumes, yogurt, fish and nuts, would not counterbalance the reduction due to lower consumption of meat and full-fat dairies (+5.4 vs. -23.9 kgCO2eq.). These results indicate that in the attempt to optimize CVD prevention by appropriate food choices, reducing the intake of red and processed meat as well as full-fat dairies and replacing them with vegetable protein sources but also with yogurt and moderate amounts of fish - particularly fatty fish - and eggs, could also reduce by almost 60% GHG emissions linked to protein-rich foods consumption in Europe.

GENETIC HETEROGENEITY OF SEVERE HYPERTRIGLYCERIDEMIA: LOW PREVALENCE OF THE FAMILIAL CHYLOMICRONAEMIA SYNDROME

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Aim. Severe Hypertriglyceridemia (HTG) is an inherited disease characterized by very high plasma levels of triglycerides (TG) and a considerably increased risk of acute pancreatitis.The monogenic autosomal recessive form (Familial Chylomicronaemia Syndrome - FCS) is due to 2 pathogenic variants in 5 known genes involved in metabolism of the TG-rich lipoproteins (LPL, APOA5, APOC2, GPIHBP1 and LMF1) and associated to very severe phenotype. High levels of TG are often due to multifactorial chylomicronaemia syndrome (MCS) caused by heterozygous rare variants in canonical FCS genes and/or in non-canonical genes recently associated with TG metabolism. We aim to compare genetic status in a cohort of patients with severe hypertriglyceridemia.
Patients and Methods. Thirty seven unrelated patients were recruited based on plasma levels TG>10mmol/L and screened for rare variants in candidate FCS genes. Multiplex ligation-dependent probe amplification was used to detect large rearrangements in the LPL gene. Recently, the genetic analysis was performed by NGS with a large panel of genes involved in lipid metabolism.
Results. Among studied patients, 9 carried two pathogenic variants in canonical genes (4 were true homozygotes, 4 were compound heterozygotes and 1 was double heterozygote). In 11 patients we detected only one variant classified as pathogenic: 9 patients with variants in canonical genes (6 LPL - 3 APOA5); 2 patients with variants in non-canonical genes (GCKR and CREB3L3). No pathogenic variants were identified in 17/37 patients (45.9%) but 5 of them were carriers of additional rare variants of uncertain significance (USV) in canonical and non-canonical genes.
Conclusions. Genetic screening revealed that only a small number of patients suffer from FCS since they presented 2 pathogenic variants. These results highlight the genetic heterogeneity of the disease and the great usefulness of NGS to define complexes genotypes including of potential pathogenic variants, also in non-canonical genes.

BODY MASS INDEX MODULATES THE IMPACT OF SHORT-TERM EXPOSURE TO AIR PARTICULATE MATTER ON HIGH DENSITY LIPOPROTEIN FUNCTION

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Background. The exposure to environmental pollution has been associated with an increased risk of ischemic heart disease and stroke, with increased susceptibility in obesity. Air pollutants trigger inflammation and oxidative stress, which may impact the atheroprotective functions of HDL. Aim of the present study was to assess the relationship between short-term exposure to air particulate matter (PM) and HDL function, and the modifying effect of BMI.
Methods. Daily exposures to PM10 and PM2.5 of 50 subjects with overweight/obesity and 41 healthy volunteers with BMI<30kg/m2 were obtained from fixed monitoring stations. HDL function was assessed as their ability to promote nitric oxide (NO) release by endothelial cells and to reduce cholesterol mass in macrophages.
Results. HDL ability to induce NO production progressively declined with the increase in BMI. No association was found between HDL-mediated NO production and PM10 or PM2.5 exposures. Nevertheless, a significant modifying effect of BMI on PM10 was observed on the day before the recruitment. In subjects with a normal BMI, a positive association between day-1 PM10 exposure and HDL-mediated NO production was found, but this compensatory response was lost in participants with higher BMI values. Similar results were obtained with HDL ability to reduce macrophage cholesterol mass, although this functional parameter was independent from BMI and the modifying effect of BMI on PM10 exposure measured the day-1 was less evident.
Conclusions. The compensatory response of HDL function after exposure to PM was progressively lost at increasing BMI levels. The impaired ability of HDL to promote NO release could contribute to the endothelial dysfunction induced by PM and could help to explain the susceptibility of subjects with obesity to the detrimental effects of pollution.

TREATMENT WITH NATRACEUTICAL IS SAFE AND EFFECTIVE IN REDUCING LDL AND REACHING THERAPEUTICAL GOAL IN PATIENTS TREATED WITH PCSK9-I AND INTOLERANT TO STATIN AND EZETIMIBE

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Background. Anti-proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9-i) are usually added to statins (S)±ezetimibe (E), but a percentage of patients cannot use S and/or E due to intolerance. Furthermore, a percentage does not reach the LDL target with PCSK9-i alone. In these subjects, the addition of a natraceutical could be helpful.
Patients and Methods. Aim of our study was to evaluate, in a series of patients treated with PCSK9-i intolerant to S and E and not in therapeutic target, the reduction of LDL, the achievement of therapeutic target on LDL and the safety by adding a natraceutical. Eighteen patients already on treatment with full dose of PCSK9-i (14 evolocumab, 4 alirocumab) unable to reach the therapeutic target and intolerant to S and E, were treated for six weeks with a natraceutical (monacolin K 10 mg plus octacosanol 12 mg). The mean age was 61.94±7.01 years, BMI 26.5±0.71 (kg/m^2). All were assessed for total cholesterol, HDL, LDL, triglycerides (mg/dl), ALT and CPK before treatment and at six weeks with natraceuticals.
Results. Initial mean values were: total cholesterol 184.11±30.07, HDL 60.94±13.20, triglycerides 132.79±46.97, LDL 98.83±30.61. After six weeks mean values were: BMI 26.6±0.20, total cholesterol 148.56±31.31 (-19.31%), HDL 64.56±13.34 (+5.94%), triglycerides 121±51.76 (-8.86%), LDL 64.33±29.06 (-34.91%); twelve (66.67%) of patients reached therapeutic goal for LDL. No adverse events or significant changes in ALT and CPK were reported.
Discussion. In our study, the addition of a natraceutical to PCSK9-i therapy was effective in reducing LDL values and achieving the target according to the 2019 guidelines. Of particular interest is the intensity of the LDL reduction (-35%), which appears to be greater than expected (24%).
Conclusions. This reduction of LDL and the absence of adverse events could justify the use of natraceuticals in this patient group, although further randomised studies are needed.

SUCCESSFUL TREATMENT WITH LOMITAPIDE IN A PATIENT WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA AND SEVERE FATTY LIVER DISEASE

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Introduction and Aims. Homozygous familial hypercholesterolemia (Ho-FH) is a rare condition due to biallelic mutations in low-density lipoprotein-receptor (LDL-R) pathway genes characterized by very high level of LDL cholesterol (LDL-c) from birth and an extremely high risk of premature atherosclerotic cardiovascular disease (ASCVD), determining low quality of life and life expectancy. Lomitapide, an oral microsomal triglycerides transfer protein (MTTP) inhibitor, is an effective therapeutic option for Ho-FH, but caution should be observed when used in patients with fatty liver disease (FLD) and increased liver enzymes since it is associated with onset/worsening of liver steatosis. Here we present a case in which lomitapide was safely used in an adult Ho-FH patient with pre-existing severe FLD.
Case presentation. A 39-year-old man with severe hypercholesterolemia since young age (LDL-c up to 405 mg/dl) and a history of premature coronary heart disease with residual stable angina, was referred to the Lipid Clinic in Modena for the suspicion of FH. He also presented an overt metabolic syndrome (visceral obesity, arterial hypertension, impaired fasting glucose) and FLD with elevated liver enzymes and elastosonographic evidence of moderate liver fibrosis. His lipid-lowering therapy (LLT) included rosuvastatin 20 mg, ezetimibe and evolocumab 140 mg twice a month without reaching the LDL-c goal. Both parents were affected by hypercholesterolemia and a history of ASCVD. Genetic analysis revealed homozygous pathogenic mutation in LDL-R gene. LLT was further enhanced by increasing evolocumab up to 420 mg twice a month and starting LDL-apheresis with a negative impact on quality of life. For this reason, lomitapide 5 mg daily together with a personalized low-fat diet were started, obtaining a significant weight loss and improvement of lipid profile without any gastrointestinal adverse event. However, liver enzymes elevation higher than 5-fold the baseline values was observed, leading to lomitapide discontinuation until exclusion of secondary causes of hypertransaminasemia and baseline liver enzymes values restoration. After one month wash-out, lomitapide was gradually reintroduced up to 5 mg daily without recurrence of hypertransaminasemia, leading to long-term LDL-c target achievement and LDL-apheresis discontinuation. The optimal adherence to low-fat diet and the weight loss resulted in improvement of FLD and fibrosis despite lomitapide therapy.
Conclusion. Ho-FH requires a complex and combined treatment. Co-existence of metabolic comorbidities makes Ho-FH management even more difficult. Lomitapide can be safely used also in Ho-FH patients with FLD and hypertransaminasemia, but its use requires strict follow-up of liver disease and a multidisciplinary approach with tailored lifestyle advices. When considering lomitapide therapy in this setting, low-fat diet should ideally be started in advance and weight stabilization should be obtained before treatment introduction.

A DIFFERENTIAL PROTEOMIC ANALYSIS ON HUH7 HEPATOCARCINOMA CELL LINE NATURALLY OVEREXPRESSING OR HYPOEXPRESSING LOW DENSITY LIPOPROTEIN RECEPTOR

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Background. LDL receptor (LDLR), chiefly expressed in liver, is the main regulator of LDL plasma levels, and carriers of LOF mutations on LDLR gene are affected by a rare disease that causes early atherosclerosis and fatal and non-fatal cardiovascular events. LDLR expression is highly regulated both at transcriptional/post-transcriptional (es. hsa-miR-140-5p) and post-translational levels (es. PCSK9, IDOL). The aim of this study was to identify new modulators of LDLR and cholesterol/triglycerides (chol/TG) metabolism.
Methods. A polyclonal HuH7 human hepatocarcinoma cell line was incubated with fluorescently labeled LDLs and sorted in two subpopulations naturally expressing, respectively, high and low LDLR expression in order to identify differentially expressed proteins (DEPs) by the means of mass spectrometry technique (cut-off: ±1.95 fold-change). DEPs involved in LDLR modulation and/or in chol/TG metabolism have been selected and Gene Ontology database has been used for the annotation analysis. Ingenuity pathway analysis by Qiagen has been used to elaborate the upregulated vs downregulated pathways in the two subpopulations of cells.
Results. The proteomic analysis on HuH7 High and Low pointed out an enrichment in 7 downregulated DEPs (CPS1, CES1, ABAT, MT1X, GLRX) and in 5 upregulated DEPs (AGR2, ACOT7, AKR1C1, FLNA, ANXA3). Among the downregulated DEPs, CES1 and CPS1 are of greatest interest. CES1 (carboxylestherase 1) is highly expressed in liver and involved in TG metabolism and in the protection against hepatic steatosis. CPS1 (carbamoil-phosphate synthase), is a mitochondrial protein involved in urea cycle and it has been recently associated with HDLc levels in a genome wide association study (GWAS). Among upregulated proteins, ACOT7 (acyl-CoA thioesterase 7), who hydrolyses acyl-CoA thioesters into free-fatty acids (FFA). Moreover, ACOT7 resulted overexpressed in biopsies from patients with HCC (where an overproduction of FFA has been observed). Interestingly, the analysis on up- and down-regulated pathways unveiled the modulation of the FXR/RXR axis, thus leading to an important modulation in cholesterol metabolism (CYP7A1), biosynthesis (CYP51A1, FDFT1), transport (ABCA1, ABCG1-5-8), efflux (APOA4, CD36), lipoprotein synthesis (LPL, CETP, PLTP) and lipogenesis (SREBP-1c, FASN, SCD1, ACACA, MLXIPL).
Conclusions. The proteomic analysis shed light on several up- and down-regulated DEPs between HuH7 with High and Low LDLR expression. The Gene Ontology functional analysis revealed that CPS1, CES1, and ACOT7 are annotated as involved in "lipid metabolism" and "fatty acid metabolism", and the Ingenuity pathway analysis confirmed the modulation of pathways involved in these processes. Even if the activity of some of these proteins is mostly studied in tissues or cell populations different from the liver or hepatocytes (es. CES1 has been widely studied in macrophages, while ACOT7 is known for it role in FFA formation in the brain), it would be of great interest to further investigate their potential contribution into the hepatic tissue in relation to life-threatening pathologies such as familial hypercholesterolemia.

ULTRASOUND AND ELASTOSONOGRAPHY: THE "EYES" OF THE DOCTOR IN PATIENTS WITH METABOLIC SYNDROME AND NAFLD UNDERWENT TO DIET INTERVENTION

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NAFLD is the most widespread liver disease, characterized by fatty acids liver accumulation and subsequent fibrosis with a prevalence that is estimated to be around 50% in patients with metabolic syndrome. There are several imaging techniques that can accurately diagnose fatty liver. Recently, ultrasound has acquired a leading role in the diagnosis and follow-up of fatty liver disease. Furthermore, elastosonography and in particular shear wave represent a valid alternative to liver biopsy. We evaluated the effects of lifestyle and nutritional interventions on hepatic steatosis through ultrasonographic and elastosonographic techniques. Thirty-two female subjects with metabolic syndrome were subjected to clinical, anthropometric, and laboratory assessments, as well as abdominal ultrasonographic/elastosonographic measurements taken from enrollment time (T0) and after 3 months (T1) of lifestyle modifications. After 3 months of lifestyle changes, significant weight loss was observed, with a marked improvement in all adiposity indices. The laboratory parameters at T1 showed significant decreases in total and LDL cholesterol, triglycerides, basal blood glucose, 120 min glycaemia, basal insulin and HOMA Index ($p<0.001$). A similar improvement was observed at T1 for steatosis degree ($p<0.01$) and elastosonographic measurements (Kpa $p<0.001$). After 3 months, the liver size showed improvement with positive correlations to all previous variables. Hepatic stiffness (Kpa) positively correlated with neck circumference, visceral fat, and ALT, with basal insulin, gamma-GT, and AST, and with waist circumference, WhtR, and fat mass. The degree of steatosis was positively correlated with more variables and with greater statistical significance at T1 with respect to T0. Particularly, the positive correlations between the degree of steatosis and neck circumference ($p<0.001$), HOMA Index, and triglycerides ($p<0.001$) appeared to be very significant. In conclusion NAFLD management may include liver ultrasonographic and elastosonographic techniques to better manage and follow-up patients.

PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 GIVES ATHEROGENIC PROPERTIES TO EXTRACELLULAR VESICLES RELEASED BY VASCULAR SMOOTH MUSCLE CELLS

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Extracellular vesicles (EVs) mediate intercellular and inter-system communication. They play a role in pathological conditions, e.g. atherosclerotic cardiovascular diseases (ASCVD), participating in atherosclerosis onset and progression. Besides endothelial, monocytes and macrophages, vascular smooth muscle cells (VSMCs) are involved in atheroma formation. They can influence neighboring cells through bioactive molecules, some of which are packed into EVs. VSMCs express and secrete proprotein convertase subtilisin/kexin type 9 (PCSK9), crucial in the pathophysiology of ASCVD and influences VSMCs differentiation, migration and proliferation.
Aim. To unveil whether the overexpression of PCSK9 in VSMCs affects the composition of EVs favoring a pro-atherogenic phenotype.
Methods. EVs were isolated from VSMCs wild-type (VSMCs-WT-EVs) or overexpressing PCSK9 (VSMCs-PCSK9-EVs) and tested on EA.hy926 endothelial cells, THP-1 monocytes, THP-1-derived-macrophages and in embryos of zebrafish. The following approaches were used: flow cytometry, WB, mass spectrometry, qPCR, nanoparticle tracking analysis, transmission electron microscopy, mitochondrial-bioenergetics analysis.
Results. VSMCs-WT-EVs and VSMCs-PCSK9-EVs expressed specific markers of EVs (tetraspanins, Alix, and β1-Integrin) with no differences in their concentrations (625.17±235.23/mL/cell count and 926.17±815.26/mL/cell count, respectively), size (235.78±29.78 nm and 233.16±16.3 nm, respectively) and morphology. VSMCs-PCSK9-EVs, expressing higher levels of PCSK9, carried a different pattern of proteins and miRNAs linked to inflammation. VSMCs-PCSK9-EVs led to a rise in the expression of adhesion molecules and pro-inflammatory cytokines in endothelial cells. In monocytes and macrophages, exposure to VSMCs-PCSK9-EVs raised the expression of MCP-1, IL-1α, IL-1β, IL-6 and IL-8 as well as the phosphorylation of the inflammatory protein STAT3. VSMCs-PCSK9-EVs enhanced the migratory capacity of THP-1, decreased that of macrophages and reduced THP-1 basal and maximal mitochondrial respiration. VSMCs-PCSK9-EVs increased the uptake of oxidized LDL in macrophages. Injection of VSMCs-PCSK9-EVs in the hindbrain ventricle of zebrafish embryos favored a local recruitment of macrophages.
Conclusion. In atheroma formation, PCSK9 seems to play an atherogenic role by means of EVs derived from VSMCs.

HOW DIFFERENT DIETS SHAPE PLASMA AND AORTA LIPIDOME: A STUDY IN THE APOE KNOCKOUT MOUSE MODEL

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Introduction. Specific lipid molecules circulating in plasma at low concentrations have emerged as biomarkers of atherosclerotic risk. The aim of the present study was that of evaluating, in an athero-prone mouse model, how different diets could affect plasma and aorta lipidome.
Materials and Methods. Thirty-six apoE knockout mice were divided in 3 groups and fed for 12 weeks with diets (standard, Western-type and Paigen) differing for cholesterol and fatty acid (FA) content. Atherosclerosis was measured at the aortic sinus and aorta. Lipids were quantified in plasma and aorta with a hybrid triple quadrupole/linear ion trap mass spectrometer equipped with an ultra-high pressure liquid chromatography system.
Results. Taking into consideration the entirety of the dataset, the variation of the bulk lipid content of each sample in the plasma was overwhelmingly large with respect to any variation in the aorta. Conversely, the diversity of the relative amount of lipids that could be found in the aorta was much larger than in plasma.The cholesterol content of the diets was the main driver of lipid accumulation in plasma and aorta. The different composition of the diets resulted in sizeable differences in plasma of essential (linoleic acid) and nonessential (myristic and arachidonic acid) FA, even though the distribution pattern of single FA moieties followed a comparable trend for all diets. We found a comparable distribution, in plasma and aorta, of the main lipid components of oxidized LDL, including cholesteryl esters and lysophosphatidylcholines. Interestingly, LacCer, Glc/GalCer and individual ceramide species known in the clinic as markers of cardiovascular disease were found to accumulate in diseased aortic segments and increase with plaque development.
Conclusions. Both the cholesterol and FA content of the diets profoundly affected plasma lipidome. Aorta lipidome was likewise affected with the accumulation of specific lipids known as markers of atherosclerosis.

MEDITERRANEAN DIET IN HYPERLIPIDEMIC CHILDREN: EFFICACY AND KIDMED SCORE APPLICATION

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Introduction. Mediterranean Diet (MD) is worldwide known for its health benefits on cardiovascular diseases (CVD) but scanty data are available in dyslipidemic pediatric patients showing increased CV risk. .The aim of the study is to evaluate MD efficacy on lipid profile and adherence by the Mediterranean Diet Quality Index for children and adolescents (KIDMED score) to verify its application in children with primary hyperlipidemia.
Methods. The study included 157 dyslipidemic children (10.01±3.54 years; M/F 73/84). Dietary intake was evaluated twice, at baseline and three months after dietary advices (MD pattern), by food week diaries and KIDMED score application. On the basis of the score, patients were classified in the three levels of adherence: optimal; needed improvement; very low diet quality. Plasma lipid levels were evaluated by standard methods at baseline and after three months since the diet counselling.
Results. After dietary advices, KIDMED score improved in 65%, was unchanged in 18.5% and worsened in 16.6%. Stratifying patients on the basis of adherence level , 33.8% improved their class, 57.3% maintained the same category and 8.9% worsened their class. In particular, out of 80 people with very low diet quality at baseline, 53.8% improved their class. Out of 90 subjects with unchanged class, 54.4% increased the score. Finally, statistical analyses highlighted that the increase of adhesion scores lead to a decrease in non-HDL and LDL-C levels ($p<0.0001$), in particular, in patients who improved their class, LDL-C levels decrease by 5.8%.
Conclusions. Present results demonstrate the MD efficacy and the applicability of the KIDMED score as tool to verify the adherence to the diet in pediatric dyslipidemic patients and it could be proposed as a valid model in order to prevent cardiovascular diseases. However, it is a qualitative evaluation method which does not take into account quantitative target of CHILD I and II dietary protocols, standard of care for dyslipidemic children.

CORRELATION BETWEEN SLCO1B1 AND ABCB1 POLYMORPHISMS AND RISK OF ADVERSE STATIN REACTIONS

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Aims. tatins are the gold standard in reducing cardiovascular risk through lowering LDL cholesterols. However, 7 to 29% of statin-treated patients experience myopathy. The SLCO1B1 and ABCB1 gene are responsible for hepatic reabsorption and biliary and renal elimination of statins, respectively. The aim of the study was to evaluate the presence of a correlation between heterozygous rs 4149056 SLCO1B1 or homozygous rs 2032582 ABCB1 mutations and adverse events in patients treated with (rosuva, prava, atorva/simva) statins.
Methods. We monitored through clinical and laboratory parameters two statin metabolism's regulatory genes (SLCO1B1,ABCB1) in patients under statin therapy.
Results. SLCO1B1 gene: in patients treated with atorvastatin/simvastatin, 7 out of 8 patients experienced adverse events such as to discontinue therapy; in those treated with rosuvastatin, 50% in a group of 10 patients required a change of therapy while for pravastatin, only 1 out of 5 patients experienced side effects.ABCB1 gene: there was a marked intolerance towards atorvastatin/simvastatin (6 out of 6 patients discontinued therapy), while for pravastatin 2 out of 3 patients discontinued therapy and, about rosuvastatin, only 1 out of 6 had to discontinue therapy.
Conclusions. Despite the small sample size, the SLCO1B1 mutation appears to be strongly associated with an increased probability of adverse reactions from statins in drugs metabolized by cytochrome CYP450 3A4 (simvastatin and atorvastatin). Instead, rosuvastatin minimizes the risk of adverse effects in patients with the ABCB1 polymorphism. Although metabolism of pravastatin is not influenced by cytochromes, a greater number of adverse events have been observed in patients carrying the ABCB1 mutation. The genotyping of the polymorphisms rs 4149056 SLCO1B1 and rs 2032582 ABCB1, could help to improve the therapeutic management of the statins in future increasing the safety of the administration, and allowing to implement more and more a "tailored therapy".

THE INFLAMMATORY POTENTIAL OF DIET PREDICTS THE DEVELOPMENT OF PRE-CLINICAL CAROTID ATHEROSCLEROSIS

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Background. An inflammatory/atherogenic effect of some nutrients has been postulated, but not confirmed by epidemiological studies which provide conflicting results how this relationship matters for Cardiovascular Disease (CVD) risk. Assessing nutrient intake is complex and few inflammatory biomarkers were analyzed so far. Hence, to overcome this gap, we harnessed a panel of 368 inflammatory biomarkers, previously related to CVDs, to unveil these relations.
Methods. During the basal visit ('99-'01), records on dietary habits of 417 subjects without pre-clinical carotid atherosclerosis (determined by carotid ultrasonography; "SCA") were collected. Dietitians analyzed the dietary records to:
1) derive the caloric intake from total, saturated, monounsaturated, and polyunsaturated fats (as percentage of total energy intake (En%)) and
2) estimate the pro-/anti-inflammatory effect of diet via the Dietary Inflammatory Index (DII).

We measured plasma expression of 368 proteins (OlinkTM) to validate the estimated pro-/anti-inflammatory effect of diet (DII below or above the cohort median). The same subjects were re-evaluated after 11 years on average (10-11, 25th-75th percentiles) for carotid ultrasound to assess the development of SCA.
Results. At the basal visit, pro-inflammatory effects of diet were associated with increased En%SFA (rho:0.25, fdr<0.0001), decreased En%PUFA (rho:-0.18, fdr<0.0001) but neither with En%Fats (rho:0.07, fdr=0.633) nor En%MUFA (rho:0.00, fdr=0.929), implying that specific dietary lipids explain the higher values of DII. DII correlated with: increased expression of interleukin-6 (rho:0.25, fdr<0.0001), leptin (rho:0.18, fdr<0.05), T-cell surface glycoprotein (rho:0.18, fdr<0.05), and decreased expression of interleukin-27 (rho:-0.14, fdr<0.1). Of note, higher DII values at the first basal vascular examination predicted the development of SCA at follow-up, as subjects that developed SCA (n=177) presented with higher basal DII (1.86[0.85-2.50]) as compared to subjects that did not develop SCA (1.46[0.71-2.20]), p=0.015).
Conclusions. We support that an inflammatory effect of diet predicts the evolution of pre-clinical atherosclerosis. Larger studies are warranted to confirm this possibility.

A NUTRACEUTICAL CONTAINING BERGAMOT CITRUS AND ARTICHOKE IMPROVES LIVER STEATOSIS IN OBESE ADULTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Introduction. Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disorder associated with obesity, which leads to an increased risk of developing cardiovascular diseases. Currently, there is no approved medication for NAFLD management. The nutraceutical containing a polyphenolic fraction of bergamot and artichoke extract (BC) reduces intrahepatic fat in diabetic and non-diabetic subjects. We aim to evaluate the effects of this nutraceutical on the intrahepatic fat content in obese with NAFLD. In addition, we investigated the molecular mechanism of the BPF extract in hepatocyte cultures.
Methods. We analyzed the data of 31 non-diabetic obese with NAFLD enrolled in a previous clinical trial (ID ISRCTN12833814). Subjects received BC (1cp/die), or placebo for 12 weeks. Liver fat content (CAP) was assessed by transient elastography. For the in vitro study, we evaluated the effect of Bergamot extract in 2D and 3D human hepatoma cells and human primary hepatocytes.
Results. At baseline, there were no significant differences between groups. After 12 weeks, we found a greater CAP reduction in obese taking BC rather than placebo (-18±11 % vs -6±17 %, p=0.02) and a greater prevalence of improvement of NAFLD degree after taking the nutraceutical compared to placebo (80% vs 37%, p=0.03). Multivariate regression analysis shows that CAP change was significantly associated with baseline serum GGT levels (B=1.07, SE=0.25 p<0.001) and BC treatment (B=-23.8, SE=10 p=0.026). The 2D in vitro study showed that incubation with BPF decreases intracellular lipid content and is associated with an increase in expression levels of β-oxidation genes (Acox1, Pparα, and Ucp2). This result was confirmed by 3D spheroids and organoids.
Conclusions. BC is a promising non-pharmacological treatment to counteract the onset and progression of NAFLD, even in obese subjects. The underlying effect of liver fat reduction could be due to an increase in β-oxidation.

EFFECTS OF THYROID HORMONES THERAPY ON AN IN VITRO MODEL OF FATTY LIVER DISEASE

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Background. Thyroid hormone has a direct effect on cholesterol levels. Hypothyroid patients have increased cholesterol levels compared to individuals with normal thyroid function and have a greater risk of non-alcoholic liver disease (NAFLD). A recent study suggested that levothyroxine treatment increased the prevalence of liver fibrosis in obese individuals, but the effect on hepatic steatosis has been poorly investigated. Our aim was to evaluate the molecular mechanism of triiodothyronine (T3) and thyroxine (T4) on an in vitro model of hepatic steatosis.
Material and Methods. We evaluated the effect of 20 and 30 nM T3 or with 20 and 60 nM T4 for 96 hours in MCA-Rh7777 hepatic cells. We evaluated the intracellular lipids and triglycerides content. In addition, to understand the mechanism underlying this increase, we evaluated the genes involved in the metabolism of triglycerides, cholesterol and β-oxidation, as well as the pathways involved.
Results. In MCA-Rh7777, the incubation with T3 and T4 hormone increased intracellular lipid (p=0.01 and 0.002, respectively) and triglycerides (p=0.03 and p=0.001, respectively) content. In addition, we shown that T3 increased genes involved in triglycerides (Srebp1c: p=0.014; Acc: 20nM T3 vs CTRL, p=0.002), cholesterol (Srebp-2: p=0.006; HmgCs1: 20nM T3 vs CTRL, p=0.04) and β-oxidation (Cpt1α: 20nM T3 vs CTRL, p=0.006) metabolism. Furthermore, T3 incubation increased p-mTor pathways (p=0.03). Incubation with T4 hormone also increased gene involved in triglycerides (Srebp-1c: 20nM T4 vs CTRL, p=0.01; Scd1: p=0.005), cholesterol (HmgCr: 20nM T4 vs CTRL, p=0.008; HmgCs1: 0.02) and β-oxidation (Pparα: p=0.001) metabolism. In addition, T4 reduced p-Erk1/2 (p=0.002) and increased p-Ampk (p=0.003) pathways.
Conclusion. We found, for the first time, that replacement therapy with thyroid hormones induced liver fat accumulation thought the alteration of the expression levels of genes and pathways involved in hepatic lipid metabolism.

A NEW NUTRACEUTICAL (LIVOGEN PLUS®) IMPROVES LIVER STEATOSIS IN ADULTS WITH HYPERTRANSAMINASEMIA AND NON-ALCOHOLIC FATTY LIVER DISEASE

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Background. Non-alcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver-test results among adults with a prevalence of 13-23%. Currently, there is no approved medication for NAFLD treatment. Pre-clinical and clinical studies showed that several bioactive molecules in plants or foods (i.e., curcumin complex, bergamot polyphenol fraction, artichoke leaf extract, black seed oil, concentrate fish oil, picroliv root, glutathione, S-adenosyl-L-methionine and other natural ingredients) have been associated with improved fatty liver disease. Starting from these evidences, our purpose was to evaluate the effects of a novel combination of abovementioned nutraceuticals as a treatment for adults with fatty liver disease.
Methods. We analyzed the data of 53 participants with NAFLD and liver enzyme alteration enrolled in a previous clinical trial (ID ISRCTN70887063). The intervention group received six softgel capsules daily of a nutraceutical containing a combination of natural bioactive components for 12 weeks. The control group received six softgel capsules daily of a placebo containing maltodextrin for 12 weeks. The primary outcome measure was the change in liver fat content (CAP score). CAP score, by transient elastography, serum glucose, lipids, transaminases, and cytokines were measured at baseline and after intervention.
Results. The CAP score reduction (%) was greater (nutraceutical: -15±3 % vs placebo: -3±3 %, p=0.006) in subjects with AST reduction after adjustment for confounding variables. In addition, we found a greater prevalence of improvement of NAFLD degree after taking the nutraceutical in the participants with AST reduction (58% vs 33%, p=0.04).
Conclusions. Our results showed that a new combination of bioactive molecules as nutraceutical was safe and effective in reducing liver fat content over 12 weeks in individuals with hepatic steatosis.

ANTI-HYPERTENSIVE DRUGS AND MORTALITY RISK IN ATRIAL FIBRILLATION PATIENTS ON ORAL ANTICOAGULANTS. THE NATIONWIDE START REGISTRY

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Background. Arterial hypertension is the most common cardiovascular comorbidity in patients with atrial fibrillation (AF). Data on the use of antihypertensive drugs are scarce as their association with mortality risk. Objective is to investigate the relation between antihypertensive drugs and mortality risk.
Methods. We analysed the use of single antihypertensive molecules in 5769 AF patients included in the nationwide Italian START registry. We also investigated the association of antihypertensive drugs with mortality risk.
Results. Mean age was 80.8 years, 46.1% were women; 80.3% of patients were hypertensive. Furosemide (30.1%) was the most frequent diuretic followed by hydrochlorothiazide (15.4%) and potassium canrenoate (7.9%). 61.1% received β-blockers: 34.2% bisoprolol, 6.2% atenolol. Additionally, 36.9% were on ACE-I: ramipril (20.9%), enalapril (5.3%) and perindopril (2.8%); 31.7% were on ARBs: valsartan (7.6) and irbesartan (6.4%). Amlodipine and lercanidipine were prescribed in 14.0% and 2.3%, respectively. ACE-I ($p<0.001$), α-blockers ($p=0.020$) and D-CCB ($p=0.004$) were more common in men, while ARBs ($p=0.008$), thiazide diuretics ($p<0.001$) and β-blockers ($p<0.001$) in women. During 22.61±17.1 months, 512 patients died. Multivariable Cox regression analysis showed that ACE-I (Hazard ratio [HR] 0.677 95% Confidence Interval [95%CI] 0.545-0.841, $p<0.001$) and ARBs (HR 0.572, 95%CI 0.447-0.732, $p<0.001$), were inversely associated with mortality. ACE-I/ARBs prevented mortality in patients with diabetes, ACE-I also in previous cardiovascular disease, and ARBs also in HF. ACE-I/ARBs prevented death both in women and men.
Conclusions. ACE-I/ARBs are inversely associated with mortality in AF. Our data suggest that ACE-I/ARBs should be considered to optimise clinical management of AF patients.

PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 (PCSK9) SERUM LEVELS AND ABNORMALLY HIGH ANKLE-BRACHIAL INDEX IN PATIENTS WITH ATRIAL FIBRILLATION

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Background. High ankle-brachial index (ABI) has been associated with increased risk of worse outcomes in the general population. Few data on atrial fibrillation (AF) do exist. Experimental data suggest that proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) contribute to vascular calcification but clinical data on this association are lacking. We want to investigate the relationship between circulating PCSK9 levels and abnormally high ABI in patients suffering from AF.
Methods. We analysed data from 579 patients included in the prospective ATHERO-AF study. An ABI ≥ 1.4 was considered as high. PCSK9 levels were measured coincidentally with ABI measurement. We used an optimized cut-off of PCSK9 >1150 pg/ml obtained from ROC curve analysis. All-cause mortality according to the ABI value was also analysed.
Results. 115 (19.9%) had an ABI ≥1.4. The mean age was 72.1 years and 42.1% of patients were women. Patients with ABI ≥1.4 were older, more frequently male and diabetic. Multivariable logistic regression analysis showed an association between ABI ≥1.4 and serum levels of PCSK9 >1150 pg/ml (Odds Ratio 1.835, 95%CI 1.133-2.970, $p=0.014$). During a median follow up of 41 months, 112 deaths occurred. At multivariable Cox regression analysis, ABI ≥1.4 was associated with an increased risk of mortality (Hazard Ratio 1.676, 95%CI 1.073-2.617, $p=0.023$).
Conclusions. In AF patients, PCSK9 levels relate to an abnormally high ABI, which is in turn associated with an increased mortality risk. This is the first clinical evidence of an association between PCSK9 and vascular calcification in AF patients.

THE GENETIC LACK OF ANGTPL3 DOES NOT ALTER HDL FUNCTIONALITY

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Aim. Individuals with loss-of-function mutations in ANGPTL3 gene express a rare lipid phenotype called as Familial Combined Hypolipidemia (FHBL2). FHBL2 individuals show reduced plasma concentration of total cholesterol and triglycerides as well as ApoB and Apo-AI -containing lipoproteins particles, including HDL.This feature is particularly remarkable in homozygotes in whom ANGPTL3 in the blood is completely absent. ANGPTL3 is a circulating inhibitor of LPL and EL and it is thought that EL hyperactivity causes plasma HDL reduction in FHBL2. Nevertheless, the consequences of ANGTPL3 deficiency on HDL functionality has been poorly explored. In this work, HDL particles isolated from homozygous and heterozygous FHBL2 carriers of Italian cohort of Campodimele (LT) were evaluated for their ability to preserve endothelial homeostasis compared with controls.
Methods. Six homozygous and 26 heterozygous carriers of LOF mutation S17* in ANGPTL3 gene, and 22 non-affected family members (controls), all belonging to the Italian families, volunteered for the study. HDLs were purified by sequential ultracentrifugation from serum and analyzed for subclasses composition. Furthermore, the ability of isolated HDL to modulate the release of nitric oxide (NO) and the expression of adhesion molecules was evaluated in cultured endothelial cells.
Results. ANGPTL3 deficiency alters HDL subclass distribution. As homozygous, but not heterozygous FHBL2 subjects have reduced content of large and increased content of small HDL with no alteration in HDL2 and HDL3 size. The plasma content of preβ-HDL was reduced in homozygotes (P=0.028 one way ANOVA) and showed a positive correlation with plasma levels of ANGPTL3 (R2=0.128 and P=0.04) with an estimated cut-point of Angptl3 level value below 138 ng/dL. However, changes in composition did not alter the functionality of FHBL2 HDL, as lipoprotein particles isolated from carriers retain their capacity to promote NO production and to inhibit VCAM-1 expression in endothelial cells. Consistently, no significant changes in circulating levels of soluble ICAM-1 and E-selectin were detected in carriers.
Conclusion. These results indicated that the reduction of HDL-C level and the alteration of HDL subclass distribution observed in subjects with genetically determined ANGPTL3 deficiency doesn't hamper the vaso-protective and the anti-inflammatory properties of this lipoprotein fraction.

ALTERATIONS OF LIPID OBSERVED IN COVID-19 PATIENTS

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Introductioni. It seems that during SARS-CoV-2 infection, total cholesterol, LDL-C, and HDL-C values decrease and lipids could play a fundamental role in viral replication.
Methods and Aim. We performed a retrospective analysis of 118 hospitalized patients with COVID-19, comparing pre-infection lipid profile (53 patients) to those measured on admission. Our aim was to evaluate whether SARS-CoV-2 infection could be involved in lipid profile alterations and study possible correlations with disease severity and clinical outcome.
Results. Median baseline values at the admission time were: total cholesterol at 136.89±42.73 mg/dL, LDL-C 81.53±30.35 mg/dL, and HDL-C 32.36±15.13 mg/dL; and triglycerides at 115.00±40.45 mg/dL, non-HDL-C 104.53±32.63 md/dL. Median values of pre-infection total cholesterol and HDL-C were significantly higher than those measured at the admission time (p value < 0.05). The C-reactive protein (CRP) negatively correlated with LDL-C (p=0.013) and HDL-C (p=0.05).
Conclusion. Our data underline suggest a possible relation between COVID-19 and lipid profile with a negative correlation between CRP, LDL-C, and HDL-C values, proposing the hypothesis that lipid lowering could follow the rising of the COVID-19 inflammatory state.

IMPACT OF Mrc1 DEFICIENCY IN BONE MARROW HOMEOSTASIS IN DYSMETABOLIC CONDITION

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Obesity and type 2 diabetes are associated with increased myelopoiesis, which involves the sinusoidal endothelium, that regulates leucocytes egression as a consequence of the presence of inflammatory molecules including the mannose receptor (Mrc1). Mrc1 is a C-type lectin receptor with high affinity for mannose, fucose and N-acetylglucosamine, which is known to regulate leukocytes trafficking on lymphatic sinusoids by direct interaction with the highly glycosylated CD44. Aim of this project was to investigate whether the Mrc1 deficiency affects obesity development in experimental models.
Methods. Mrc1 KO mice and wt littermates were feed with a high fat diet (HFD, 45% Kcal/die) for 20 weeks. Weight gain was monitored during the diet regimen and glucose and insulin tolerance were assessed. Extensive flow cytometry profiling and histological analyses were performed.
Results. After HFD feeding, Mrc1 KO mice presented a different hematopoiesis profile with a reduced number of neutrophils progenitors ($p<0.05$), mature neutrophils ($p<0.05$) and macrophages ($p<0.05$) in the bone marrow(BM) compared to WT mice. BM adipocytes, known to maintain HSC quiescence by supporting CXCL12 expression, increased in Mrc1 KO mice ($p<0.01$), as was the number of adipocytes expressing CXCL12 ($p<0.05$). The reduced innate branch hematopoiesis in KO mice was reflected in the reduction of circulating neutrophils and pro-inflammatory monocytes ($p<0.05$) and it is paralleled by a decreased infiltration of macrophages in the liver and visceral adipose tissue. The deficiency of Mrc1 results in a less marked liver steatosis compared to WT (3.370%±2.610 vs 7.970%±1.891, average±SEM; $p<0.05$) and with better insulinic and glucidic response. Furthermore, plasma proteomics analysis confirmed reduced transendothelial migration of leucocytes (z-score= -1.414, $p<0.05$). Overall our data show that Mrc1 deficiency, in an experimental model of obesity, promotes a less inflammatory BM phenotype, associated with reduced systemic inflammation and protection from metabolic dysregulation.

FIBRATE USE IS ASSOCIATED WITH LOWER INCIDENCE OF HEART FAILURE AMONG PEOPLE WITH TYPE 2 DIABETES IN THE REAL WORLD

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Background. Fenofibrate, a PPAR-alpha agonist mainly known for its triglycerides lowering effect, has been recently shown to reduce the incidence of heart failure (HF) in a post-hoc analysis of the ACCORD trial. The effect on HF was independent from fenofibrate's action on lipids and was mainly seen among subjects on standard glycemic control. We aimed to validate such findings in a real-world clinical setting.
Methods. This observational study was conducted on patients with type 2 diabetes evaluated at the University Hospital of Padua between 2008 and 2018. Detailed clinical information were linked to administrative databases with death certificates and hospital discharge codes. The association of fibrate prescription with the composite primary outcome of hospitalization for HF and cardiovascular mortality was tested with Cox proportional hazard models with time-dependent co-variates or Cox marginal structural models (MSM, with inverse-probability of treatment weighing evaluated at each visit) that allows to adjust for multiple confounding factors and biases. The interactions of baseline and follow-up HbA1c with fibrate treatment were tested in Cox models. A similar "falsification" analysis was run for omega-3 fatty acids, which have a similar indication as fibrates.
Results. We included 5419 patients, 41% women, with an average age of 66 and a diabetes duration of 7.6 years. Only 10% had pre-existing cardiovascular disease and 5.6% had prior HF. During a median follow-up of 7.3 years, patients were seen 12 times at the clinic, and we recorded 1710 events in 1136 patients. During the study, ≈5% of the population (n=265) was treated with fibrates. Fibrate use was associated with younger age, male sex, higher BMI, worst glycemic and lipid profile, greater use of antidiabetic medications, higher prevalence of NAFLD, but a lower prevalence of macrovascular disease and HF at index date. After accounting for these confounding factors, fibrate use was associated with a 39% lower risk of the primary endpoint (HR 0.61; 95% C.I. 0.41-0.92, p=0.02). The finding was confirmed in a more extensively adjusted model (HR 0.65; 95% CI 0.43-0.98; p=0.04) and similar results were obtained with Cox MSM models. We found no interactions between fibrate treatment and glycemic control. The effect of omega-3 fatty acids on the primary outcome was neutral (HR 1.03; 95% CI 0.85-1.23; p=0.8).
Conclusions. Our findings support the possible beneficial effect of fibrates on HF in patients with type 2 diabetes. Further studies are warranted to identify the mechanism of action and confirm whether fibrates might be considered a treatment option against the HF burden in diabetes.

FIXED ASSOCIATION OF MONACOLINE K AND OCTACOSANOL IS EFFECTIVE, AT ONE YEAR, IN MAINTAINING THE REDUCTION OF LDL IN PATIENTS AT MODERATE CARDIOVASCULAR RISK AND UNWILLING TO STATINS

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Background. Natraceuticals are bioactive elements that are used to reduce LDL values. After one and half months, treatment with monacolin K 10 mg combined with octacosanol 12 mg reduced LDL values by approximately 24% without side effects (B. Napolitano, SISA 2021). This may support use of natraceuticals to reduce LDL. In literature, however, there are insufficient data on the maintenance of effects on LDL and the safety of long-term natraceutical use.
Patients and Methods. We wanted to evaluate the efficacy and safety in reducing LDL values in 46 patients with pure hypercholesterolaemia, with moderate cardiovascular risk (SCORE >0.9% and <5% at 10 years), unwilling to take statin therapy, with a 54-week treatment with monacolin K at 10 mg and octacosanol at 12 mg once daily. All patients were assessed for body mass index (BMI in kg/m2), total cholesterol, HDL, LDL, triglycerides (in mg/dl), ALT and CPK before and at the end of treatment. At baseline: mean age 58±11.57 years, BMI 25±3.1, cholesterol 211.78±36.53, HDL 59.52±14.84, triglycerides 122.17±57.40, LDL 131.51±30.36 mg/dl.
Results. Six weeks after the start of therapy: BMI 25±1.2, cholesterol 178.14±40.38, HDL 57.09±16.54, triglycerides 116.62±39.19, LDL 95.29±32.01 (-27.48%). After fifty-four weeks: BMI 24±1.4 (-4.17%), cholesterol 170.17±33.93, HDL 58.04±15.38, triglycerides 96.7±35.21, LDL 91.13±27.69 (-30.53% compared to the start of therapy and -4.5% compared to six weeks after the start of therapy). No adverse events or significant changes in ALT and CPK were reported.
Discussion. In our study, a 54-week natraceutical therapy successfully reduced LDL levels without side effects. Six weeks after the start of therapy, the average reduction was 27%, 30.53% after 54 weeks and 4.5% compared to the first six weeks.
Conclusions. Treatment with a combination therapy of monacolin K and octacosanol usefully reduces LDL values and keeps them reduced for one year.

DISTINCTIVE PROATHEROGENIC LIPOPROTEIN PROFILE IN CHILDREN AND ADOLESCENTS WITH HIGH TRIGLYCERIDE-TO-HIGH DENSITY LIPOPROTEIN CHOLESTEROL RATIO: A MAGNETIC RESONANCE STUDY ON LIPOPROTEIN SUBCLASSES SIZE AND DISTRIBUTION

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Background and Aims. A high triglyceride-to-high density lipoprotein cholesterol (TG/HDL) ratio is used to refine cardiovascular risk estimation with high prognostic power even in healthy youths; nonetheless, the underlying mechanisms are debated. We hypothesized that these might reside in unfavorable combinations of lipoprotein subclasses, since the traditional density-based classification of lipoproteins overlooks the heterogeneity of particle subclasses differing in dimensions and atherogenic potential.
Methods. We examined data from the Yale Pathogenesis of Youth Onset Type 2 Diabetes study cohort including 630 children and adolescents of different ethnicity with overweight/obesity and devoid of other cardiovascular risk factors, undergoing a thorough metabolic characterization comprising 3-hour oral glucose tolerance test and abdominal magnetic resonance imaging. Lipoproteins were analyzed with a 400-MHz proton nuclear magnetic resonance (NMR) analyzer.
Results. Analysis was performed on 592 individuals aged 13±3 years, 58% females, BMI z-score 2.1±0.8, divided in quartiles of TG/HDL ratio. Independently from sex, age, ethnicity, BMI, fasting glucose, and insulin sensitivity, the highest TG/HDL quartile showed increased visceral adiposity, intrahepatic fat, worse metabolic profile, and increased absolute concentration of very-low-density (VLDL; +178%, $p<0.0001$), intermediate-density (IDL; +338%, $p<0.0001$), and low-density (LDL; +42%, $p<0.0001$) lipoprotein particles. TG/HDL ratio positively correlated with the average particle size of VLDL ($r=0.37$, $p<0.0001$), and negatively with size of both LDL ($r= -0.51$, $p<0.0001$) and HDL ($r= -0.69$, $p<0.0001$). The relative and absolute frequency of the proatherogenic large VLDL, very small LDL, and small HDL progressively increased across TG/HDL quartiles.
Conclusions. Children and adolescents with a high TG/HDL ratio display a distinctive, unfavorable lipoprotein profile characterized by high levels of ApoB-containing lipoproteins mainly driven by a relative and absolute increase in proatherogenic subclasses. This novel may partly explain the increased cardiovascular risk associated with high TG/HDL ratio, justifying its use to stratify atherosclerotic cardiovascular risk beyond traditional risk factors.

LIPOPROTEIN(A) DOES NOT HAVE A CLINICAL ARTERIAL OR VENOUS PROTHROMBOTIC EFFECT

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Background and Aim. Over the past decade, lipoprotein(a) [Lp(a)] has been the subject of controversy and debate about its physiological functions and roles in atherogenesis, thrombogenesis, and development of cardiovascular diseases. Lp(a) is an apoB-containing lipoprotein covalently bound to an apolipoprotein(a) [apo(a)], and is causally associated with the risk of cardiovascular disease. Because the apo(a) moiety has sequence homology with plasminogen, it has been suggested that Lp(a) may exert a prothrombotic effect. However, evidence on its role as a risk factor for venous thromboembolic events remains controversial. We therefore sought to determine whether Lp(a) has a clinically significant venous or arterial prothrombotic effect, carrying out a Mendelian randomization study.
Methods. An Lp(a) genetic score was calculated for each UK Biobank participant by summing the number of increasing alleles inherited at rs3798220 and rs10455872 variants, which are the main polymorphisms in the LPA gene influencing Lp(a) plasma values. To verify the procoagulant and prothrombotic effect of Lp(a) we performed the following analyses. First, we conducted a Mendelian randomization analysis to evaluate the causal effect of Lp(a) on the risk of venous thromboembolism (VTE), as a composite of deep vein thrombosis (DVT) and pulmonary embolism (PE), among participants in the UK Biobank. Then, we evaluated the causal effect of Lp(a) on the risk of myocardial infarction (MI) in the entire study sample. Finally, we repeated the latter analysis stratifying by two genetic scores that mimic the effect of antiplatelet and antithrombin therapies. The effect of increased Lp(a) on VTE and MI was assessed using Cox proportional hazard models adjusted for age, sex, and the first 10 principal components of ancestry, with age as the time scale, and expressed as hazard ratios (HR) and 95% confidence intervals (95%CI). The risk was estimated using both Lp(a) genetic score and measured Lp(a) concentrations (each 100 nmol/L increase in measured levels).
Results. Among 445,719 participants (mean age 57.3 years, 54% female), a total of 17,432 subjects had an incident VTE event, and 21,868 had a first MI. The median [IQR] level of Lp(a) [nmol/L] increased with increasing value of the genetic score (13.6 [6.2-35.0], 146.3 [104.8-200.2], 261.8 [190.2-336.0]), as expected. Lp(a) was not associated with the risk of VTE (HR: 1.00, 95%CI: 0.97-1.03,), neither with the risk of DVT (HR: 1.00, 95%CI: 0.96-1.05) or PE (HR: 1.00, 95%CI: 0.96-1.04) evaluated separately. By contrast, Lp(a) was strongly associated with the risk of MI (HR: 1.32 per 100 nmol/L higher Lp(a), 95%CI: 1.26-1.38), with the effect of increased Lp(a) concentrations on the risk of atherosclerotic events that did not change with the type of atherosclerotic event considered. Furthermore, despite the antithrombin score was associated with a dose-dependent step-wise decrease in the risk of VTE and the antiplatelet score was associated with a dose-dependent step-wise decrease in the risk of MI (Figure E), as expected from antiplatelet and antithrombin therapies, the effect of Lp(a) on the risk of MI was not diminished by either genetically determined thrombin or platelet inhibition, suggesting that the effect of Lp(a) against atherosclerotic disease is not modulated by these treatments.
Conclusions. Lp(a) does not have a clinically significant venous or arterial prothrombotic effect. Indeed, genetically determined and measured Lp(a) concentrations were not associated with thrombotic events. Moreover, the effect of increased Lp(a) levels on MI was not attenuated by antithrombotic therapies. Therefore, the increased risk of MI caused by elevated Lp(a) is unlikely to be reduced by treatment with an antiplatelet or antithrombin therapy, emphasising that drugs specifically targeting Lp(a) are extremely needed.

THE SODIUM-GLUCOSE COTRANSPORTER-2 (SGLT2) INHIBITORS REDUCE PLATELET ACTIVATION AND THROMBUS FORMATION BY LOWERING NOX2-RELATED OXIDATIVE STRESS: A PILOT STUDY

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Introduction. Sodium-glucose cotransporter-2 inhibitors or Gliflozins, the newest anti-hyperglycemic class, induce cardioprotective benefits in patients with type 2 diabetes (T2D). As platelet activation and oxidative stress play a key role in atherothrombotic-related complications, we hypothesized that gliflozins might modulate oxidative stress, platelet activation, and thrombus formation.

Methods. We performed an interventional open-label single-arm before-after study in 32 T2D patients on top of ongoing metformin therapy. The population was divided into two groups: treatment with GLP-1 receptor agonists (GLP-1RA, Group A) and gliflozins (Group B). Oxidative stress, platelet activation, and thrombus growth were assessed before and after 15 days of treatment.

Results. Compared to baseline, gliflozins treatment significantly decreased sNOX2-dp (-45,2%,p<0.001), H2O2 production (-53.4%,p<0.001), TxB2 (-33.1%,p<0.001), sP-selectin (-49.3%,p<0.001) and sCD40L levels (-62.3%,p<0.001) as well as thrombus formation (-32%,p<0.001), whereas potentiated antioxidant power(H-BA,+30.8%,p<0.001). Moreover, a significant difference in oxidative stress, platelet activation, and thrombus formation across groups A and B was found. In addition, in vitro study on stimulated platelets treated with gliflozins (10–30μM) showed a reduction in oxidative stress, platelet activation, and thrombus growth.

Conclusion. Our results showed that gliflozins have an antiplatelet and antithrombic activity related to NOX2 downregulation suggesting a new mechanism responsible for cardiovascular protection.

TREATMENT WITH PCSK9 INHIBITORS IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA LOWERS PLASMA LEVELS OF PLATELET-ACTIVATING FACTOR AND ITS PRECURSORS: A COMBINED METABOLOMIC AND LIPIDOMIC APPROACH

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Background. Familial hypercholesterolemia (FH) is characterized by extremely high levels of circulating low-density lipoprotein cholesterol (LDL-C) and is caused by mutations of genes involved in LDL-C metabolism, including LDL receptor (LDLR), apolipoprotein B (APOB), or proprotein convertase subtilisin/Kexin type 9 (PCSK9). Accordingly, PCSK9 inhibitors (PCSK9i) are effective in LDL-C reduction. However, no data are available on the pleiotropic effect of PCSK9i. To this end, we performed an untargeted metabolomics approach to gather a global view on changes in metabolic pathways in patients receiving treatment with PCSK9i.

Methods. FH patients starting treatment with PCSK-9i were evaluated by an untargeted metabolomics approach at baseline (before PCSK9i treatment) and after 12 weeks of treatment.

Results. 25 FH subjects were enrolled on maximal tolerated lipid-lowering therapy prior to study entry. After a 12 week treatment with PCSK9i, we observed an expected significant reduction in LDL-cholesterol levels (from 201.0±69.5 mg/dL to 103.0±58.0 mg/dL, p<0.001). The LDL-C target was achieved in 36% of patients. After peak validation and correction, after 12 weeks of PCSK9i treatment as compared to baseline, we observed increments in creatine (p-value =0.041), indole (p-value =0.045), and indoleacrylic acid (p-value= 0.045) concentrations. Conversely, significant decreases in choline (p-value =0.045) and phosphatidylcholine (p-value < 0.01) together with a reduction in platelet activating factor (p-value =0.041) were observed.

Conclusions. Taking advantage of untargeted metabolomics, we first provided evidence of concomitant reductions in inflammation and platelet activation metabolites in FH patients receiving a 12 week treatment with PCSK9i.

CHANGE OVER TIME OF LIPID PROFILE RELATES TO STEROID TREATMENT BUT NOT TO AN INFLAMMATORY STATE IN GRANULOMATOSIS WITH POLYANGIITIS (GPA)

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Introduction. Granulomatosis with polyangiitis (GPA) is a small vessel vasculitis. Anti-Neutrophils cytoplasm Antibodies (ANCA) characterize the disease in a large number. Inflammation of the vessel wall may induce multiple vascular damages, mainly in the lung and kidneys. Lipid and metabolic profile and cardiovascular risk are far to be determined in these patients. However, atherosclerosis is accelerated during vasa inflammation. Thus, Cardiovascular atherosclerotic disease (ASCVD) may represent a risk for patients' outcomes.The purpose of the study is to evaluate ASCVD risk and lipid profile in GPA over time during disease follow-up.
Methods. We retrospectively evaluated 37 patients (22 Females and 15 Males, aged 51.45 ± 17.15) who received a diagnosis of GPA (T0) according to the recent international guidelines. Patients were evaluated at 1 (T1) and 2 (T2) year follow-up. All patients were treated with high steroid dose followed by a one year tapering, associated to another immunosuppressant. Lipid profile included total cholesterol, HDL, LDL and Triacylglycerols. To evaluate inflammatory activity, we evaluate erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and neutrophil to lymphocyte ratio (NLR) at the same time points. ANOVA for repeated values was used to evaluate the trend over time and Tukey's multiple comparisons test was a second step evaluation.
Results. At one year follow-up there was an increase in total cholesterol compared to baseline (T0 181.60 ± 38.33 vs T1 259.50 ± 65.76 mg/dl, $p<0.05$) and T2 (T1 259.50 ± 65.76 mg/dl vs T1 186.30 ± 51.45 mg/dl, $p<0.05$). Similarly, LDL (T1 259.50 ± 65.76 mg/dl vs T0 117.70 ± 47.65 mg/dl, $p<0.05$; vs T2 95.00 ± 9.89 mg/dl) presents the same trend, while Triacylglycerols increased in T1 compared to baseline (T1 253.50 ± 216.20 mg/dl vs T0 112.20 ± 27.43 mg/dl, $p<0.05$), but no difference there was in T2 compared to T1 or T0. Moreover, no difference was found in HDL between the different time points. CRP was no different in the different time points, despite a reduction being noticed. On the contrary, we found a reduction at T2 but not in T1 in ESR (T1 259.50 ± 65.76 mg/dl vs T0 117.70 ± 47.65 mg/dl, $p<0.05$) and NLR (T1 259.50 ± 65.76 mg/dl vs T0 117.70 ± 47.65 mg/dl, $p<0.05$).
Conclusion. Our data suggest that a change in lipid profile may not relate to better control of inflammation. On the contrary, the increase in the first year of follow-up should be a consequence of steroid treatment needed to spread disease control. These data may be helpful in the evaluation of both cardiovascular disease and lipid metabolism due to the connection between the two parameters with vessel inflammation. Further studies are needed to better evaluate the cardiovascular effect of vasculitis and consequent treatment.

MACROPHAGE POLARIZATION MARKERS IN SUBCUTANEOUS, PERICARDIAL AND EPICARDIAL ADIPOSE TISSUE ARE ALTERED IN PATIENTS WITH CORONARY HEART DISEASE

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Background. Epicardial and pericardial adipose tissue (EAT, PAT) surround the heart, with EAT sharing the microcirculation with the myocardium, possibly presenting a distinct macrophage phenotype that might affect the inflammatory environment in coronary heart disease (CHD). This study aims to investigate the expression of genes in different AT compartments driving the polarization of AT macrophages towards an anti- (L-Galectin 9, CD206) or pro-inflammatory (NOS2) phenotype.
Methods. EAT, PAT and subcutaneous (SAT) biopsies were collected from 52 CHD patients undergoing coronary artery bypass grafting and 22 CTRLs undergoing aortic valve replacement. L-Galectin 9, CD206 and NOS2 AT gene expression and circulating levels were analysed through RT-PCR and ELISA, respectively.
Results. The measured markers were similarly expressed in all AT compartments in CHD and CTRLs, as also L-Gal9 and CD206 circulating levels, while NOS2 serum concentration was higher in CHD ($p=0.012$ vs CTRLs). In CTRLs, NOS2 expression was lower in EAT compared to SAT ($p=0.007$), while in CHD patients CD206 expression was lower in SAT and EAT compared to PAT ($p=0.003$, $p=0.006$, respectively). In CHD patients NOS2 expression in SAT correlated to that in PAT and EAT ($r=0.556$, $p=0.007$, both), suggesting an overall inflammatory milieu driven by CHD. CD206 expression correlated positively to L-Gal9 ($r=0.561$, $p<0.0001$) only in EAT. These associations weren't observed in CTRLs. Among CHDs, subjects with LDL-C>1.8mmol/l showed higher EAT and PAT NOS2 expression vs subjects with LDL-C<1.8mmol/l ($p<0.05$, both), possibly increasing the cardiac AT pro-inflammatory activation. BMI correlated with CD206 expression in SAT and PAT in CHD and CTRLs ($p<0.05$, all), and with L-Gal9 in EAT in CHD ($r=0.503$, $p=0.02$). Altogether, the lower EAT CD206 expression in CHD patients suggests a macrophage reprogramming towards a pro-inflammatory phenotype, also supported by the lower EAT NOS2 expression in CTRLs. Hence, EAT macrophage polarization might represent a promising field of investigation to target the altered inflammation in CHD.

EFFICACY AND SAFETY OF VASCULAR DOSE OF RIVAROXABAN IN PATIENTS WITH CARDIOVASCULAR DISEASE: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Background. Low dose rivaroxaban 2,5 mg twice daily (LDR) has been approved as secondary prevention in patients with coronary artery disease (CAD) and peripheral artery disease (PAD).
Aims. To assess the efficacy and safety of LDR in patients with CAD and/or PAD in RCTs.
Methods. Systematic review and meta-analysis of randomized controlled trials (RCTs) including CAD and/ or PAD patients treated with LDR. Efficacy endpoints were cardiovascular events (CVEs), myocardial infarction, stroke, all-cause and cardiovascular death. Any, major and fatal bleeding, and intracranial haemorrhage (ICH) were safety endpoints. Number needed to treat (NNT) and number needed to harm (NNH) were calculated for LDR+ASA vs ASA treatment.
Results. 9 RCTs were included with 45,836 patients: 34,276 with CAD and 11,560 with PAD. Overall, 4,247 CVEs and 3,082 bleedings were registered. LDR in association with either any antiplatelet drug or ASA alone reduced the risk of CVEs (Hazard Ratio [HR] 0.86, 95% Confidence Interval [95%CI] 0.78-0.94) and ischemic stroke (HR 0.68, 95%CI 0.55-0.84). LDR + ASA increased the risk of major bleeding (HR 1.71, 95%CI 1.38-2.11) but no excess of fatal bleeding or ICH was found. The NNT to prevent one CVE for LDR was 63 (43-103) and the NNH to cause a major bleeding was 107 (77-193).
Conclusion. LDR reduces CVEs and ischemic stroke in patients with CAD/PAD. There was an increased risk of major bleeding but no excess of fatal or ICH was found. LDR seems to have a favourable net clinical benefit compared to ASA treatment alone.

MORTALITY RISK OF ATRIAL FIBRILLATION PATIENTS WITH AN INDICATION TO STATIN TREATMENT BUT LEFT UNTREATED: INSIGHTS FROM THE START NATIONWIDE REGISTRY

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Background. Statins are mainstream drugs for cardiovascular prevention. Use of different statins in atrial fibrillation (AF) patients is barely known. We also investigated the association between underuse of statins and mortality risk in a large AF cohort.
Methods. 5,477 patients from the ongoing nationwide START registry were included. The prevalence of different statins was reported and the association with all-cause and cardiovascular mortality investigated. We also studied mortality risk of patients with an indication to but not taking statins.
Results. Mean age was 80.2 years, and 2,539 patients (46.4%) were women. Overall, 1,578 (28.8%) of patients were on statins. The most prescribed statins were atorvastatin (45.3%), simvastatin (35.4%) and rosuvastatin (15.3%). In a mean follow-up of 22.5±17.1 months, 491 all-cause and 106 cardiovascular deaths occurred. At multivariable Cox regression analysis, statin use was inversely associated with all-cause and cardiovascular mortality (HR 0.755, 95%CI 0.598-0.953, p=0.018 and HR 0.398, 95%CI 0.241-0.660, p<0.001). In our cohort, 24.1% of high cardiovascular risk patients were not taking statins despite an indication to treatment. Of these, 4.5% had more than one indication to statin. Under prescription of statins was associated with higher risk of all-cause mortality (multivariable HR 1.283, 95%CI 1.032-1.595, p=0.025) and cardiovascular death (HR 1.986, 95%CI 1.339-2.945, p=0.001).
Conclusions. AF patients with an indication to statins but left untreated disclose a high risk of all-cause mortality. Statin prescription should be implemented in the AF population to reduce the residual cardiovascular risk of these patients.

CARDIOVASCULAR RISK ASSESSMENT IN A COHORT OF CHILDREN WITH SEVERE PRIMARY HYPERCHOLESTEROLEMIA

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Introduction. Familial hypercholesterolemia (FH) is one of the most common genetic dyslipidemias characterized by marked hypercholesterolemia that exposes affected patients to a high risk of cardiovascular disease (CVD). The identification of cardiovascular risk factors starting from pediatric age allows the identification of those FH patients for whom earlier and more aggressive treatment should be reserved, as they are exposed to a higher risk.
Patients and Methods. We conducted an observational analysis of a cohort of 126 children and adolescents (9,8±3,5 years) with FH (median LDL-C value: 191.5 mg/dl). The aim is to define the main risk factors that can be identified in pediatric age, and we then attempted to stratify the analyzed population according to the identified risk factors. The first stratification was performed considering only LDL-C values and the score given according to the modified DLCN SCORE for Italian pediatric population. We subsequently added the most significant risk factors identified: presence of CVD or premature CVD (pCVD, before 55 and 60 years in male and female, respectively) in first- or second-degree relatives, Lp(a)>30 mg/dl, overweight or obesity, MTHFR TT genotype.
Results. Considering only LDL-C values, patients were stratified as follows: 25 at moderate risk, 16 at medium risk, 21 at high risk, and 64 at very high risk. Adding up the additional risk factors, patients were reclassified as: 19 moderate, 15 medium, 15 high risk, and 77 patients at very high risk. Overall, by applying the proposed score 24 patients (19% of the cohort) were reclassified to a higher risk class than using LDL-C value only.
Conclusions. We can speculate that a careful identification of risk factors could allow identifying patients who required more aggressive treatment starting from pediatric age.

MAFLD AND COMORBIDITIES: AN OBSERVATIONAL, RETROSPECTIVE STUDY OF PHENOTYPES AND DISEASE PREDICTORS

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Metabolic Associated Fatty Liver Disease (MAFLD) is characterized by the association of fatty liver with metabolic alteration. Aim of this study was to identify MAFLD's different phenotypes and the predictive power of clinical and biochemical variables towards the potential evolution to cirrhosis. We analysed 418 patients with newly diagnosed MAFLD gathering anthropometric and bio-humoral data, calculating steatosis (FLI, HSI) and fibrosis (FIB-4, APRI, NFS) indices, and measuring hepatic stiffness and fatty involvement (Controlled Attenuation Parameter, CAP) through FibroScan. In the MAFLD cohort, compared to non-diabetic overweight/obese and lean subjects, subjects with type 2 diabetes (T2D, n=68, 16.3%) were older (50.0±0.7, 48.2±1.5 and 57.7±1.5 years, p<0.0001), with higher waist circumference (103.4±10.5, 88.9±8.3 and 180.7±16.2 cm, p<0.0001) and slightly lower eGFR (98±1, 100±2 and 91±2 ml/min/1.73 m2, p=0.001). T2D showed higher BMI and triglycerides only with respect to lean subjects. No difference emerged among groups in term of sex, hepatic function (albumin, prothrombin time, platelets, bilirubin) and cytolisis (AST, ALT, GGT) indices, HDL and LDL-cholesterol, ferritin. Steatosis (FLI e HSI) and fibrosis (NFS) scores, hepatic stiffness and CAP were higher in obese and in T2D than lean; FIB-4 was higher in T2D; APRI did not differ among groups. In the whole cohort, the negative effect of BMI on hepatic stiffness was more marked in T2D than in non-T2D (p for interaction 0.001). Biochemistry performed 59 [33-74] months before MAFLD diagnosis was retrieved in 144 participants. In such subset, higher ALT (r=0.28, p=0.0007) and AST (r=0.33, p<0.0001), reduced platelets count (r=-0.20, p=0.02), older age (r=0.18, p=0.03) and higher FIB-4 (r=0.30, p=0.0008) were associated with increased liver stiffness at diagnosis. These data confirm that T2D and obese patients are characterized by a more advanced form of MAFLD, supporting the indication of a systematic use of FIB-4 for a deep screening of these high-risk individuals.

PLASMA HDL PATTERN, CHOLESTEROL EFFLUX AND CHOLESTEROL LOADING CAPACITY OF SERUM IN CARRIERS OF A NOVEL MISSENSE VARIANT (Gly176Trp) OF ENDOTHELIAL LIPASE

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Background. Loss of function variants of LIPG gene encoding endothelial lipase (EL) are associated with primary hyperalphalipoproteinemia (HALP), a lipid disorder characterized by elevated plasma levels of high density lipoprotein cholesterol (HDL-C). Objective. Aim of the study was the phenotypic and genotypic characterization of a family with primary HALP.
Methods. HDL subclasses distribution was determined by polyacrylamide gradient gel electrophoresis. Serum content of preβ-HDL was assessed by (2D)-electrophoresis. Cholesterol efflux capacity (CEC) of serum mediated by ABCA1, ABCG1 or SR-BI was assessed using cells expressing these proteins. Cholesterol loading capacity (CLC) of serum was assayed using cultured human macrophages. Next generation sequencing was used for DNA analysis. Plasma EL mass was determined by ELISA.
Results. Three family members had elevated plasma HDL-C, apoA-I and total phospholipids, as well as a reduced content of preβ-HDL. These subjects were heterozygous carriers of a novel variant of LIPG gene [c.526 G>T, p.(Gly176Trp)] found to be deleterious in silico. Plasma EL mass in carriers was lower than in controls. CEC of sera mediated by ABCA1 and ABCG1 transporters was substantially reduced in the carriers. This effect was maintained after correction for serum HDL concentration. The sera of carriers were found to have a higher CLC in cultured human macrophages than control sera.
Conclusion. The novel p.(Gly176Trp) variant of endothelial lipase is associated with changes in HDL composition and subclass distribution as well as with functional changes affecting cholesterol efflux capacity of serum which suggest a defect in the early steps of reverse cholesterol transport.

VOLANESORSEN TO TREAT SEVERE HYPERTRIGLYCERIDEMIA: A POOLED ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Background. Patient with severe hypertriglyceridemia (sHTG) are often refractory to lipid lowering therapy. Apolipoprotein (Apo) CIII inhibition could be promising to treat subjects with sHTG. The antisense oligonucleotide against APOC3 mRNA volanesorsen was recently introduced to treat sHTG. We performed a systematic review and meta-analysis of RCTs on efficacy and safety of volanesorsen as compared to placebo treatment in patients with severe HTG.
Methods. Studies were systematically searched in the PubMed, Web of Science, Scopus databases according to PRISMA guidelines. Last search performed on 07thFeb2022.
Results. Four studies showed significant reduction in TG after 3 months of treatment with volanesorsen as compared with placebo (MD: -73.9%; 95%CI: -93.5%, -54.2; P<0.001 I2=89.05%; P<0.001); VLDL-C level (MD: -71.0%; 95%CI: -76.6%, -65.4%; P<0.001 I2= 94.1 %; P<0.001); Apo-B48 level (MD: -69.03%; 95%CI: -98.59.4%, -39.47%; P<0.001, I2=93,51%; P<0.001); Apo-CIII level (MD: -80.0%; 95%CI: -97.5 %, -62.5; P<0.001 I2=94.1 %; P<0.001) with an increase in HDL-C level (MD: +45.92.5%, 95%CI: +37.24%, +54.60%; P<0.001 I2= 94.34%; P<0.001) and in LDL-C level (MD: +68.6%, 95%CI: +7.0%, +130.1%; P<0.001 I2=96.18%; P<0.001) without a significant elevation of Apo-B100 level (MD: +4.58%, 95%CI: -5.64%, +14.79%; P=0.380 I2= 95.09%; P<0.001) in 139 volanesorsen patients as compared to 100 placebo-treated controls. Most of adverse events were mild and related to local injection site reactions.
Conclusions. In patients with severe HTG, volanesorsen is associated with a significant reduction in TG, VLDL-C, Apo-B48, non-HDL-C, and increment of HDL-C as compared to placebo. Documented efficacy is accompanied by an acceptable safety profile.

PREVALENCE OF OBESITY AND LIPID PHENOTYPE IN A COHORT OF DYSLIPIDEMIC PATIENTS: A RETROSPECTIVE STUDY

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Background. Obesity (Body Mass Index or BMI> 30 kg/m^2), classified into 3 stages with increasing severity, is a chronic disease and is associated with multiple comorbidities including the metabolic syndrome characterized by atherogenic dyslipidemia, hypertension and fasting hyperglycemia. Dyslipidemias (hypercholesterolemias, hypertriglyceridemias and mixed dyslipidemias) are classified into primary and secondary and most of them determine an increase in cardiovascular risk.
Objectives. The study aim is to evaluate the prevalence of obesity in a cohort of dyslipidemic patients. Secondary objective is to evaluate the relationship between BMI and lipid phenotype.
Patients and Methods. A sample of 479 patients referred for the first time to the Dyslipidemias Outpatient Clinic of the San Martino Hospital (Genoa) from 2020 to 2022, selecting those who are overweight and obese and classifying them according to their BMI. Of these patients were recorded age, sex, blood pressure, blood tests (glycemia, total cholesterol, HDL, LDL, triglyceridemia).
Results. Of the dyslipidemic patients evaluated in the clinic 44.69% are normal weight, 33.4% overweight, so the prevalence of obesity is 21.91% (in general population 14% ISS data): 16.7% I degree, 3.54% II degree, 1.67% III degree. There is no significant correlation between the increase in BMI and the severity of dyslipidemia, on the contrary, higher lipid levels are found in overweight subjects in comparison to obese patients.
Conclusions. Prevalence of obesity in dyslipidemic patients is higher than in general italian population. Despite excess weight, the patient must always be framed from a lipidological point of view in order to correctly diagnose primary dyslipidemias (FH, polygenic hypercholesterolemia, FCHL), in particular those with high cardiovascular risk.

CARDIOVASCULAR RISK IN SARCOIDOSIS: A PROGNOSTIC STRATIFICATION MODEL BASED ON SUBCLINICAL ATHEROSCLEROSIS EVALUATION

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Sarcoidosis is a chronic granulomatous disease that can affect any organ and that can lead to increased risk of atherosclerosis and cardiovascular (CV) disease. The aim of our observational study was to define a prognostic stratification model of sarcoidosis patients based on CV risk assessment. A cohort of 53 sarcoidosis patients and a cohort of 48 healthy volunteers were enrolled. Sarcoidosis patients were divided in four subgroups based on different organ involvement; CV risk evaluation was performed through the analysis of hemodynamic and morphological parameters of common carotid ultrasound and using CV risk scores. Results showed CV risk scores significantly higher in sarcoidosis cohort than in the control group (Framingham score, American Heart Association/American College of Cardiology score and Heart score: p=0,008, p=0,000 and p=0,034, respectively). The assessment of common carotid doppler parameters showed the presence of subclinical atherosclerosis significantly more pronounced in sarcoidosis group: peak systolic velocity (PSV) and end diastolic velocity (EDV) were significantly lower in sarcoidosis cohort (p=0,045 and p= 0,017, respectively), whereas intima media thickness (IMT) showed higher values in sarcoidosis group than in controls (p=0,016). The analysis of sarcoidosis phenotypes showed no significative differences of CV risk among them when CV risk scores were considered, while partial differences emerged by evaluating surrogates of subclinical atherosclerosis. A relationship between CV risk score and carotid doppler ultrasound parameters was detected: EDV showed an inverse correlation with Framingham score (R=-0,275, p=0,004), whereas IMT showed a direct one (R=0,429; p=0,001); furthermore, an inverse correlation between PSV and EDV and illness duration (R=-0,298, p=0,030 and R=-0,406, p=0,002, respectively) was found, suggesting a higher CV risk in patients with a longer story of disease. Our study suggests a useful role of subclinical atherosclerosis evaluation in clinical and prognostic phenotyping of sarcoidosis patients.

PRO-INFLAMMATORY EFFECT OF ANGIOPOIETIN-LIKE 3 ON THP-1 MACROPHAGES

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Background and Aim. ANGPTL3 is an hepatokine acting as negative regulator of lipoprotein lipase (LPL) with its N-terminal domain. Besides this activity, the C-terminal domain of ANGPTL3 interacts with integrin αVβ3. Since integrins are involved in inflammation and in the initiation of the atherosclerotic plaque, the aim of our study was to evaluate the potential direct pro-inflammatory action of ANGPTL3 through the interaction of fibrinogen-like domain (FLD) and integrin αVβ3.
Methods. We utilized cultured THP-1 derived macrophages and evaluated their pro-inflammatory phenotype in response to treatment with human recombinant ANGPTL3 (hANGPTL3). By western blot, RT-qPCR, biochemical analysis and ELISA assays, we determined the expression of genes and proteins involved in lipid metabolism and inflammatory response as well as intracellular cholesterol and triglycerides levels.
Results. Incubation of THP-1 derived macrophages with 100ng/mL of hANGPTL3 increased the mRNA expression of proinflammatory cytokines IL-1β, IL-6 and TNFα (respectively 1.87±0.08, 1.35±0.11, 1.57±0.49 fold vs control), thus demonstrating the positive involvement of ANGPTL3 on inflammation in THP1 derived macrophages. The secretion of TNFα, determined by ELISA assay, was also induced by hANGPTL3 (1.98±0.4 fold vs control). The pro-inflammatory effect of hANGPTL3 was counteracted by the co-treatment with RGD peptide, inhibitor of integrin αVβ3, reducing the mRNA levels of IL-1β. Moreover, intracellular triglyceride and cholesterol concentration after incubation with hANGPTL3 increased respectively by 30% and 18% compared to control.
Conclusions. ANGPTL3 is an important liver-derived regulator of plasma lipoprotein metabolism, and overall, our results allow us to define an additional important role of ANGPLT3 in promoting the inflammatory response that leads to plaque formation through the integrin αVβ3. The originality of this work is the identification of a possible new pathophysiological action of ANGPTL3 in the context of atherosclerosis. Its localization within the atherosclerotic plaque could be linked to the disease even independently of the action on lipid metabolism. This new evidence could have significant implications in evaluating the efficacy of new anti-AGPTL3 therapies such as evinacumab and this needs to be further investigated.

SENESCENCE AND ATHEROSCLEROSIS: CHARACTERIZATION OF A REPLICATIVE SENESCENCE MODEL IN VASCULAR SMOOTH MUSCLE CELLS

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Cellular senescence is characterized by growth arrest, senescence-associated secretory phenotype (SASP), and oxidative stress. Accumulation of senescent vascular smooth muscle cells (SMCs) contributes to aging and cardiovascular disease. Senescent SMCs are present in atherosclerotic plaques and contribute to their instability. We aimed at establishing the molecular signatures of replicative senescence (RS) in SMCs.
Human aortic SMCs were serially passaged to represent different stages of RS and used from 5th to 7th passages (young cells) and from 15th to 17th passages (old cells). We measured SA-β-gal activity (a marker of senescence), genes, proteins, and long non-coding RNA (lncRNAs) expression by qPCR and western blot analysis, morphological and nuclear changes by immunofluorescence, and cell proliferation by cell counting.
More than 40% of old cells stained positive for SA-β-gal compared to 10% of young cells and showed an increased β-galactosidase-1 expression. Old cells have a diminished proliferation rate (doubling time of 42 hours compared to 29 hours in young cells), a migratory activity reduced by 50%, a downregulation of contractile markers (α-actin, calponin), but increased cell cycle inhibitors (p21/p16) expression. Senescent/old cells showed a flattened appearance and enlarged and irregular nuclei. The expression levels of LMNB1 and HMGB1 were downregulated in old cells, indicating an altered nuclear membrane. Old cells showed an increased expression of SASP molecules (e.g.NF-kB, IL1β, MMP-1,-2,-3), and of "Related glycolysis inhibitor and calcium channel regulator" (RRAD), recently associated in cellular senescence as a negative regulator. Also, a set of lncRNAs (PURPL, SENEBLOC, NEAT1, MIR31HG and ANRIL) was modulated in old cells. The modification of markers specific for the contractile state and migratory activity, together with low-level proliferation in old SMCs, could contribute significantly to inefficient plaque repair and instability. The detection of novel genes/lncRNAs deregulated in senescent SMCs could be helpful for future studies on potential anti-aging factors.

COMBINATION OF LIPOPROTEIN APHERESIS AND ALIROCUMAB THERAPY IN A PATIENT WITH ELEVATED LIPOPROTEIN(A) LEVELS AND RADIATION-INDUCED CARDIOVASCULAR DISEASE: A CASE REPORT

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Introduction. Lipoprotein apheresis (LA) is effective in acutely lowering concentration of low-density lipoprotein cholesterol (LDL-C) and lipoprotein(a). PCSK9 inhibitors have potential to improve performance of LA, dampening the lipid rebound effect and possibly reducing its frequency. However, no data are available on the effectiveness and safety of combination therapy in patients with radiation-induced atherosclerotic cardiovascular disease (ASCVD) and concurrent Lp(a)hyperlipoproteinemia.
Case presentation. We present the case of a 62 years-old male patient with non-familial hypercholesterolemia and Lp(a)hyperlipoproteinemia, complicated by symptomatic and recurrent ASCVD. Medical history included Hodgkin lymphoma treated with radiotherapy at age 28, middle cerebral artery stroke at age 46, unstable angina for critical stenosis of left anterior descending artery treated with percutaneous transluminal angioplasty (PTA) at age 49, unstable angina for intrastent restenosis at age 54, right carotid artery PTA for 80% stenosis at age 56. At baseline LDL-C was 150 mg/dl and Lp(a) 104 mg/dl. No xanthomas or corneal arcus were detected. Because of statin/ezetimibe intolerance, he was initially treated with LA, obtaining mean inter-apheresis LDL-C level of 136.2±14.9 mg/dl (-9.3%) and Lp(a) of 73.1±8.2 mg/dl mg/dl (-29.7%). Then alirocumab 150 mg/2 weeks was added, obtaining mean inter-apheresis LDL-C level of 59.1±9.9 mg/dl (-60.6%), proximal to the recommended target, and Lp(a) of 62.9±11.4 mg/dl (-39.5%). After 5 years of follow-up, he is still on biweekly LA and alirocumab, reporting no cardiovascular events or side effects, demonstrating a good efficacy and safety profile of combination therapy.
Conclusion. This report suggests that combination therapy with LA and PCSK9 inhibitors may have synergic effects on lipid levels, reducing concentration of LDL-C by approximately 60% and Lp(a) by almost 40% in our patient. Its relevance as a highly effective and safe treatment in patients with documented ASCVD, Lp(a)hyperlipoproteinemia and a history of thoracic radiation therapy warrants further investigation in larger studies.

REAL-WORLD USE OF PCSK9 INHIBITORS: ANALYSIS OF DATA FROM 180 PATIENTS AND 5 YEARS OF EXPERIENCE IN A SINGLE LIPID CENTRE

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Background. Elevated levels of low-density lipoprotein cholesterol (LDL-C) and lipoprotein(a) are well established cardiovascular risk factors. Clinical trials have shown that proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) are able to reduce LDL-C by 60% and Lp(a) by 25-30%. However, data regarding the use of PCSK9i in real-world practice are limited.
Methods. Monocentric observational cohort study aiming at evaluating the efficacy of PCSK9i therapy in reducing LDL-C and Lp(a) levels in a real-world setting.
Results. We recruited 180 patients (112 males, 68 females, mean±DS age 63.3±10.9 years): 35.6% had familial hypercholesterolemia (FH), 63.3% coronary heart disease, 7.2% stroke, 48.9% peripheral vascular disease, 67.2% hypertension, 40% impaired fasting glucose, 18.9% diabetes mellitus (DM), 23.9% multiple cardiovascular events, 57.8% statin intolerance. Baseline mean±DS LDL-C levels were 149.8±52.5 mg/dl. 35% of patients had hyperLp(a), median baseline levels 89 mg/dl [IQR 62-127]. FH patients had higher mean LDL-C levels (179.8 vs 133.2 mg/dl). Lower mean LDL-C levels were observed in patients with DM (134.5 vs 153.4 mg/dl) and hyperLp(a) (143.5 vs 160.5 mg/dl). LDL-C levels were reduced between -46.8% and -60.8%, while Lp(a) levels in hyperLp(a) patients between -2.8% and -12.3%. LDL-C target in very high-risk patients (<55 mg/dl) was reached in 60.4% at 3 months, 55.2% at 6 months, 58.2% at 12 months, 52.6% at 24 months, 52.3% at 36 months, 55.4% at 48 months, 56% at 60 months, 46.2% at 72 months. During follow-up, 14 patients suspended PCSK9i, 5 patients interrupted apheresis. Side effects were reported in 13 subjects, mostly injection-site reactions or flu-like symptoms.
Conclusion. We observed a significant reduction in LDL-C and a high percentage of patients achieving recommended targets, with results comparable to those reported in clinical trials. Lp(a) reduction was noticed, but in a less pronounced way. Even in real-world practice, PCSK9i represent an effective and safe therapy.

CHANGES IN HDL SUBFRACTIONS IN RELATION TO THE PRESENCE OF OVERWEIGHT, HYPERTENSION, AND METABOLIC SYNDROME IN A POPULATION OF WOMEN

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Background. Obesity, hypertension, and metabolic syndrome are comorbidities associated with increased cardiovascular disease (CVD) risk. Although there is an inverse correlation between HDL cholesterol (HDL-C) levels and CVD risk, this parameter is not sufficiently predictive. In contrast, HDL functionality, which in turn is closely related to the size of these particles, is emerging as a key factor in dictating their cardio-protective properties. Therefore, assessment of the concentration of HDL-C subfractions could provide more insights into the hypothesized protective activity of HDL-C.

Scope. To assess changes in HDL lipoprotein subfractions in relation to overweight, hypertension, and metabolic syndrome in a population of women.MethodsSeventy-two women aged 35-65 years (52±6), with 25,1±3,9 kg/m^2 and Caucasian race, were enrolled in this study. Fasting blood samples were collected to assess biochemical parameters. Serum HDL-C subfractions were quantified using the Lipoprint HDL System, which allows identification of 10 subclasses.

Results. The large HDL-C 1, 2, 3, 4 and 5 subfractions were found to be significantly higher in normal-weight women than in overweight women. Furthermore, nonhypertensive women showed significantly higher levels of HDL-C subfractions 1, 2 and 3 (large) and, in parallel, a decrease in HDL-C 10 (small) levels compared to women affected by hypertension. In parallel, HDL-C 1, 2, 3 and 4, were significantly more abundant among women without metabolic syndrome versus patients diagnosed with the metabolic syndrome, as opposed to HDL-C 10, which was higher in women with metabolic syndrome.

Conclusions. Conditions linked with impaired cardio-metabolic health are associated with a decrease of large HDL-C subfractions, suggesting that these subfractions correlate with better metabolic profiles and potentially lower cardiovascular risk. In contrast, an increase of the small HDL-C subfraction 10, which occurs in the presence of hypertension and metabolic syndrome, is associated with unfavourable cardio-metabolic profiles.

POSITIVITY OF STATIN-ASSOCIATED MUSCLE SYMPTOMS – CLINICAL INDEX IN A HYPERTENSIVE POPULATION CANDIDATED TO LIPID-LOWERING THERAPY BUT NOT TAKING STATINS

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Background. Statin use has been claimed to be associated with muscle-related symptoms, called SAMS (Statin-Associated Muscle Symptoms). The SAMS-Clinical Index (SAMS-CI) is an approved questionnaire to assess the probability that muscle symptoms are related to statin. Aim: evaluate the difference in prevalence and characteristics of muscle symptoms between hypertensive patients taking statins and hypertensive patients candidates for statins.

Methods. Observational study on 390 outpatients referred to our Hypertension Centre: 250 patients were already on statin therapy and 140 who took at least one other drug different from statins. Patients underwent a modified version of SAMS-CI for patients not taking statins in which the items referred to any other drug or nutraceutical taken by the patients.

Results. Mean age: 60.5±13.6 years; male prevalence: 53.8%; mean SAMS-CI score: 3.33±2.17 points. In the statin group, the main statins taken were rosuvastatin (52.0%) and atorvastatin (37.6%). Patient-reported episodes of muscle symptoms was reported by 50.8% of patients in the group taking statins and by 44.3% in the group not taking them (p=0.217). Within patients with reported episodes of muscle symptoms, a slightly higher score at SAMS-CI emerged in the statin group (3.6±2.4 vs 2.8±1.6 points, p=0.004). Regarding SAMS-CI items, no significant difference emerged in the localization of muscle pain (p=0.170) and timing of symptoms onset in relation to drug (p=0.067). A slightly higher score in the item "resolution timing of muscle symptoms after drug/statin withdrawal" was showed in the statin group (p=0.002).

Conclusion. In our study no significative differences emerged in the prevalence of patient-reported episodes of muscle symptoms between hypertensive patients taking statins and hypertensive patients not taking them. This finding is in line with the growing evidence that most subjective muscle-related adverse effects are misattributed to statins and occurring because of the nocebo/drucebo effect or due to other common conditions.

COMORBIDITY IN LIPOPROTEIN APHERESIS: THEIR ROLE IN THE ERA OF NEW LIPID-LOWERING THERAPIES

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Introduction. Lipoprotein apheresis (LA), decreasing the plasma and tissue pools of lipoproteins, plays a leading role in the management of severe hypercholesterolemia and in atherosclerosis prevention. Recent advances in lipid-lowering therapy can reduce the use of LA which, however, it remains an important therapeutic option such as the impact of comorbidities in effectiveness of therapeutic plan/outcomes.
Methods. Aim of this study was to retrospective evaluate Charlson Comorbidity Index (CCI), presence of major comorbidity and/or concomitant polypharmacy (definite as 5+ drugs daily assumptions) in patients with inherited dyslipidemias on chronic LA.
Results. Since 1994 a total of 83 patients (mean age 55±12 years, male 75%) was treated. In these subjects we recorded a progressive increase in the time-course of CCI / number-of-patient-ratio (from 4.00 to 5.00), consistent with a progressive increase in the care burden. In subjects with more than 5 years of LA treatment (38 patients, mean age 52±12 years, male 66%), we evaluated comorbidity and concomitant polypharmacy: at the end of observation time, they had higher CCI (3.5±1.6 vs 6.0±2.4; p<0.001), polypharmacy (18 vs 53; <0.001), anemia (0 vs 11; p<0.05), heart failure (0 vs 7; p< 0.05), peptic ulcer disease (6 vs 16; p<0.05) and benign prostatic hyperplasia (1 vs 10; p<0.05).
Conclusions. Even in the era of new lipid-lowering therapies, the LA treatment, considered for decades like a therapeutic "Cinderella", established itself as a safe and lifesaving intervention. Patients on chronic LA require a multidisciplinary approach to face their comorbidities and the apheresis unit's medical staff (doctors and nurses) play a pivotal role in creating a bridge with general practitioner and other specialists to overcome the clinical issues management.

PARADOXICAL MACES INCREASE IN LONG-TERM PCSK9-INHIBITORS THERAPY: A TUSCANY COST EFFECTIVENESS STUDY

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Aim. Proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) represent a breakthrough in the treatment of hypercholesterolemia as efficiently and safely reduce LDL-C and cardiovascular events. Aim of this study was to perform a multicentre prospective analysis on the effects of PCSK9i, introduced in Italy on 2017.
Methods. During the study period (July 2017 - February 2022) 246 patients (mean age 61±11 years, male 73%) were enrolled in the CERTI (Costo Efficacia Regione Toscana terapia Inibitori PCSK9) study (evolocumab 142/246; alirocumab 104/246). Lipid value before and after PCSK9i therapy, major cardiovascular events (MACE), intima-media thickness evaluation and adverse events (AEs) recorded during the follow-up were analysed.
Results. PCSK9i therapy allowed a significant improvement in patient's lipid profile (total cholesterol -35%, p<0.001; triglycerides -9%, p<0.05; LDL cholesterol -51%, p<0.001; Lp(a) levels -4%, p<0.05) maintained in the follow-up. No significant variation in intima-media thickness were observed.In the subgroup of patients with more than one year of PCSK9i therapy (165/246 patients) we observed:
1) a 66% reduction in MACEs compared to the year before recruitment,
2) a progressive increase in MACEs during the follow-up (MACEs event/rate at first year 0.08 vs MACEs event/rate at year 5: 0.47),
3) patients with late MACEs are older, with higher prevalence of hypertension, smoking habit and peripheral vascular disease. Moreover, during the follow-up, we recorded AEs in 31% of patients which led to reduction/discontinuation in back-bone lipid-lowering therapy.

Conclusions. Our data agree with the large evidences on effectiveness and tolerability of PCSK9i therapy, however, despite PCSK9i represent a good dyslipidaemias therapeutic option, our study show a progressive increase in MACEs during the follow-up.

DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA IN A LARGE COHORT OF ITALIAN GENOTYPED HYPERCHOLESTEROLEMIC PATIENTS

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Introduction. Familial Hypercholesterolemia (FH) is the most relevant genetic cause of early cardiovascular disease (CVD). FH is suspected when LDL-C levels exceed the 95th percentile of the population distribution, but family and clinical history support the diagnosis. Different scoring systems have been developed, as the Dutch Lipid Clinic Network (DLCN) score, used worldwide to diagnose FH. The aim of the study is to describe the characteristics of FH patients of a large cohort of more than eight hundred genotyped subjects enrolled in an Italian Lipid Clinic and evaluated the DLCN score performance applied retrospectively to the case study.
Materials and Methods. 836 hypercholesterolemic patients with LDL-C > 4.88 mmol/L were genotyped for FH causative mutations in the LDLR, PCSK and APOB genes. Relatives of mutated patients were also analyzed by cascade screening.
Results and Conclusions. Mutation carriers were younger, presented higher LDL-C and DLCN score and lower HDL-C levels in comparison with hypercholesterolemic (HC) noncarriers and presented a five-fold higher prevalence of previous CV events. Carotid US data available in 490 subjects (FH n=195, HC n=295), showed that mutation carriers had an odds ratio of 3.66 (1.43-10.24) for atherosclerotic plaques in comparison with noncarriers. Scoring system were evaluated by ROC analysis in 203 subjects without missing DLCN items and with available pre-therapy LDL-C levels, and LDL-C levels (A.U.C.=0.737) resulted more performing than the DLCN score (A.U.C.=0.662), even including carotid US data (A.U.C.=0.641) in a modified DLCN score version. The DLCN scoring systems failed to demonstrate a clear superiority in predicting FH mutations in comparison with the measure of LDL-C levels in a retrospective case study. The results enforce the need for more performant tools to detect FH.

NOVEL MISSENSE VARIANTS IN THE LMF1 GENE: IDENTIFICATION BY NEXT GENERATION SEQUENCING AND FUNCTIONAL CHARACTERIZATION

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Introduction. Hypertriglyceridemia (HTG) is a common form of dyslipidemia associated with an increased risk of cardiovascular disease and pancreatitis. The severe forms are characterized by very high plasma levels of triglycerides (TG) (>1000 mg/dL -11.2 mmol/l). Monogenic autosomal recessive forms are characterized by homozygous or compound heterozygous loss-of-function mutations of genes involved in the intravascular lipolysis of the triglyceride-rich lipoproteins, namely lipoprotein lipase (LPL), apolipoprotein C2 (APOC2), apolipoprotein A5 (APOA5), glycophosphatidylinositol (GPI)-anchored high-density lipoprotein-binding protein 1 (GPIHBP1), lipase maturation factor 1 (LMF1), and glycerol-3-phosphate dehydrogenase 1 (GPD1). LMF1 has been shown to be essential for the maturation of both LPL and hepatic lipase (HL) to their fully functional forms.
Materials and Methods. We performed Next Generation Sequencing (NGS) analysis on Ion GeneStudio S5 Plus to study the coding exons and intron/exon boundaries of genes affecting the main pathways of triglyceride synthesis and metabolism.
Results and Conclusion. In the majority of subjects no functionally relevant mutations in the LPL, APOC2, APOA5, GPIHBP1 genes were detected. Four patients were found to be carriers of unknown missense variants in LMF1 gene: a) one compound heterozygous carrier for c.787C>T (p.His263Tyr) and c.1381C>T (p.Arg461Cys); b) one homozygous carrier for c.874 G>A (p.Gly292Arg). The other two were heterozygous carriers for c.1351 C/T (p.Arg451Trp) and c.428 C/T (p.Thr143Met) respectively. A functional analysis was carried out to assay LMF1 activity, protein expression and specific activity. The results showed that the Arg461Cys and Gly292Arg dramatically impair LMF1 function, the Arg451Trp does not have an impact, whereas His263Tyr and Thr143Met exhibit moderate effects.

BIGLYCAN INVOLVEMENT IN HEART FIBROSIS: MODULATION OF ADENOSINE 2A RECEPTOR IMPROVES DAMAGE IN CARDIAC FIBROBLASTS

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Cardiac fibrosis is a common pathological feature of different cardiovascular diseases characterized by an aberrant deposition of extracellular matrix (ECM) proteins in the cardiac interstitium and myofibroblasts differentiation stimulated by Transforming Growth Factor (TGF)β. Biglycan (BGN), a small leucine-rich proteoglycan, plays a key role in matrix assembly and phenotypic control of cardiac fibroblasts. Moreover, BGN is involved in the pathological cardiac remodelling through TGF-ß binding. BGN is also acknowledged as a biomarker of atherogenic process, and its role in atherogenesis is currently investigated. Adenosine receptors (ARs), and in particular A2AR, may play a key role in stimulating fibrotic damage through collagen production/deposition. For this reason, A2AR modulation could be though useful tool to manage cardiac fibrosis and reduce fibrotic scar deposition in heart tissue. Therefore, the aim of the present study was to investigate the possible crosstalk between A2AR modulation and BGN in an in vitro model of TGF-β-induced fibrosis. Immortalized Human Cardiac Fibroblasts (IM-HCF) were stimulated with TGF-β (10 ng/ml) for 24 hours to induce a fibrotic phenotype. After TGF-β stimulus, cells were treated with two different A2AR antagonists, Istradefylline (10 µM) and ZM241385 (1 µM) for additional 24 hours. Both A2AR antagonists were able to regulate the oxidative stress induced by TGF-β through intracellular reactive oxygen species (ROS) reduction. Moreover, Collagen1a1, MMPs 3/9, BGN, caspase-1 and IL-1β gene expression appeared to be markedly decreased following A2AR antagonists treatment. The results obtained for Collagen1a1 and BGN were also confirmed when protein expression was evaluated; phospho-Akt protein levels were also reduced following Istradefylline and ZM241385 use, thus suggesting that collagen production involves AKT recruited by A2AR.These results suggest that A2AR modulation might be an effective therapeutic option to reduce the fibrotic processes in the heart. The potential role of this pathway in atherogenesis and atherosclerotic vascular damage should be further investigated.

DIETARY FATTY ACID QUALITY IS AT THE NEXUS BETWEEN IL-18 CIRCULATING LEVELS AND INSULIN RESISTANCE

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Metabolic inflammation not only represents a key feature of obesity, but has also been implicated in the pathogenesis of its comorbidities, including type 2 diabetes. In this regard, the pro-inflammatory cytokine IL-18 has been consistently linked with obesity and insulin resistance. As such, it represents a biomarkers of the metabolic aberrations underpinned by obesity and possibly one of the effectors of metabolic inflammation on insulin resistance. Despite dietary lipids representing a major player in shaping inflammatory responses, their impact on IL-18 circulating levels remains to be fully elucidated. Thus, the aim of this study was to investigate how saturated, unsaturated fatty acids and their ratios affected IL-18 circulating levels. Using a cross-sectional study design a total of 403 individuals aged 66±5 years and with a BMI of 26.5±3.7kg/m2 were characterised with regard to their metabolic health status and their dietary intakes. Particularly, insulin resistance was assessed using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and the metabolic syndrome defined according to the National Cholesterol Education Program-Adult Treatment Panel III criteria. Dietary intakes were investigated using 24h recalls. Additionally, circulating IL-18 levels were measured using an enzyme-linked immunosorbent assay (ELISA).First, it was confirmed the relationship between IL-8 and impaired metabolic health in our cohort. Indeed, IL-18 correlated positively with insulin resistance ($p<0.001$) and individuals with a HOMA-IR >2.5 displayed higher circulating IL-18 levels compared to subjects with a lower HOMA-IR ($p<0.001$). The same held true for the metabolic syndrome, with higher IL-18 plasma levels occurring in individuals affected by the metabolic syndrome ($p<0.001$). In terms of the effect of dietary lipids, the intake of saturated fatty acids tended to positively correlate with IL-18 ($p=0.068$). On the contrary, the monounsaturated/saturated fatty acid ($p<0.001$), omega-3/saturated fatty acid ratio ($p<0.001$) and the intake of eicosapentaenoic ($p=0.0445$) as well as docosahexaenoic ($p=0.006$) acids correlated negatively with IL-18. However, this cytokine did not correlate with total energy, carbohydrate, total lipid or fibre intake ($p>0.05$). Not surprisingly, considering the Mediterranean diet being low in saturated and high in monounsaturated and omega-3 fatty acids, adherence to this dietary pattern also negatively correlated with IL-18 levels ($p=0.044$). Finally, individuals with a HOMA-IR >.5, apart from having higher IL-18 levels, consumed a lower ratio of monounsaturated/saturated fatty acid and omega-3/saturated fatty acids.Thus, the downregulation of IL-18 may be key in underpinning, at least in part, the beneficial metabolic effects of substituting monounsaturated or omega-3 for saturated fatty acids with this cytokine potentially representing a biomarker linking dietary lipids and metabolic outcomes.

STRENUOUS PHYSICAL EXERCISE INDUCES AN INCREASE IN HIGH DENSITY LIPOPROTEIN CHOLESTEROL IN A POPULATION OF OBESE MEN

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Background. Regular exercise increases HDL-cholesterol (HDL-C), but changes induced by a single bout of strenuous physical exercise such as half-marathon or marathon race remain to be fully elucidated. Indeed, the studies conducted so far only focused on athletes or normal weight subjects.
Aim. To evaluate changes in HDL-C induced by strenuous exercise in a population of trained obese men.
Methods. Seventeen obese men aged 40±6 years and with BMI 31.3±2.8 kg/m^2, were enrolled in this study. Participants were trained for 6 months, in preparation to a race of 21.1, 30 or 42.2 km depending of their physical conditions. Fasting blood samples were collected before (pre-race), immediately after (post-race) and three days after (3- post-race) the race to measure biochemical parameters. Heart rate, average speed, maximal aerobic speed (MAS) and VO2 max were measured or estimated during the race.
Results. No changes were observed in the serum levels of total cholesterol, LDL-C, triglycerides, glucose and insulin. Instead, the concentration of HDL-C and cortisol were significantly increased after the race ($p<0.001$ and $p=0.001$, respectively). Cortisol levels returned to baseline three days after the race, HDL-C remained elevated even at 3-post-race relative to pre-race levels ($p=0.041$). Correlation analysis showed a positive association between Δ HDL-C and the MAS (Coef. Pearson= 0.511, $p=0.036$) and VO2max (Coef. Pearson= 0.549, $p=0.022$). Non association was found between Δ HDL-C and race distance or pre-race BMI. Stepwise regression analysis indicated that only MAS was an independent predictor of Δ HDL-C ($\beta=1.475$, $R2=0.261$, $p=0.036$).
Conclusions. Strenuous exercise induced an increase of HDL-C in trained obese men that could be predicted by MAS.

PLASMA LEVELS OF PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 ARE INVERSELY ASSOCIATED WITH N-TERMINAL PRO B-TYPE NATRIURETIC PEPTIDE IN OLDER POPULATION

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Background and Aims. Cardiac natriuretic peptides (NPs) exert several effects on lipid metabolism. Higher NPs levels are likely to be associated with a favorable lipid profile. In in vitro studies, NPs have been found to modulate low-density lipoprotein receptor (LDLR) trafficking by preventing proprotein convertase subtilisin/kexin type 9 (PCSK9) overexpression. The aim of our study is to investigate a possible association between plasma levels of PCSK9 and N-terminal pro B-type natriuretic peptide (NT-proBNP) in vivo.
Methods. We performed a cross-sectional study on 160 consecutive older male and female patients hospitalized for medical conditions. Patients taking lipid-lowering drugs and patients with an admission diagnosis of acute heart failure were excluded. Fasting blood samples were collected after clinical stabilization of the acute illness, the day before discharge.
Results. The mean age was 87.8±6.4 years with a female prevalence (62.5%). The median NT-proBNP was 2340 (814-5397) pg/mL. The mean plasma PCSK9 was 275.2±113.2 ng/mL. We found an inverse correlation between plasma PCSK9 and NT-proBNP ($r=-0.280$; $p=0.001$). This association was confirmed after taking into account NT-proBNP tertiles (plasma PCSK9 levels: 317.4±123.6 ng/mL in the first tertile, 283.3±101.8 ng/mL in the second tertile, 231.3±99.0 ng/mL in the third tertile, $p=0.001$) and even after an adjustment for confounding factors (beta=−0.361, $p=0.001$ for ln(NT-proBNP); beta=−0.330, $p=0.001$ for NT-proBNP tertiles). The strength of the correlation between plasma PCSK9 and NT-proBNP was likely greater in patients affected by type 2 diabetes mellitus ($r=-0.483$; $p=0.006$) and in male patients ($r=-0.431$, $p=0.001$).
Conclusion. The inverse association found between PCSK9 and NT-proBNP plasma levels in our real-life clinical study supports the hypothesis that NPs may play a role in cholesterol metabolism, possibly through an inhibitory action on circulating PCSK9 concentrations, thus increasing the availability of LDLR.

GENETIC PREDISPOSITION PROFILE UNDERLYING FAMILIAL HYPERCHOLESTEROLEMIA

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Background. As many familial hypercholesterolemia (FH) subjects (about 60%) did not demonstrate functional mutations in major candidate genes (LDLR, APOB, PCSK9, LDLRAP1), we assessed FH patients genetic profile by high-throughput sequencing (HTS).
Methods. We analysed 90 FH patients[adults with possible/probable/definite FH according to Dutch Lipid Clinic Network Score (DLCN)]. Targeted HTS (57 genes including those involved in lipid metabolism, supposed to be involved in dyslipidaemia, pharmacogenetics of statins, related to FH polygenic forms, HDL and triglycerides related diseases) was assessed by Illumina technology.
Results. Among 90 patients, 41 carried a rare variant in LDLR gene, whereas 49 patients were LDLR-negative. Talmud score evaluation (Talmud 2013) showed a higher mean value in patients without LDLR mutations, with respect to LDLR-positive (0.972±0.207 vs 0.941±0.175). HTS analysis revealed that 14 LDLR mutation-positive patients also carried likely pathogenetic/uncertain significance mutations in APOB or LDLRAP1 genes. In patients without LDLR mutations, at least 2 rare variants were identified in 24 patients (49%), and at least 3 rare variants were identified in 18 patients (37%). In these patients, a total of 117 rare variants with uncertain significance/conflicting interpretation of pathogenicity have been identified in 44 different genes (APOB, PCSK9, LDLRAP1, ABCB1, ABCG2, ABCG5, ABCG8, ANGPTL3, APOA4, CELSR2, CETP, CREB3L3, DAB2, GCKR, GHR, HFE, ITIH4, LCAT, LIPC, LIPI, LMF1, LPA, LPL, LRP1, MTTP, NPC1, NYNRIN, PON1, PP1R17, SCARB1, SLCO1B1, SLC12A4, SREBF1, SREBF2, SLC22A1, EPHX2, GPD1, OSBPL5, STAP1, ABCA1, DGAT1, INSIG2, NPC1L1, APOA5). Among FH patients, 29 were younger than 18 yrs. Among adults, LDL-cholesterol levels were comparable between LDLR-positive and LDLR-negative group, whereas in younger subjects significantly higher LDL-cholesterol levels were observed among LDLR-positive. As concerns DLCN score, performed in adult population, significantly higher values in subjects carrying LDLR mutation were found.
Conclusions. Present data support the involvement of multiple loci beyond LDLR gene in the modulation of lipid profile, as well as cardiovascular risk. Expansion of genetic analysis might allow a better comprehension of the role of further major/modifier genes, as well as of accumulation of common small-effect LDL-C raising alleles in determining LDL-C levels and cardiovascular events.

IMPACT OF ASIALOGLYCOPROTEIN RECEPTOR AND MANNOSE RECEPTOR DEFICIENCIES ON MURINE PLASMA N-GLYCOME PROFILES

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Background. Recent studies have found that glycan moieties can modulate the progression of atherosclerosis due to the functional impact on cytokines and other inflammatory mediators implicated in the disease. Glycan-binding receptors carry out the clearance of circulating glycoproteins based on the selective affinity for the glycan moiety. Two well described receptors for their selective recognition and clearance of circulating glycoproteins, are the asialoglycoprotein receptor (ASGPR) which recognizes galactose and N-acetylgalactosamine and the mannose receptor C-type 1 (MRC1) which recognizes mannose, fucose and N-acetylglucosamine. In mice models, lack of ASGR1 and MRC1 promotes an improved cardiometabolic phenotype. Yet, the role of those receptors on modulating the glycome and the potential role of glycans in CVD has not been well exported.
Methods. ASGR1-/-, MRC1-/- mice and their WT littermates, were fed in a high-fat diet (45% Kcal fat) for 20 weeks. Plasma was collected and processed for N-glycan release and for linkage-specific sialic acid derivatization. Ultra-high-resolution matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry (MALDI-FTICR-MS) was used for glycan assessment and MassyTool software was used for spectra analysis.
Results. In both plasma from ASGR1-/- and MRC1-/- mice, 78 glycan compositions were assigned. The most prevalent structures were complex glycans, represented for more than 80% by di- and triantennary glycans, with almost complete galactosylation. No change is observed on the galactose in ASGR1-/- mice neither in mannose in MRC1-/-. ASGR1-/- presented a 32% increase in O-acetylation compared to the WT mice (WT 8.2%±0.2%, ASGR1-/- 10.8%±0.4%, P-value<0.001). MRC1 deficient mice did present a 24% reduction in core fucosylation (WT 34.3%±2.2%, MRC1-/- 26.2%±1.1%, P-value=0.002). Also, a trend towards reduction in antennary fucosylation was observed for MRC1-/- mice (WT 12.8%±1.1%, MRC1-/- 10.4%±0.4%, not significant).
Conclusions. This study suggests that a tight control of the glycome is so important for an organism that significant redundance exists in terms of plasma glycoprotein clearance receptors with glycan-epitope specificity. Whether those glycan changes can predict the cardiometabolic state of the disease is under investigation.

LONG-TERM ADVERSE EFFECT OF LIVER STIFFNESS ON GLYCAEMIC CONTROL IN TYPE 2 DIABETIC PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE: A PILOT STUDY

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Background. Currently, there is limited data regarding the long-term effect of liver stiffness on glycaemic control in patients with type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD).
Methods. We prospectively followed an outpatient sample of 61 consecutive post-menopausal women with T2DM and NAFLD, who had baseline data on liver ultrasonography and Fibroscan®-measured liver stiffness (LSM) in 2017 and who underwent follow-up in 2022. Hemoglobin A1c (HbA1c) was measured both at baseline and follow-up.
Results. At baseline, 52 patients had NAFLD (hepatic steatosis) alone and 9 had NAFLD with coexisting clinically significant fibrosis (defined as LSM ≥7 kPa on Fibroscan®). At follow-up, 16 patients had a worsening of glycaemic control (arbitrarily defined as a HbA1c increase ≥0.5% from 2017 to 2022). The prevalence of NAFLD and coexisting significant fibrosis at baseline was at least three times greater among patients who developed worse glycaemic control at follow-up, compared with those who did not (31.3% vs. 8.9%; p=0.030). In logistic regression analysis, the presence of NAFLD and significant fibrosis was significantly associated with an approximately 4.5-fold increased likelihood of developing worse glycaemic control at follow-up (odds ratio 4.66, 95% confidence interval 1.07-20.3), even after adjustment for age, body mass index and baseline use of some glucose-lowering agents that may positively affect NAFLD and liver fibrosis.
Conclusions. Our results suggest that the presence of Fibroscan®-assessed significant fibrosis was associated with a higher risk of developing worse glycaemic control in post-menopausal women with T2DM and NAFLD.

THE AGING OF NEUTROPHILS IS A CRITICAL DETERMINANT OF HIGH FAT DIETS-INDUCED METABOLIC ALTERATIONS

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Aim. High-Fat diet (HFD) promotes metabolic alterations and hyper-activation of myeloid precursors. Neutrophils, the main short-living innate immune responders, physiologically age in circulation, thanks to the signaling of CXCR2 after being released as "fresh" from the bone marrow (BM) by the signaling of CXCR4. We here studied whether the aging is accelerated by HFD and whether it contributes to the development of metabolic alterations.
Methods. We immunophenotyped and metabolically profiled mice with constitutively aged neutrophils (conditional CXCR4 deletion by Cre recombinase on MRP8; CXCR4fl/flCre+), mice with constitutively fresh neutrophils (CXCR2fl/flCre+) and wild-type mice (WT) fed 20 weeks a HFD versus a standard diet (Standard Fat Diet "SFD").
Results. Metabolic alterations developed after feeding a HFD WT resulted in a striking infiltration of neutrophils in liver and visceral adipose tissue (VAT) as compared to SFD.CXCR4fl/flCre+ mice were affected by comparable metabolic alterations to those observed in WT while, CXCR2fl/flCre+ mice were protected from obesity, hepatic steatosis and displayed insulin sensitivity. This phenotype observed of CXCR2fl/flCre+ mice associated with more hepatic utilization of fatty acids and was explained by reduced infiltration of CXCR2fl/flCre+ in the liver, eliciting less inflammatory pathways. Besides, CXCR4fl/flCre+ neutrophils also infiltrated significantly more in VAT, resulting into local inflammation and impaired proresolving skewing of inflammatory macrophages (M1). Notably, these effects were abrogated in presence of CXCR2fl/flCre+ neutrophils, which infiltrated less in VAT and reduced the accumulation of M1 macrophages. In humans Cxcl1 (CXCR2 ligand) and Cxcl12 (CXCR4 ligand) were related to circulating neutrophils counts, accumulation of visceral adiposity in abdominal area and hepatic steatosis indices.
Conclusion. The aging of neutrophils appears as a critical determinant for the development of HFD-induced metabolic alterations and could represent a future target against metabolic diseases.

PCSK9 PLASMA LEVELS ARE ASSOCIATED WITH MECHANICAL VASCULAR IMPAIRMENT IN FAMILIAL HYPERCHOLESTEROLEMIA SUBJECTS WITHOUT A HISTORY OF ATHEROSCLEROTIC CARDIOVASCULAR DISEASE: RESULTS OF SIX-MONTH ADD-ON PCSK9 INHIBITOR THERAPY

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Proprotein convertase subtilisin/kexin type-9 (PCSK9) is a key regulator of low-density lipoprotein (LDL) metabolism and of low-density lipoprotein receptor (LDLR) degradation. Changes in PCSK9 plasma levels have been recorded in subjects who were prescribed lipid lowering therapy (LLT). Few data exist regarding the role of PCSK9 in vascular damage. The aim of the study was to evaluate the impact of PCSK9 plasma levels on pulse wave velocity (PWV), as well as the effect of PCSK9 inhibitors (PCSK9-i) on circulating PCSK9 concentration and PWV in a cohort of heterozygous familial hypercholesterolemia (HeFH) subjects. Biochemical analyses and PWV assessment were performed at baseline (T0), after 6 months of high-efficacy statin plus ezetimibe (T1) and after 6 months add-on therapy with PCSK9- i (T2). PCSK9 levels were evaluated in 26 selected HeFH subjects at T0, T1 and T2. 26 subjects were enrolled as healthy controls for the reference value for PCSK9 plasma levels. PWV values decreased at each time point in HeFH subjects after starting LLT (8.61 ± 2.4 m/s, –8.7%; $p<0.001$ vs. baseline at T1, and 7.9 ± 2.1 m/s, –9.3; $p < 0.001$ vs. both T1 and baseline). This was correlated to PCSK9 levels ($r = 0.411$, $p = 0.03$). PCSK9 levels increased on statin/EZE therapy (+42.8% at T1) while it decreased after PCSK9-i was started (–34.4% at T2). We noted a significant relationship between PCSK9 levels and PWV changes at T1 and T2. In conclusion, PCSK9 levels were associated with baseline PWV values in HeFH subjects; moreover, we found that PCSK9 level variations seemed to be correlated with PWV changes on LLT. A longer observation time and wider sample size are needed to assess the potential role of PCSK9 plasma levels on the vascular function and remodeling, and to clarify the effects of PCSK9-i in these pathways.

KIDNEY DISEASE, A NEW COMPONENT OF FCS PHENOTYPE: PRELIMINARY EVIDENCE FROM A COHORT STUDY

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Background. Familial Chylomicronemia Syndrome (FCS) is a very rare monogenic autosomal recessive disorder of lipid metabolism determining severe hypertriglyceridemia (HTG). The hallmarks of FCS are fasting chylomicronaemia (TGs levels greater than 885 mg/dl), poor response to conventional lipid lowering medication and high risk of acute pancreatitis. It has been reported also kidney complication in FCS, but the data are sparse.
Aim and methods. The study population comprises 20 patients defined as FCS by NGS sequencing that have been included in the LIPIGEN study. Among this, 11 were females and 9 males with a median age of 51 years (IQR 38.2-63.7). Clinical and biochemical information were collected retrospectively. In most cases (85%) patients were carrying homozygous mutations in LPL. The majority (85%) of them had experienced at least one episode of acute pancreatitis (AP) that was recurrent in 12 patients (75%). Glomerular filtration rate (eGFR) was estimated with CKD-EPI and diagnosis of CKD was performed based on the more recent Kidney Disease Improving Global Outcomes (KDIGO) guideline. Hyperfiltration was defined as an increase in eGFR greater than 75° percentile. Proteinuria was defined as protein in the urine spot ≥30 mg/dl or ≥ 150 mg/day in the 24-hour urine sample.
Results. The median GFR values was 99.5 (IQR 93.8-113.7) ml/min. Four (20.0%) patients had hyperfiltration whereas 3 (15.0%) were exhibiting an eGFR below 90 ml/min. Overall, 5 (25%) have had proteinuria in at least one occasion. Among hyperfiltrating, two had also proteinuria in at least one occasion during life. One patient with eGFR below 90 ml/min and proteinuria had a biopsy-proven diagnosis of glomerulonephritis. In two patients, kidney data were missing. The impairment in kidney function was independent from age, diabetes, hypertension, median TGs, AP, sex.
Conclusions. In our cohort, 9 out of 20 patients (45%) had evidence of renal impairment. Further studies are needed to better clarify if kidney disease might be a hallmark of FCS in broader population and understand the patho-physiological mechanism, if any.

THROMBOCYTOPENIA AS A POSSIBLE HALLMARK OF FCS PHENOTYPE: PRELIMINARY EVIDENCE FROM A COHORT STUDY

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Background. Familial Chylomicronemia Syndrome (FCS) is a rare monogenic autosomal recessive disorder of lipid metabolism determining severe hypertriglyceridemia (HTG). A recent study reported the occurrence of spontaneous, transient thrombocytopenia in 55.2% of FCS patients. As the use of volanesorsen, a novel FCS treating drug, has been associated with thrombocytopenia, the relationship between FCS and low platelets counts should be firmly estabilshed. To this aim, we have retrospectively evaluated the spontaneous variation of platelet counts in a cohort of patients with FCS.
Methods. The population comprises 20 FCS patients (16 index cases) equally distributed among sex (F/M 11/9). Most were Caucasian (95) and carrying homozygous mutation in LPL (85%). The median age at enrolment was 51 years (IQR 38.2-63.7) and 17 patients (85%) had experienced cumulatively 65 episodes of acute pancreatitis (AP). None had history of atherosclerotic cardiovascular disease, but one patient has had Tako-Tsubo. Two patients reported history of skin and pancreatic cancer, respectively. The occurrence of thrombocytopenia was defined as mild, moderate, or severe if platelet count (PLTs) were below 140000, 100000 or 50000, respectively.
Results. Across the study population, the median PLT count was 180,225 platelet/mcL (IQR 158,404-213,624). During follow-up, 8 (44.4%) patients experienced at least one episode of mild and 1 (5%) of moderate thrombocytopenia. None had severe thrombocytopenia. The median on treatment TG levels in the whole cohort was 1309 (IQR 820-1701) mg/dl Changes in platelets counts did not correlate with variation of TG nor was associated with history of AP.
Conclusions. The present analysis confirmed that thrombocytopenia might be a clinical characteristics of FCS phenotype. No association with changes of TG levels was detected suggesting the other mechanisms not involved in TG-rich lipoprotein metabolism might be involved.

PARAOXONASE-1 AND MYELOPEROXIDASE ACTIVITIES ARE IMPAIRED IN MENOPAUSE WOMEN AFFECTED BY DIABETES: A PILOT STUDY

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Background. Type 2 diabetes (T2D) is a metabolic disease characterized by an increased risk for cardiovascular diseases (CVDs). Women in menopause and affected by T2D have a higher risk of cardiovascular events, that may be related to an impaired atheroprotective function of High Density Lipoprotein-cholesterol (HDL) particles. These pleiotropic activities are mostly related to HDL accessory proteins, like Paraoxonase-1 (PON-1, antioxidant) and myeloperoxidase (MPO, pro-oxidant).
Aims: To determine whether menopause in the presence of diabetes impairs the atheroprotective function of HDL as assessed by PON-1 and MPO activities.
Methods. A total of 148 women (n=63 pre-menopause/no diabetes, n=62 post-menopause/no diabetes, n=23 post-menopause/yes diabetes) were included in this pilot study. The activity of PON-1 and MPO were evaluated by spectrophotometric or spectrofluorimetric assays in serum samples from the subjects.
Results. Pre- and post-menopause women did not differ in PON-1 and MPO activities. Post-menopause women with diabetes showed decreased PON-1 and increased MPO activities compared to pre-menopause/no diabetes ($P<0.05$). MPO was positively correlated with the duration of menopause only in women with diabetes ($r=0.395$, $P<0.05$), whereas PON-1 was negatively correlated with the duration of the disease ($r=-0.470$, $P<0.05$). By separating post-menopause diabetic women in two groups depending on the duration of menopause (<10 years or >10 years), we found that a longer duration of menopause was associated with lower levels of PON-1 and higher activity of MPO ($P<0.05$).
Conclusion. Menopause seems able to negatively affect HDL atheroprotective functions in conjunction with diabetes.

EFFECTS ON LIPIDS AND PHARMACOKINETICS PARAMETERS OF PCSK9I MONOCLONAL-ANTIBODIES IN A REAL LIFE SETTING HIGH CARDIOVASCULAR RISK PATIENTS

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Aim. To investigate PCSK9-inhibitor mAbs kinetics and possible correlations between blood levels of total, free and monoclonal antibodies (mAbs)-bound forms of PCSK9 in a group of high CV risk patients.
Methods. Blood samples were obtained from 56 patients (32 men, 24 women) 7 days after administration of PCSK9-i mAbs (Evolocumab or Alirocumab), and again after a wash-out period of 3-4 weeks or before starting therapy. Full lipid profile and total/free-PCSK9 plasma levels were measured by two ELISA assays: a standard ELISA assay for total PCSK9, and in house developed ELISA assay for free-PCSK9. The treatment effects were evaluated as Δ and Δ% of the means. Data were analyzed by paired t-test and the Wilcoxon test.
Results. PCSK9 mAbs decreased TC by 38%; LDL-C by 53%; TG by 17%; non-HDL-C by 50%, and Lp(a) by 15% ($p<0.05$ for all variables); HDL-C increased by 5%. On treatment circulating total PCSK9 values increased by 72% ($p<0.05$), and free-PCSK9 decreased by 20% ($p<0.05$) with some differences between the two drugs. Four patients were "hypo-responders" with an LDL-C reduction <15%.
Conclusions. In a real-life setting, mAbs effects on LDL-C, non-HDL-C and Lp(a) were comparable to those observed in large clinical trials (FOURIER, ODYSSEY OUTCOMES). Interestingly, PCSK9 plasma values measured 3-4 weeks after last injection show a significant residual effect of PCSK9 mAbs. It is plausible to hypothesize the development of an algorithm using total/free/bound PCSK9 assays to define both adherence to therapy and hypo-responders patients.

GENETIC PROFILE OF FAMILIAL HYPERCHOLESTEROLEMIA AND RESPONSE TO LIPID LOWERING THERAPY

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Background. Familial hypercholesterolemia (FH) is the most frequent Mendelian disorder among genetic diseases and is characterized by the plasma accumulation of cholesterol in the form of LDL.The early genetic diagnosis of FH is essential in the fight against atherosclerosis also thanks to biotechnological drugs with monoclonal antibodies.
Aim. The aim of the study was to evaluate the efficacy of therapy with PCSK9i drugs, both in terms of overall reduction of the lipid profile and in terms of reaching the LDLc target, in a population of patients with a clinical phenotype suggestive of familial hypercholesterolemia (FH), confirmed by genetic testing. In particular, we stratified patients on the base of genotype to study the correlation with the great phenotypic variability (both in terms of the severity of plasma LDLc levels and the prevalence of major cardiovascular events related to atherosclerosis) and with the response to therapy.
Methods. The subjects involved in the study (195 subjects, divided into three groups: Group I = patients with confirmed FH, Group II = patients negative to the genetic test and Group III = patients with high cardiovascular risk) were evaluated with clinical and laboratory parameters and the lipid profile was analyzed at baseline and 1 month after the introduction of lipid-lowering therapy (with Statins, Ezetimibe and PCSK9i alone or in various combinations). Furthermore, within the population affected by genetically confirmed FH (90 patients), the molecular profile of the various "major candidate genes" implicated in the disease (LDLR, APOB, PCSK9, LDLRAP1, LIPA) was examined and the correlation was analyzed between the different mutations found and the changes in the lipid profile before and after treatment.
Results. In the analyzed population (patients with FH + patients with high CV risk), the PCSK9i biotechnological drugs show good efficacy. Patients with confirmed FH appear to be more responsive to Alirocumab than to Evolocumab (54% vs 44% reduction in LDLc), while those at high risk appear to be more responsive to Evolocumab compared to Alirocumab (67% vs 52% reduction in LDLc).The most responsive patients to PCSK9i drug therapy are those carrying mutations on the PCSK9 gene (overall reduction of LDLc, after treatment, by 64%), as expected based on the intrinsic mechanism of the molecule. Patients less responsive to PCSK9i drugs are those carrying mutations on the LDLRAP1 gene (overall LDLc reduction, after treatment, by 30%); in only one case this mutation was present as a single one, whereas in most cases it was associated with other genes' mutations (double heterozygosity in 12 out of 13 patients with mutation of LDLR).Patients with mutations on the other genes (LDLR, APOB, LIPA), on the whole, are on average responsive to treatment with PCSK9i (LDL reduction of about 40-50%).
Conclusions. The efficacy of PCSK9i drugs could be conditioned by the each individual's "genetic pedigree"; therefore, in daily clinical practice, therapeutic choices should also converge towards "tailored therapy" interventions, related to the mean expected response to the different treatments we could choose.

PLASMA EXCHANGE REDUCES LIPOPROTEIN X IN FAMILIAL LCAT DEFICIENCY

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Familial LCAT deficiency (FLD) is a rare recessive disease of lipid metabolism with no cure. The major cause of morbility and mortality in FLD is renal failure due to the nephrotoxicity of an abnormal lipoprotein, called lipoprotein X (LpX). Therapeutic plasma exchange (TPE) was tested in a FLD patient for the first time to remove LpX and to prevent kidney failure. The plasma exchange was performed once per week for 8 weeks using 5% human albumin solution. Plasma samples were collected at baseline and after 1 and 2 months of TPE, immediately before plasma exchange, and 4 months after TPE interruption. A complete lipid-lipoprotein profile was determined using a Roche Integra c311 analyzer. Cholesterol esterification rate (CER) and LCAT activity were tested by in-house assays. The 1.020-1.063 g/mL lipoprotein fraction was separated at each timepoint by ultracentrifugation and analyzed by fast performance liquid chromatography (FPLC).The patient was genotyped as a compound heterozygote of the Leu187Pro and Thr270Ile mutations in LCAT gene; CER and LCAT activity were null, confirming the FLD phenotype. After 1 month of treatment, all plasma lipids decreased and the unesterified/total cholesterol ratio (UC/TC) decreased from 0.84 to 0.74, suggesting a partial removal of LpX. TPE produced a remodelling of the lipoprotein profile, reducing circulating LpX as confirmed by FPLC. Unfortunately, after 8 weeks kidney function worsened and TPE was stopped; the lipoprotein profile 4 months after TPE interruption showed a subsequent reoccurrence of LpX.The present results demonstrate that TPE induces lipoprotein remodelling in FLD by reducing nephrotoxic LpX. When the therapy was stopped, due to worsening of the kidney function, lipoprotein abnormalities reoccurred. In conclusion, plasma exchange could represent an option to normalize lipoprotein profile in LCAT deficiency. More investigations are needed to clarify the effects on renal outcomes.

PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 AND PLASMA/ CEREBROSPINAL FLUID LIPIDS IN PATIENTS WITH COGNITIVE DECLINE

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Cerebral cholesterol homeostasis impairment seems related to the pathogenesis of Alzheimer's disease (AD). We previously found higher levels of Proprotein convertase Subtilisin/Kexin type 9 (PCSK9) in the cerebrospinal fluid (CSF) of AD patients compared to control subjects. This study aims to evaluate the levels of PCSK9, as well as several markers of cholesterol metabolism, in both CSF and plasma of subjects with different degrees of cognitive decline. The 83 subjects were divided into three groups: patients with AD (AD; n=27), patients with mild cognitive impairment that furtherly developed AD at follow-up (MCI-AD; n=28), patients with mild cognitive impairment stable at follow-up (MCI; n=28). PCSK9 levels were determined with ELISA assay. CSF and plasma cholesterol concentration was determined with a fluorometric assay; oxysterols (24-, 25-, 27-hydroxycholesterol, OHC) were quantified through liquid chromatography associated with mass spectrometry.PCSK9 levels in CSF were similar in all three groups, but higher compared to a control group analyzed in our previous work. Among AD, PCSK9 levels were increased in carriers of the ε4 isoform of apolipoprotein E (ApoE) compared to non-carriers (+38%, $p<0,05$). In AD we found a positive correlation between CSF and plasma levels of PCSK9 ($r=0.521$; $p=0.004$), particularly robust in ε4 carriers ($r=0.755$; $p=0.001$). The concentrations of cholesterol and oxysterols were comparable among the groups, both in plasma and in CSF. Only in ε4 carriers, a negative correlation between 24-OHC and PCSK9 levels in CSF was observed ($r=-0.34$; $p=0.03$).In conclusion, our data suggest that PCSK9 levels may be already increased since the early phases of the disease, particularly in the presence of the ε4 isoform of ApoE. The plasma/CSF positive correlation of PCSK9 levels suggests a major permeability of blood brain barrier occurring specifically in AD. Moreover, the relationship between 24-OHC and PCSK9 supports an involvement of this protein in sterol homeostasis that is worth to be further investigated.

RNA-SEQ ANALYSIS OF HUMAN ATHEROSCLEROTIC PLAQUES AND THEIR ADJACENT REGIONS: A FOCUS ON LNCRNAS

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Aim. Atherosclerosis is a chronic inflammatory disease of the arterial wall. Pro-inflammatory factors and lipoprotein accumulation trigger the development of atherosclerotic plaques. In the last decade the role of non-coding RNAs in the physiopathology of this disease has been emerging, but long non-coding RNAs (lncRNAs) and their association with atherosclerosis is still poorly investigated. In this contest, this work has been developed with the aim to evaluate the role of lncRNAs in plaque development and find novel therapeutic and/or diagnostic biomarkers.
Materials and Methods. Human carotid atherosclerotic plaques and their respective adjacent regions (with a lower lesion grade) from 15 patients undergoing carotid endarterectomy were collected (30 samples). All samples were homogenized with liquid nitrogen and immediately processed for RNA extraction, by a combined protocol with Trizol and MirVana kit. After quality check, NGS libraries were prepared using the Illumina Stranded Total RNA Prep kit. The sequencing reactions were performed on the Illumina NextSeq550Dx instrument.
Results. After a quality check, Dragen RNA bioinformatic tool by Illumina was used for the alignment, the RNA quantification and the quality controls. The iDEP.96 web-based bioinformatic tool was used for the differential expression analysis. Clustering analysis showed a clear separation between the two sample groups, highlighting 214 downregulated and 756 upregulated genes in the plaques respect to the adjacent regions. Moreover, we found 108 upregulated and 42 downregulated lncRNAs in the plaques respect to their adjacent regions; among these, SAMMSON and LINC00670 have been already reported as downregulated, whereas HAGLR, LINC01480, LINC00528, IFNG-AS1, and HAGLROS as upregulated in cardiovascular diseases.
Conclusions. Even if these preliminary data need to be further evaluated by a functional point of view, also by increasing the sample number, they suggest a different expression pattern of lncRNAs in plaques samples that may be considered as potential biomarkers/therapeutic targets.

ASSOCIATION BETWEEN CIRCULATING LEVELS OF LIPOPROTEIN(A) AND RISK OF CORONARY ARTERY DISEASE, CEREBROVASCULAR DISEASE AND PERIPHERAL ARTERY DISEASE IN A DYSLIPIDEMIC POPULATION

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Background. Hyperlipoproteinemia (a) represents a widespread public health problem: levels of Lp(a) greater than 30 mg/dL have been found in 10-30% of the world population. High Lp(a) levels are a strong, causal and independent risk factor for atherosclerotic cardiovascular disease (ASCVD) through different pathogenetic mechanisms.
Objective. Investigation of associations between circulating Lp(a) levels, risk of coronary (CAD), cerebrovascular (CVD) and peripheral (PAD) artery diseases. Methods: in this retrospective case-control study 519 dyslipidemic outpatients were included between January 2019 and June 2022. We collected lipid profile, Lp(a) concentration and Dutch Lipid score.
Results. 519 dyslipidemic patients in total, 282 female (54%) and 237 male (46%), aged 53–70 years. 268 patients (52%) were cases with Lp(a)>30 mg/dL, and 251 were controls (48%) with Lp(a)<30 mg/dL. Cases showed higher levels of Lp(a) (8 mg/dL vs 88.1 mg/dL), while controls have higher concentration of total cholesterol, LDLc and lower levels of HDL. Subjects developing ASCVD were 101 (19%), with a higher prevalence among cases (21% vs 18%). Instead, controls have a higher prevalence of CVD (48% vs 35%), while cases of CAD and PAD were 45% vs 37% and 20% vs 3%, respectively. Moreover, individual vascular events were analyzed by gender, and we observed that high Lp(a) levels are significantly associated with incidence of PAD in women (p-value 0.024), but it is no longer significant in men (p-value 0.635).
Conclusions. Our study confirms a strong association between Lp(a) levels and PAD. Our analysis highlights the role of gender difference, in particular women with hyperlipoproteinemia (a) exhibit a greater risk of developing PAD than men. In this context we may propose an ecographical screening to identify presence of PAD in women with higher levels of Lp(a).

A LATE DIAGNOSIS OF FAMILIAL CHYLOMICRONEMIA SYNDROME

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Background. Familial Chylomicronemia Syndrome (FCS) is a rare genetic disease characterized by severe hypertriglyceridemia (sHTG) non-responding to standard lipid-lowering therapy (LLT). The worst clinical manifestation of FCS is HTG-induced acute pancreatitis because of its severity and potentially life-threatening consequences.
Case Report. A 70 years-old man was referred to our lipid-clinic with sHTG refractory to diet and LLT, diabetes mellitus type 2 with micro and macrovascular complications and severe chronic iperCPKemia. He reported chronic abdominal pain, steatorrhea. At physical examination the patient was asthenic, with hypotrophic muscular masses, legs pitting edema, mild ascites, mild pleural effusion. Patient was hospitalized and blood tests confirmed the sHTG and malnutrition compatible alteration. The abdominal CT-scan showed a complete substitution of pancreatic tissue by adipose tissue and laboratory examination demonstrated a severe reduction of exocrine pancreatic function. Genetic test showed a pathogenetic homozygous mutation in lipoprotein-lipase gene (LPL c.844G>T p.(Glu282)). An appropriate therapy was settled, including Volanesorsen, an antisense oligonucleotide inhibitor of apolipoprotein CIII.
Conclusions. Delayed diagnosis of FCS led to exocrine pancreatic impairment that resulted in a severe protein energy malnutrition complicated by severe sarcopenia and anasarcatic state. The presented case shows that rare genetic disorder, despite the typical presentation is reported in childhood, may also be diagnosed in adult age. A delayed diagnosis could determine both the development of irreversible complications and retard an adequate treatment.

META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS COMPARING THE IMPACT OF LIPID-LOWERING THERAPIES ON C-REACTIVE PROTEIN LEVELS

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Lipid-lowering therapies (LLTs) may have an effect also on inflammatory mediators, with potentially better clinical outcomes. However, evidence from clinical studies is conflicting. Our study aimed to assess the effect of LLTs on C-reactive protein (CRP), in addition to lipid reduction. We conducted a meta-analysis according to the PRISMA guidelines, and databases were searched from inception to June 2022. Inclusion criteria were:
1) randomized controlled trials (RCTs) in humans, phase II, III, or IV;
2) English language;
3) reporting the effects on CRP levels;
4) with intervention duration of more than 3 weeks;
5) and sample size over than 100 subjects.

Meta-regression analysis was conducted to assess the correlation between mean absolute change in low-density lipoprotein cholesterol (LDL-C) or triglyceride (TG) and CRP. A total of 173,939 subjects from 47 RCTs were included in our meta-analysis. An additional -0.65 mg/L (95%CI -0.87 to -0.43) absolute reduction of CRP concentration was observed for statins. CRP was also decreased by -0.24 mg/L (95%CI -0.37 to -0.11) and -0.26 mg/L (95%CI -0.52 to -0.01) with ezetimibe and omega-3 fatty acids (omega3FAs), respectively. In addition, a -0.40 mg/L (95%CI -1.17 to 0.38) decrease in CRP level was observed in patients treated with fibrates, although not statistically significant. A slight increase of CRP concentration was found for PCSK9 inhibitors (0.06 mg/L [95%CI -0.04 to 0.15]) and CETP inhibitors (0.05 mg/L [95%CI 0.00 to 0.10]), but none of these differences were statistically significant. Meta-regression did not show a significant correlation between changes in CRP and LDL-C or TG across LLTs even after adjustment by age, sex, and intervention duration (LDL-C, slope: 0.0048, P=0.1536; TG, slope: 0.0015, P=0.7420).In conclusion, among LLTs, statins, ezetimibe, and omega3FAs seemed to reduce serum CRP concentration. The impact of this anti-inflammatory effect in terms of cardiovascular prevention needs further investigation.

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