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COMUNICAZIONI ORALI

HUMAN SIMPSON-GOLABI-BEHMEL SYNDROME (SGBS) ADIPOCYTES MOUNT A METABOLIC ADAPTIVE RESPONSE TO PREVENT PALMITIC ACID-INDUCED INSULIN RESISTANCE

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Adipocytes contribute to systemic metabolic health by buffering excess energy and secreting endocrine mediators, including extracellular vesicles (EVs), which influence the metabolism of other tissues. Besides protein and nucleic acids, EVs also carry lipids which act as additional metabolic messengers of adipocytes. However, it remains to elucidate if saturated fatty acids, particularly in the form of palmitic acid (PA), are able to disrupt adipocyte metabolic health and modulate EVs lipid cargo. This study aimed at evaluating the effects of PA overload on SGBS adipocytes metabolic health and the putative repercussions on the fatty acid profile of EVs secreted by these cells. SGBS adipocytes were treated with 1000 µM PA for 48h followed by the assessment of intracellular triglyceride accumulation, mitochondrial membrane potential using the JC-10 fluorescent probe and oxygen consumption rate (OCR) assessed by MitoXpress assay. Insulin-mediated phosphorylation of protein kinase B (Akt) and insulin receptor substrate (IRS), the activation of AMP-activated protein kinase (AMPK) and NF-kB signalling were assessed by Western blot. EVs were isolated via ultracentrifugation and both intracellular and EVs fatty acid profile characterised using gas chromatography coupled with mass spectrometry. PA induced an increase in intracellular triglyceride accumulation (p<0.001). However, it did not hamper insulin-mediated AKT or IRS1 phosphorylation (both p>0.05) or trigger the activation of NF-κB signalling (p>0.05). Additionally, relative to controls, PA increased mitochondrial membrane potential (p<0.01) and OCR (p<0.001) in the presence of PA as an energy substrate, despite no changes in AMPK activation. Finally, PA increased SFA content in both adipocytes and EVs (p<0.001). SGBS adipocytes undergo a metabolic rewiring marked by increased mitochondria function and secretion of SFA in EVs. The latter may be a compensatory mechanism to export excess SFA which, however, may contribute to systemic metabolic dysregulation.

BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) IN CARDIOVASCULAR AGING

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Aging brings about biological changes that increase the risk of atherothrombosis; understanding the underlyng mechanisms is crucial for better prevention and treatment. Recent studies suggest Brain-Derived Neurotrophic Factor (BDNF) play a key role in the cardiovascular system by influencing platelet activity, coagulation, endothelial growth, vascular remodeling, and inflammation. Low BDNF levels have been detected in older adults and associated with poorer cardiovascular outcomes. In this study we explore whether an unbalance between matureand pro-BDNF levels contributes to vascular aging predisposing to thrombosis. We found that in aorta tissue of wild-type (WT) mice, the ration of mature to pro-BDNF decreased with age. reaching levels at 12 months comparable to those measured in a young BDNFMet/Met mice, a model with naturally low mature-BDNF levels. As expected, older WT mice (18-22 months) showed an increase in leucocyte and platelet count, platelet/leucocyte aggregates and enhanced arterial thrombosis compared to young WT mice. Interestingly, young BDNFMet/Met mice had a higher number of circulating blood cells, a greater tendency to form platelet/leucocyte aggregates, and developed carotid occlusion in response to FeCl3 faster than young WT mice, exhibiting a prothrombotic profile similar to old WT mice. Gene expression analysis revealted that middle-age BDNFMet/Met mice and old WT mice shared similar aging markers (eg. p16, p19, p21, p53, SIRT1, and Nfil3CLOCK-gene expression), with a negative correlation between mature-BDNF levels and aging related genes. Additionally, BDNFMet/Met mice had significantly higher mortality rates than WT mice (35% vs. 1%). Notably, administration of pro-BDNF accelerate aging, while m-BDNF delays it. In conclusion, our findings suggest that BDNF acts as a senolytic hormone, and its reduction contributes to vascular aging and a prothrombotic state. Whether BDNF modulation could prevent aging and atherothrombosis in human requires further investigation.

STATIN INTOLERANCE AND APPROPRIATENESS OF PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 INHIBITORS PRESCRIPTIONS: A POST-MARKETING, OBSERVATIONAL, REGION-WIDE STUDY

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Background: Statins are the cornerstone of cardiovascular prevention in patients at risk of atherothrombotic events. However, intolerance frequently hampers patients' adherence in clinical practice. Alongside statins, therapeutic options such as proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) have been flourishing over the past years, paving the way for statin-free lipid-lowering strategies that hold the potential of improving cardiovascular risk prevention in intolerant individuals. Because these are burdened by significant costs, refining the identification of truly intolerant patients may have profound impact on health-care systems.

Aims: To appraise the appropriateness of PCSK9i prescription and estimate the prevalence of statin intolerance in a contemporary cohort of patients prescribed on lipid-lowering medication. **Methods:** Prescriptions of lipid-lowering drugs between 2017 and 2023 were retrieved from region-wide, post-marketing databases patronized by the Agenzia Italiana del Farmaco (AIFA) and the Italian Ministry of Health. The prevalence and appropriateness PCSK9i prescription was appraised using the AIFA recommendations as a reference. Statin-intolerant patients were defined according to AIFA reimbursement criteria. The incidence of adverse cardiovascular events (ACE) in patients taking PCSK9i was also assessed.

Results: Prescription of non-statin lipid-lowering drugs increased steadily across treatments over the analyzed time-window. Among PCSK9i users, 2135 (65%) met the criteria for appropriate prescription whereas 1159 (35%) did not, and were therefore considered a pool of intolerant individuals and patients inappropriately prescribed on the medication. Average statin-intolerant in individuals' prevalence was estimated to be 3.7%, progressively increasing from 2.2% in 2017 to 5.2% in 2023. Prescription of non-statin lipid-lowering medication increased over time, paralleled by a reduction of ACE and of the number of patients experiencing an event.

Conclusions: In this region-wide, regulatory registry-based, observational study, statin intolerance was estimated to occur in 3.7% of patients on average over a seven-year period, whereas possible inappropriate prescription of PCSK9i occurred in as many as one out of three cases.

ANGPTL3 AND PCSK9 MODULATE THE UPTAKE OF LDLS IN LIVER CELLS

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Background and Aim: We have already proven the existence of an ANGPTL3-PCSK9 complex both present intracellularly and extracellularly in cellular models. This complex is modulated by nutrient availability and is more represented in the feeding state. In the literature, patients harboring homozygous LOF of ANGPTL3 show hypobetalipoproteinemia and lower PCSK9 levels. This study aims to characterize the function of the ANGPTL3-PCSK9 concerning LDL uptake.

Methods: A liver cell model (HepG² cells) was used to investigate the interaction between ANGPTL3 and PCSK9 and LDL uptake. We generated cells transiently over expressing ANGPTL3, PCSK9, or both. Human LDLs were isolated through ultracentrifugation and then labeled with red oil-O. Cells were treated with red-oil-labeled LDLs, and intracellular red-oil accumulation was measured in a timelapse for 24h. ANGPTL3 and PCSK9 were co-localized in liver cells treated or not with a standard concentration of human LDLs.

Results:

- HepG2 cells overexpressing ANGPTL3 showed reduced intracellular Red-LDL accumulation and increased LDL turnover. PCSK9 overexpressing cells show increased intracellular LDL entrapment. Cells overexpressing both ANGPTL3 and PCSK9 show an LDL uptake similar to the control.
- 2) ANGPTL3 and PCSK9 co-localize in a lipid-free medium in all experimental conditions. However, LDL treatment is a sufficient stimulus to separate the ANGPTL3-PCSK9 complex; the separation is complete when ANGPTL3 and PCSK9 are overexpressed, ANGPTL3 and PCSK9 separation remains incomplete in the control condition, and in cells overexpressing both ANGPTL3 and PCSK9.

Conclusion: ANGPTL3 and PCSK9 modulate LDL uptake in opposite ways. The first increases LDL particle turnover, and the other reduces it. The dissociation of the ANGPTL3-PCSK9 complex allows ANGPTL3 and PCSK9 to regulate LDL uptake.

LOMITAPIDE IN PEDIATRIC PATIENTS WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: A POTENTIAL GAME CHANGER FOR A RARE DISEASE

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Introduction: Homozygous Familial Hypercholesterolemia (HoFH) is a rare disease characterized by LDL cholesterol (LDL-C) levels >400 mg/dL due to mutations in the LDL receptor (LDLR), apo-B, PCSK9 and LDLRAP1 genes. If untreated, HoFH patients have a life expectancy of less than 20 years. Pharmacological approaches currently used in pediatric patients (statins, ezetimibe, and PCSK9 inhibitors) are insufficient to achieve optimal LDL-C control and it is often necessary to resort to an invasive method such as lipoprotein apheresis. Nevertheless, most patients do not reach the therapeutic goal of LDL-C <115 mg/dL. Lomitapide is an oral inhibitor of microsomal triglyceride transfer protein (MTP), which reduces the production of apo-B-containing lipoproteins in the liver and intestine. The drug, already approved in adults, reduces LDL-C regardless of residual LDLR activity.

Aim of the Study: To evaluate the safety and efficacy of lomitapide use in HoFH pediatric patients on stable lipid-lowering therapy.

Methods: 4 HoFH children, aged 8 to 12 years, were evaluated. These patients, originally selected for a 2-year multicenter open-label international study, continued to take lomitapide as part of a compassionate-use follow-up trial. Lomitapide was added to statins, ezetimibe and lipoprotein apheresis and progressively titrated up to the maximum tolerated dose. Subjects were monitored regularly with outpatient visits, blood tests, ECG and imaging.

Results: In our cohort, lomitapide, at the dose range of 15 to 30 mg daily, in addition to standard therapy, reduced LDL-C by an average of 38.4% (324±52 *vs.* 198±82 mg/dL; mean±SD), allowing lipoprotein apheresis to be reduced or discontinued. The effect remained stable over 2 years. The drug was well tolerated and neither gastrointestinal nor hepatic adverse events were observed.

Conclusions: The use of lomitapide is safe, effective and well tolerated in HoFH children. Lomitapide resulted in a reduction/discontinuation of apheresis sessions, leading to a significantly improved quality of life.

HETEROZYGOUS FAMILIAL HYPOBETALIPOPROTEINEMIA: DESCRIPTION OF PHENOTYPE IN AFFECTED CHILDREN AND ADOLESCENTS IN THE ERA OF OBESITY

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Background and Aim: In childhood, the diagnosis of familial hypobetalipoproteinemia (FHBL) is often underestimated, especially if heterozygous, and guidelines are still lacking. The aim of the study is to describe the phenotypic features of a cohort of children and adolescents with a genetic confirmed FHBL attending our pediatric lipid clinic.

Methods: This is a monocentric, observational study collecting anamnestic, anthropometric, biochemical and instrumental (liver ultrasound and elastographic profile) data in children and adolescents with a genetically confirmation of heterozygous FHBL. Statistical analysis was carried out on STATISTICA (Stat-Soft Inc, Tulsa, OK, USA).

Results: 12 children and adolescence (4 females), aged $12.14\pm$ 1.80 years, were genetically diagnosed with heterozygous FHBL (9/12 carrying a mutation on ApoB, 2/12 on ANGPTL8 and one case on MYLIP). At diagnosis, overweight and obesity were identified in 7/12 cases. Only one patient was fully asymptomatic. In all cases, lipid assessment was suggestive of FHBL (mean TC 80.41±14.81 and LDL-C 31.54±12.88 mg/dl) independently from BMI SDS (obese *vs.* normal-weight patients: p 0.62 and 0.39, respectively). In 6/12 patients, steatosis was graded from moderate to severe, mainly when accompanied by overweight and/or obesity (p 0.05). Moreover, transient elastography, an indirect surrogate of fibrosis, was more elevated in FHBL patients if overweight and/or obes (5.65±0.71 *vs.* 4.60±0.28, p 0.06).

Conclusions: Our data document an unexpectedly wide phenotype of FHBL in childhood and adolescence with a high percentage of overweight or obesity. Moreover, we document a frequent precocious hepatic involvement in FHBL children, especially if obese and overweight, with a potential rapid evolution in fibrosis. In conclusion, even if these preliminary data require the confirmation from sizable cohort, they support the need of a precocious diagnosis of FHBL in childhood that could let to early plan a personalized follow up.

TRIGLYCERIDE/HDL RATIO AND SUBCLINICAL MYOCARDIAL DAMAGE IN PATIENTS WITH CARDIOVASCULAR RISK FACTORS

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Cardiovascular diseases (CVD) are the leading cause of death around the world. As is well known, high triglycerides (TG) and low high-density lipoprotein (HDL) cholesterol are important CV risk factors. Recently, many studies reported that triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio, a simple surrogate markers of insulin resistance, is a useful predictor of CV diseases. The aim of the present study was to evaluate TG/ HDL ratio as a CV risk factor and its possible correlation with subclinical myocardial damage examined by speckle tracking echocardiography. We enrolled 470 Caucasian patients (mean age 60.0±11.7, 293 male and 177 female) referring to Catanzaro Metabolic Risk Factors (CATAMERI) Study. Main exclusion criteria were secondary hypertension, clinical evidence of CV complications, endocrinological and malignant disease, alcohol or smoking abuse. All patients underwent to Oral Glucose Tolerance test (OGTT). Plasma glucose was measured by the glucose oxidation method and plasma insulin concentration was determined by a chemiluminescence-based assay. Insulin sensitivity was evaluated using the Matsuda index (Matsuda/ISI). Renal function was tested by measurement of estimated glomerular filtration rate (e-GFR) with CKD-Epi formula. TG and total cholesterol, LDL and HDL concentrations were measured by enzymatic methods (Roche Diagnostics, Mannheim, Germany). TG/HDL ratio values were assessed by arithmetic ratio between plasma TG and HDL concentrations. Echocardiographic recordings were performed using an E-95 Pro ultrasound system. ANOVA test was used to test the differences between groups. A linear correlation analysis was performed to compare values of global longitudinal strain (GLS) with different covariates. Patients were divided into 4 quartiles based on increasing values of TG/HDL ratio: I quartile (0.5-1.79) (n=120), II quartile (1.80-2.63) (n=115), III quartile (2.65-3.75) (n=118) and IV quartile (≥3.77) (n=117). From the I to the IV quartiles, there was a progressive increase in the prevalence of male gender, type 2 diabetes mellitus (T2DM), obesity. From the I to the IV quartile, there was a significant increase of TG values (p<0.0001), LDL cholesterol (p=0.008) and a significant reduction of HDL cholesterol (p<0.0001). Moreover, there was a progressive deterioration of glucose metabolism as reported by the increasing values of fasting plasma glucose (FPG) (p<0.0001), 2h-glucose (p<0.0001), fasting plasma insulin (FPI) (p<0.0001), 2-h insulin (p<0.0001), HbA1c (HbA1c) (p<0.0001) and a worsening of insulin sensitivity accounting for the reduction in MAT-SUDA/ISI (p<0.0001). In addition, we observed a statistically significant increase in high-sensitivity C-reactive protein (Hs-PCR) values (p<0.0001) and a reduction of e-GFR (p<0.0001). The left global systolic function, evaluated as myocardial deformation and GLS, appeared progressively deteriorated proceeding from the I to the IV quartile (p<0.0001). Moreover, for similar values of ejec-

tion fraction (EF), IV quartile subjects presented increased GLS compared to I quartile (p<0.0001). In the whole study population. the linear regression analysis showed that GLS was significantly and directly correlated with TG/HDL (r=0.233, p<0.0001), hs-PCR (r=0.110, p=0.009), E/e' (r=0.408, p<0.0001), left ventricular mass index (LVMI) (r=0.381, p<0.0001) and inversely correlated with Matsuda Index (r= -0.198, p<0.0001) and e-GFR (r= -0.121, p=0.005). Subsequently, stepwise multivariate linear regression model showed that E/e' was the major predictor of GLS justifying 16.5% of its variation (p<0.0001) and LVMI and TG/HDL added respectively 7.3% and 1.2%. The present study demonstrated that patients with increased TG/HDL ratio present functional alterations of myocardial contractile fibres, evaluated as GLS, in the absence of symptoms as well as pathological reductions in EF. In conclusion, the evaluation of TG/HDL ratio can be a useful, simple and inexpensive indicator of CV risk.

ADIPOCYTE METABOLIC RESILIENCE TO CHRONIC PALMITIC ACID (PA) OVERLOAD IS IMPAIRED IN THE PRESENCE OF HIGH-GLUCOSE CONCENTRATIONS

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Overconsumption of long-chain saturated fatty acid, mainly in the form of palmitic acid (PA), as well as refined sugar has been implicated in impairing metabolic health. However, the metabolic effects of these nutrients on isolated adipocytes remain elusive. The aim of this study was to evaluate the impact of PA overload, alone or in combination hyperglycaemic condition, on human adipocyte metabolic health. SGBS adipocytes were treated with 1000 µM BSA-conjugated PA, alone or in combination with high-glucose concentrations (Glucose 13mM), for 96 hours. Following the treatments, mitochondrial function was assessed by quantifying mitochondrial membrane potential (MMP) using the fluorescent probe JC-10 and oxygen consumption rate (OCR) via MitoXpress assay kit. Western blot was employed to investigate insulin-mediated phosphorylation of insulin receptor substrate-1 (IRS) and protein kinase -B (Akt) as well as the activation of NK-KB signalling pathway and AMP-activated protein kinase (AMPK). Real-time qPCR was performed to quantify the expression of IL-6 and IL-16.96h PA exposure did not impair insulin-mediated phosphorylation of IRS and Akt and failed to trigger the NK-kB inflammatory pathway and induce the expression of IL-6 and IL-1ß cytokines (all p>0.05) in SGBS adipocytes. Additionally, PA did not hamper MMP but increased OCR (p<0.001) relative to controls, despite no changes in AMPK activation. However, PA combined with hyperglycaemic milieu, lowered MMP (p<0.001) and decreased OCR compared to PA alone-treated cells (p<0.001). PA alone did not compromise adipocyte metabolic health, and this may be dependent on the preserved metabolic flexibility to lipids which allows adipocytes to cope with excess PA. However, the supply of PA in an hyperglycaemic milieu, appears to compromise adipocytes metabolic adaptation to fatty acid availability which, in turn, may be at the basis of adipocyte metabolic health deterioration.

SEX-RELATED DIFFERENCES IN RESPONSE TO LOMITAPIDE IN HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Objectives: Homozygous familial hypercholesterolemia (HoFH) is an inherited disorder of lipid metabolism characterized by severe elevation of low-density lipoprotein cholesterol (LDL-C) and a high risk of early atherosclerotic cardiovascular disease (ASCVD). Lomitapide, an inhibitor of microsomal triglyceride transport protein, has been shown to be effective in reducing LDL-C levels, albeit with a variable response in a real-world setting. Sex-based differences in the efficacy and safety of this treatment have not yet been investigated.

Methods and Rsesults: Through a retrospective analysis of the "Pan-European Lomitapide Study", sex-specific differences in the efficacy and safety of lomitapide in patients with HoFH (N=38 women and N=37 men) were examined. Data from HoFH patients treated with lomitapide across Europe were collected. Clinical characteristics, lipid profile and adverse events were compared between women and men. Baseline characteristics and ASCVD risk factors were comparable between the sexes. Both groups achieved a significant reduction in LDL-C compared to baseline with lomitapide, but a trend towards a greater reduction emerged in women compared to men, particularly evident after 6 months of treatment (-53.0% vs. -32.9% P=0.051). No sex differences were observed in the median dose of lomitapide or the intensity of concomitant lipid-lowering therapies. Univariate regression analysis showed that LDL-C reduction could be predicted by pre-treatment plasma LDL-C concentration. Among adverse events, gastrointestinal disorders were more frequent in women than in men (78 events vs. 32, P=0.0002), although in most cases they were mild/moderate. The analysis of survival without ASCVD events showed no significant differences between the two sexes (P=0.363).

Conclusion: Lomitapide demonstrates comparable efficacy in reducing LDL-C levels in men and women with HoFH, with potential sex-specific differences in tolerability.

ROLE OF CIRCULATING ENDOTHELIAL PROGENITOR CELLS AND CORRELATION WITH VASCULAR STIFFNESS AND ORGAN DAMAGE

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Background and Aims: Statins are still one of the most common and effective treatments to reduce high plasma concentration of LDL, preventing atherosclerosis progression in cardiovascular (CV) risk. Endothelial dysfunction correlates to a reduced availability of circulating endothelial progenitor cells (EPCs), physiologically recruited from bone marrow in response to vascular damage. EPCs contribute to re-endothelialization and vascular integrity. Several other beneficial effects have been proposed, including statins protective effects on vascular inflammation. We investigate whether statins might help to ameliorate EPCs number and function, therefore helping to elucidate potential additional mechanisms for statins protective effects on CV disease-prone patients.

Methods: Patients with CV diseases and administered (STAT, n=51, 24F, aged 67.7 ± 9.6) or not (NO-STAT, n=24, 13F, aged $63.1\pm$ 11.3) with statins will undergo routine hematological and biochemical tests as well as instrumental examinations to assess arterial stiffness and organ damage (ABI, PWV). Quantitative (flow cytometry) and functional (wound healing) analysis will be performed on EPCs isolated from both groups. Small RNAseq will be carried out to identify potential changes in miRNAs expression under statins treatment. Data presented are those with P less 0.05, that was considered significant.

Results: STAT group has a significantly decreased level of total Cholesterol, LDL and HDL. On the contrary, PWV estimated vascular age was significantly increased in STAT group. Total number of EPCs was lower in NO-STAT and their migration (measured by wound healing assay) was significantly improved in STAT. Concomitantly, miRNAseq analysis suggests that miR-3960, mir-328-3p, mir-10400-5p, all involved in invasion and inflammation, were downregulated in STAT compared to NO STAT patients.

Conclusion: Obtained preliminary data support the improved EPCs frequency and their functional role both representing an additional mechanism by which statins improve vascular protection. Modulation of specific miRNAs expression should have a role in this mechanism. Moreover, our results emphasize the importance of EPCs as a specific readout of tissue damage in patients at risk for cardiovascular complication.

ELDERLY PEOPLE EXPERIENCE A LESS SEVERE METABOLIC DYSFUNCTION ASSOCIATED STEATOTIC LIVER DISEASE: A SURVIVORSHIP BIAS?

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Aim: Aim of the study was to evaluate MASLD prevalence and its characteristics across age groups, in a population of metabolic patients. MASLD is a disease characterized by liver accumulation of lipids due to metabolic disorders. Little data exists on MASLD characteristics in elderly patients. Previous data showed that MASLD is less prevalent in the elderly and that it associates with a less severe liver disease and metabolic burden. **Methods:** In the Plinio Study we assessed the prevalence of MASLD across ages. To minimize confounders, MASLD characteristics were evaluated in patients aged below and above 65 years after propensity score matching (PSM) for sex and specific therapeutic interventions (antidiabetic, antihypertensive, antiplatelet, and statins). PRO-C3 was measured as a marker of collagen III deposition during liver fibrogenesis, sNOx2-dp and oxidated-LDL (ox-LDL) as markers of oxidative stress.

Results: The study included 1080 patients. 78,6% of them had a diagnosis of MASLD. In the older patient the prevalence of MASLD was lower than the youngest group (81.1% in patients aged below 65 years, 58.8% in those aged 80 years or older). After PSM, a subset of 374 patients was selected. Among patients aged 65 years or older, there was a more favorable lipid profile (HDL-C: 48.5 [42.0-61.0] *vs.* 46 [39.0-56.7], p=0.022; LDL-C: 108.0 [81.2-132.0] *vs.* 118.0 [96.4-141.7], p=0.005), lower HOMA-IR (2.9 [0.6-4.9] *vs.* 3.7 [2.5-5.6], p=0.001), and pro-C3 levels (6.1 [5.2-7.8] *vs.* 6.8 [5.7-8.3], p=0.022). However, despite the most favorable lipid profile, elderly patients with MASLD presented no-difference es in sNOx2-dp (p=0.126) and higher ox-LDL (24.9 [16.5-35.6] *vs.* 21.4 [16.7-29.1], p=0.011).

Conclusions: Our data confirm a lower prevalence of MASLD in elderly with less severe liver disease and most favourable lipid profile. However, the data on the oxidative stress markers show that the elderly should not be assumed to have less severe disease overall.

INVESTIGATING PLASMA AND BRAIN CHOLESTEROL ESTERIFICATION IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, with metabolic alterations, abnormal cholesterol and lipid levels in the bloodstream and CNS reported in patients. Similar to plasma, cholesterol in the cerebrospinal fluid (CSF) is carried by lipoproteins known as "HDL-like particles," due to their close similarity in density and composition to plasma HDL. Lecithin cholesterol acyltransferase is a key enzyme in HDL metabolism. It catalyses the cholesterol esterification both in plasma and CSF, thus leading to HDL maturation. A recent study conducted in our laboratory demonstrated a hampered cholesterol esterification in the CSF of patients with Alzheimer disease (AD). For this reason, the purpose of this work was to investigate cholesterol esterification in plasma and CSF, and to characterize HDL subclass distribution in patients with ALS. The study included 20 ALS patients and 20 controls, in whom lipoprotein profile and cholesterol esterification were evaluated both in plasma and CSF. Plasma lipids are similar between patients and controls; however, the amount of discoidal preß-HDL is significantly reduced in patients compared to controls (8.5±4.9% vs. 13.6±4.1%, p<0.0001). A significant increase in CSF unesterified cholesterol levels is observed in ALS patients compared to controls (0.22±0.07 mg/dL vs. 0.15±0.04 mg/dL, p<0.01), leading to an increased unesterified/total cholesterol ratio in ALS patients (0.52±0.12 vs. 0.40±0.12, respectively). Stratification for the presence of SOD1 variant shows that the UC/TC ratio does not depend on the genetic background (0.23±0.06 vs. 0.21±0.07, in carriers vs. non-carriers). ALS patients do not show alterations in plasma cholesterol esterification, but the cholesterol esterification rate is significantly reduced in the CSF of the patients (0.16±0.10 vs. 2.41±1.98, p<0.01). In conclusion, these results suggest an impairment of cholesterol esterification in the CSF of ALS patients and support the evidence that this is a common feature in neurodegenerative diseases.

THE LONG-TERM, REAL-WORLD EFFECTIVENESS AND SAFETY OF LOMITAPIDE IN HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLAEMIA (HoFH): NINE-YEAR DATA FROM THE LOMITAPIDE OBSERVATIONAL WORLDWIDE EVALUATION REGISTRY (LOWER)

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Aim: To evaluate the long-term effectiveness and safety of the microsomal triglyceride transfer protein inhibitor lomitapide in patients with HoFH.

Methods: LOWER is a global, prospective, observational cohort study of the long-term safety and effectiveness of lomitapide in clinical practice across sites in the US, Europe, Canada, Argentina and Taiwan. Enrolment began in March 2014 and data collection will continue for 10 years from enrolment of the 300th patient.

Results: As of February 2023, 226 patients (43.4% male) were enrolled in LOWER, with a mean±SD [range] age of 51.4 ± 15.1 [18–83] years and baseline LDL-C level of 237.1 ± 105.4 [71–632] mg/dL. Post-lomitapide data were available for 223 patients (98.7%); mean lomitapide [range] exposure was 38.5 [0.3–118.1] months, with 78.9% receiving lomitapide for ≥ 12 months and 25.6% for $5\leq10$ years. Lomitapide doses ranged from 2.5–50 mg/ day and the global mean dose was 13.0 mg/day (17.4 mg/day in patients treated in Europe). Figure shows LDL-C reductions from baseline in the full analysis set and in patients who remained on treatment.



Figure - Percentage change in LDL-C from baseline.

Patients in the registry were included at each time point where evaluable LDL-C data values were available.

Mean LDL-C reduction in patients who remained on treatment was 43.2%. At any time after lomitapide initiation, 73.1% (n=163) patients achieved an LDL-C level <100 mg/dL and 47.1% (n=105) achieved <70 mg/dL. The most common events of special interest (ESI) were gastrointestinal and hepatic in nature. During nine years follow-up, hepatic steatosis was reported in 6.3% of patients (n=14). Asymptomatic elevated aminotransferase levels $\ge 3 \le 5x$ and $\ge 5x$ the upper limit of normal were observed in 15.0% and 6.5% of patients, respectively.

Conclusions: Data from the LOWER registry support the sustained lipid-lowering effect and safety of lomitapide, with ~50% patients achieving LDL-C <70 mg/dL at some point during follow-up. ESIs in this large cohort were consistent with the known safety profile of lomitapide.

IMPACT OF HIGH NEUTROPHIL-TO-LYMPHOCYTE RATIO ON THE CARDIOVASCULAR BENEFIT OF PCSK9 INHIBITORS IN FAMILIAL HYPERCHOLESTEROLEMIA SUBJECTS WITH ATHEROSCLEROTIC CARDIOVASCULAR DISEASE: REAL-WORLD DATA FROM TWO LIPID UNITS

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Background and Aims: Neutrophil-to-lymphocyte ratio (NLR) is a novel inflammatory biomarker strongly associated with atherosclerotic cardiovascular disease (ASCVD). Our aim was to evaluate the role of NLR on pulse wave velocity (PWV) after adding-on proprotein conver- tase subtilisin/kexin type 9 inhibitor (PCSK9-i) in familial hypercholesterolemia (FH) subjects with ASCVD.

Methods and Results: In this prospective observational study, we evaluated 45 FH subjects with ASCVD on high-intensity statins plus ezetimibe and with an off-target LDL-C. Study population was divided into two groups according to the mean value of NLR. All patients received PCSK9-i therapy and obtained biochemical analysis as well as PWV evaluation at baseline and after six months of PCSK9-i. After six months of add-on PCSK9-i therapy, a significant reduction of TC, LDL-C, Non-HDL-C, Lp(a) and ApoB plasma levels was observed in the two groups; while low-NLR group exhibited a significant PWV reduction after six month therapy with PCSK9-i (D 16.2%, p<0.05), no significant changes in PWV were observed in the high-NLR group.

Conclusions: Only FH subjects with low-NLR experienced a significant reduction of PWV after PCSK9-i. Our findings suggest a role of NLR in predicting PCSK9-i effect in FH subjects with ASCVD.

THE INTERACTION OF APOB AND ANGPTL3 LOSS OF FUNCTION VARIANTS WITH RISK FACTORS IN SHAPING THE RISK OF CHRONIC LIVER DISEASE

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Background: Reducing LDL-C is essential for cardiovascular prevention, but achieving this through inhibition of apoB or ANGPTL3 may raise concern about liver safety. Recently we have reported that cholesterol lowering variants in APOB, but not ANGPTL3, associates with increased risk of liver disease in a large cohort of UK Biobank. Whether this effect may be modulated by the common risk factors is undetermined.

Objective: To investigate how metabolic risk factors may modulate the hepatic consequences of genetic inhibition of the APOB and ANGPTL3 genes.

Methods: The association of inactivating variants in APOB and ANGPTL3 with liver disease was tested in 910 carriers and 410,721 non-carriers from the UK Biobank. Chronic liver disease (CLD) was defined as a composite of alcoholic and non-alcoholic fatty liver, liver cirrhosis and liver cancer. Patients were stratified according to diabetes (yes/none), obesity (BMI<25kg/m² – BMI 25-29.9 kg/m² - BMI ≥30kg/m²) and PNPLA3 rs738409 genotype (dominant model). Multivariable regression analyses were used to assess the risk of liver disease. Kaplan-Meier curves and multivariable Cox proportional hazards regression models with covariates were employed to estimate the lifetime risk of incident CLD. Two-tailed P values ≤0.05 were considered to indicate statistical significance. Python (v3.10.13) and R software (v4.3.1) were used for all statistical analyses.

Results: A total of 411,631 UK Biobank participants (aged 57 [49-62] years, 177,793 [43%] male) were included in the study. Of these, 241 were identified as carriers of inactivating variants in APOB, 669 in ANGPTL3 and 410,721 as non-carriers. After adjustment for potential confounders, individuals with the APOB variants had a fourfold increased risk of chronic liver disease (OR 4.34, 95%CI 2.56–7.37; Padj=4.8E-8). In contrast, inactivating variants in ANGPTL3 did not demonstrate a statistically significant association with the risk of any liver-related outcomes. After stratifying for the presence or absence of metabolic risk factors, the occurrence of CLD was higher in the APOB group compared to non-carriers, independently of diabetes and obesity status or PNPLA3 genotype (all P<0.005). It is noteworthy that within both APOB and ANGPTL3 groups, patients with diabetes exhibited a higher prevalence of CLD compared to hose without

(P=1.9E-8 and P=0.004, respectively). However, in a multinomial regression analysis, only APOB carriers demonstrated the highest risk of CLD within each risk factor category (all Padj<0.04). Similar results were obtained in the analysis of lifelong event-free survival.

Conclusions: Genetic inhibition of APOB, but not ANGPTL3, is associated with an increased risk of liver disease, independently of the presence or absence of metabolic risk factors.

DECIPHERING THE MECHANISM OF LIPID FAT ACCUMULATION IN RESPONSE TO siRNA ANTI ANGPTL3

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Background: Angiopoietin-like 3 (ANGPTL3) is an hepatokine acting as negative regulator of lipoprotein lipase (LPL). Vupanorsen, ANGPTL3 directed antisense oligonucleotide, showed an unexpected increase in liver fat content in humans and was discontinued from clinical trial. We investigated the molecular mechanism linking ANGPTL3 silencing to hepatocyte fat accumulation.

Methods: Human hepatocarcinoma Huh7 cells were treated with siRNA-ANGPTL3, siRNA-PCSK9, or the combination of the two siRNA. Western blot, Oil red-O, RTqPCR, biochemical and ELISA assays were performed to assess changes in lipid metabolism. RNAseq was performed on the same experimental conditions. Livers from PCSK9 KO mouse in standard diet were analyzed by western blot.

Results: Oil red-O analysis demonstrated that lipid content increased significantly after ANGPTL3 or PCSK9 silencing as well as the combination of both (respectively 3.5, 0.45, 1.40 fold) compared to siRNA scramble control. When ANGPTL3 gene expression was reduced by 99.5% we observed an increase in main genes involved in de novo lipogenesis (FAS +36%, ACC1 +54%, ACLY +18%, SCD1 +89%) and in SREBP1 (+49%), SREBP2 (+27%) and PCSK9 (+163%). We also found a significant increase in FAS and SCD1 proteins (2.81 and 3.16 fold vs. basal, respectively) after siRNA-ANGPTL3. Moreover, PCSK9 silencing increases ANGPTL3 expression by 1.58 fold, and vice versa, ANGPTL3 silencing induces PCSK9 by 2.63 fold. The modulation of ANGPTL3 expression in absence of PCSK9 was confirmed also in PCSK9 KO mice. RNA sequencing identified 338 differentially regulated genes (DEGs) between siRNA scramble and siR-NA-ANGPTL3, including LMAN1 and SERPINA1.

Conclusions: Silencing of ANGPTL3 may reduce the intracellular concentration of free cholesterol determining the activation of the SREBP pathway. ANGPTL3 and PCSK9 are inversely regulated *in vitro* and *in vivo* but fat accumulation occurs independently from PCSK9. 338 DEGs were found significantly regulated in response to siRNA-AGPTL3, including LMAN1 and SERPINA1. De novo lipogenesis and SERPINA1 deficiency represents possible mechanisms contributing to the hepatic fat accumulation observed after ANGPTL3 ASO treatment.

IDENTIFICATION OF CREB3L3 VARIANTS IN PATIENTS WITH HYPERTRIGLICERIDEMIA

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Introduction: Hypertriglyceridemia (HTG) is a prevalent form of dyslipidemia, strongly associated with an increased risks of cardiovascular diseases and pancreatitis. Current guidelines define normal fasting plasma TG levels as <1.7 mmol/L (150 mg/ dL), while levels ranging from 2 to 10 mmol/L (175-885 mg/dL) and >10 mmol/L (885 mg/dL) classify mild-to-moderate and severe hypertriglyceridemia, respectively. Very severe HTG, defined as TG levels >20 mmol/L (>1770 mg/dL), is extremely rare, with a prevalence of only 0.014%. Autosomal recessive monogenic forms typically involve loss-of-function variants in key genes that regulate triglyceride metabolism, such as LPL, APOC2, APOA5, GPIHBP1, and LMF1. In addition, variants in genes such as CREB3L3 and GCKR have been implicated in dominant forms of familial hypertriglyceridemia. CREB3L3 variants may play a role in a broader spectrum of lipid metabolism disorders involved in phenotypic heterogeneity associated with hypertriglyceridemia.

Materials and Methods: Targeted Next Generation Sequencing (NGS) was employed to analyze the coding regions and intron-exon junctions of genes implicated in the synthesis and metabolic pathways of triglycerides. NGS Analysis was performed on a cohort of 19 patients who had various degrees of hypertriglyceridemia, ranging from moderate to severe and very severe HTG.

Results and Conclusion: Several variants of CREB3L3 were identified in 15 patients belonging to this cohort. Five distinct variants associated with phenotypic variability ranging from mild to severe hypertriglyceridemia were identified. Four patients were identified as heterozygous carriers of the nonsense c.610C>T mutation (p.Arg204Ter) and had hypertriglyceridemia of varying severity, ranging from mild to severe. One patient with mild hypertriglyceridemia was identified as carrying the previously characterized pathogenic c.718G>A (p.Glu240Lys) variant, while three other patients carrying the same variant had moderate and severe forms of the condition. Another patient with mild hypertriglyceridemia was a carrier of a loss-offunction variant, c.732_733insG (p.Lys245GlufsTer130), while two patients with moderate and severe hypertriglyceridemia were identified as heterozygous carriers of the c.742C>T variant (p.Arg248Cys). In addition, three patients were found to be carriers of the c.333delG variant (p.Lys111fsX20), although functional studies on this variant are still ongoing. The phenotypic heterogeneity associated with CREB3L3 variants underscores the complexity of the genotype-phenotype relationship in hypertriglyceridemia. These findings highlight the need for further functional studies to elucidate the specific impact of CREB3L3 variants on triglyceride metabolism, suggesting the involvement of additional genetic or environmental modifiers, which require further investigation.

ACUTE PANCREATITIS AND SEVERE HYPERTRIGLYCERIDEMIA: THE RARE CASE OF A YOUNG PATIENT AMIDST DIAGNOSTIC CHALLENGES AND CLINICAL MANAGEMENT

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Introduction: Acute pancreatitis is a potentially life-threatening condition, often caused by gallstones or alcohol abuse. However, the rising rates of obesity and diabetes have led to an increase in cases of pancreatitis induced by hypertriglyceridemia (HTG), a less common but clinically significant condition. We present a rare case of edematous acute pancreatitis associated with severe hypertriglyceridemia in a 26-year-old patient with no history of alcohol use or a family history of pancreatitis, but with a family background of mixed dyslipidemia.

Case Report: In September 2022, a 26-year-old man presented at the Regional Center for Dyslipidemias of the A.O.U. of Napoli Federico II due to severe hypertriglyceridemia (Triglycerides 5900 mg/dl, Total Cholesterol 1012 mg/dl) associated with edematous acute pancreatitis. The patient reported no alcohol consumption or family history of cardiovascular events or pancreatitis, but he did have a family history of mixed dyslipidemia (father and brother). His medical history included moderate hypertriglyceridemia (Max TG 372 mg/dl) and nodular thyroid disease in euthyroid status. His BMI was 28 kg/m².

Laboratory tests showed the following values: Total cholesterol 429 mg/dl, triglycerides 710 mg/dl, blood glucose 235 mg/ dl, elevated inflammatory markers (fibrinogen >1000 mg/dl, CRP 431 mg/L), and neutrophilic leukocytosis (WBC 11420/uL). An abdominal ultrasound revealed hepatic steatosis without biliary calculi and splenomegaly. Therefore, the patient began parenteral nutrition, insulin therapy, and lipid-lowering treatment. Genetic testing for hypertriglyceridemia yielded negative results. At the 6-month follow-up, lipid profiles were normalized, and the pancreatic pseudocyst showed reduction, but blood glucose was slightly elevated (112 mg/dl, HbA1c 6.2%). A decision was made to implement strict monitoring of glucose levels, with subsequent reassessment in 3 months, revealing a worsening glycometabolic profile (blood glucose 225 mg/dl, HbA1c 10.8%). Further tests ruled out type 1 diabetes, except for the presence of anti-IA2 antibodies (1832.29 uI/ml), suggesting an autoimmune type, for which insulin therapy was initiated with a basal-bolus regimen. Genetic testing for MODY diabetes was positive for the PDX1 mutation associated with MODY4 diabetes. Given the complex glycometabolic condition, a decision was made to continue close clinical and laboratory monitoring of the patient, who currently, due to normalization of glucose values, has discontinued insulin and lipid-lowering therapy.

Conclusions: This case highlights the complexity of managing patients with severe hypertriglyceridemia and the diagnostic challenges associated with conditions like autoimmune diabetes. Therefore, a multidisciplinary approach and careful follow-up are crucial to ensure proper clinical management and prevent complications.

HDL SUBFRACTIONS AS AN ADDITIONAL BIOMARKER OF HDL METABOLISM IN HYPERCHOLESTEROLEMIC PATIENTS

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Introduction: Familial Hypercholesterolemia (FH) is an autosomal dominant disease mainly caused by pathogenic variants in LDLR, APOB and PCSK9 genes. It is characterized by high levels of LDL-cholesterol (LDLc) and increased risk of coronary artery disease (CAD). We aim to investigate additional biomarkers useful in the evaluation of HDL metabolism in FH patients with (FH/V+) and without (FH/V-) pathogenic variants.

Methods: Lipid profile of each patient includes total cholesterol, LDL-c, HDL-c, triglycerides and ApoA1. We analysed the distribution of HDL subfractions in 192 patients (116 FH/V+ and 76 FH/V-) with clinical suspicion of FH using a method based on polyacrylamide gel electrophoresis. This method allows to separate 10 subfractions of HDL that are grouped in: HDL Large, HDL Intermediate and HDL Small.

Results: No difference of HDL-c and HDL-c/ApoA1 were observed between FH/V+ and FH/V- patients, whereas ApoA1 levels resulted higher in FH/V+. This group of patients also showed higher levels of the ratio between HDL Large and Small (HDL L/S): 2.23 (1.38-3.74) in FH/V+ and 1.63 (1.10-2.45) in FH/V-patients (p<0.001). The association of this ratio with the presence of pathogenic variants remains significant independently from age, sex, LDL-c, HDL-c and ApoA1 levels OR=1.507 (95% CI 1.174 - 1.933; p=0.001). We observed that HDL L/S correlated positively with HDL-c (Spearman coefficient = 0.444; p<0.001) and negatively with triglycerides (Spearman coefficient = -0.577; p<0.001) in the whole population. A positive correlation of HDL L/S with ApoA1 (Spearman coefficient = 0.386; p<0.001) was observed only in FH/V- patients.

Conclusions: HDL L/S ratio could represent an additional biomarker of HDL metabolism that differentiate FH/V- and FH/ V+ patients independently from standard HDL biomarkers. The altered size of HDL could explain the increased predisposition to cardiovascular disease of FH/V+ patients compared to FH/Vpatients.

COULD BE AN ECHOCARDIOGRAPHIC "CALCIUM-TARGETED" SCORE A GOOD SURROGATE OF CT-scan AGATSTON CALCIUM SCORE TO PREDICT CARDIOVASCULAR RISK IN HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA?

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Background: Heterozygous familial hypercholesterolemia (HeFH) determines a high risk of atherosclerotic-based cardiovascular disease. Coronary artery calcification (CAC) score by Ct-scan Agatston calcium score (ACS) >100 classifies FH as severe disease. Echocardiographic calcium score (ECS) evaluates aortic valve calcifications and is considered a good predictor of atherosclerotic burden and cardiovascular outcome.

Objective: To test ECS as predictor of ACS >100 in a HeFH cohort.

Methods: Coronary CT-scan with determination ACS and at rest-transthoracic echocardiogram with ECS evaluation were performed in 81 HeFH patients. Patients were divided into 2 groups according to ACS: high-risk ACS patients (high-ACS) with Agatston value >100 and low risk ACS patients (low-ACS) with Agatston value <100. Patients were stratified according to ECS=0 or ECS >0.

Results: High-ACS patients were older and exhibited higher atherosclerotic burden at carotid US exam (p<0.032) than Low-ACS patients; BMI, waist circumference and blood systolic pressure were significantly higher (p<0.001) in high-ACS patients. ECS predicted a ACS >100 with sensitivity = 0.84, specificity = 0.89, accuracy = 0.86, precision = 0.76.

Conclusion: ECS could be a good surrogate of CT-scan CAC evaluation in the specific subset of HeFH patients.

GENETIC LYSOSOMAL ACID LIPASE DEFICIENCY AFFECTS THE IMMUNE LANDSCAPE: EVIDENCE FROM NAÏVE AND SEBELIPASE-TREATED PATIENTS

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Lysosomal acid lipase (LAL) hydrolyzes cholesterol esters and triglycerides in the lysosome. Genetic LAL-deficiency (LAL-D), a rare autosomal-recessive lysosomal storage disorder, induces hepatic steatosis and hypercholesterolemia resulting in an increased cardiovascular risk. An enzyme replacement therapy (ERT) with sebelipase-alfa is available, which was shown to reduce liver steatosis and fibrosis and to correct dyslipidemia. Little is known about the impact of LAL-D, and consequently of ERT, on other organs and systems. In this context, experimental evidence suggests that LAL-D may affects both innate and acquired immune response. Therefore, whether LAL-D causes immune alterations contributing to the cardio-metabolic dysfunction and whether ERT is effective in normalizing these alterations have not yet been investigated. Thus, aim of the project was to characterize the immunophenotype of ERT-naïve and ERT-treated LAL-D patients versus controls. A trend of reduction in circulating levels of leukocytes in ERT-naïve LAL-D patients was observed, when compared to controls. No difference was observed in the frequency of T lymphocytes. Neutrophils were significantly increased in LAL-D naïve patients, compared to controls, and normalized by ERT. More interestingly, in LAL-D patients we reported a 75% decrease of NK cells, with a shift in the distribution from CD56dim (cytotoxic) towards CD56bright NK subset (cytokine-releasing). Consistently, the functional characterization showed an impaired cytotoxic and degranulation capacity in NK cells from ERT-naïve patients compared to controls. The frequency of NK population persists even in ERT-treated patients, with a partial correction of the subset distribution. Thus, our data suggest that LAL-D affects the distribution of immune cells and particularly that of NK cells, which

seems to be only partially corrected by ERT. Further studies are needed to understand the underlying molecular mechanisms linking LAL to NK biology.

CYTOTOXIC AND DYSMETABOLIC IMPACT OF A NEW POTENTIAL ATHEROSCLEROTIC CARDIOVASCULAR RISK FACTOR, NANO-PLASTICS, ON A STEATOSIS MODEL OF HepG2 CELLS

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Introduction and Aim: The pervasiveness of nano-plastics (NPs) gave raise to serious concerns on the environmental, animal, and human health linked to the One Health principle. Most research activity was performed in animal models, showing how NPs can induce macrophage lipid accumulation, leading their differentiation into foam cells observed in atheromas. In cardiometabolic disease-mouse models polystyrene (PS)-NPs were found to disrupt lipid metabolism and induce weight gain by promoting adipogenesis. In vitro PS-NPs induced migration of mouse aortic smooth muscle cells, promoting atherosclerotic plaques formation. Microplastics were recently found in human atheromas associated with reduced collagen production and increased pro-inflammatory cytokine expression. Nevertheless, the knowledge gap about the adverse impact of NPs in human beings remains. This project aims at unveiling the potential cytotoxic and dysmetabolic acute effects induced by PS-NPs in hepatocytes, since the liver plays a major role in the clearance of xenobiotics and supports the cardiometabolic health.

Methods: Human hepatoma HepG2 cell line and the steatosis model of HepG2 cells differentiated with oleic acid (HepG2-OA) were selected. Both *in vitro* models were exposed to fluorescent and non-fluorescent PS-NPs (20, 100, 200, 500 nm) at concentrations of 10, 100, and 200 ug/mL. NPs uptake was quantified by cytofluorimetric analysis. Cell viability was evaluated by MTT assay after 24/48 hours. Metabolic derangements were assessed by flow cytometry, namely glucose uptake and reactive oxygen species production.

Results: PS-NPs reduced viability mainly in HepG2-OA after 24/48h NP-treatment. PS-NPs uptake increased in a dose-response fashion after 24/48h-exposure as revealed by cytofluorimetric analysis. Exposure to NPs resulted in an impaired glucose uptake.

Conclusions: These findings highlighted PS-NPs-driven cytotoxic and dysmetabolic effects in HepG2 and HepG2-OA cells. Considering the impact of PS-NPs in the liver may help to better understand the pathological mechanisms exhausting liver steatosis and leading to MASLD and atherosclerotic cardiovascular disease.

INSULIN RESISTANCE SURROGATE BIOMARKERS AND 24-HOUR BLOOD PRESSURE MONITORING IN A POPULATION OF OVERWEIGHT NON-DIABETIC HYPERTENSIVE PATIENTS

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Background and Aim: Insulin resistance (IR), a pathological condition associated with visceral adiposity excess and characterised by impaired glucose-lipidic metabolism and compensatory hyperinsulinemia, promotes hypertension and dyslipidemia through multiple pathophysiological mechanisms. The triglycerides-HDL cholesterol ratio (TG/HDL-C), the TG/ glucose index (TyGi), and the metabolic score for insulin resistance (METS-IR) are surrogate biomarkers of IR based on routinely available anthropometric parameters. Regarding office blood pressure (BP), higher values of these biomarkers are invariably associated with an increased risk of hypertension and poor BP control. However, no investigation has been conducted to compare the IR surrogate biomarkers and the 24-hour ambulatory blood pressure monitoring (ABPM). and data on IR surrogate biomarkers in patients undergoing anti-hypertensive treatment is lacking. Our study evaluated the association between TG/HDL-C, TyGi and METS-IR with 24-hour blood pressure control in a non-diabetic hypertensive outpatient population.

Methods: We designed an observational cross-sectional study on 1514 consecutive outpatients who were consecutively evaluated for the diagnosis and treatment of hypertension at our ESH Excellence Centre for Hypertension and Cardiovascular Disease, IRCCS INRCA Internal Medicine and Geriatric Unit of Ancona, and who had a recent and valid ABPM available. TG/ HDL-C, TyGi, and METS-IR were calculated based on available anthropometric and metabolic parameters. Anti-hypertensive therapies were assessed considering the number of drugs and the treatment intensity score (TIS), calculated as the ratio of the administered dose to the maximum dose. Participants were compared on the base of the 24-hour blood pressure control. Multivariate logistic regression models (Model 1: adjusted for age, sex, and BMI; Model 2: further adjusted for the number of anti-hypertensive drugs and TIS) were used to evaluate the association between TG/HDL-C, TyGi and METS-IR quartiles (referenced to the first quartile) and the prevalence of uncontrolled 24-hour BP, in the overall population and subgroups based on the anti-hypertensive therapy.

Results: 1.514 patients with a mean age of 56 ± 14 years, 59.1% male. The mean body mass index (BMI) was 28 ± 5 kg/m². The median TG/HDL-C ratio was 2.17 (IQR 1.44-3.40), the median TyGi was 8.56 (IQR 8.21-8.93), and the median METS-IR was 40 (IQR 35-47). Type 2 diabetes mellitus (T2DM) was diagnosed in 11.8% of patients, and 72.2% were on anti-hypertensive treatment(s), predominantly renin-angiotensin system inhibitors (RASi, 72.5%). The median number of anti-hypertensive drugs was 2 (IQR 1.3), and the median TIS was 1.35 (IQR 0.75-2). Twenty-four-hour BP with hypertensive BP criteria despite therapy was found in 62.9% of the (n=953). Patients with uncontrolled 24-hour BP were younger, predominantly male, with

lower rates of cardiovascular comorbidities (obesity, T2DM, dyslipidemia, chronic kidney disease) and use of anti-hypertensive drugs and TIS. There were no differences between groups in median TG/HDL-C, TyGi, or METS-IR levels. In Model 1, higher METS-IR quartiles were significantly associated with an increased risk of uncontrolled 24-hour BP compared to the first quartile (OR 1.62, 95% CI 1.18-2.23 for the second quartile, OR 1.46, 95% CI 1.07-1.99 for the third quartile, and OR 1.39, 95% CI 1.03-1.86 for the fourth quartile, p=0.018). In patients not on anti-hypertensive treatment, only the higher TG/HDL-C quartile showed a higher risk of uncontrolled BP (OR 2.40, 95% CI 1.27-4.55). In Model 2, compared to the number of anti-hypertensive drugs and TIS, none of the three IR surrogate biomarkers was independently associated with an increased risk of uncontrolled 24-hour BP.

Conclusions: Surrogate biomarkers IR based on simple and readily available anthropometric and metabolic parameters, especially the METS-IR, suggest a common dysmetabolic (adipo-centric?) origin of both IR and higher BP with increased cardiovascular risk. In treated hypertensives, the independent association between surrogate biomarkers of IR and 24-hour BP control was lost when the model included the number of anti-hypertensive drugs and TIS, further highlighting the crucial role of adequate pharmacological treatment.

IMPACT OF IMMUNE SYSTEM HUMANIZA-TION ON ATHEROSCLEROSIS IN DYSLIP-IDEMIC IMMUNOCOMPROMISED MICE

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Background and Aim: Immune cells are key players in atherosclerosis development and interest in targeting cardiovascular immune response has grown lately. Indeed, the availability of immunomodulatory biologics targeting human proteins has prompt the need of experimental models to investigate molecular mechanisms and test therapeutic approaches to cardiovascular disease.Hence, we provide an immune and metabolic characterization of a novel immunodeficient mouse model humanized with human hematopoietic cells on an atheroprone background. **Methods:** LDLR-KO mice were crossed with the immunodeficient C57BL/6J strain Rag2-KO/IL2rg-KO/CD47-KO (TKO, IMSR_JAX:025730) to generate an immunocompromised dyslipidemic mouse (TKO-LDLR KO mice) recipient of human hematopoietic stem cells (hCD34+).

Results: TKO mice present a deficiency in mature lymphocytes and NK cells. This characteristic is conserved in TKO-LDLR KO mice with an added atheroprone background. When fed with a high cholesterol diet, TKO-LDLR KO develop marked dyslipidemia and atherosclerosis. This profile confirms the suitability of TKO-LDLR KO mice for atherosclerosis studies. We, then, tested the impact of immune system humanization on disease progression. TKO-LDLR KO were irradiated with a low-dose irradiation (200 cGy) at births and thereafter underwent intrahepatic injection of 2 x 10⁵ hCD34+. Engraftment of human leukocytes (hCD45+) was evaluated starting from two months after injection by flow cytometry from tail blood. This approach allowed the reconstitution of 10-30% of hCD45+, mainly T and B cells. hCD45 were also successfully engrafted in the thymus (95%), spleen (20%), and liver (25%). The humanization process did not affect cholesterol levels (939,8±94,41 vs. 987,4±48,82 mg/ dL) and didn't seem to contribute greatly to the worsening of atherosclerosis development and lesion area compared to internal non-injected controls.

Conclusion: We have generated and characterized the humanized dyslipidemic TKO-LDLR KO model. It presents human B and T cells and represents a promising tool to investigate the impact of human adaptive immune cells modulation as a therapeutic strategy in the context of atherosclerosis.

ROLE OF KUPFFER CELL-DERIVED ApoE ON HDL COMPOSITION AND FUNCTIONALITY

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Background and Aim: Apolipoprotein E (ApoE) is one of the main proteins associated with lipoproteins packaging and transport as well as cholesterol efflux regulation. Hepatocytes are the main producers of ApoE, despite myeloid cells, especially macrophages including Kupffer cells, liver-resident macrophages, have been shown to contribute to ApoE production. This suggests an immuno-modulatory role of the protein, promting the investigation of the role of Kupffer cell-derived ApoE (KCs-ApoE) in the modulation of immunometabolic response at systemic and cellular level.

Methods: Kupffer cells selective ApoE-KO mice were generated by crossing ApoE flox/flox mice with Clec4f-Cre (ApoE KC-KO) mice. They were fed a normal chow diet for 12 weeks and FPLC, western blot, 2D-electrophoresis and flow cytometry analyses were performed to characterize the metabolic and immunophenotype profile of the mice.

Results: Cholesterol (53,93±9,313 vs. 49,59±12,16) and triglycerides (90,33±30,89 vs. 82,27±22,67) levels resulted comparable between ApoE KC-WT and KO. However, a western blot analysis on total plasma revealed a significant reduction of circulating ApoE in KO mice, suggesting that a portion of ApoE in normo-cholesterolemic conditions may also derive from KCs. An FPLC analysis revealed no changes in cholesterol distribution, with the majority of cholesterol in HDL. To asses whether KC-derived ApoE contribute to the total amount of lipoproteins ApoE, a western blot on HDL plasma fractions was performed and showed a significantly low level of HDL-ApoE in KO mice. We also performed a more in-depth HDL characterization by 2D-electrophoresis. We observed an increase in the percentage of immature pre-beta HDL (6,5% vs. 2,1%) compared to WT. No changes in the liver and circulation were reported in immune cell distribution.

Conclusion: We highlighted the contribution of Kupffer cell-derived ApoE on total HDL ApoE levels which seem to affect their composition. Although molecular mechanisms are still to elucidate, our data unveil cell-specific hepatic functions of ApoE.

TRANSCRIPTOME APPROACH UNRAVELS POTENTIAL BIOMARKERS IN ACUTE ISCHEMIC STROKE PATIENTS

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Background and Aims: Acute ischemic stroke (AIS) is a cerebrovascular disease leading to death and disability. Recent advancements in omics technologies suggest relevant information into clot composition, stroke mechanisms and etiology. The main goal of the study is to delve genetic aspect of AIS through the application of transcriptomic approaches. Our focus centers on the exploration of molecular signatures within cerebral thrombi (CT) and venous peripheral blood (PB) related to etiology.

Methods: The transcriptomic profiles from CT and PB from 92 ischemic stroke patients were analyzed with Affymetrix technology, followed by a Gene Ontology (GO) and Reactome enrichment analysis. Olso, we performed a Cibersort analysis, to evaluate the immune system.

Results: Analysis of CT data unveiled significant differences in gene expression profiles when comparing strokes of LAA origin with CE and cryptogenic strokes. Notably, LAA strokes exhibited overexpression of 301 genes compared to CE strokes, with differential expression of 209 genes compared to cryptogenic strokes. GO and REACTOME enrichment analysis, showed, respectively, biological processes involved in inflammation. Genes such as S100A12, S100A9 and S100A8, associated with inflammation and atherosclerotic plaque instability, were overexpressed in LAA strokes. Genes involved in inflammation, including MMP9, IL-1 β and VNN2. Concerning microRNA, we found overexpression of miRNA-223 in atherosclerotic-origin strokes. The Cibersort analysis, suggest a similar immune cell composition in both CT and PB, specifically, a predominance of neutrophil.

Conclusions: Transcriptome profiling has provided valuable insights into the molecular landscape of AIS. The overexpression of genes such as MMP-9, S100A12, S100A9 and S100A8 in atherosclerotic strokes underscores their association with plaque instability and adverse neurological outcomes. Dysregulation of genes such as IL-1 β exacerbates ischemic injury, highlighting their crucial role in AIS pathophysiology. Transcriptome signatures hold promise in distinguishing between stroke etiologies, paving the way for personalized approaches to secondary stroke prevention.

CROSSTALK BETWEEN LDL RECEPTOR AND MITOCHONDRIA: THE ROLE OF CARBAMOYL PHOSPHATE SYNTHETASE 1

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Background and Aim: LDL receptor (LDLR) is the main regulator of LDL-cholesterol plasma levels. The aim of study was to identify proteins associated to differently expressed levels of LDLR.

Methods: HuH7 cells were incubated with fluo-LDLs and sorted by FACS in 2 subpopulations expressing high and low levels of LDLR. Proteomic analysis was performed, and some of proteins found differentially expressed (DEPs) have been knocked down by siRNA in HuH7 cells. RT-qPCR and WB assays were performed to check for LDLR (and LDLR-turnover related proteins) expression. 10% or 0.4% FBS, as well as increasing concentrations of simvastatin, were used to stimulate SREBP2 pathway and investigate for any changes in the DEP identified. Jaspar database was interrogated for the presence of SREBP1 and SREBP2 responsive elements on the promoter of the identified DEP. HepG2 mock and overexpressing PCSK9 (HepG2PCSK9) were used as in-house in-vitro model for differential expression of LDLR.

Results: The proteomic analysis on HuH7 High and Low pointed-out an enrichment in several mitochondrial proteins differentially expressed. Among them, carbamoyl phosphate synthase 1 (CPS1), that catalyzes the rate limiting step of the urea cycle, resulted significantly upregulated in cells with higher expression of LDLR. siRNA anti CPS1 in HuH7 cells resulted in a significant decrease in LDLR mRNA levels (-40%, p<0.05 vs. scr). Surprisingly, we observed a significant decrease in PCSK9 (-50%, p<0.05), HMG-CoA red (-90%, p<0.001), and SREBP2 (-70%, p<0.01) mRNA levels vs. scr cells. Simva induced the expression of CPS1 mRNA (+25% at 40µM, p<0.05 vs. ctr) and protein (+40% at 40µM, p<0.001 vs. ctr) after 24h of treatment in HuH7 cells. Challenging HepG2 cells with starving medium±simva 40µM brought us to observe a significant 1.5-fold increase in CPS1 mRNA in starving conditions vs. ctr cells, with an additive effect upon simva treatment. These results suggest an involvement of SREPB2 pathway in the regulation of CPS1. Indeed, the search for SREBP1 and SREBP2 responsive elements (RE) on human CPS1 promoter revealed the presence of 6 SREBP1 RE and 5 SREBP2 RE. HepG-2PCSK9 cells showed a nearly total decrease in CPS1 protein and mRNA compared to HepG2 cells (p<0.001).

Discussion and Conclusions: modulation of the LDLR either by selection of cells with high capacity of lipoprotein uptake or by overexpressing PCSK9 led to the identification of CPS1 as a coregulatory protein. This association suggest a feed-forward regulatory loop between LDLR and CPS1 (and mitochondria), thus paving the way to new therapeutic scenarios in cholesterol dysfunctional diseases.

IMPACT OF BEMPEDOIC ACID ON SKELETAL MUSCLE MITOCHONDRIAL ACTIVITY IN ApoE-/- MICE – A PRELIMINARY ANALYSIS

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Lipid-lowering therapy is a cornerstone in preventing coronary disease. Bempedoic acid (BA) can be prescribed when statin-associated muscular side effects are observed. Based on the hypothesis that statins impact skeletal mitochondrial activity, this study aimed to evaluate the effect of BA on this aspect in a murine model of hypercholesterolemia and compare it to the results obtained using atorvastatin. ApoE-/- mice were fed a high-fat high-cholesterol (HFHC) diet for 12 weeks, either alone (n=15) or with atorvastatin (40 mg kg-1 day; n=13) or BA (30 mg kg-1 per day; n=15). The primary outcome measured was skeletal muscles mitochondrial functionality. Secondary outcomes were locomotion and riding time. Tertiary outcomes included changes in lipids, plaque deposition, energy expenditure and oxygen consumption. Safety was assessed by evaluating organ weights and glycemia. After 12 weeks, body weight, food intake, glycaemic profile, and organ weights were unaffected by BA vs. HFHC diet alone. BA significantly reduced HDLc and neutral lipid accumulation in thoracic and abdominal aorta. The necrotic core of aortic sinus was also reduced with BA (all p<0.05). Mitochondrial functionality of skeletal muscles (tibialis anterior, extensor digitorum longus, soleus, gastrocnemius, guadriceps, and biceps brachii) in mice receiving BA (Mito stress analysis) was not reduced vs. HFHC alone, whereas mice receiving atorvastatin showed a significant 22% reduction in basal and maximal mitochondrial respiration. BA did not affect proteins of mitochondrial dynamics and of OX-PHOS complexes (WB). No changes were found in locomotion (stride width and length, distance to opposite foot) and riding time in mice with BA or atorvastatin vs. HFHC. Metabolic cages revealed a significant 12% increment in pedestrian locomotion in mice given BA vs. those on HFHC diet, which was also associated with a significant rise in oxygen consumption rate. BA positive impacts plaque burden while preventing skeletal muscle mitochondrial functionality and locomotion.

CORRELATION BETWEEN CHOLESTEROL BURDEN AND CORONARY ATHEROSCLEROSIS IN PATIENTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA (HeFH)

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Introduction to the project and state of the art Heterozygous Familial Hypercholesterolemia (HeFH) is an autosomal dominant disorder causing high LDL cholesterol and increased risk of premature atherosclerosis and cardiovascular disease, including aotic stenosis. Cholesterol burden (CB) is key in developing atherosclerosis, though its exact relationship with atherosclerotic burden (BA) remains uncertain. Computed tomography coronary angiography (CCTA) is a valuable tool for assessing BA and aortic valve disease. Recent studies indicate that early lipid-lowering therapy reduces coronary plaque volume, linking CB to plaque size.

Project objectives: The main objective of this study is to investigate the association between CB and BA in patients with genetically confirmed HeFH. Secondary objectives include the evaluation of the impact of lipid-lowering on the reduction of BA. Methodology: The is a retrospective-prospective, observational, non-randomized study on 250 patients with molecularly confirmed HeFH carrying pathogenic variants in LDLR, included in the LIPIGEN study and undergoing CCTA for routine clinical practice. Among these patients, 92 have already undergone CCTA and will be included in the present preliminary analysis. Medical records were retrospectively revised to collect clinical, biochemical, pharmacological and imaging data. BA was estimated by a comprehensive assessment of plaque phenotype, assessment of the degree of coronary stenosis by Coronary Artery Disease-Reporting and Data System (CAD-RADS score from 0 to 5) and evaluation of calcium quantity by Agatston score (expressed in HU, Hounsfield Units)].

Preliminary results: The cohort of patients undergoing CCTA (n=92, 59.8% male) had a mean LDL-C levels of 150,22 mg/dl corresponding to a CB equal to 6276 mg-year/dl (4769.25 - 8636.25). 44.6% (n=41) had a CAD-RADS score of 0, while more than half of the patients (n=51, 55.5%) had a CAD-RADS ≥1. The entire cohort of patients also had a median Agatston score of 8.85 (0 - 125.3). Regression analyzes highlighted a positive correlation between CB and CAD-RADS (Beta=0.26, P=0.009) which remained significant even after adjustment for major confounding factors, such as sex, BMI and smoking habits (Beta=0.33, P=0.001). Furthermore, the CB emerged as a predictor of the increase in the Agaston score (Beta=0.25 P=0.016), even after adjustment for confounding factors (Beta=0.27 P=0.008)

Conclusions: This preliminary analysis confirmed a positive correlation between cholesterol burden and atherosclerotic burden. Further investigation will enable us to assess whether pharmacological modulation of LDL cholesterol can lead to a significant reduction in atherosclerotic plaque burden.

NEONATAL SCREENING FOR LYSOSOMAL ACID LIPASE DEFICIENCY IN THE SARDINIA REGION, ITALY

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Cholesterol Ester Storage disease (CESD) is a term used to group lysosomal acid lipase deficiencies associated with a less severe clinical phenotype than that of Wolman disease, a fatal disease within one year of the newborn's life. This is because in CESD the enzymatic defect is subtotal, while in Wolman the enzymatic deficiency is practically totally. In both cases the transmission is autosomal recessive. The enzyme deficiency is due to mutations in the acid lipase gene (LIPA or LAL). This enzyme plays a fundamental role in the hydrolysis of lipoproteins at the endosomal and lysosomal levels. In CESD, lipid storage abnormalities mainly concern the accumulation of esterized cholesterol, with abnormalities in the accumulation of triglycerides less evident than in Wolman's disease. The age of onset and progression of CESD are extremely heterogeneous, with a continuous spectrum of the disease between almost asymptomatic forms, early-onset and severe forms which may be related to a change in the level of residual acid lipase activity. The estimated prevalence of the enzyme deficiency in the Caucasian population causing Wolman is not known, while the estimate varied between 1/40,000 and 1/300,000 in CESD. In the latter pathology, hepatomegaly is the main, and sometimes the only, clinical sign. The age of onset varies but is usually early (generally before the age of 10-12), although the appearance of first symptoms in adulthood has been described. Splenomegaly is also present in a third of cases. Liver disease may remain asymptomatic for a long period, but tends to progress to steatosis, liver failure, fibrosis and even micronodular cirrhosis in severe cases. In the majority of patients, plasma hyperlipidaemia with hypercholesterolemia is present, associated with an increase in LDL cholesterol levels, a reduction in HDL cholesterol levels with variable hypertriglyceridemia (Phenotype IIB). Adul patients have a significant risk of atherosclerosis. Radiographs can detect adrenal calcifications in Wolman disease, but not in CESD. In both forms the diagnosis can be made by measuring the enzymatic activity on Dry Blood Spot (DBS). Genetic analysis can be used to confirm the diagnosis. The differential diagnosis of CESD must be made with IIB related dyslipidaemias (Combined Hyperlipidaemia) and with liver diseases that lead to micronodular cirrhosis. For both disorders, replacement therapy of acid lipase (Sebelipase Alpha) is available intravenously once every two weeks with results of remission of the disease. The aim of the present study, financed by Alexion and approved by local ethics committee, was the early identification in Sardinian newborns, by means of a drop of blood obtained with a heel stick and normally used for neonatal screening for congenital hypothyroidism and phenylketonuria, of Wolman disease and CESD, respectively, due to a total or subtotal acid lipase enzyme deficiency. Every year approximately 6,000 babies born in Sardinia. In spite of a discontinuously activity due to the Covid-19 pandemia, from February 2020 until October 2023, 4,889 enzymatic assays on DBS have

been performed. Until now, no samples with zero activity and no sex differences were found; 14 samples with very low activity have been obtained (<0.20). The considered normal range of LAL activity is 0.20–0.80 nmol/spot/h; the mean normal LAL activity is 0.44; The genetic analysis of the 14 samples with low enzymatic activity revealed only already known SNPs variants for all 10 exons. At the present time, neonatal LAL screening analysis is still in progress. The goal of an extension of the study would be to screen more than 40,000 newborns.

STRUCTURE AND TRAFFICKING OF PCSK9 IN LDL BINDING

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Introduction: Circulating PCSK9 is known to interact with the LDL-R thus promoting its degradation and blunting the uptake of LDL from the circulation. In this context, anti-PCSK9 mAbs and siRNAs are approved for the treatment of hypercholesterolemia. Previous studies have demonstrated that a significant proportion of circulating PCSK9 associates to LDL. The purpose of our research is to better understand the basis of the PCSK9-lipoprotein interaction and its synthesis.

Methods: A three-layered iodixanol gradient was used to isolate lipoproteins fractions from patients' plasma before and after treatments. Lipoproteins components were studied by spectrophotometric, lipidomic and proteomic approaches.

Results: The LDL-C levels decreased from 103±50 to 42±17 mg/dL after siRNA treatment and from 126±50 to 57±31 mg/dL after mAbs therapy. Circulating PCSK9 decreased 65% after siRNA, while plasma PCSK9 levels increased 10-fold after mAbs (n=18 and 30 respectively; p<0.05). Independent of the therapy, PCSK9-bound to LDL was on average 10% (n=30; p<0.01). Immunoblotting analysis demonstrated that PCSK9 binds to LDL with its active form. Lipidomic and LC-MS analyses revealed that PCSK9 associates with a subfraction of LDL that has a lower density and contains higher amounts of ApoE, ApoCs than average LDL. In post-prandial subjects, PCSK9 levels decreased from 468.6 to 407.2 ng/mL, by 10%.

Conclusions: Our study identified an LDL subfraction more buoyant than classical LDL (IDL-like lipoproteins) that is involved in PCSK9 binding. Although the therapies significantly modify the total circulating levels of LDL and PCSK9, the percentage of PCSK9-bound LDL remains constant. The active form of PCSK9 appears to be involved in binding both LDL and the LDL receptor. The analysis of post-prandial samples requires further investigation due to the observed decrease in PCSK9 levels.

FACTORS INFLUENCING THE INITIATION OF LIPID-LOWERING TREATMENT IN HOSPITALIZED PATIENTS AFTER A CARDIO-CEREBROVASCULAR EVENT: A RETROSPECTIVE COHORT STUDY

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Background: Current European guidelines on cardiovascular prevention recommend the use of lipid-lowering therapies (LLTs) in patients who have experienced an atherosclerotic cardiovascular event (ASCVD).

Objectives: This study aims to provide updated data on the prescription of LLTs in patients discharged after an ASCVD event and to investigate which demographic and clinical characteristics are associated with a higher likelihood of receiving such therapy after the event.

Methods: Using administrative data from Lombardy, individuals aged \geq 40 hospitalized for an ASCVD event in the first 9 months of 2022 were identified. The prevalence of those prescribed LLTs within 90 days was assessed. A multivariate logistic regression model was then applied to evaluate the impact of various factors on the likelihood of initiating treatment (odds ratio [OR] and 95% confidence intervals [95% CI]).

Results: Out of a cohort of 16,025 subjects with an incident AS-CVD event, 41.14% did not receive a prescription for LLTs. The likelihood of initiating lipid-lowering treatment was higher in subjects who had a cardiovascular event compared to a cerebrovascular event (OR 2.22, 95%CI 2.07-2.38), in patients aged 51-60 years (OR 1.30, 95%CI 1.16-1.46, vs. 61-70 years), and in patients previously treated with antidiabetic (OR 1.42, 95%CI 1.25-1.62), antihypertensive (OR 1.96, 95%CI 1.80-2.13), and antihypothyroidism medications (OR 1.34, 95%CI 1.10-1.63). Conversely, older age (71-80 years: OR 0.79, 95%CI 0.71-0.87; >80 years: OR 0.47, 95%CI 0.42-0.52), female sex (OR 0.73, 95%CI 0.68-0.79), previous exposure to antithrombotic medications (OR 0.65, 95%CI 0.59-0.72), and polypharmacy (OR 0.90, 95%CI 0.81-0.99 for 5-9 medications, OR 0.61, 95%CI 0.52-0.72 for ≥10 medications) were associated with a lower likelihood of initiating treatment after the event

Conclusions: The study reveals suboptimal initiation of LLTs in patients discharged after an ASCVD event. Additionally, it underscores the importance of understanding influencing factors to enhance patient management in secondary prevention.

INFLUENCE OF APOE4 GENOTYPE ON PCSK9-LIPIDS ASSOCIATION IN CEREBROSPINAL FLUID AND SERUM OF PATIENTS IN THE ALZHEIMER'S DISEASE CONTINUUM

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Alterations in factors involved in cholesterol homeostasis are critical in Alzheimer's Disease, but the stage of occurrence, their association, and the possible relationship with the APOE4 genotype are not clarified. We aimed to quantify and correlate specific lipid factors in patients with different degrees of cognitive decline, namely patients with Alzheimer's dementia (AD) and patients with mild cognitive impairment due to AD (MCI-AD), carriers or non-carriers of the APOE4 genotype. We evaluated PCSK9, cholesterol and 24-, 25-, 27-hydroxycholesterol (HC) in the cerebrospinal fluid (CSF) and serum of AD (n=28) and MCI-AD (n= 27) patients. Human neuroblastoma IMR-32 cells were incubated with recombinant ApoE4; PCSK9 expression was analysed by WB. CSF and serum PCSK9 and lipids were similar, except for higher serum PCSK9 and triglycerides in MCI-AD compared to AD. AD APOE4-carriers showed higher CSF PCSK9 expression (+61.3%, p=0.027), suggesting the existence of a mechanistic link between cerebral PCSK9 and ApoE4, confirmed in vitro in IMR-32 cells by the increase in PCSK9 expression following ApoE4 exposure (+12%, p=0.018). CSF 24-HC was higher among AD APOE4-carriers (+32.7%, p=0.037 compared to non-carriers). A negative association was observed between CSF PCSK9 and 27-HC in AD (r=-0.444, p=0.049) and, exclusively in AD APOE4-carriers, a negative association between CSF PCSK9 and 24-HC (r=-0.786, p=0.028). A positive correlation was found between CSF and serum PCSK9 in AD (r=0.520, p=0.004), driven by APOE4-carriers (r=0.544, p=0.038), suggesting PCSK9 exchange between brain and periphery. A positive correlation was found between serum and CSF 27-HC (r=0.465, p=0.039) in AD. None of these observations were found in MCI-AD. We identified an altered pattern, evident in AD and particularly in APOE4-carriers, involving CSF PCSK9 and 24-HC, suggesting a mechanistic association between ApoE4 isoform and lipid markers, possibly contributing to the complex interplay between alterations in cerebral lipid metabolism and AD pathogenic mechanisms.

STATIN-INTOLERANT PATIENTS ARE CHARACTERIZED BY A DECREASED MUSCLE QUALITY INDEX INDEPENDENT OF LIPID LOWERING THERAPY

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Background and Aims: Statin-associated muscle symptoms (SAMS) is a frequent side effect of statin therapy (2-3% of population), limiting its clinical use and, therefore, increasing cardio-vascular risk. Its nature is not completely understood, particularly muscle performance and quality. The aim of our study was to retrospectively compare muscle quality, body composition and lipid profile in dyslipidemic patients according to their tolerance for statins.

Material and Methods: In a secondary level outpatient clinic for hypercholesterolemia, cardiovascular risk factors, body composition, handgrip (as a marker of muscle quality) and lipid profile were analyzed in 148 statin-intolerant (SI) and in 145 sex and age matched statin tolerant (ST) patients in a four-year follow-up. Results: At baseline, Body Mass Index (BMI), fat mass, cholesterol and triglycerides in SI patients were higher than in ST patients. While, during follow-up, BMI further increased and total and low-density lipoproteins (LDL)-cholesterol remained significantly higher in SI than in ST patients. At the end of the follow-up, BIA-assessed fat mass percentage was higher in SI than in ST. Handgrip absolute value or standardized for BMI, fat free mass and appendicular muscle mass were significantly lower in SI patients (P<0.001) but, in a sub-analysis, this was confirmed only in their non-dominant arm (P<0.01 for all arms). Circulating Creatine Kinase (CK) levels were significantly higher in SI patients at baseline (P<0.001) and decreased during follow-up. but remained higher in those who never restarted statins after re-challenge (0.029).

Conclusion: Statin intolerance is clinically associated with lower muscle quality, particularly in less exercised arms, suggesting a role for physical activity in prevention of muscle deterioration. Circulating muscle enzyme patterns suggests that the effects of SAMS persist also long time after statin discontinuation.

CLINICAL BURDEN OF ELEVATED LEVELS OF LIPOPROTEIN(a) ON PATIENTS WITH ATHEROSCLEROTIC CARDIOVASCULAR DISEASE: AN ANALYSIS IN THE ITALIAN REAL CLINICAL PRACTICE

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Background and Aims: Elevated Lp(a) is independently and causally associated with increased risk of atherosclerotic cardiovascular disease (ASCVD). This real-world analysis compared the characteristics of ASCVD patients with normal (<30 mg/dL) or elevated Lp(a) (≥50 mg/dL) levels and assessed the impact of elevated Lp(a) on future cardiovascular event occurrence.

Methods: A retrospective cohort study was conducted on patients with available Lp(a) measurement and established ASCVD between 2012 and March 2023 in laboratory and administrative databases of a pool of Italian healthcare entities. The index-date was the time of the first detected ASCVD hospitalization during the observation period. After stratification by normal and elevated Lp(a) levels, the groups were compared for demographics, clinical characteristics, comorbidities, medications at baseline, and occurrence of cardiovascular events within 2 years from the index-date (first ASCVD event).

Results: At index-date, patients with elevated Lp(a) levels (N=719) *vs.* those with normal levels (N=2585) were younger (67.5±12.9 *vs.* 69.2±13.4 years, p<0.010), had similar sex distribution (nearly 70% males), higher likelihood of ischemic heart disease as index ASCVD hospitalization (73.9%, *vs.* 68.5% p<0.010) and higher rates of previous atrial fibrillation. Patients with elevated Lp(a) levels showed a larger utilization of lipid-lowering therapies and antihypertensives, and had higher LDL-cholester-ol levels (107.1±42.0 *vs.* 99.5±39.4 mg/dL, p<0.001). At 2 years of follow-up, the rates per 100 person-year for ASCVD-events were higher among patients with elevated Lp(a) than those with normal levels (9.7 *vs.* 5.8, p<0.001).

Conclusion: This real-world analysis showed that ASCVD patients with elevated Lp(a) levels are at increased risk of experiencing a second ASCVD event within two years from the first event. These findings corroborate the importance of Lp(a) testing/screening to implement more effective cardiovascular risk assessment and prevention of future events in high-risk ASCVD patients, which in turn might alleviate the economic burden sustained by the healthcare system for disease management.

SLCO1B1 IS A GENETIC PREDISPOSITION FOR STATIN INTOLERANCE IN PATIENTS WITH HYPERCHOLESTEROLEMIA INDEPENDENTLY FROM FH MUTATION

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Background and Objectives: The aim of this study is to analyze lipid target achievement in patients attending our Lipid Clinic using real-life data. The presence of the SLCO1B1 mutation were evaluated to identify potential differences in achieving lipid targets and statin intolerance, in patients with hypercholesterolemia with and without FH.

Methods: The study involved 185 patients (96 F, 89 M), stratified based on the presence or absence of familial hypercholesterolemia (FH+ and FH-). All patients underwent genetic analysis as part of the LIPIGEN project, screening for mutations in LDLR, APOB, PCSK9, LDRAP1, LIPA, SLCO1B1, and ABCB1. Data were collected and analyzed to assess lipid target achievement and statin intollerance, comparing results by gender and SLCO1B1 mutation status.

Results: Females showed greater difficulty compared to males, associated with a higher rate of statin intolerance. Patients with a positive SLCO1B1 mutation demonstrated slightly more difficult in achieving lipid targets. Mutations in LDRAP1, LIPA, and ABCB1 genes were found in several patients, distributed evenly regardless of gender or the presence of the SLCO1B1 mutation. We further subdivided our patients as follows:

- Men with FH+ and SLCO1B1 mutation failed to achieve the target in 14.29% of cases, while those without the mutation failed in 11.63% of cases. Additionally, there was a significant increase in statin intolerance among men with FH+ and the SLCO1B1 mutation compared to those without it (33% vs. 7%, respectively).
- Women with FH+ and SLCO1B1 mutation failed to achieve the target in 43.48% of cases, compared to 22.73% in those without the mutation. However, the degree of statin intolerance was not significantly affected by the presence of SLCO1B1 mutation, with 22% in mutated women vs. 25% in non-mutated women.
- Men with FH-, the presence of the SLCO1B1 mutation led to greater difficulty in reaching the target (14% failed compared to 8% without the mutation). However, there was little difference in statin intolerance between those with and without the mutation (14% vs. 19%, respectively).
- Women with FH- showed a similar rate of target achievement regardless of SLCO1B1 mutation, with approximately 20% failing to reach the target in both groups. However, the presence of the SLCO1B1 mutation significantly increased statin intolerance, with 50% of mutated women experiencing intolerance compared to 30% of non-mutated women.

Conclusions: Likely, female gender and the presence of the SLCO1B1 mutation are two independent risk factors both for achieving lipid targets and for the degree of statin intolerance. Although limited to a single-center study, these results suggest that older individuals, women, obese patients, and carriers of the SLCO1B1 mutation face greater challenges in reaching lipid goals. Further multicenter studies are needed to confirm these trends and improve dyslipidemia management in more vulnerable patient subgroups.

EXECUTIVE COGNITIVE DYSFUNCTION IN PEOPLE LIVING WITH SEVERE HYPERTRIGLYCERIDEMIA INDEPENDENT OF CARDIOVASCULAR RISK FACTORS

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Background: Patients with Hypertriglyceridemia (HTG) often report dizziness and confusion, especially during triglyceride spikes. Recent studies have indicated a potential relationship between triglyceride levels and cognitive function. Brain blood barrier dysfunction or amyloid metabolism alterations may represent some of the mechanisms involved in this process. The causal link between dyslipidemia and neurodegeneration or cognitive decline remains uncertain and debated. Our aim was to investigate the relationship between triglycerides and neuroemotional functions in HTG.

Methods: This cross-sectional study included 36 adults (aged: 30-53 years) of both genders. Exclusion criteria included: neurological/psychiatric diagnoses, uncorrected hearing/vision deficits, brain injury and diabetes. Participants were classified into three groups based on their fasting triglyceride levels: severe HTG (≥500 mg/dL), moderate HTG (150-500 mg/dL) and control group (<100 mg/dL). All participants underwent the following neuropsychological assessments: Frontal Assessment Battery (FAB), Trail Making Test (TMT), Digit Span Test (DGS, forward and backward), Digit Symbol Test (DST), and State Trait Anxiety Inventory (STAI-Y).

Results: Individuals with severe HTG exhibited significantly lower mean scores in total FAB ($13.1\pm3 vs.$ CTR: 15.5 ± 0.9 ; p=0.03), backward DGS (p=0.04), DST (p=0.02), and STAI-Y (p=0.01) compared to the moderate HTG and control groups, while also showing significantly higher TMT scores than the other groups (p=0.001). Severe executive dysfunction was observed in 33% of the severe HTG group, 7% of the moderate HTG group, and none of the controls (p=0.02). There was no significant correlation between FAB scores, hypertension, creatinine, and LDL cholesterol. In contrast, only the FAB (r=-0.72; p<0.001), backward DGS (r=-0.61; p<0.001), and DST (r=-0.54; p=0.001) tests showed significant correlations with serum triglyceride levels.

Conclusions: These findings suggest that severe hypertriglyceridemia is linked to impaired cognitive performance in relatively young adults, independent of cardiovascular risk factors. Further research is needed to prevent neuropsychological dysfunction in individuals with hypertriglyceridemia.

ACUTE AND CHRONIC EFFECTS OF TRADITIONAL CIGARETTE, TOBACCO HEATING PRODUCT AND ELETRONIC CIGARETTE ON VASCULAR SMOOTH MUSCLE CELL AGING

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Aging and smoking are major risk factors for cardiovascular diseases (CVD). Cellular senescence, a process characterized by permanent proliferative arrest, senescence-associated secretory phenotype (SASP), and high levels of reactive oxygen species (ROS) in cells, has emerged as a potentially important contributor to aging and age-related diseases. Senescent vascular smooth muscle cells (VSMCs) are present in atherosclerotic plaques and contribute to their instability. Traditional cigarette (TC) smoke is a known exacerbator of age-related diseases. To reduce the harm associated with TC, alternative next-generation tobacco products (NGTPs), such as tobacco heating products (THP) and electronic cigarettes (ECIG), have been developed. We studied the acute and chronic effects caused by exposure to aqueous extracts (AEs) of smoke from TC, THP, or ECIG on VSMC aging compared to doxorubicin-induced senescent (DIS) human VSMCs. Cells were incubated for 48 hours or 7 days with 10% TC, THP, ECIG AEs, or doxorubicin 100nM. Then, we measured SA-β-gal activity (senescence marker), senescence-associated gene and protein expression, cell proliferation, cell cycle, and ROS production. After 48h of exposure to TC, VSMCs behaved similarly to aged cells, showing an increased expression of senescence markers, DNA damage, inflammation, oxidative stress, and decreased proliferation. On the contrary, THP and ECIG did not affect VSMC aging. However, after 7 days of exposure to AEs, also NGTPs exacerbated the aging effect in VSMCs, while TC further increased its aging effects. Our results show that TC AE accelerates aging in VSMCs after both acute and chronic smoke exposure. Instead, THP and ECIG did not show any significant effect on VSMC aging after acute smoke exposure, but chronic THP and ECIG exposure did affect VSMC senescence. In conclusion, our preliminary results give new insights into the real long-term effects of these NGTPs.

TREATMENT WITH INCLISIRAN DURING PERITONEAL DIALYSIS: A CASE REPORT

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Introduction: Inclisiran is a small interfering RNA that prevents PCSK9 synthesis, lowering low-density lipoprotein cholesterol (LDL-C). In ORION trials, efficacy and safety outcomes were similar among different renal impairment degrees, therefore no dose adjustments are necessary. However, the effect of peritoneal dialysis on inclisiran pharmacokinetics has not been studied and no data are available in literature regarding the use of inclisiran in patients undergoing peritoneal dialysis.

Case Report: We present the case of a 47-year-old female patient with non-familial hypercholesterolemia and Lp(a)hyperlipoproteinemia, complicated by severe atherosclerotic cardiovascular disease (ASCVD). Medical history included acute myocardial infarction in 2011, chronic kidney disease stage 5 secondary to acute renal failure on post-streptococcal glomerulonephritis background, for which she has been on peritoneal dialysis since 2019, quadruple coronary artery bypass graft (CABG) surgery in 2021, subsequent CABG failure in 2022 for restenosis of 3 grafts. When she came to our attention, lipid-lowering therapies included atorvastatin 80 mg and ezetimibe 10 mg, with mean LDL-C and Lp(a) concentrations of 76 mg/dl and 102 mg/dl, respectively. A baseline lipid profile was taken before the start of inclisiran treatment and repeated at 1 and 3 months. Following a single subcutaneous injection, LDL-C levels significantly decreased from 63 mg/dl to 40 mg/dL (-35%) at 1 month and 28 mg/dL (-55%) at 3 months. As expected, we observed significant reductions in ApoB concentrations. Lp(a) levels were also remarkably reduced to 62 mg/dL (-26%) at 3 months. No side effects were reported.

Conclusion: This report suggests that inclisiran therapy may have a significant effect on lipid levels in patients on peritoneal dialysis, with results comparable to those reported in clinical trials and a good tolerability profile. Its relevance as a highly effective and safe treatment in patients on peritoneal dialysis with documented ASCVD and Lp(a)hyperlipoproteinemia warrants further investigation in larger studies.

IMPACT OF CLINICAL FEATURES AND LIPID LOWERING THERAPIES ON VASCULAR AND COGNITIVE PROFILE IN FAMILIAL HYPERCHOLESTEROLEMIA SUBJECTS

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Aims: Familial hypercholesterolemia (FH) is a monogenic lipid disorder characterized by high levels of LDL-C that is a well-known causative factor of atherosclerotic cardiovascular diseases (ASCVD). Few data exist about the association of an increased LDL-C plasma level and cognitive abnormalities, thus an early assessment of FH patients' cognitive condition is essential. The Short Blessed Test (SBT) is a 6-item orientation-memory concentration neuropsychological test that has been validated as a measure of cognitive impairment and has been shown to discriminate among mild and sever cognitive deficits. The aim of this study was to evaluate cognitive impairment, clinical features and medications in FH patients and the association between early cognitive impairment (evaluated via the SBT) and CV risk stratified with arterial stiffness (Pulse Wave Velocity, PWV).

Methods: All patients underwent a physical examination, a review of clinical history, assessment of smoking status and medications, and the SBT. PWV, intima-media thickness (IMT), carotid and femoral atherosclerosis were evaluated in 253 subjects with FH aged 18-75 years, stratified according to SBT results into three groups: normal cognition (n=202), mild cognitive impairment (n=35), dementia (n=16).

Results: Age and BMI were significantly higher in the dementia than the normal cognition group. The groups were homogeneous for sex and smoking status, total and LDL cholesterol, and triglycerides. Diabetes and hypertension were significantly more prevalent in groups with impaired cognitive function. Regarding treatments, we found statistically significant differences among the groups, with a greater use of all lipid-lowering drugs in dementia group, especially statins and ezetimibe. Dutch score calculated at the diagnosis of FH showed statistically significant higher values in patients who developed cognitive decline. PWV was significantly increased from normal cognition to severe cognitive impairment groups (6.7±1.1 m/s, 8.2±1.9 m/s, 11.2±3.2 m/s). No statistically significant results were obtained for IMT and atherosclerosis. Furthermore, multiple regression analyses revealed that PWV was independently associated with cognitive impairment (β =0.16, P=0.039) and age (β =0.44, P<0.001)

Conclusions: In our study population, the cognitive impairment was present in 20% of subjects. Patients with dementia were found to be the most medicalised. We found increasing PWV in FH patients with progressively worse cognitive decline. This result suggests a potential underlying association between vascular damage and the cognitive decline in FH patients.

CARDIOVASCULAR RISK IN FAMILIAL HYPERCHOLESTEROLEMIA MAY EXTEND BEYOND CAUSATIVE MUTATIONS: A RETROSPECTIVE ANALYSIS FROM THE LIPIGEN DATABASE IN FERRARA

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Introduction: Familial hypercholesterolemia (FH) is an autosomal disease characterized by high LDL cholesterol levels (LDL-C) and increased cardiovascular risk. While some mutations have been clearly linked with FH, other genetic variants are considered "not causative" (NCG). This study aimed at investigating the impact of different genetic profiles on the occurrence of major adverse cardiovascular events (MACE) in hypercholesterolemic patients.

Methods: A cohort of 347 adult patients (age 48,7±13.4 years) enrolled in the LIPIGEN Project in Ferrara between 2016 to 2021 were genotyped into three groups: individuals negative for mutations on candidate genes (NEG, n=156), positive for NCG variants (n=77) and positive for FH-causing mutations (POS, n=114). Demographic and clinical data were obtained from outpatient visits or clinical records along with blood samples. MACE included cardiovascular mortality, myocardial infarction, stroke and unstable angina. Statistical analyses were performed through contingency tables or ANOVA test using SPSS software. Results: Subjects with NEG and NCG at presentation were older (50.9 vs. 48.2 vs. 45.8 years, p=0.008), had a lower Dutch Lipid Score (6.0 vs. 7.0 vs. 8.3, p<0,001), LDL-C levels (200.0 vs. 209.1 vs. 225.2 mg/dl, p=0.047) and were diagnosed later (36 vs. 31 vs. 27 years, p<0.001) than POS patients. The latter experienced their first MACE earlier compared to NCG and NEG (48.7 vs. 50.3 vs. 54.2 years, p=0,017). Although not statistically significant (p=0,554), MACE prevalence appeared highest in NCG patients (16,9%) followed by NEG (13,5%) and POS (11,4%).

Conclusions: Despite differences are more pronounced between NEG and POS patients, NCG appears to have an intermediate risk between the two extremes. However, their limited access to specific lipid lowering therapies reserved for FH may exacerbate their risk for MACE. Further studies with increased sample size are needed to further examine this possibility.

ANGIOPOIETIN-LIKE 3 (ANGPTL3) DEFICIENCY PROMOTES METABOLIC SUBSTRATE REROUTING

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ANGPTL3, a hepatokine which inhibits lipases, acts by sparing triglyceride-rich lipoproteins from hydrolyzation and controlling the postprandial triglycerides partitioning. This project aims to unravel the connection between hypolipidemia-derived metabolic alterations and possible hepatic responses according to substrate availability in ANGPLT3 deficiency setting. Angptl3-deficient (KO) mice and WT littermates, were fed chow or a High-Fat Diet (HFD, 60% kcal from fat) for 16 weeks. Metabolic adaptation to different diets was assessed by indirect calorimetry. Lipid tolerance test and lipoprotein production assay were performed. As expected, Angptl3 KO mice were hypolipidemic when fed and at fasting, on both chow diet and HFD. Following oral oil gavage, ANGPTL3 KO mice showed lower triglycerides absorption when fasting or postprandially; this was coupled with a decreased rate of hepatic lipoprotein production at 4 hours after Poloxamer injection in KO mice compared to WT (triglycerides HO:401.1mg/ dL±669.0 vs. WT:2209.7mg/dL±106.0; p=0.009), and numerically on HFD (triglycerides HO:444.6mg/dL±566.9 vs. WT:808.1mg/ dL±541.6; p=0.330). Glucose metabolism was not impaired. Data from indirect calorimetry in KO mice indicate a decrease in the Respiratory Exchange Ratio, with more oxidative metabolism when KO mice were fed a chow diet. To investigate a metabolic alteration, we assessed the activation of hepatic mTOR pathway, a sensor for caloric restriction; downstream effectors phosphorylation was assessed by western blotting and a reduction in S6K (fold on housekeeping: HO:0.28±0.122 vs. WT: 0.647±0.119; p=0.021) and 4E-BP1 (fold on housekeeping: HO:0.503±0.193 vs. WT:1.043±0.270, p=0.048) activation was observed, suggesting a lower protein synthesis. Liver RNA sequencing data confirmed that KO mice experience a different metabolic setting when compared to WT, facing an upregulation of urea cycle, higher lipid oxidation and synthesis, especially of bile acids, while hepatic signalling pathways (NR1H2/NR1H3) are shut down. The latter changes were common with HFD KO mice, ANGPTL3 deficiency affects the metabolic landscape by reducing circulating lipemia. Parallel changes occur in systemic metabolism, depending on the dietary setting

RECLASSIFYING CARDIOVASCULAR RISK BASED ON LIPOPROTEIN(a) LEVELS: TIME TO RE-EVALUATE LDL-C TARGETS?

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Lipoprotein(a) (Lp(a)) is a low-density-lipoprotein(LDL)-like lipoprotein, with an additional apolipoprotein calledapolipoprotein(a). Elevated Lp(a) levels are an independent significant additional risk for cardiovascular diseases, including coronary artery disease(CAD) and stroke. Lp(a)levels are largely genetically-determined and marginally-influenced by lifestyle and diet. Thus, the higher the Lp(a)level, the lower LDL-C-level should ideally be. The recently-developed tool Lp(a)clinical guidance risk ristratification tool allows to estimate lifetime CAD/stroke risk. However, little is understood about how to effectively incorporate Lp(a)values into 10-year cardiovascular(CV) risk stratification and how to adjust LDL-C targets.

Our study aimed to:

- assess the concordance of different validated clinical scores for CVrisk estimation;
- explore Lp(a) distribution in moderate, high and very-high CVrisk patients;
- 3) reclassify CVrisk based on Lp(a)levels;
- identify an Lp(a) threshold that identifies patients at higher CVrisk.

We selected 422 patients (55±15 years, 51% female) attending our tertiary-care outpatient clinics. We collected: biometric parameters; data on smoking habits, diabetes, hypertension and AS-CVD; glycemic and lipid profiles -including Lp(a). The following scores were calculated when appropriate: SCORE2/SCORE2-OP/SCORE-2-Diabetes, ESCU-prevent 10 years/lifetime score, Lp(a) CGtool. We found a concordance between SCORE-2/ SCORE2-OP/SCORE2-Diabetes and the 10-year ESC-U-preventscore, and between the lifetime ESC-UPrevent and the lifetime Lp(a) CGtool -before and after adjustment forLp(a). Lp(a) distribution in our patients' cohort mirrored that of Caucasians. The cohort's average estimated lifetime CV risk increased after adjustment for Lp(a)values (22.36%±18.82 vs. 28.38%±22.04). The Lp(a) threshold associated with higher CV risk was set at ≥ 114 mg/dL (ROC curve). These values characterized 64 (15.2%) patients; out of them, 40 (62.5%) also had baseline LDL-C ≤160mg/ dL. In conclusion, while it is easier to focus on lipid targets in high/very - high CVrisk patients - with ambitioustherapeutic goals leading to low circulating levels of LDL poor in cholesterol content- it seems mandatory to consider Lp(a) contribution in patients classified at lower CVrisk.

AN EARLY ONSET OF DEMENTIA: A DELAYED DIAGNOSIS OF CEREBROTENDINOUS XANTHOMATOSIS

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Background: Cerebrotendinous Xanthomatosis (CTX) is a rare autosomal recessive lipid storage disorder caused by congenital abnormalities of bile acid synthesis. Cholesterol and cholestanol deposition could affect virtually all tissues. A delayed diagnosis can lead to devastating complications including severe cognitive impairment and dementia.

Case Report: A 74-year-old female referred to our clinic for a subcutaneous mass of right Achilles tendon with the size of an apricot, progressively grown over the last three months and causing walking disability. Tendon ultrasound and CT scan confirmed the xanthoma. Her parents were first cousins. Medical history included chronic diarrhea, cataract surgery at 33 years old, osteoporosis, neuropsychiatric manifestations with a previous suicide attempt and dementia from 55 years old. She had urine incontinence and dysphagia with a previous respiratory arrest due to suffocation. She was never investigated with neuroimaging, and she never undergone a neurologic assessment. Physical examination showed deteriorated general conditions with cachexia. Patient appeared poorly oriented with slurred speech; she was not able to walk in a straight line. Multiple smaller xanthomas were also present in left Achilles tendon and bilateral knees. Routine laboratory exams were normal, including serum cholesterol levels. These elements were suggestive of CTX. Molecular genetic testing is still underway.

Conclusions: CTX is characterized by a pleomorphic clinical phenotype and variable penetrance. Despite most diagnosis occur in young adulthood, in some patients this is missed until advanced age, with severe neurologic consequences. Improved diagnostic algorithms could be an efficacy strategy to anticipate the treatment and slow down the progression of CTX.

ASSOCIATION OF CORONARY COMPUTED TOMOGRAPHY ANGIOGRAPHY AND STRESS ECHOCARDIOGRAPHY WITH LONG-TERM CARDIAC OUTCOME: A COMPARISON STUDY

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Aims: This study aimed to assess which variables on coronary computed tomography angiography (CTA) and vasodilator stress-echocardiography (SE) are best associated with longterm cardiac outcome in patients presenting for suspected chronic coronary syndrome (CCS) who performed both tests. **Methods:** We identified 397 patients with suspected CCS who,

between 2007 and 2019, underwent both SE and CTA within 30 days. Coronary artery calcium score (CACS) and the number of coronary arteries with diameter stenosis >50% were assessed on CTA. The presence of reversible regional wall motion abnormalities (RWMA) and reduced Doppler coronary flow velocity reserve in the left-anterior descending coronary artery (CFVR) were assessed on SE. The association of SE and CTA variables with cardiac outcome (cardiac death or myocardial infarction) was evaluated using Fine and Gray competing risk models.

Results: During a median follow-up of 10 years, 38 (9.6%) patients experienced a nonfatal myocardial infarction and 19 (4.8%) died from a cardiac cause. RWMA (HR 7.189, p<0.001) and a lower CFVR (HR 0.034, p<0.001) on SE, along with CACS (HR 1.004, p<0.001) and the number of >50% stenosed coronary vessels (HR 1.975, p<0.001) on CTA, were each associated with cardiac events. After adjusting for covariates, only CACS and CFVR remained associated (both p<0.001) with cardiac outcome.

Conclusion: Our data suggest that only CFVR on vasodilatory SE and CACS on CTA are independently and strongly associated with long-term cardiac outcome, unlike RWMA or the number of stenosed coronary arteries, usually considered the hallmarks of coronary artery disease on each test.

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PCSK9 INHIBITION AS A POTENTIAL STRATEGY TO TREAT ALZHEIMER'S DISEASE: IN VITRO AND IN VIVO STUDIES

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Alteration of cerebral cholesterol homeostasis and neuroinflammation are typical features of Alzheimer's disease (AD). The proprotein convertase subtilisin/kexin 9 (PCSK9), beyond regulating plasma cholesterol, is also expressed in the central nervous system (CNS), where a pathogenetic role in AD has been postulated. We previously demonstrated in vitro that PCSK9 enhances the β-amyloid (Aβ)-induced neurotoxicity and neuroinflammation, while PCSK9 genetic deletion in an AD-mice model showed an improvement in cognitive performance. This research aims to investigate the potential protective effect of PCSK9 pharmacological inhibition with newly-synthesized small molecules through in vitro and in vivo studies. Two novel PCSK9 inhibitors (MR-532, MR-533) were tested in human microglial cells (HMC3) to assess: cerebral PCSK9 inhibition (Western blot). the modulation of Aβ-induced neurotoxicity (MTT assay), cytokines' secretion (ELISA assay). The two compounds were subcutaneously injected at 40mg/Kg for 7 days in C57BL6/J mice evaluating macroscopic (weight and locomotion monitoring) and hepatic toxicity (histological analysis), plasma and brain inhibitors concentrations (LC-MS/MS), plasma cholesterol concentrations (colorimetric assay). In vitro, MR-532 and MR-533 [10µM] significantly decreased PCSK9 expression in HMC3 cells. Microglial viability, significantly reduced after incubation with Aβ-fibrils (-28%; p<0.0001), was dose-dependently restored by PCSK9 inhibitors (p>0.05 vs. basal condition). Furthermore, MR-532 and MR-533 [10µM] significantly reduced microglial IL-6 release (-60%, -32% respectively; p<0.05). In vivo treatment with MR-532 and MR-533 showed absence of macroscopic and hepatic toxicity. Moreover, the two molecules were significantly detected in plasma and brain samples; the treatment showed a downward trend of plasma cholesterol concentrations. In conclusion, our in vitro results show the protective role of PCSK9 pharmacological inhibition in Aß-induced neurotoxicity and neuroinflammation. The in vivo treatment exhibited a good tolerance of these compounds, their ability to reach the CNS and a cholesterol-lowering effect. Although further studies will be necessary, this pharmacological strategy may potentially open the way for novel therapies in AD.

LncRNA H19: A CIRCULATING BIOMARKER OF ATHEROSCLEROSIS REFLECTING OVEREXPRESSION ON HUMAN CAROTIDS PLAQUES

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Aim: The influence of long non-coding RNAs (lncRNAs) in the onset of several diseases is emerging over the last decade. However, their regulatory role in the development of atherosclerosis is still under investigation. The aim of this study was to evaluate the role of lncRNAs as effector and biomarker of carotid atherosclerosis in humans.

Materials and Methods: Carotid atherosclerotic plaques, their adjacent regions (AR) with a lower grade lesion and plasma samples were collected from 15 patients undergoing endarterectomy and processed for total RNA-Seq analysis by next generation sequencing (NGS). Circulating RNA was extracted by MirVana PARIS kit from additional 34 plasma samples from therosclerotic patients and 19 plasma samples from healthy controls (in total 48 cases and 19 controls). Quantitative real-time PCR (qRT-PCR) experiments were conducted on QuantStudio 6 PRO Real-Time PCR System with SYBR Green PCR Master Mix and primers were designed for lncRNA H19 and GAPDH as house-keeping gene. Data analysis was performed by using Design and Analysis (DA2) Software and SPSS.

Results: Among the differentially expressed genes at NGS analysis, IncRNA H19 caught our attention, showing a very high fold change between plaque and AR and a positive correlation with plasma levels. qRT-PCR results about tissues confirmed that IncRNA H19 is overexpressed in plaques samples [16.5 (6.6-40.5)] respect to AR [0.12 (0.07-1.82) - p=0.001]. Circulating IncRNA H19 levels were higher in plasma from atherosclerotic patients [0.35 (0.21-0.74)] than in plasma from healthy controls [0.22 (0.11-0.32) - p=0.004].

Conclusion: We demonstrated the overexpression of lncRNA H19 in human advanced atherosclerotic carotid plaques respect to low-grade lesions from the same subjects, confirming its role in the development of atherosclerotic plaque, previously reported only in animal models. Additionally, our results confirmed that lncRNA H19 can be considered a circulating biomarker, since its high levels in plasma were associated with plaque presence.

POSTER

THE USE OF BEMPEDOIC ACID IN DAILY CLINICAL PRACTICE, DATA SUGGESTIVE OF EXCELLENT FUTURE PROSPECTS

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Introduction: Bempedoic acid is the first of a new class of adenosine triphosphate citrate lyase (ACL) inhibitor drugs, which is used as a lipid-lowering agent in patients with hypercholesterole-mia who do not have an adequate response to specific therapy already in place or who are intol-erant to conventional statin therapy. This innovative molecule, on 27 January 2023, was approved by AIFA with class A reimbursement for patients with absolute contraindication or proven intolerance to statins and/ or ezetimibe or not at target despite 1st and 2nd level treatment with statins at the maximum tolerated dose in combination with ezetimibe.

In addition to the demonstrated effectiveness in reducing LDL levels, the drug appears to be ex-tremely safe, including among its most frequent side effects (>10% of patients treated): hyperu-ricemia (16%), increase in serum creatinine levels (11%) and thrombocytosis (19%).

Methods: We performed a descriptive observational study in our Level III 'Dyslipidemia Center', enrolling 168 patients of both sexes and Caucasian ethnicity. Of these, 123 were being treated with a lipid-lowering drug (statin - ezetimibe - iPCSK9) and, among them, 35 subjects were al-ready undergoing combination therapy with bempedoic acid. We measured the baseline total and LDL cholesterol of all subjects before the introduction of bempedoic acid therapy (Total cholesterol 229.21 \pm 51.75 mg/dL; LDL cal 140.47 \perp 43.24 mg/dL). Subsequently, serial blood tests showed a reduction in both total and LDL cholesterol levels three months after the start of treatment (total cholesterol 177.12 \perp 32.84 mg/dL; [p<0.0001]; LDL cal 95.16 \perp 30.29 mg/dL; [p<0.0001]), confirmed six months into therapy (total cholesterol 180.25 ⊥ 33.59 mg/dL; [p<0.0001]; LDL cal 99.29 ⊥ 29.46 mg/dL; [p<0.0001]). Analyzing the different subgroups, we ob-served that in patients treated only with ezetimibe for manifest intolerance to the statin, the in-troduction of bempedoic acid caused a clear reduction in LDL at 3 months, confirmed at 6 months of treatment (n 41; baseline LDL cal 141.41 \perp 42.96; LDL cal 3 months 96.22 \perp 28.67; LDL cal 6 months 101.54 ± 29.05 ; [p<0.0001]). This data was also superimposable for those patients treated only with statins (n 11; baseline LDL cal 140.66 \perp 43.06 vs. LDL cal 3 months 93.99 \perp 30.29 vs. LDL cal 6 months 99.29 ± 31.65; [p<0.0184]). Regarding side effects, in ac-cordance with the available scientific data, we recorded an increase in uric acid levels 3 months after the start of treatment (baseline value $4.75 \pm 1.1 \text{ mg/dL}$ vs. $5.72 \pm 1.27 \text{ mg/}$ dL at 3 months; [p<0.0001]) which, however, with new plasma titration at 6 months, remains unchanged $(5.69 \pm 1.44 \text{ mg/dL})$; [p<0.0001]). We also observed an increase in plasma creatinine levels at 3 months from the start of bempedoic acid therapy compared to baseline values (baseline 0.84 ± 0.15 mg/dL vs. 0.92 ± 0.19 mg/dL at 3 months; [p<0.0001]) but this showed a stabilization over time (value at 6 months 0.87 ± 0.16 mg/dL [p<0.0771]).

Conclusions: the adoption of bempedoic acid in clinical practice is depicted as an effective strategy in hypercholesterolemia both in conjunction with traditional therapies and for that fringe of patients who present an absolute intolerance or a poor response to therapy with statins or ezetimibe. In addition to the proven efficacy and interesting prospects, this new molecule ap-pears to be extremely safe and free of significant side effects, impacting minimally on the quality of life of patients.

VESICLE-MEDIATED ROLE **OF MMP-9 IN TUMOR PROGRESSION.** NEW HIGHLIGHTS ARISING FROM SUBPOPULATIONS OF VESICLES SECRETED BY DIFFERENT TUMOR **CELL LINES**

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The comprehension of extracellular vesicles (EVs)' cargo role in tumor progression is hampered by their intrinsic heterogeneity and suboptimal separation methods. Several data demonstrate that metalloproteinase-9 (MMP-9, a member of extracellular matrix degrading enzymes), which is involved in melanoma invasion/metastatization processes, is part of EVs cargo. In order to unravel the function(s) of EVs and MMP-9 in cancer development, we utilized our in vitro size-separation method, which allowed us to obtain five EVs subfractions (dimensional range >200nm/<50nm) secreted from cancer cell lines by sequential ultracentrifugazions, following the algorithm developed by Livshits et al. (2015). After method validation, fractions have been characterized by light-scattering, electron transmission/ atomic force microscopy techniques (size), fatty acid profile, cholesterol/phospholipid (lipidomics). Proteomics (LC-MS, western blot) revealed that in the melanoma metastatic line LM-16. MMP-9 is exclusively present and functional in the <50nm fraction, which is constituted by "non-vesicular extracellular particles (NVEPs)" or "exomeres" (MISEV 2023 Guidelines). Interestingly, we found that MMP-9 is present and functional not only in melanoma-derived NVEPs, but also in those secreted by metastatic mammary adenocarcinoma MDA-MB-231 and bone metastatic prostate cancer PC-3, as assessed by western-blot and zymography, suggesting a common involvement of MMP-9 in tumor development. Altogether, these data suggest a possible utilization of MMP-9 as an exomer biomarker, but the mechanism(s) of its shuttling to membrane after cellular biosynthesis and the interplay with its physiological inhibitor TIMP-1 are still to be understood. We are actually performing functional assays in order to address this point and to assess whether MMP-9 matrix-digesting activity is directly due to the protein carried by NVEPs and subsequently released in the extracellular fluids or is mediated by target cell effectors. Pharmacological studies will be designed to unravel MMP-9 roles in metastatic niche formation, with the goal to find a valuable pathology biomarker and new innovative therapeutical strategies

HYPERCHOLESTEROLEMIA: GENETIC PROFILE AND CARDIOVASCULAR DISEASE

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Background: Many familial hypercholesterolemia (FH) subjects did not demonstrate functional mutations in major candidate genes. According to this observation, we assessed FH patients genetic profile by high-throughput sequencing (HTS).

Methods: We analysed 129 patients [with possible/probable/ definite FH according to Dutch Lipid Clinic Network Score (DLCN)]. Targeted HTS (57 genes involved in lipid metabolism, dyslipidaemia, pharmacogenetics of statins, related to polygenic forms, HDL and triglycerides related diseases) was assessed by Illumina technology.

Results: Among 129 patients, 52 (40%) carried a rare variant in LDLR gene [42 (33%) carrying a likely pathogenic/pathogenic (LP/P) variant, 6 (5%) carrying an uncertain significance variant (VUS), and 4 (3%) carrying a likely benign/benign variant]. Talmud score evaluation showed a significantly higher median value in patients LDLR-negative for the presence of a VUS or LP/P variant, with respect to LDLR-positive [median(IQR): 1.015(0.897-1.090) vs. 0.887(0.754-1.038),p=0.012]. Ten out of 42 LDLR-positive patients also carried a rare variant in another gene. In patients without LDLR mutation, at least 2 rare variants were identified in 44 patients (54%), and at least 3 rare variants were identified in 31 patients (38%). A total of 170 rare variants have been identified in 41 different genes (ABCA1, ABCB1, ABCG2, ABCG5, ABCG8, ANGPTL3, APOA4, APOA5, APOB, APOC3, APOE, CELSR2, CREB3L3, DAB2, EPHX2, GCKR, GHR, GPD1, HFE, HMGCR, INSIG2, ITIH4, LDLRAP1, LIPC, LIPI, LMF1, LPA, LRP1, MTTP, NPC1, NPC2, NYNRIN, PCSK9, PON1, PPP1R17, SCARB1, SLC22A1, SLC01B1, SREBF1, SRE-BF2, ST3GALA). Among FH patients, 32 were younger than 18yrs. 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, significantly higher values in adults carrying LDLR mutation were found.

Conclusions: Present data suggest the involvement of multiple loci beyond LDLR in the modulation of lipid profile, as well as cardiovascular risk.

RESIDUAL PLATELET ACTIVATION IN CHRONIC CAD PATIENTS WITH DEPRESSION

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Depression has been linked to cardiovascular events and predicts mortality more effectively than other risk factors, comorbidities, or follow-up conditions. This suggests that standard antiplatelet therapy might not be enough to prevent poor outcomes in patients with this condition. To date, no specific studies have investigated the impact of depression on standard antiplatelet therapy in coronary artery disease (CAD) patients. In our study, we aimed to determine whether chronic CAD patients with depression (CAD-DEP) exhibit hyper-reactive platelets compared to CAD patients without depression. Additionally, we explored the potential relationship between platelet function and circulating brain-derived neurotrophic factor (BDNF), a marker for depression. We evaluated 232 chronic CAD patients on standard aspirin treatment for depression using the BDI-II scale. Of these, 96 patients were enrolled (37 with depression and 59 without). CAD-DEP patients had significantly higher residual serum thromboxane levels compared to CAD patients (p<0.007). They also exhibited enhanced platelet aggregation in response to different doses of collagen (0.5, 1, 2 and 4 mg/L; p<0.01) and ADP (0.5, 1 and 2 µM, p<0.03), though their response to serotonin and TRAP was similar to CAD patients. Moreover, CAD-DEP patients had a higher percentage of platelet/leukocyte aggregates (p<0.0001), increased P-selectin exposure and GPIIbIIIa activation, and abnormal platelet calcium homeostasis (p<0.02). Circulating BDNF was lower in CAD-DEP patients compared to CAD patients (p<0.002), with BDNF levels in CAD group positively associated with P-selection exposure (r:0.367 p<0.01) and platelet count (r:0.398 p<0.002). In our population, CAD-DEP patients underwent clinical percutaneous coronary Intervention more frequently than CAD patients during a five-year follow-up period (p<0.002; HR: 3.91). In conclusion, our study highlights the presence of hyper-reactive platelets and lower BDNF levels in CAD-DEP patients, raising the question of whether standard aspirin alone is sufficient to prevent future thrombotic events in these individuals.

ALAGILLE SYNDROME: CHARACTERIZATION OF LIPID PROFILE AND EVALUATION OF LIPOPROTEIN NEPHROTOXICITY

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Alagille Syndrome (ALGS) is a rare genetic disease (1/30.000) due to heterozygous variants in genes involved in the Notch signaling pathway. This syndrome exhibit with chronic cholestasis. stenosis of the peripheral pulmonary arteries, anomalies in vertebral segmentation, unique facial characteristics, pigmentary retinopathy, kidney dysfunction and xanthomas. The aim of the study is to investigate lipid alterations in patients with ALGS and to assess the relationship between these changes and renal complications. Sixteen pediatric patients (11.17±5.67) with a diagnosis of ALGS were enrolled. Lipid and lipoprotein profile were assessed and in vitro experiments on renal cells carried out. The results indicated a wide variability in lipid profiles, with LDL-C levels ranging from 41 to 301 mg/dL and HDL-C levels from 13 to 126 mg/dL. The activity of the enzyme lecithin-cholesterol acyltransferase (LCAT), which is responsible for cholesterol esterification in plasma, was found to be reduced on average (18.79±11.28 nmol/mL/h, reference range 25-55 nmol/mL/h). However, plasma LCAT concentrations was within the normal range (5.41±0.62, reference range 3.1-6.7 µg/mL), suggesting normal enzyme synthesis. An inverse correlation was observed between LCAT activity and the apoB/LDL-C ratio (r=-0.532, p=0.0411), suggesting the presence of Lipoprotein LpX, an abnormal lipoprotein rich in unesterified cholesterol and phospholipids. Indeed, LpX was detected in the plasma of nine subjects. In vitro experiments on podocyte cell cultures incubated with plasma fractions containing LDL+LpX from six patients demonstrated the nephrotoxic effects of LpX. In particular, it was observed an increase in cellular necrosis (+25.55%) and a reduction in podocin expression (-66.86%). Conversely, no changes were detected in the gene expression of key inflammatory mediators (VCAM-1, IL-6, MCP-1, TGF_β).In conclusion, the presence of LpX may help explain the renal complications observed in ALGS patients, and reducing its plasma levels may represent a potential therapeutic target.

REAL-WORLD EXPERIENCE OF EFFICACY AND SAFETY OF EVINACUMAB IN PATIENTS WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: CHECKMATE TO SEVERE HYPERCHOLESTEROLEMIA?

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Background: Homozygous familial hypercholesterolemia (HoFH) is a rare genetic disease characterized by high low-density lipoprotein cholesterol (LDL-C) levels and premature atherosclerotic cardiovascular disease. The availability of novel lipid-lowering therapies (LLTs) has changed the clinical management of HoFH. Evinacumab, an inhibitor of angiopoietin-like 3 protein, was recently introduced in Italy, offering a new approach to achieving guideline-directed LDL-C control in this very high-risk population.

Objective: This study investigates the safety and efficacy of evinacumab in adults and adolescents with HoFH not undergoing lipoprotein apheresis (LA) in real-world clinical practice.

Methods: Evinacumab was administrated intravenously (15 mg /kg every 4 weeks) to 6 patients with genetically confirmed HoFH, of whom 2 adolescents started therapy on a compassionate-use basis due to being <12 years of age. At each visit, patients underwent medical examinations, and blood samples were collected to assess lipid profiles and to evaluate liver and kidney function for safety monitoring.

Results: 6 patients were studied, of whom 3 were adults (mean age 64.4 years) and 3 adolescents (mean age 11.4 years). All adults had a history of coronary artery disease and aortic valve replacement. All patients had discontinued LA, and were receiving the best standard LLTs, including lomitapide. The mean treatment duration was 19 weeks. Evinacumab treatment decreased LDL-C by 58±15% (mean±standard deviation) in the overall population from baseline to week 24; mean LDL-C reduction in adults and adolescents was 61±17% and 56±18%, respectively. No serious treatment-related adverse events occurred in any patient.

Conclusion: In this small cohort, evinacumab was associated with a robust and persistent LDL-C lowering effect, enabling the permanent discontinuation of LA, and an excellent safety profile. Evinacumab is a potential game changer in the clinical management of HoFH, with predictable significant clinical cardiovascular benefits related to the magnitude of LDL-C reduction.

EVALUATIONS OF METABOLIC AND INNATE IMMUNITY PROFILES IN SUBJECTS WITH FAMILIAL HYPERCHOLESTEROLEMIA WITH OR WITHOUT SUBCLINICAL ATHEROSCLEROSIS

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Background: Familial hypercholesterolemia (FH) is a genetic condition characterized by high low density lipoprotein cholesterol (LDL-C). The presence of risk modifiers could promote the atherosclerotic injury beyond LDL-C. Our aim was to evaluate metabolic and innate immunity profiles in FH subjects with or without subclinical atherosclerosis.

Methods: In this cross-sectional observational study, we evaluated 211 genetically confirmed FH subjects on LDL-C target and without cardiovascular diseases. Biochemical analyses, LDL-C burden (LCB) calculation and vascular profile evaluation were obtained from all subjects. Study population was divided into two groups according to subclinical atherosclerosis presence.

Results: Exposed group had higher LDL-C at diagnosis (288.35 \pm 24.52 vs. 267.92 \pm 23.86, p<0.05) and LCB (13465.84 \pm 3617.46 vs. 10872.63 \pm 3594.7, p<0.001) than the non-exposed. Exposed group had larger amounts of White blood cell count (WBCC, 6.9 \pm 1.66 vs. 6.1 \pm 1.16), neutrophil count (NC, 4.2 \pm 1.3 vs. 3.6 \pm 1.11), monocyte count (MC, 0.8 \pm 0.2 vs. 0.4 \pm 0.1) than the non-exposed (p value for all <0.01). Multivariate logistic regression analysis showed that LCB (p<0.01), WBCC (p <0.01), NC (p<0.05) and MC (p<0.05) were associated with subclinical atheresclerosis. Simple linear regression analyses showed that LCB was associated with WBCC, NC and MC (p value for all <0.01).

Conclusion: An increased LCB and an impaired innate immunity profile were found in FH subjects with subclinical atherosclerosis and they were independently associated with atherosclerotic injury. LCB could modulate the innate immunity profile.

GENETIC CHARACTERIZATION OF SUBJECTS WITH AND WITHOUT CAD CARRYING "EXTREME LIPID PHENOTYPES"

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Introduction: Coronary artery disease (CAD) is the leading cause of mortality and morbidity in the world. The increase in circulating low-density lipoproteins cholesterol (LDL-C) and triglyceride (TG)-rich lipoproteins, while the reduction of high-density lipoproteins cholesterol (HDL-C) are associated with an increased or reduced risk of developing CAD. All these factors in practice can be combined to identify clusters of populations that have a higher risk of developing CAD and would benefit most from preventive therapies.

Material and Methods: The study sample includes patients enrolled in the Verona Heart Study (VHS) with angiographically documented CAD (positive CAD) and subjects without CAD (negative CAD). We selected patients with extreme LDL-C, TG and HDL-C phenotype with cut-offs >98th percentile and <2nd percentile. We also recruited patients with extreme phenotype of ApoC-III (<5° percentile and >95° percentile). For genetic analysis, a large-scale targeted sequencing analysis was performed on Ion Torrent technology.

Results and Conclusion: Among the patients with LDL-C> 98th percentile, the NGS analysis revealed the presence of a pathogenetic mutation in LDLR gene. The analysis of patients with low levels of LDL-C allowed the identification of two causative mutations in the APOB gene and one missense variant in PCSK9. Among the patients with ApoC-III levels <5° percentile, the NGS analysis revealed the presence of a pathogenetic mutation in APOC3 gene. Among subjects with low HDL-C we identified an ABCA1 gene mutation. We analyzed shared variants among these patients and classified them as rare, polymorphism or unknown using the minor allele frequency (MAF). Although most of these variants are classified as polymorphism and individually cannot explain the phenotype, the presence of multiple variants in different genes could contribute to determine the clinical and biochemical phenotype.

LYSOSOMAL ACID LIPASE DEFICIENCY: THE FOLLOW-UP OF FIVE CASES WITH MILD PHENOTYPE

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Background: Lysosomal acid lipase deficiency (LAL-D) late-onset phenotype, cholesteryl esterstorage disease (CESD), is a rare disorder potentially underdiagnosed. The mid/long term follow-up of 5 CESD subjects is here shown.

Methods: 3 children and 2 adults were suspected of LAL-D as affected, as first signs, by dyslipidemia (n. 1), liverenlargment (n. 1), selected screening as a patient sibling (n. 1), lymphadenopatia (n. 1) andgastroenterogical symptoms (n. 1). LAL-D was confirmed by LAL activity measurement(<0.03nmol/punch/h) and LIPA gene analysis. Treatment with ezetimibe (10 mg/day, meanduration 8.4 years, range 3-20 years) was started, the outcome monitored focusing on lipid andtransaminase changes, liver steatosis/fibrosis by liver transient elastography (TE) and magneticresonance (MR).

Results: All patients carry the variant c.894 G>A, showed variability of clinical presentation and the efficacy of treatment. The mean decrease on treatment was 19.2% and 26.6% for LDL-C and ALTrespectively. Mean HDL-C increase was 13.4%. No evident progression of liver fibrosis wasobserved during the follow-up. MR and TE detected steatosis and F0-F1 fibrosis stage. At the lastevaluation the TE, liver stiffness measurement was 5.1, 5.5, 5.9 kPa in children and 5.1, 6.2 kPa inadults.

Conclusion: Late-onset LAL-D presentation mimics common disorders, then it should bemisdiagnosed, despite diagnostic tools are easily available. The treatment is challenging as theout-come is difficult to be established. Ezetimibe should be a valid option but precocious diagnosisand strict follow-up are needed before considering the enzyme replacement therapy.

SEX DIFFERENCES IN LIPID PROFILE OF PEDIATRIC PATIENTS AFFECTED BY FAMILIAL HYPERCHOLESTEROLEMIA AND TREATED WITH STATIN

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Background: Differences between males and females in lipid profile are observed in distribution and trajectory during pediatric age, and are more pronounced in hereditary lipid disorders such as familial hypercholesterolemia (FH) when absolute cholesterol levels are higher from birth onwards. However, little is known about the response to statin therapy.

Methods: Sex-specific analyses for this retrospective cohort study was performed using data from pediatric patients referring to Rare Diseases and Medical Genetics Unit, Ospedale Bambino Gesù, Rome, Italy. Data from a total of 152 patients, age 8-17 years, M/F ratio 78/74, were collected. For each patient, lipid profile ad diagnosis and 1 year after the start of statin therapy was registered.

Results: The value of total cholesterol (TC), HDL- cholesterol (HDL-C), LDL- cholesterol (LDL-C) and triglycerides (TG) at diagnosis was compared to the results after 1 year of treatment, according to sex and age. A different rate of response was observed in females more than males in all lipid fractions.

Conclusions: These findings, although preliminary, highlight the importance of personalized therapy according to sex and age also in pediatric age.

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COMMON VARIABLE IMMUNODEFICIENCY AND CARDIOVASCULAR DISEASE: MULTICENTER EPIDEMIOLOGICAL ANALYSIS OF RISK FACTORS AND MORTALITY

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Background: Common Variable Immunodeficiency (CVID) is a rare immunological disease characterized by a deficiency in antibody production. Clinical manifestations are variable and range from increased susceptibility to infections to non-infectious manifestations secondary to immune dysregulation. In particular, the presence of non-infectious complications identifies the so-called "complicated" phenotype. The relationship between CVID and the risk of developing atherosclerosis and its impact on cardiovascular (CV) mortality is currently debated.

AIM of the Study: To evaluate the frequency of CV risk factors, the prevalence of atherosclerotic disease

(AsCVD) and its role as cause of death within an Italian cohort of CVID patients.

Materials and Methods: We conducted a retrospective-prospective multicentre observational study on Italian patients affected by CVID, enrolled between 1/1/2018 and 12/31/2023 in 5 Italian centres (Cagliari, Naples, Treviso, Rome and Turin). For each patient, anamnestic information, anthropometric data, biohumoral parameters and immunological characteristics were collected. Finally, we analysed the main causes of death of patients who died during the study period.

Results: We enrolled 411 CVID patients, predominantly female (n=229, 56%), with a mean age of 52 ± 15.8 years. Of these, 210 (51%) had a complicated disease phenotype and 42 were affected by AsCVD. We compared the prevalence of CV risk factors and immunological parameters of AsCVD patients (n=42) with the rest of the cohort (n=369). As expected, hypertension, dyslipidaemia, diabetes and advanced age were associated with the presence of AsCVD. Furthermore, the presence of bronchicctasis and replacement therapy with intravenous immunoglobulin were associated with the presence of AsCVD. There were 62 deaths with a mean age of 61 ± 15.5 years. The main cause of death (40%) was the presence of neoplasms, followed by infections (18%) and terminal lung disease (14.5%). 6 patients (10%) died from CV disease.

Conclusions: Our study analyses for the first time the prevalence of AScVD disease and the distribution of cardiovascular risk factors in a large cohort of CVID patients. We found no significant differences in immunological parameters between patients with and without history of AsCVD. Although the prevalence of common cardiovascular risk factors in our cohort is not different from that of the general population, CV disease is only the fourth cause of death. These preliminary data suggest that CVID patients do not have an increased risk of CV disease.

IMPACT OF WESTERN DIET AND PCSK9 ON THE EXPRESSION OF AMYOTROPHIC LATERAL SCLEROSIS-PREDISPOSING GENES

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Background and Aim: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive degeneration of motor neurons. Mutations in three genes, C9orf72, SOD1, TARDBP, account for the disease in about 70% of patients with familial ALS. Since cholesterol may play a role in the onset/progression of ALS, the impact of PCSK9, as well as that of different dietary lipid contents, on the expression of genes relevant in ALS and cholesterol metabolism was evaluated in brain and liver.

Methods: Six-week-old C57BL/6J WT and PCSK9-KO female mice were fed for 16 weeks either standard chow diet (CD), or Western-type diet (WD). After sacrifice, gene expression in liver and brain was evaluated via qPCR.

Results: When comparing dietary treatments, in the liver of both genotypes, WD significantly increased the expression of Sod1, Tardbp and C9orf72, as well as that of Dhcr24, Cyp27a1, Cyp46a1 and Soat1 *vs.* CD. In the brain, WD did not alter the expression of any of the genes considered. When comparing genotypes, the hepatic expression of Sod1, Tardbp and C9orf72 was comparable in PCSK9-KO and WT when fed the same diet. On WD, increased hepatic expression of Dhcr24, Dhcr7, Msm01, Hmgcr and Ldlr was observed in PCSK9-KO *vs.* WT. Conversely, in the brain, PCSK9-KO mice showed increased expression of C9orf72, Tardbp, Ldlr and Hmgcr, *vs.* WT when fed the same diet.

Conclusions: WD administration increases the expression of ALS-predisposing genes only in the liver but not in the brain in a genotype-independent manner. The lack of PCSK9 consistently results in increased expression of C9orf72 and Tardbp in the brain. Additional studies may shed light on the role of PCSK9 in the onset of ALS.

INTESTINAL METABOLITES OF HIGH AMYLOSE WHEAT PHENOLIC EXTRACT IMPROVE ENDOTHELIAL FUNCTION: ROLE OF FERULIC ACID AND ITS METABOLITES

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Atherosclerosis and inflammatory bowel diseases share common pathophysiological mechanisms in terms of their genetics, immunology, and contributing environmental factors, with endothelial cells playing a central role in both conditions (1). Dietary polyphenols, known for their antioxidant and anti-inflammatory properties, represent a promising approach to preserve endothelial function. Newly developed high-amylose durum wheat cv. Svevo (Svevo-HA), an innovative material characterized by a high content of resistant starch and phenolic acids, is attracting interest as a functional food ingredient (2). Given the extensive metabolic processing of wheat in the intestine, this study aimed to evaluate the effects of the intestinal metabolites of Svevo-HA phenolic extract (HAPE) on endothelial activation and to analyze underlying mechanisms of action. To this aim, we established a transwell intestinal epithelial-endothelial co-culture system with Caco-2 and HMEC-1 cells mimicking the gut-vascular barrier (3). In detail, Caco-2 were grown on semi-permeable membrane until fully differentiated into an enterocyte-like phenotype, and then shifted to plates containing HMEC-1 in the lower compartment. HAPE (1-10 µg/mL GAE), or its main component ferulic acid, were added to the upper compartment for 2h. Alternatively, the ferulic acid metabolites, ferulic acid 4-O-sulfate and dihydroferulic acid (5-10 µmol/L) were added to the lower compartment. Afterwards, TNF (10 ng/mL) was added to HMEC-1 for 16h to recreate the vascular inflammatory milieu. The results showed that HAPE intestinal metabolites dose-dependently suppressed TNF-stimulated leukocyte-endothelium interaction, reduced the expression of endothelial adhesion molecules ICAM-1 and VCAM-1 and decreased the release of inflammatory mediators IL-6 and MCP-1. These protective effects were attributed to ferulic acid and its derivatives and were linked to a significant reduction in intracellular ROS production and NF-KB activation. In conclusion, our findings highlight Svevo-HA's potential as a functional food ingredient, rich in ferulic acid, capable of reducing endothelial inflammation and supporting intestinal and vascular health.

References

- Cainzos-Achirica M., et al. JACC 2020, 76, 2895, https://doi.org/ 10.1016/j.jacc.2020.10.027.
- Romano G., et al. Foods 2022, 11, 4070, https://doi.org/10.3390/ foods11244070.
- Calabriso N., et al. Nutrients 2022, 14, 1175, https://doi.org/10.3390/ nu14061175.Funding: PRIMA Section 1, 2020 Agrofood Value Chain IA, MEDWHEALTH, grant n.2034.

RELATIONSHIP BETWEEN GUT PERMEABILITY AND PCSK9 IN HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Background and Aims: Gut dysbiosis is a major determinant of low-grade endotoxaemia via dysfunction of the intestinal barrier scaffold, which is a prerequisite for Lipopolysaccharide (LPS) translocation into the systemic circulation. Endotoxaemia is associated with atherosclerotic burden and its clinical sequelae. Moreover, current data show that LPS is cleared from the circulation via low-density lipoprotein receptors (LDLR) on hepatocytes, which are downregulated by proprotein convertase subtilisin/kexin type 9 (PCSK9), protein directly associated with circulating LDL cholesterol (LDL-C) level and in the onset of hypercholesterolemia.The aim was to analyze the relationship between PCSK9, gut permeability and endotoxemia after the treatment with PCSK9 inhibitors (PCSK9i).

Methods: We performed a before-after study including 40 patients with heterozygous familial hypercholesterolemia (HeFH) on treatment with maximum tolerated statin dose±ezetimibe before and after six months of PCSK9i therapy. We analysed plasma PCSK9 levels, intestinal permeability marker such as zonulin, endotoxemia marker such as LPS and oxidized-LDL (ox-LDL) that play a central role in atherosclerotic process, by enzyme-linked immunosorbent assay (ELISA). To study a potential mechanism of intestinal permeability, Caco2 cells, a well characterized intestinal *in vitro* model, were treated *in vitro* with PCSK9, and the expression of occludin, an integral membrane thigh junction protein, and zonulin levels were evaluated.

Results: cWe observed a significant decline in LDL-C, zonulin, LPS, and ox-LDL levels after six months of PCSK9i compared to baseline. Furthermore, linear regression analysis showed that LPS levels were associated with LDL-C and zonulin reduction, suggesting a relationship between gut permeability and PCSK9 levels. *In vitro*, PCSK9, at concentration of 50-150-300 ng/ml, significantly reduced the protein levels of occludin compared to unstimulated cells whereas increased levels of zonulin. The treatment with NOX2ds-tat and PCSK9 is ginificantly improve occludin expression and reduced zonulin levels in cell media.

Conclusions: This study provides evidence that PCSK9i could improve gut permeability by reducing circulating endotoxemia as well as oxLDL production by counteracting the atherosclerotic process in HeFH patients.

DIFFERENCES BETWEEN FRIEDEWALD AND MARTIN-HOPKINS EQUATIONS FOR LDL-C ESTIMATES AND CONSEQUENT EFFECTS ON RISK CATEGORIZATION IN A DIABETIC POPULATION

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Introduction: Concordance of LDL-C estimating equations with direct enzymatic assay is lower in diabetes. Previous studies have demonstrated that the Martin-Hopkins (MH) equation provides better concordance than the Friedewald (F) equation. In our prior analysis of a population of inpatients, we found that LDL-C target achievement declines progressively in higher-risk categories, with only 32.1% of diabetic patients reaching their LDL-C targets.

Methods: Retrospective real-world data were extracted from the Hospital Information System using automated data extraction strategies and stored in a patient-centered repository (the Dyslipidemia Data Mart). Patients were excluded if triglycerides were >400 mg/dl. LDL-C was calculated using both the MH and F equations, and Pearson correlation was performed. Differences in LDL-C (ALDL-C) between the two equations were calculated for the entire population, as well as the diabetic and non-diabetic subgroups. LDL-C target achievement was assessed based on the 2019 ESC/EAS guidelines.

Results: 13.834 patients were included (30,5% with diabetes). The Pearson correlation coefficient between MH and F equations was 0.99 (p<1x10-16). The mean LDL-C was higher when calculated using MH compared to F. Δ LDL-C was 2.66 mg/dl, 2.16 mg/dl and 3.78 mg/dl (p<0.001) for the entire population, non-diabetic subgroup and diabetic subgroup, respectively. Overall, on-target patients were 35.8% with MH compared to 88.9% with F. The percentage of on-target patients (according to F) switching to off-target (according to MH) was significantly higher in diabetics compared to non-diabetics (5.1% vs. 2.8%, p<0.001). Distance-to-target (DTT) was higher when LDL-C was calculated with MH than with F. The increase in DTT observed comparing MH vs. F was higher in the diabetic subgroup than in non-diabetics (3.8 mg/dl vs. 2.2 mg/dl, p<0.001).

Conclusions: Cardiovascular risk management is pivotal in diabetes and the methodology for LDL-C estimation could have a significant impact on LDL-C target achievement and therapeutic choices.

COMPARISON OF METHODS FOR THE QUANTIFICATION OF SMALL DENSE LDL-c

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Aim: Increased small dense low-density lipoprotein-cholesterol (sdLDL-c) is recognized as a risk factor for atherosclerotic cardiovascular disease (ASCVD). Traditional methods (ultracentrifugation) used to measure sdLDL-c are labor intensive and cannot be utilized for routine diagnostic testing. We aim to evaluate the agreement between an automated direct method for measurement of sdLDL-c (d-sdLDL-c), and the estimation by the Sampsons equation (E-sdLDL-c - Clin. Chem. 2021: 67, 987-997).

Patients and Methods: We analyzed data of 99 patients with clinical suspect of Familial Hypercholesterolemia. Levels of sdLDL-c were measured by automated direct homogeneous method (Denka Seiken) on Cobas c-503 (Roche Diagnostics). Traditional lipid parameters, used for evaluation of the estimation of sdLDL-c, (LDL-c, total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-c), and triglyceride (TG)) were measured by standard enzymatic methods.

Results: We observed a good correlation between E-sdLDL-c and d-sdLDL-c (Spearman coefficient r=0.812). The greater discordance was observed for very high values (>80 mg/dl), that were measured by the direct method and greatly underestimated by the Sampson equation. In fact, the maximum observed value for E-sdLDL-c was 81 mg/dl and for d-sdLDL-c was 100 mg/dl. We searched for possible explanations related with this underestimation, and we identified a possible role of high LDL-c and TC levels, whereas no impact of TG was observed.

Conclusion: There is a good agreement between the values obtained from the Sampson equation and ones directly measured. However, since there is an underestimation of high values of sdLDL-c by the formula estimation, the homogenous methods could be a more appropriate choice for measuring sdLDL-c. A correct identification of patients with very high sdLDL-c levels can improve the assessment of residual cardiovascular risk.

THE NATURAL AND GREEN EXTRACT OF RED GRAPE POMACE PROMOTES VASCULAR HEALTH BY REDUCING ENDOTHELIAL CELL SENESCENCE AND INHIBITING ENDOTHELIAL DYSFUNCTION

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The essential role of endothelium in maintaining vascular homeostasis can be undermined by cell senescence and aging, which can lead to endothelial dysfunction and age-related diseases. Due to the biological and clinical importance of health endothelium, it is necessary to identify and therapeutically target senescent endothelial cells (1). Grape pomace is an important source of bioactive polyphenols with potential beneficial properties for the endothelium, but the effects on endothelial senescence are not clear (2, 3).

The aim of this research is to enhance grape pomace, by applying green chemistry, to achieve high-value materials, with healthful properties for the prevention of vascular aging. Negroamaro grape pomace was extracted in distilled water at 90°C and dehydrated under spray dryer, before being chemically characterized by HPLC/DAD and NMR and finally used for its bioactivity in primary endothelial cell cultures. Human umbilical vein endothelial cells (HUVECs) were pretreated with grape pomace extract (GPE) and exposed to H_2O_2 , as an *in vitro* aging model, detected by SA-β-gal staining, or to TNF, to induce endothelial dysfunction, analyzed by the expression of inflammato-ry markers, at protein (EIA/ELISA/Western) and mRNA levels (qRT-PCR). MTT, DCFH-DA and Griess assay were used to evaluate cell viability, reactive oxygen species (ROS) and nitric oxide (NO) levels, respectively.

Our results showed that H_2O_2 exposure promoted endothelial senescence, which was significantly alleviated by pre-treatment with 5µg/ml GPE. Furthermore, GPE ameliorated TNF-stimulated endothelial dysfunction by increasing eNOS expression and NO production and reducing ROS levels, and COX-2 expression.

The GPE effects could be mainly explained by the presence of bioactive polyphenols including anthocyanins, catechins, epicatechins and resveratrol.

Overall, the results show that a natural extract of red grape pomace, as obtained by green chemistry, inhibits endothelial dysfunction and senescence and may contribute to the prevention of age-related diseases.

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References

- Katsuumi G., et al. Front Cardiovasc Med. 2018 Mar 5; 5: 18. doi: 10.3389/fcvm.2018.00018.
- Gerardi C., et al. Front Bioeng Biotechnol. 2020 Jun 26; 8: 645. doi: 10.3389/fbioe.2020.00645.
- 3) Calabriso N., et al. Nutrients. 2022; 14 (6):1175. doi: 10.3390/nu14061175.

IDENTIFICATION OF A NEW LIKELY PATHOGENIC VARIANT IN APOA5

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Introduction: Severe Hypertriglyceridemia is a genetic disease characterized by high levels of triglycerides caused by pathogenic variants in the LPL, APOA5, APOC2, LMF1, GPIHBP1 and CREB3L3 genes. Two forms of the disease were defined, one usually caused by 2 pathogenic variants (Familial Chylomicronemia Syndrome - FCS) and one usually caused by a single variant (Multifactorial Chylomicronemia Syndrome - MCS). We report the case of a patient that received the genetic confirmation of FCS after the analysis of her relatives.

Methods: We analyzed a patient with a maximum triglycerides value of 6500 mg/dL and clinical diagnosis of FCS. The genetic analysis was made by Next Generation Sequencing (NGS) using a panel including 43 genes related to lipid metabolism. The pathogenicity classification of variants was carried out according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

Results: We identified 2 variants at the heterozygous state in the APOA5 gene: c.427delC - p.(Arg143Alafs*57), classified as Pathogenic, and c. 49+5 G>A, classified as uncertain significance variant (USV). The identification of the first variant in the mother and the second one in the father allowed to establish that the patient is a compound heterozygote for variants in APOA5 gene. After 2 years also the patient's sister (TG max of 5000 mg/dL) was analyzed, and the genetic analysis revealed that she is a compound heterozygote like the proband. Based on the segregation analysis and on the identification of the variant in another FCS patient in the family, the c. 49+5 G>A variant was reclassified as Likely Pathogenic.

Conclusion: The analysis of this family comprising two FCS patients allowed to define a pathogenic role for the variant c. 49+5 G>A in the APOA5 gene. We reported one of the few cases of patients with biallelic variants in APOA5 gene present in the literature.

PERSONALIZED APPROACHES TO SEVERE HYPERTRIGLYCERIDEMIA IN PEG-ASPARAGINASE THERAPY: THE ROLE OF GENETIC TESTING

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Background: PEG-Asparaginase or Pegaspargase (PEG) is an asparagine-specific enzyme that selectively kills leukemic cells by depleting plasma asparagine and is approved for the firstline treatment of acute lymphoblastic leukemia (ALL). PEG is associated with several adverse effects, including thrombosis, hypersensitivity reaction, hepatotoxicity, and severe hypertriglyceridemia (HTG), complicated with acute pancreatitis. HTG-associated PEG-based mechanisms are poorly understood. Case Presentation: We present the case of a 31-year-old woman with T-cell acute lymphoblastic leukemia who developed severe hypertriglyceridemia (triglyceride levels >5000 mg/dL) following treatment with PEG-asparaginase. In the emergency department, initial management included fasting, fenofibrate, and omega-3 fatty acids, which effectively reduced triglyceride levels during her hospitalization. In addition, she was found to have a significant elevation in liver function tests (LFTs, >5 times the upper limit of normal). A review of her medical records revealed no prior history of plasma lipid disturbances or LFT elevations. It is noteworthy that she had a history of systemic lupus erythematosus (SLE), for which she was not receiving treatment due to remission. Additionally, a family history of cardiovascular disease and diabetes suggested a possible genetic predisposition to lipid metabolism disorders. After informed consent signing, she underwent blood drawn for genotyping within the LIPIGEN study. Genetic testing identified a rare heterozygous missense variant (rs268) in the LPL gene (NM_000237: exon 6: c.A953G: p.N318S), classified as a variant of uncertain significance but associated with familial combined hyperlipidaemia. The patient was also homozygous for the c.162-43G>A variant (rs2072560) in the APOA5 gene, which is known to increase triglyceride levels. Sequencing of the APOC3 gene is pending to further investigate potential genetic factors. After withdrawal of PEG-asparaginase, the patient's lipid profile normalised, allowing triglyceride-lowering therapies to be discontinued.

Conclusion: This case highlights the importance of personalised medicine approaches in oncology to identify and manage genetic susceptibilities that may exacerbate treatment-related side effects. In the new era of targeted therapy and cardio-oncology, further studies are needed to achieve a personalised treatment approach tailored to the patient.

References

- Heo Y.A., Syed Y.Y., Keam S.J. Pegaspargase: A Review in Acute Lymphoblastic Leukaemia. Drugs. 2019 May; 79 (7): 767-777. doi: 10.1007/ s40265-019-01120-1.
- Lau K.M., Saunders I.M., Goodman A. Pegaspargase-induced hypertriglyceridemia in a patient with acute lymphoblastic leukemia. J Oncol Pharm Pract. 2020 Jan; 26 (1): 193-199. doi: 10.1177/1078155219833438.
- Shmookler A., Smith S. Pegaspargase-induced hypertriglyceridemia. J Clin Apher. 2018 Feb; 33 (1): 97-98. doi: 10.1002/jca.21571.
- 4) Bender C., Maese L., Carter-Febres M., Verma A. Clinical Utility of Pegaspargase in Children, Adolescents and Young Adult Patients with Acute Lymphoblastic Leukemia: A Review. Blood Lymphat Cancer. 2021 Apr 19; 11: 25-40. doi: 10.2147/BLCTT.S245210.

- Vyas N., Ching Companioni R., Tiba M., Alkhawam H., Walfish A. Pegaspargase Induced Hypertriglyceridemia Resulting in Severe Fatal Pancreatitis. Case Rep Gastrointest Med. 2015; 2015: 753062. doi: 10.1155/2015/753062.
- 6) Iannuzzi A., Annunziata M., Fortunato G., Giacobbe C., et al. Case Report: Genetic Analysis of PEG-Asparaginase Induced Severe Hypertriglyceridemia in an Adult With Acute Lymphoblastic Leukaemia. Front. Genet. 2022; 13: 832890. doi: 10.3389/fgene.2022.832890.
- 7) D'Erasmo L., Di Costanzo A., Cassandra F., Minicocci I., Polito L., Montali A., Ceci F., Arca M. Spectrum of Mutations and Long-Term Clinical Outcomes in Genetic Chylomicronemia Syndromes. Arterioscler Thromb Vasc Biol. 2019 Dec; 39 (12): 2531-2541. doi: 10.1161/ ATVBAHA.119.313401.

USE OF LIPID-LOWERING DRUGS IN CLINICAL PRACTICE: A RETROSPECTIVE SURVEY AT A METABOLIC DISEASE OUTPATIENT CLINIC

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Dyslipidemias are a leading cause of cardiovascular disease, with pharmacological interventions playing a crucial role in preventing adverse events. Statins are the first-line treatment, but many patients experience side effects, particularly muscle-related symptoms or hepatic issues, leading to reduced compliance and long-term efficacy. I patients who are stati intolerant, at high cardiovascular risk, or unable to achieve target lipid levels, alternative therapies like bempedoic acid and PCSK9 inhibitors are viable options.

This study aimed to evaluate the use of lipid lowering drugs in a clinical setting, focusing on statin intolerance and subsequent treatments. Furthermore to assess the correlation between age, sex, and the likelihood of developing statin intolerance among patients with dyslipidemia.

We performed a two-month retrospective analysis of 286 consecutive dyslipidemia patients (51.4% women) with a mean age of 64.2 (\pm 13.8) years. Statins were prescribed to 65% of patients, while 30% discontinued use due to intolerance. Statin-intolerant patients were older, with a mean age of 65.5 (\pm 12.8) years, and had a higher prevalence among women (58.3%), though the difference was not statistically significant (p = n.s.). Second-line treatments were common, with 20% of all patients using bempedoic acid, although 5% experienced intolerance. A PCSK9 inhibitor was used primarily by 12% of statin-intolerant patients.

Statin intolerance was most frequent in patients under 60: 14 women and 9 men (mean age 48.2) and over 70: 19 women and 12 men (mean age 74.9).

The study highlights that statins remain the cornerstone of dyslipidemia treatment but emphasizes the significant challenge of intolerance, particularly in women and older patients. This underscores the need for personalized therapy, with valuable alternatives like

bempedoic acid and PCSK9 inhibitors. Further research is needed to optimize treatment strategies based on individual patient characteristics.
SARCOPENIA AND PROGNOSIS IN MIDDLE-AGED HEART FAILURE PATIENS: ROLE OF SGLT2 INHIBITORS

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Sarcopenia is common in patients with heart failure (HF), and it is frequently associated with other comorbidities. The prevalence of sarcopenia in HF patients varies significantly depending on the population considered: sarcopenia rates are notably higher among hospitalized patients compared to outpatients. Sarcopenia could potentially worsen the prognosis observed in patients with HF; in fact, sarcopenia has been linked to an increased risk of all-cause mortality and MACE in patients with HF. SGLT2 inhibitors (SGLT2i) are antidiabetic drugs indicated to improve glycaemic control and reduce MACE in patients with type 2 diabetes mellitus (T2DM). The aim of this study was to evaluate the impact of sarcopenia on prognosis and the potential role of SGLT2 inhibitors on the incidence of MACE in outpatients with HF. In this retrospective study, 670 outpatients with HF (369 males and 301 females, average age of 65.6±12.5 years) were enrolled and stratified based on the presence of sarcopenia. The median follow-up was 4.7±2.8 years. MACE were identified as study endpoints. MACE included non-fatal ischemic stroke, nonfatal coronary events, and cardiovascular (CV) death. Additionally total mortality during follow-up was also evaluated. The entire population was stratified by sarcopenia diagnosis (340 sarcopenic patients and 330 non-sarcopenic patients). Between the two groups, statistically significant differences were observed for male gender (63.5% in the sarcopenia group and 46.4% in the non-sarcopenia group, p=0.0001), body mass index (BMI) (p=0.0001), vitamin D levels (p=0.0001), albumin levels (p=0.0001). A total of 245 (7.8 events/ 100 patient-year) MACE were observed. In particular, 78 (4.9 events/100 patient-year) were non-fatal coronary events, 198 (6.3 events/100 patient-year) non-fatal stroke events, and 88 (2.8 events/100 patient-year) CV deaths. In the sarcopenia group, MACE observed were 213 (13.3 events/100 patient-year) while in the no sarcopenia group they were 32 (2.1 events/100 patient-year) (p<0.00001). Non-fatal coronary events were 78 (4.9 events/100 patient-year) in the sarcopenia group and 12 (0.8 events/100 patient-year) in the no sarcopenia group (p<0.00001). Furthermore, non-fatal stroke events were 181 (11.3 events/100 patient-year) in the sarcopenia group and 17 (1.1 events/100 patient-year) in the no sarcopenia group (p<0.00001). CV death events were 84 (5.3 events/100 patient-year) in the sarcopenia group and 4 (0.3 events/100 patient-year) in the no sarcopenia group (p<0.00001). Non-CV mortality was not significantly different between the sarcopenia group (18, 1.1 events/100 patient-year) and the no sarcopenia group (24, 1.5 events/100 patient-year) (p=0.291). Total mortality was higher in the sarcopenia group (102, 6.4%) compared to the no sarcopenia group (28, 1.8%) (p<0.00001). Cox regression analysis showed that SGLT2i reduced the risk of MACE by 89%. In contrast, Sarcopenia significantly increase the risk of MACE, with a hazard ratio of 5.82, indicating a nearly sixfold increase in risk. In conclusion, we provide evidence that sarcopenia significantly increases incidence of MACE, thus early detection and treatment is mandatory to improve outcome of these patients. For a deeper understanding, additional sources and detailed study results are necessary to confirm these findings and elucidate the underlying biological mechanisms. Furthermore, this study highlights the protective effect of SGLT2i in patients with HF. Assumption of SGLT2i reduced the composite endpoint of non-fatal stroke, non-fatal coronary event and cardiovascular death for HF by 89%; in addition, we also observed a reduction of total mortality.

ACHIEVING LDL-C GOALS IN STATIN-INTOLERANT PATIENTS: INSIGHTS FROM BEMPEDOIC ACID TREATMENT

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Background: Statins remain the gold standard for treating hypercholesterolemia. The introduction of ezetimibe and PCSK-9 inhibitors allowed the therapeutic goals, set for each risk class, to be achieved more easily. However, LDL-targets are not always met but therapeutic goals are not always met, especially in high-risk patients, such as those with familial hypercholesterolemia or statin intolerance. In these cases, bempedoic acid offers an additional therapeutic option. By inhibiting ATP-citrate lyase, bempedoic acid triggers the upregulation of low-density lipoprotein particles and LDL-C reduction.

Methods: We conducted a real-world study to assess the efficacy and safety of bempedoic acid in patients with statin intolerance that didn't reach the therapeutic target, yet. We evaluated the lipid profile, liver enzymes, at baseline and after 12 weeks and of treatment.

Results: We preliminarily enrolled 52 patients, with a mean age of 59 years: 69,2% were hypertensive, 42,3% had carotid atherosclerosis and hepatic steatosis, 13,5% were diabetic, only three patients reported cardiovascular events. After 12 weeks. we observed a significant reduction in LDL-C levels (from 134.63±42,083 mg/dl to 85.44±41,846 mg/dl, p<0.001). No adverse events, including significant elevations in liver and muscle enzymes; however, there was an increase in triglyceride levels (123,730±50,976 mg/dL to 142,08±72,116 mg/dL). The increasing of Creatinine and uric acid, effects commonly reported in the literature, are not statistically significant (p>0,05) in our sample Conclusions: These preliminary data show that bempedoic acid could be an additional therapeutic option for patients, who have failed other therapies, such as those who are intolerant to statins. The fact that there were no events in our patients, although the observation period is still too short, makes bempedoic acid a valid additional therapeutic option, also in terms of safety.

CLINICAL CHARACTERISTICS AND GENETIC PREDISPOSITION **OF DYSLIPIDEMIC PATIENTS** WITH STATIN INTOLERANCE

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Background: Statin therapy represents the gold standard in lipid lowering therapy, although it is associated with an increasing rate of therapeutic abandonment especially due to the onset of muscle symptoms (statin associated muscle symptoms SAMS).In literature, a higher incidence of SAMS in the female population has already been documented, probably attributable to differences in pharmacokinetics and pharmacodynamics between genders. A relevant element in this condition would seem to reside in the SLCO1B1 gene (1), responsible for the tissue transport of statins, whose mutation would determine an increased plasma concentration of the same with consequent development of SAMS.

Aim: The aim of our study was to evaluate the real-life prevalence of statin intolerance in patients referred at our Center and how this determines whether or not the 2019 ESC guideline's LDL target was reached. The influence of genetic factors (specifically the SLCO1B1 mutation) and of patients' general and clinical characteristics (gender, age, BMI) on the achievement of the target was also analyzed.

Methods: We selected a population of 185 patients attending our Center and enrolled in the LIPIGEN project (96 F; 89 M), of whom 131 FH+ (67 F; 64 M). In 97% of the total population it was possible to evaluate the SLCO1B1 gene, which was found to be mutated in 67 out of 179 patients (37 F; 30 M). The mean age of our patients was 35 years (18 to 74 years; 41 f, 30 m), the mean BMI was 24.2 (23.9 f; 24.5 m).

Discussion: In accordance with the literature, our data showed a greater statin intolerance in female (58% f vs. 42% m). In particular, Atorvastatin was the worst tolerated, with predominantly SAMS development even in the absence of CPK elevation (only 2 patients). If intolerance was referred by the patient, we preferred to shift to Rosuvastatin, generally characterized by better tolerability.Intolerance showed a continuous growth trend in relation to age in both sexes, more significant in female (5-fold increase from 35 to 75 years). The evaluation of the BMI was affected by the different numerical representation between classes, given the prevalence of normal weight and overweight population. From our preliminary data, the BMI would seem to be directly correlated with the development of statin intolerance: less significant the correlation with the achievement of the target since the poor representation of some groups could determine confounding results. Of the 179 patients analyzed for mutations of the SLCO1B1 gene, 37% presented its mutation. By stratifying the data based on sex, the influence of this mutation on the development of statin intolerance in female was confirmed, independently of the diagnosis of familial hypercholesterolemia, particularly in female FH+.

Conclusions: Statin intolerance still represents an obstacle to therapeutic compliance and the achievement of the LDL target. Particular importance seems to be related to age and the presence of SLCO1B1 mutations; the role of BMI/waist circumference is still uncertain.

Reference

Bosco G., Di Giacomo Barbagallo F., Di Marco M., et al. The impact of SLCO1B1 rs4149056 on LDL-C target achievement after lipid lowering therapy optimization in men and women with familial hypercholesterolemia. Front Endocrinol (Lausanne). 2024 Feb 8; 15: 1346152. doi: 10.3389/fendo 2024 1346152

NEUTROPHIL CATHEPSIN G AND PLATELET ACTIVATION IN TYPE 2 **DIABETES: ROLE OF GLIFOZINS**

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Background and Aims: Type 2 diabetes (T2D) is frequently complicated by cardiovascular disease (CVD). Platelet dysfunction plays a central role in the development of CVD in patients with T2D, as these patients have an increased thrombotic state associated with endothelial dysfunction and increased platelet reactivity. Neutrophils may contribute to platelet activation through the release of cathepsin G, a serine protease released upon neutrophil activation. There is also evidence that gliflozins reduce platelet activation, but their role in modulating neutrophil-derived cathepsin G release has never been studied in T2D. In this study, we investigated whether serum cathepsin G is elevated in patients with type 2 diabetes mellitus (T2DM) and the relationship between cathepsin G levels and platelet activation.

Methods: In 32 patients with T2D on ongoing metformin therapy before and after Gliflozin therapy and in 20 healthy subjects (HS), Cathepsin G levels and platelet activation markers including soluble platelet selectin (sP-selectin), serum thromboxane B2 (TxB2) and soluble CD40 ligand (sCD40L) were analysed. The association between serum cathepsin and platelet activation was also investigated. We also performed an in vitro study to compare the effect of gliflozin on the release of cathepsin G and then on the interaction between platelets and neutrophils.

Results: Compared to HS, T2D patients had significantly higher blood levels of cathepsin G (p<0.0001). Furthermore, reduced circulating levels of cathepsin G and reduced markers of platelet activation were observed after gliflozin treatment.In addition, the reduction in serum cathepsin G levels significantly correlated with TxB2 (rS=0.474, P=0.047), sP-selectin (rS=0.589, p<0.010) and sCD40L (rS=0.507, p<0.032) delta in T2D patients. Finally, an in vitro study on platelet-neutrophil mixture treated with gliflozin (10-30uM) resulted in higher cathepsin G levels in the medium than untreated platelet-neutrophil.

Conclusions: This study suggests that cathepsin G contributes to platelet activation in patients with T2D and that Gliflozins, in addition to their hypoglycemic effects, may have a beneficial effect on cathepsin G-mediated platelet-neutrophil interaction.

IMPACT OF DIETARY CHOLINE SUPPLEMENTATION ON PLASMA METABOLOME AND HEPATIC TRANSCRIPTOME IN APOLIPOPROTEIN E KNOCKOUT MICE

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Epidemiological studies proved a positive correlation between cardiovascular risk and TMAO plasma levels, a microbiota-derived metabolite of choline. With the aim of investigating if dietary choline could modulate cardiovascular risk affecting additional metabolic pathways, a multi-omics approach was performed. Ten-weeks-old EKO female mice were fed two chow diets, differing for a standard (0.09%, STD) or high (1.2%, HC) choline content. To test whether the choline-shaped microbiota might impact host metabolism, after 16 weeks of dietary treatment, the cecal content of STD and HC microbiota-donor mice was transplanted into antibiotic-treated, microbiota-recipient, EKO female mice (FMT-STD and FMT-HC), fed STD for 16 weeks. In all mice, atherosclerosis development, targeted plasma metabolome and hepatic transcriptome were evaluated. In microbiota-donor mice, HC diet worsened atherosclerosis development. Moreover, HC increased TMAO plasma levels together with those of methionine, sarcosine, glycine, carnitine, propionylcarnitine and butyrylcarnitine and lowered homocysteine. Consistently, liver transcriptome showed that the expression of 1247 genes was upregulated in HC mice. These genes enriched pathways mainly involved in the amino acid and one-carbon metabolism. In microbiota-recipient mice atherosclerosis development was comparable, but plasma metabolome of those that received the HC-shaped microbiota showed a lower concentration of several triglyceride species. Again, consistently with metabolomic findings, the analysis of liver transcriptome revealed that several among the 477 genes downregulated in FMT-HC mice were involved in pathways linked to metabolic processes, in particular lipid metabolism. In conclusion, dietary choline supplementation impacted on the hepatic transcriptional profile and influenced atherosclerosis development not only by increasing TMAO levels, but also by modifying several metabolites belonging to one-carbon metabolism. Finally, the gut microbiota shaped by the HC diet per se was able to influence liver metabolism by leading to reduced synthesis of several triglyceride species. These results suggest new choline-dependent mechanisms implicated in atherosclerosis development which deserve further investigation.

PRUNUS DOMESTICA EXTRACT TREATMENT IMPROVES MASDL-RELATED METABOLIC PARAMETERS IN A STEATOTIC-LIKE MODEL BASED ON THE HepG2 CELL LINE

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Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a widespread chronic liver condition linked to an increased risk of atherosclerotic cardiovascular disease (ASCVD). Current treatment strategies focus on lifestyle changes, such as weight loss and regular exercise, while pharmaceutical solutions are still under investigation. Nutraceuticals, particularly Prunus domestica extracts rich in hydroxycinnamic acids, flavanols, and glycosylated flavonols, show promise. These extracts have been shown to inhibit enzymes like α -amylase, α -glucosidase, pancreatic lipase, and HMG CoA reductase, and reduce inflammation, indicating potential benefits for MASLD treatment.

Aim: This study aims at developing and characterizing an *in vitro* model of hepatic steatosis and at evaluating the effects of Prunus domestica extract on cellular pathways involved in MASLD pathogenesis.

Methods: Human hepatoma HepG2 cells and a steatotic-like HepG2 cell model (HepG2-OA), differentiated with 100 μ M oleic acid for 7 days, were used. Both models were characterized by evaluating glucose uptake, lipid droplet accumulation, reactive oxygen species (ROS) production, triglyceride levels, gene expression and NMR metabolomics. Different concentration of Prunus domestica extract (0.01, 0.1, 0.5, and 1 mg/mL) at different time points (6, 24, and 48 hours) were tested in both cell lines.

Results: The characterization confirmed the steatotic-like phenotype in HepG2-OA cells. Prunus domestica extract did not show toxicity after 24/48 hours (MTT assay) and increased glucose uptake in a dose-dependent manner after 6 and 24 hours. The extract improved glucose absorption by 64.1% in HepG2 cells and 39.1% in HepG2-AO cells. Additionally, it reduced ROS and triglyceride levels and decreased the expression of genes related to lipid metabolism and oxidative stress.

Conclusion: These results demonstrate that the HepG2-OA cell model effectively mimics hepatic steatosis and that Prunus domestica extract positively influences key mechanisms involved in MASLD. This suggests its potential as a nutraceutical for managing MASLD and reducing the associated ASCVD risk.

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AN INNOVATIVE FUNCTIONAL PASTA WITH OAT β-GLUCAN, PHYTOSTEROLS, AND CHITOSAN REDUCE LDL-CHOLESTEROL IN INDIVIDUALS WITH HYPERCHOLESTEROLEMIA

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Background: There is a growing interest in reducing serum lipid levels thought lipid-lowering agents from natural source especially in intolerant individuals. Evidence suggests that β -glucan, phytosterols, and chitosan are safe and effective in reducing lipids, but no studies have specifically investigated their synergistic effect on cholesterol levels when carried by foods. Pasta is the main dish for Italian people and is gaining popularity worldwide. The aim of this study was to evaluate the lipid-lowering effect of an innovative functional pasta containing a combination of oat β -glucan, phytosterols, and chitosan in individuals with polygenic hypercholesterolemia.

Methods: Eighty patients with hypercholesterolemia were randomized into four groups: control (CTR), pasta with oat beta-glucans and chitosan (PBC), pasta with oat beta-glucans and phytosterols (PBF), and pasta with oat beta-glucans, chitosan, and phytosterols (PBCF). The intervention consisted of 100 g/day of pasta for 4 weeks. The control group receiving only Mediterranean Diet advice. Serum lipid levels were measured at baseline and at the follow-up visit.

Results: A total of 69 subjects completed the protocol. After 4 weeks of treatment, participants who consumed PBCF showed a significantly greater reduction in LDL cholesterol compared to those who consumed PBC, PBF, and CTR (-34 mg/dL, -22 mg/dL, -21 mg/dL, and -9 mg/dL, respectively; p<0.001). Furthermore, a higher percentage of patients in the PBCF group achieved an LDL cholesterol reduction of \geq 20 mg/dL compared to the other groups (56%, 37%, 28%, and 5%, respectively; p=0.004). **Conclusions:** These preliminary results demonstrate that the consumption of an innovative functional pasta containing a combination of oat β -glucan, phytosterols, and chitosan significantly reduces LDL-cholesterol. This innovative dietary intervention could represent a promising natural alternative to improve lipid profiles in individuals with hypercholesterolemia.

LCAT DEFICIENCY LEADS TO SEVERE MYOCARDIAL HYPERTROPHY AND MITRAL STENOSIS

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Introduction: LCAT (lecithin-cholesterol acyltransferase) deficiency is a rare genetic disorder characterized by a deficiency or malfunction of the enzyme involved in cholesterol esterification. The impaired or absent activity of this enzyme leads to the accumulation of free cholesterol and phospholipids in blood, due to undosable HDL and ApoA.

Case Report: A 54-year-old patient with chronic kidney disease, history of two kidney transplants, familiar deficiency of LCAT, corneal opacities, atrial fibrillation and hypertrophic cardiomyopathy was admitted to our hospital. The patient underwent a series of tests, particularly focusing on his renal and cardiovascular conditions, such as blood tests, including complete blood count, metabolic panel, lipid profile, proteinuria levels (>1 g/ die), immunologic tests that detected the presence of antibodies anti-DSA (DQ7, DQ8, and DQ9). Moreover, eve examination revealed corneal opacities. HDL was indosable and ApoA was strongly under laboratory reference value as normal. Echocardiography showed severe concentric hypertrophy of the left ventricle, suspected for infiltrative cardiomyopathy, post-capillary pulmonary hypertension. Right heart catheterization (RHC) confirmed the value. The patient underwent Cardiac Magnetic Resonance Imaging (MRI) that confirmed the diagnosis of hypertrophic cardiomyopathy with obstructive features. Extensive calcification was described including posterior mitral leaflet down to papillary muscle with severe transmitral gradient. Furthermore, dynamic severe subaortic stenosis was found. Myocardial biopsy showed focal sarcoplasmic vacuolizations and mild increase in myocellular size but no sign of infiltrative material. Renal biopsy described glomerular sclerosis.

Conclusion: Lcat patients may have a complex medical history with multi-organ involvement. Cardiovascular and Kidney complications are challenging management and they need a multi-disciplinary care.

FUNCTIONAL CHARACTERIZATION OF VARIANTS OF UNCERTAIN SIGNIFICANCE (VUS) IN PATIENTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA (HeFH) USING A FLOW CYTOMETRY ASSAY

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Background: Familial hypercholesterolemia (FH) is an autosomal lipid disorder increasing cardiovascular risk. It is primarily caused by pathogenic variants in the LDLR gene. However, some variants are classified as variants of uncertain significance (VUS), which presents a challenge in diagnosing FH. Functional tests are recommended to confirm variant pathogenicity.

Aim: To assess the functional impact of VUS in LDLR identified in FH patients using a flow-cytometry technique.

Methods: PBMCs were isolated from 33 FH patients and stimulated with CD3/CD28 beads in a lipoprotein-deficient serum (LPDS). The entire LDLR cycle (cell surface expression, LDL binding, and uptake) was assessed using flow-cytometry. Results were expressed as the ratio of the mean fluorescence intensity (gMFI) of activated CD4 T-lymphocytes to gMFI of wild-type controls. Measurements were repeated three times and presented as median (25-75th percentiles). ACMG guidelines were followed to classify variant function as normal or abnormal based on activity thresholds of >95% and <85% of wild-type levels.

Results: Out of 19 unique VUS tested, 14 (73.7%) showed deleterious effects on at least one of the LDLR functions. The c.1530_1532del, c.*34C>T and c.1007A>G variants disrupted the entire LDLR cycle. The remaining variants exhibited defects in LDLR expression on the cell surface localization (n=5-35.7%) or a combination of binding/expression or binding/uptake (n=6-42.8%). Thus, 28 of 33 FH-VUS patients (85%) showed at least one LDLR cycling defect. Interestingly, patients with damaging VUS had slightly higher untreated LDL-C levels than those with non-damaging variants (220 \pm 72.8 vs. 180.1 \pm 89.0 mg/dl; P=0.06). Furthermore, damaging VUS were more prevalent among patients with a Dutch Lipid Clinic Network (DLCN) score ≥ 6 (81.5%), suggesting that DLCN may serve as valuable predictor of biological effects.

Conclusions: This study illustrates the significance of functional testing for LDLR variants to clarify biological effects and include VUS in FH diagnosis.

STATIN TREATMENT REDUCES LONG-TERM RISK OF REVISION SURGERY FOLLOWING TOTAL HIP ARTHROPLASTY IN OSTEOARTHRITIS PATIENTS: A REGISTRY-BASED STUDY

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Background: Statins, commonly prescribed for lowering cholesterol, have been linked to various beneficial effects beyond lipid control. Research conducted both *in vitro* and *in vivo* has indicated a potential connection between statins and bone metabolism. Observational studies in humans also suggest a reduced fracture risk in individuals using statins. Total hip arthroplasty (THA) revision is a significant and costly medical procedure, but it remains unclear whether statins influence the risk of THA failure. This study aims to investigate whether preoperative statin use affects the likelihood of THA revision in patients with hip osteoarthritis.

Methods: We conducted a retrospective study of patients who underwent THA for osteoarthritis (OA) from the RIPO registry. Data on comorbidities and statin prescriptions were gathered from electronic health records. Propensity score matching was used to pair statin users with non-users in a 1:1 ratio, adjusting for factors like age, sex, and follow-up duration. THA survival was compared between the two groups, and secondary analyses examined the influence of mortality, sex, statin therapy indications, and statin potency or lipophilicity.

Results: A total of 10927 patients were classified as statin users (SU) and matched with statin non-users (SNU) using propensity score matching. Statin users exhibited a 24% reduced risk of THA revision over a 15-year period (adjusted HR 0.76, 95% CI: 0.67–0.88; p<0.001). This reduction in risk was consistent regardless of the indication for statin therapy or the specific type of statin used, with the effect being more significant among male patients (adjusted HR 0.64, 95% CI: 0.52–0.80, p<0.001).

Conclusions: This study suggests that statin therapy is associated with a reduced risk of long-term THA revision in osteoarthritis patients, regardless of the original indication for statin use.

INCLISIRAN AND PCSK9 INBITORS: ONGOING REAL WORL EVIDENCE STUDY ON THE THERAPEUTIC EFFICACY OF THESE TWO TOOLS

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Backgound: PCSK9-inhibiting monoclonal antibodies optimized the management of hypolipidemic therapy, reducing LDL-C and cardiovascular events. Inclisiran silences PCSK9 mRNA and is administered less frequently.

Methods: 52 patients were enrolled for the real-world evidence study, which consists of 1 enrollment visit (V0), mid-term (V1) and long-term (V2) visits, and assigned to 2 groups. Group 1 involves patients who switched to Inclisiran (284 mg every 6 months) after at least 6 months of PCSK9i (Alirocumab or Evolocumab). 26 patients on PCSK9i treatment were assigned to Group 2 (13 on Alirocumab 150 mg and 13 on Evolocumab 140 mg every 2 weeks). Risk factors, lipid profile (total cholesterol, C-TOT, and LDL), and clinical history (V0) were assessed for each patient. At follow-up, blood C-TOT and LDL levels were reassessed.

Results: Group 1 consisted of 12 females and 14 males, with average age of 56.1 ± 14.8 years; 13 in secondary prevention. Mean C-TOT value was 179.6 ± 67.5 mg/dl, while the mean LDL value was 105.7 ± 62.2 mg/dl. At V1, which only 22 patients underwent, C-TOT values were 175.4 ± 53.1 mg/dl and LDL values were 97.6 ± 47.9 mg/dl (Δ =-8.9%). Group 2 consisted of 15 females and 11 males, with average age of 61.5 ± 12.4 years; 12 in secondary prevention. At V0, mean C-TOT value was 232.9 ± 67.0 mg/dl, while mean LDL value was 151.8 ± 59.2 mg/dl. At V1, C-TOT values were 137.19 ± 48.96 mg/dl and LDL values were 63.61 ± 44.93 mg/dl (Δ =-41.9%) with a reduction of LDL values in both cohorts. The two cohorts LDL values at V1 show 30.73 ± 52.25 mg/dl difference between the two groups (p=0.12).

Conclusions: The comparison of the two groups shows that in the medium term, PCSK9 mab inhibitors can bring a quicker LDL reduction. Although the study is still ongoing, inclisiran is an effective tool in reducing LDL, especially for lower therapeutic adherence patients, requiring less frequent administrations.

INCLISIRAN: AN EFFECTIVE APPROACH IN THE TREATMENT OF HYPERCHOLESTEROLEMIA

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Background: Lipid-lowering therapy is a crucial aspect in reducing cardiovascular risk. The most recent guidelines require us to achieve increasingly ambitious therapeutic targets, depending on the patient's cardiovascular risk.

Objective: Our study aims to evaluate the effects of lipid-lowering therapy with Inclisiran 284 mg in patients who had not reached the therapeutic target with oral lipid-lowering agents treatment (such as statins and ezetimibe).

Methods: The evaluation was based on a short-term (3 months), medium (6 months) and long-term (12 months) follow-up system, during which data were collected on total cholesterol (C-TOT), triglycerides (TGL), HDL and LDL. For each patient, cardiovascular risk was calculated according to the SCORE charts.

Sample composition: The sample consisted of 26 patients, 12 women (46.2%) and 14 men (53.8%), aged 56.1 \pm 14.8 years. 17 patients were initially treated with statins: 5 with low-intensity statins (19.2%) and 12 with high-intensity statins (46.2%). Among the patients, 19 individuals had familial hypercholesterolemia (73.1%) with a DUTCH-score of 14.6 \pm 3.9. Finally, 11 patients were found to be smokers (42.3%) and only 1 patient had type 2 diabetes mellitus (3.8%).

Results: Data on the first visit and short-term follow-up for the first 22 patients enlisted are reported. At the initial visit, the C-TOT values were 105.7+62.2 mg/dl, while LDL values were 94.9+39.3 mg/dl. At short follow-up, 22 patients were reduced by 6.1+12.5* mg/dl C-TOT and 9.6+11.5*mg/dl (*standard error).

Conclusions: Although the data collected refer only to the short follow-up, the introduction of inclisiran represented a valid tool in the study population, contributing significantly to the reduction of C-TOT and LDL values.

PHARMACOTOXICOLOGICAL EVALUATION OF 'AGLIANICO DEL VULTURE' RED WINE POLYPHENOLIC EXTRACT AS A PREVENTIVE DIETARY SUPPLEMENT FOR PROTECTION AGAINST THE ONSET OF ATHEROSCLEROSIS

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Background and Aims: Evidence suggests that polyphenolcontaining nutraceuticals can prevent obesity and cardiometabolic disease (CMD) by modulating signaling pathways in endothelial and inflammatory cells of the vascular system. Recent studies have shown that the polyphenolic extract of "Aglianico del Vulture" red wine (RWP) exhibits significant in vitro effects, including modulation of polarization, inhibition of lipid peroxidation, regulation of histone acetylation, and reduction of proinflammatory ROS, NO-, and PGE2 production in human macrophages, indicating its potential protective role against atherosclerosis. This study aims to evaluate the pharmaco-toxicological activities and immunometabolic effects of RWP on spontaneously hypertensive rats (SHR), an established animal model of metabolic syndrome, as well as on endothelial cells (ECs) from patients with CMD (obesity, dyslipidemia, and insulin resistance).

Methods: SHR 9 weeks old (n=20) were randomized into four groups and treated with either vehicle or RWP (200, 400, or 800 mg/kg/day) via gavage for 3 weeks. Body weight and food intake were recorded daily, and systolic blood pressure (SBP) was measured every 3 days using a tail cuff. At the end of the treatment period, plasma glucose levels and pro-inflammatory cytokine concentrations were assessed. Vasodilation responses to acetylcholine (ACh, 10 nM to 3 μ M/30 seconds) and RWP (5 to 20 µg/mL/30 seconds) were evaluated in mesenteric arteries (MVA) isolated and pre-constricted with noradrenaline (5 μ M), both before and after preincubation with N-nitro-L-arginine methyl ester (L-NAME, 100 µM/20 minutes).ECs isolated from 12 patients with cardiometabolic disease (CMD) were treated with varying doses of RWP (0, 100, 200, 400, 800, 1600, and 3200 µg/ mL) for different time points (0, 24, 48, or 72 hours) to initially assess toxicological effects (apoptosis via flow cytometry) and functional effects (migration using a wound healing assay).

Results: In *in vivo* studies with SHR, RWP treatment for 3 weeks significantly reduced SBP across all doses (p<0.001 RWP-SHR vs. vehicle treated-SHR), without evidence of organ toxicity, and without significant changes in body weight or fasting blood glucose levels. Additionally, levels of pro-inflammatory cytokines (CCL5, CXCL7) and adhesion molecules (TIMP1) showed a decreasing trend. Ex vivo, RWP treatment (400 to 800 mg/kg/day) significantly enhanced ACh-mediated vasorelaxation in MVA

compared to vehicle treatment (p<0.05). Direct administration of RWP to MVA from 12-week-old vehicle-treated SHR produced a dose-dependent and reversible vasorelaxation effect, which was abolished by preincubation with L-NAME (p<0.001 *vs.* respective control), indicating that RWP-mediated vasodilation is dependent on nitric oxide (NO). Preliminary results on human ECs indicate that RWP treatment induced apoptosis only at the highest concentrations (800 to 3200 µg/mL for 72 hours), did not affect ECs migration at concentrations up to 400 µg/mL, and was able to modulate pro-inflammatory cytokine levels.

Conclusions: Data obtained so far indicate that RWP may offer significant protection against cardiometabolic risk. Underway research involving co-cultures of ECs and macrophages from CMD patients will elucidate the cellular mechanisms through which RWP may confer protection against the onset of atherosclerosis. This research was supported by "European Research Council (ERC) STARTING GRANT" (codice identificativo progetto 2023-UNBACLE-0245596 to Vanessa Desantis).

IMPACT OF TREATMENT WITH EVOLOCUMAB ON BIOMARKERS OF CHRONIC SYSTEMIC AND VASCULAR INFLAMMATION

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The aim of this study was to evaluate the impact of treatment with Proprotein Convertase Subtilisin/Kexin type 9 inhibitor (PCSK9i) Evolocumab on biomarkers of chronic systemic and vascular inflammation, in a real-life clinical setting. For this purpose, we pooled data from 202 outpatients (Men: 111; Women: 91) clinically evaluated at baseline and every six months after starting Evolocumab. Individuals who had been on PCSK9i for less than 36 months and nonadherent patients were excluded from the analysis. Over time, absolute lymphocyte concentrations, monocyte-to-HDL-C ratio (MHR) and platelet-to-monocyte ratio (PMR) did not change in the entire sample and between-sex. According to the nonparametric analysis of longitudinal data in factorial experiments, the main effects and the interaction of the sex was found to be statistically significant in the MHR and in the PMR (P<0.001 always). Our data partially reinforce the presence of differences in response to treatment to PCSK9i between men and women. Further research will clarify whether these sex-related significant differences translate into a meaningful difference in the long-term risk of atherosclerotic cardiovascular disease.

TRIGLYCERIDES TO HIGH DENSITY LIPOPROTEIN CHOLESTEROL RATIO (TG/HDL), BUT NOT TRIGLYCERIDES AND GLUCOSE PRODUCT (TyG) INDEX, IS ASSOCIATED WITH ARTERIAL STIFFNESS IN PREDIABETES

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Background: Individuals with prediabetes (preD) are at increased risk of cardiovascular (CV) events in comparison to persons with normal glucose tolerance (NGT). Insulin resistance is a hallmark of prediabetes, linked to CV risk. Thus, CV risk assessment is critical in preD. Recently, triglycerides to high-density lipoprotein cholesterol ratio (TG/HDL) and TG and fasting glucose (FG) product (TyG) have emerged as surrogate markers of insulin resistance. Our aim was to evaluate the association of these indexes with CV risk assessed through arterial stiffness in prediabetic individuals.

Methods: 377 individuals without prior diagnosis of diabetes underwent:

- 1) metabolic evaluation via oral glucose tolerance test, FG, HbA1c, cholesterol, and TG dosage;
- 2) CV risk assessment through tonometry measured arterial stiffness (i.e. pulsed wave velocity – PWV – and augmentation index – AugI) and intima-media thickness (IMT). Participants were split into three groups according to current guidelines: NGT (n=100), preD (n=216), and newly-diagnosed type 2 diabetes (ND-T2D) (n=61).

Results: TG/HDL and TyG were higher in preD than NGT (respectively, 2.9±2.0 vs. 2.3±1.7, P=0.008 and 4.6±0.2 vs. 4.5±0.2, P=0.001), with no difference between preD and ND-T2D regarding TG/HDL. In addition, PWV was higher in preD than NGT (7.8±1.6 vs. 7.3±1.8 m/s, P=0.03), as well as AugI (29.9±11.7 vs. 25.2±12.9, P=0.001) and IMT (0.75±0.12 vs. 0.68±0.11 mm, P<0.0001). Moreover, individuals with preD showed no difference in terms of AugI in comparison with ND-T2D. In preD, after adjusting for age, sex, and body mass index, PWV was correlated with TG/HDL and homeostasis model assessment insulin resistance (HOMA-IR), but not with TyG. Further adjusting for smoking status, systolic blood pressure, low-density lipoprotein cholesterol, C-reactive protein, FG, and HbA1c, PWV was still correlated with TG/HDL (β =0.16; P=0.02) and there was a trend toward association with HOMA-IR (β=0.17, P=0.08). Furthermore, in logistic regression analysis, being in the higher tertile of TG/HDL was associated to an increased risk of having a higher PWV (odds ratio 2.54, 95% CI 1.02-6.29, P=0.04).

Conclusions: In individuals with preD, TG/HDL was independently associated with PWV, suggesting it could be a simple, cost-effective tool for assessing CV risk in this population.

REAL WORLD EVALUATION OF LDL-C IN ELDERLY INPATIENTS

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Introduction: Hospitalized patients are often frail, and frailty is associated with all-cause and cardiovascular (CV) mortality. However, elderly patients have even a greater CV risk since age is an important CV risk factor and is linked to a higher co-morbidity burden and frailty. We have previously analyzed an entire adult population of inpatients and found relevant gaps in LDL-C control. Otherwise, polypharmacy is common in elderly patients, raising the risk of adverse drug effects, while late initiation of lipid-lowering therapies has limited impact on prolonging CV disease-free life expectancy.

Methods: Disease-related real-world data were collected retrospectively from Hospital Information System using automated data extraction strategies and through the implementation of a patient-centered data repository (the Dyslipidemia Data Mart). Patients were divided in two groups: non elderly (<80 years old) and elderly (>80 years old). CV risk profiles, LDL-C target achievement were assessed based on the 2019 ESC/EAS guidelines.

Results: The elderly subgroup included 2,945 patients (21.3% of the entire population): 0.3%, 20.3% and 79.4% were moderate (M), high (H) and very high-risk (VH) patients, respectively. Although mean LDL-C resulted significantly low (79.9 \pm 32.0 vs. 89.4 \pm 35.0 mg/dL), the percentage of patients not achieving LDL-C targets was significantly higher in the elderly group compared to the non-elderly group (74.3 % vs. 58.1%, p<0.001). Specifically, percentage of on-target elderly patients was progressively lower towards the worst CV categories (87.5% in moderate risk, 43.1% in high risk and 21.2% in very high risk).

Conclusions: Gaps in LDL-C target achievement were even more pronounced among elderly inpatients. These data underscore the need for improved CV risk stratification and personalized therapy. Optimizing LDL-C control in this high-risk population could improve clinical outcomes and reduce the burden of risk on healthcare systems.

SPECTRUM OF HOFH IN CAMPANIA: A SINGLE CENTER EXPERIENCE

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Introduction: Homozygous Familial Hypercholesterolemia (HoFH) is a rare genetic disease characterized by very high levels of LDL-cholesterol (LDL-c) and increased cardiovascular risk. It is caused by two pathogenic variants in the LDLR, APOB and PCSK9 genes. We are here reporting the experience on HoFH patients based on the data from a single centre for genetic diagnosis of dyslipidemias.

Methods: We analysed 815 patients with clinical suspicion of Familial Hypercholesterolemia (FH). Genetic screening was made firstly by traditional sequencing of causative genes, analyzed sequentially, thereafter analysing all FH causative genes (including large rearrangements in the LDLR gene) by next generation sequencing (NGS - Devyser's FH v.2 kit). Pathogenicity evaluations of identified variants was made according to the ACMG guidelines with the most recent suggestions to adapt them to the genetic characteristic of FH.

Results: We identified 32 unrelated patients with 2 pathogenic variants in FH-causative genes: 5 true homozygotes (15.5%), 22 compound heterozygotes (69%), and 5 double heterozygotes (15.5%). All homozygotes and compound heterozygotes carried LDLR pathogenic variants, whereas double heterozygotes carried a pathogenic variant in LDLR and a pathogenic variant in APOB (4 patients) or a pathogenic variant in PCSK9 (1 patient). All double heterozygotes were identified after the introduction of NGS. Considering only the 25 patients with 2 variants in LDLR originating from the Campania region, the HoFH prevalence was calculated as 1:240,000. Among all the 3 genetic statuses, many of the identified HoFH patients didn't reach the cut-off of 400 mg/dL used for the clinical diagnosis.

Conclusions: A high prevalence of HoFH was identified in our region. A wide genetic screening allows the identification of HoFH patients, being particularly relevant in those that do not show a very severe phenotype that otherwise would be underdiagnosed.

MILD HYPERCHOLESTEROLEMIA IN OTHERWISE HEALTHY POST-MENOPAUSAL WOMEN DISPLAYS AN EPIGENETIC SIGNATURE

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Women in the post-menopausal state are often characterized by a progressively worsening lipid profile. DNA methylation in several genes has been associated with regulatory mechanisms of cholesterol metabolic pathways; in detail, when located in a gene promoter, DNA methylation can repress gene transcription, while its reduction promotes gene expression. After exclusion of secondary causes of dyslipidaemia, 457 healthy women (no previous CVD, no diabetes, age 58±4.39, BMI 25.42±4.24) attending the outpatient "Donna-Cuore" clinic for cardiovascular prevention of post-menopausal women in the years 2017-2023 formed the study cohort. They were divided in 135 NC (LDL cholesterol <130 mg/dl). 220 HC (LDL>130 mg/dl and no lipid lowering therapy [LLT]), and 102 HC-LLT (LTT: statin or nutraceuticals). Participants underwent clinical evaluation, complete routine biochemical analyses heart and carotid ultrasonography. DNA methylation of genes involved in the regulation of cholesterol metabolism (SIRT6, HMGCR, CYP27A1) and storage (SOAT1, ABCG1) was measured by Methylation-sensitive high-resolution melting (MS-HRM), a post-PCR analytical methodology. Student's t-test, ANOVA or Kruskal Wallis/Mann Whitney test, according to variables distribution, were applied to compare differences between groups. Percent of methylation of SIRT6, HMGCR and SOAT1 was significantly reduced in HC and HC-LLT vs. NC (p<0.0001, p<0.02 and p<0.02, respectively), with no difference between statins and nutraceuticals. Previous hormone replacement therapy was associated only with increased SOAT1 methylation, while no relation emerged between current FSH and LH levels and methylation level of such genes. When participants were stratified according to absence or presence of IMT ≥0.9 and/or plaque, HMGCR methylation was significantly reduced and SOAT1 methylation was increased in women with carotid atherosclerosis. In the whole study group intima-media thickness (IMT) was positively related with SOAT1 (p=0.03), SIRT6 (p=0.008) and CYP27A1 (p=0.03) and negatively with HMGCR methylation (p<0.0001). Collectively, these data suggest the presence of an epigenetic regulation potentially overexpressing HMGCR, the rate-limiting enzyme in the de novo synthesis of cholesterol; this is coupled with increased expression of genes involved in the negative regulation of cholesterol synthesis (SIRT6) or elimination (SOAT1), as a potential defensive mechanism. A link with carotid atherosclerosis was also observed. Epigenomic studies are an opportunity to expand the knowledge of the molecular basis of polygenic hypercholesterolemia, and to identify novel biomarkers potentially useful for prognosis and follow-up of post-menopausal women carrying this cardiovascular risk factor.

ROLE OF MITOCHONDRIAL DYNAMISM IN KUPFFER CELLS ON SYSTEMIC METABOLISM

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Background: Kupffer cells (KCs) are hepatic resident macrophages essential for liver physiology that contribute to the development of metabolic associated fatty liver disease (MAFLD). The liver of patients with MAFLD shows different expression of some key regulators of the inner mitochondrial membrane fusion compared with healthy subjects, including OPA1 protein, which is a mitochondrial dynamin whose activity promotes mitochondrial fusion and modulation of oxidative phosphorylation. **Aim:** Given the close interaction that KCs have with the other cells in the hepatic niches, they play both a crucial immune and metabolic role, which is why their mitochondria are critical for their functions. This project aims to investigate how the modulation of OPA1-driven mitochondrial fusion in KCs can affect lipid metabolism and immune response at the systemic and hepatic levels.

Methods: Mice selectively lacking OPA1 in KCs were fed a Standard Diet or a High-Fat Diet for 20 weeks. The immune phenotype was assessed by cytofluorimetry while the metabolic profile was evaluated by *in vivo* indirect calorimetry and with plasma and tissue lipid profile analysis. Single cell RNA sequencing was also performed to profile the impact of OPA1 deficiency on KCs function and possible paracrine effects on hepatocytes.

Results: Under Standard dietary conditions, mice selectively lacking OPA1 in KCs exhibit a metabolic substrate preference toward carbohydrates, with an immunophenotype characterized by a higher proportion of pro-resolving KC2s than pro-inflammatory KC1s. Functionally, KCs also exhibit different phagocytic and proliferative capacity. During the High-Fat Diet, we observed a significant reduction in liver fibrosis.

Conclusions: Taken together, these data suggest that OPA1 plays a key role in the function of Kupffer cells and that the lack of OPA1, by causing metabolic reprogramming, affects their interaction with resident liver cells, thus influencing the development of fibrosis and the progression of MAFLD.

HIGH INTERINDIVIDUAL VARIABILITY IN LDL-CHOLESTEROL REDUCTIONS AFTER INCLISIRAN ADMINISTRATION IN A REAL-WORLD SETTING

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Introduction: High levels of low-density lipoprotein cholesterol (LDL-c) are a risk factor for atherosclerotic cardiovascular disease. Statins remain the cornerstone of lipid lowering treatment, but many patients fail to reach their LDL-C goal. Inclisiran is a small ribonucleic acid molecule that interferes with the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9), approved by the Food and Drug Administration (FDA) in 2021, which reduces PCSK9-mediated LDL receptor degradation and promotes the elimination of LDL-c from the bloodstream.

Material and Methods: Twenty-six patients ≥18 years old received inclisiran in the lipid clinic at the University Hospital of Palermo, Italy, for elevated LDL-C levels. We describe baseline characteristics, LDL-C changes (%) and side effects in these patients 3 months (n=26) and 9 months (n=10) after inclisiran. We assayed total cholesterol (CT), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c) levels by automated analyzer using colorimetric-enzymatic methodology, while LDL-c values were calculated by Friedewald's formula.

Results and Conclusion: We included twenty-six patients (50% women, median age 59.5). 34% had a genetic diagnosis of familial hypercholesterolemia (FH), 31% had a history of coronary artery disease. The median LDL-c levels was 127 mg/dL at baseline. We observed a wide interindividual variability in LDL-C change from baseline. At 3 months, first follow-up evaluation, the twenty-six patients showed a median LDL-C reduction of -34% (-4,4; -86,2). Ten patients who received the third dose of inclisira and 9 months showed a median LDL-C reduction of -32% (-22; -60,8). Inclisiran was well-tolerated, and side effects were not reported in the period of observation. Our results showed an high interindividual variability in LDL-cholesterol reductions.

PREDICTORS OF LDL-C TARGET ACHIEVEMENT IN PATIENTS WITH DYSLIPIDEMIA TREATED WITH PCSK9-I THERAPY: A RETROSPECTIVE ANALYSIS

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Introduction: Atherosclerotic cardiovascular disease (ASCVD) remains a leading cause of death globally, with dyslipidemia being a major risk factor. Proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9-I: alirocumab and evolocumab), have enhanced the management of hypercholesterolemia. However, some patients fail to reach LDL-C targets. This study aims to assess the impact of overweight and obesity, and other contributing factors on the effectiveness of PCSK9-I therapy.

Methods: A retrospective cohort study was conducted on patients treated with PCSK9-I at the Lipid Clinic of Policlinic San Martino Hospital, Genoa, between January 2018-September 2023. Demographic, clinical, and anthropometric data were collected. Lipid profile was measured at baseline and after 6, 12, 24. and 36 months. The primary outcome was LDL-C target achievement according to ESC/EAS guidelines.

Results: Among the 180 patients (94 females, 86 males, median age 67 years), approximately 45% achieved the recommended LDL-C target. Patients with overweight and obesity showed significantly lower LDL-C reductions (47.8%, 50.0%, and 44.0% for overweight; 25.6%, 29.4%, and 28.6% for obesity) compared to normal-weight patients at 12 (54.5%, p=0.021), 24 (62.5%, p=0.024), and 36 months (65.4%, p=0.024). Multivariate analysis confirmed these findings (OR 0.18, 95%CI 0.06-0.53, p=0.002) during the 36-months follow-up and highlighted that heterozygous familial hypercholesterolemia (He-FH) was associated with lower likelihood of achieving LDL-C targets (OR 0.35, 95%CI 0.15–0.82, p=0.015). Conversely, patients with coronary artery disease were more likely to reach LDL-C targets (OR 4.54, 95%CI 1.98-10.41, p<0.0001) during the 36-month follow-up. Additionally, the use of concomitant oral lipid-lowering therapy was linked to a higher probability of achieving LDL-C goals compared to PCSK9-I monotherapy (OR 14.5, 95%CI 2.26-92.9, p=0.005) over the 36-month follow-up.

Conclusion: It is essential to consider factors such as overweight condition, diagnosis of He-FH and use of concomitant oral lipid-lowering therapy to optimize the achievement of LDL-C targets.

EFFECT OF APOLIPOPROTEIN **A-I ON THE LIVER TRANSCRIPTOME OF APOLIPOPROTEIN E KNOCKOUT MICE**

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Fatty liver disease is a condition characterized by lipid accumulation in the hepatocytes and it is generally accompanied by a dyslipidemic condition characterized by low HDL levels. Being the interrelation between fatty liver disease and low HDL not clearly understood, in the present study, the impact of different apoA-I/ HDL levels on the transcriptome of diet-induced fatty liver was investigated. Eight-week-old mice lacking apoA-I/HDL (DKO) and mice with physiological levels of apoA-I/HDL (DKO/hA-I) were fed either a standard rodent diet (SRD) or a Western diet (WD) for 22 weeks. Steatosis was evaluated, and the hepatic transcriptome was analyzed by RNAseq. The lack of apoA-I/ HDL in DKO mice fed SRD was not associated to hepatic histological alterations. The transcriptome of DKO and DKO/hA-I mice fed SRD diverged in a relatively small number of genes, suggestive of a greater activation of the PPAR signaling pathway and the retinol metabolism pathway in DKO/hA-I mice. Following WD, both genotypes comparably showed marked lipid accumulation in the liver. Transcriptomic analysis highlighted an upregulated expression of immune/inflammatory genes and a reduced activation of the retinoid metabolism in both DKO and DKO/hA-I mice. The evaluation of the hepatic response of the two genotypes to the dietary switch from SRD to WD revealed strong divergences in genes involved in metabolic pathways only in the presence of apoA-I/HDL, with reduced endogenous sterol biosynthesis, together with increased glucose metabolism. In conclusion, although not histologically overt, apoA-I/ HDL seems to exert a substantial impact on hepatic metabolism during steatosis.

LIPID-LOWERING TREATMENT STRATEGIES IN PATIENTS HYPERCHOLESTEROLEMIC: A REAL-LIFE EXPERIENCE WITH BEMPEDOIC ACID

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Background: Bempedoic acid, an ATP citrate lyase inhibitor, reduces low-density lipoprotein cholesterol (LDL-C) levels and is associated with a low incidence of muscle-related adverse events; its effects on cardiovascular outcomes remain uncertain. **Objectives and Methods:** To assess the efficacy and safety profile of bempedoic acid in a real-life setting. We performed a retrospective analysis in 35 subjects who have been received bempedoic acid in monotherapy or in combination with other hypolipidemic drugs. Lipid profile was evaluated at baseline, at 3 months and 6 months follow-up. Medical history, background LLT and side effects were also evaluated.

Results: 35 subjects (25 females and 10 males, age 65±12,4 years) were treated with bempedoic acid. 37.1% were heterozygous familial hypercholesterolemia (HeFH), 62.9% were non-FH. 20% had suffered at least one episode of MACE. At baseline, 60% of subjects were receiving high-intensity statin (rosuvastatin 20 mg or atorvastatin 40 mg) plus ezetimibe, 40% reported a history of statin intolerance and no subjects were ezetimibe intolerant. 5 patients were also on PCSK9 inhibitors. Mean LDL-C levels at baseline were 108.5±35.22 mg/dl. Overall, LDL-C percent reduction was 22.93% (range +50.85%/-80.76%) at 12 weeks and 33.59% (range +35%/-69%) at 26 weeks. LDL-C levels decrease, was more pronounced in statin intolerant (-31%) than in statin users -14.2% vs. LDL-C goals in high (< 70 mg/dl) and very-high risk patients (< 55 mg/dl) was reached in 32.1 % and 14.3 % respectively at 6 months. No side effects were reported.

Conclusions: Bempedoic acid reduces LDL-C levels in patients at high or very-high CV risk with high interindividual differences in a real-world experience. Bempedoic acid was safe and background LLT influences the effectiveness of this new therapeutic option.

MANAGEMENT OF A PATIENT WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA (HeFH) AND CANCER: CASE REPORT AND CHALLENGE IN LIPID-LOWERING TREATMENT IN REAL LIFE

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Background: Familial Hypercholesterolemia (FH) is a co-dominant inherited disorder of lipid metabolism characterized by elevated plasma levels of LDL-C and early atherosclerotic cardiovascular disease (ASCVD). The aim of treatment of FH is to reduce the cumulative burden of elevated LDL-C levels and prevent or delay the development of ASCVD. Intercurrent conditions may complicate the management of this condition.

Methods: We describe the clinical management of a patient with severe hypercholesterolemia while receiving chemotherapy for endometroid carcinoma.

Results: The proband is a 55-year-old woman who has been diagnosed with FH (heterozygous carrier of the pathogenic mutation c.1285G-A V408M (V429M), known as Afrikaner-2. Treatment with high-efficacy statin at the maximum tolerated dose (atorvastatin 40 mg/dl) in association to ezetimibe did not allow to reach the suggested LDL-C goal (LDL-C <70 mg/dl). Therefore, treatment with PCSK9-i (Alirocumab 75 mg biweekly) was started and the goal was achieved. In 2022, the patient was diagnosed with a metastatic synchronous endometrioid carcinoma of the ovary and endometrium. Hence, she underwent surgery and adjuvant maintenance chemotherapy with bevacizumab. Although potential interaction between bevacizumab an alirocumab are not anticipated, the oncologist suggested to halt alirocumab. This prompt us to switch the treatment to inclisiran was started in combination with the background LLT. However, the association between inclisiran and background LLT was not as effective as alirocumab in lowering LDL-C levels.

Conclusions: This case points out possible difficulties that clinicians may encounter in the treatment of FH patients with cancer or other acute or chronic intercurrent conditions and the need of collegial management with other medical consultants for the management of complex cases.

DO GENETICALLY DETERMINED VERY HIGH AND VERY LOW LDL LEVELS CONTRIBUTE TO Lp(a) PLASMA CONCENTRATION?

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Background and Aims: Lipoprotein (a) [Lp(a)] is a well-recognized risk factor for atherosclerotic cardiovascular disease (AS-CVD). Few data are available on the distribution of Lp(a) levels among subjects at different cardiovascular risk and in subjects with monogenic and polygenic dyslipidemias (familial hypercholesterolemia, FH and familial hypobetalipoproteinemia type 1, FHBL1). The aim of this study was to investigate the distribution of Lp(a) plasma levels in subjects with high and low LDL-C levels (FH and FHBL1) and in the general population.

Methods and Results: The study cohorts included 356 hypercholesterolemic patients, 212 carrying a FH causative mutation, 144 with clinical FH (mutation negative - FHneg), 52 FHBL1 and 797 free-living subjects. Lp(a) levels were significantly higher in FH subjects (both FH and FHneg) (median 12.46 mg/dl and 14.0 mg/dl, respectively) compared with FHBL1 and free-living subjects (7.68 mg/dl and 7.18 mg/dl, respectively). More, Lp(a) levels were similar in FH subjects carrying LDLR defective and null mutations and FHneg. Subjects at high and very high CV risk exhibited significant higher Lp(a) levels (median 10.68 mg/ dl and 9.20 mg/dl, respectively) compared with low and moderate CV risk (median 5.72 mg/dl and 7.80 mg/dl, respectively) (p<0.0008).

Conclusions: FH subjects exhibit higher Lp(a) levels than FHBL1 and general population. Lp(a) slightly contribute to hypercholesterolemia in FH patients. Subjects at high and very high CV risk exhibited significant higher Lp(a) levels compared with low and moderate CV risk. Combined evaluation of Lp(a) levels in FH subjects with other traditional risk factors could identify very high-risk individuals who may benefit from early aggressive treatments to avoid premature CV events.

C-PEPTIDE REPLACEMENT THERAPY IMPROVES CARDIAC FUNCTION IN DIABETIC MODELS

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Background: Diabetic cardiomyopathy consists of left ventricular hypertrophy with systolic dysfunction or diastolic dysfunction, which may present as heart failure with preserved ejection fraction, fatty acid accumulations in cardiomyocytes, oxidative stress, generation of lipid intermediates and accumulation of lipid droplets. Unfortunately, insulin therapy does not prevent cardiac dysfunction while improving glucose levels. Proinsulin C-peptide, important for insulin biosynthesis and long considered biologically inert, has beneficial effects on several tissues. However, its role in regulating cardiac lipid metabolism and reducing oxidative stress is not well understood. The aim of this study is to explore the protective effects of C-peptide replacement therapy on lipid metabolism, oxidative stress and cardiac contractility in diabetic models.

Methods: Twenty-three male Wistar rats were randomized into three groups: normal control (CTR), diabetic group (D-CTR) and diabetic group plus C-peptide (C-PEP). Diabetes was induced with a streptozotocin injection, and C-peptide was administered subcutaneously for six weeks. We evaluated proteins involved in lipotoxicity and mitochondrial stress, such as Sirt1, PGC-1 α , ATGL, and PLIN2, as well as proteins implicated in cardiac remodeling, such as Troponin I and Vimentin, in cardiac muscle. Additionally, we assessed contraction marker and hypertrophy cardiac in a model of diabetes induced by high glucose using the H9C2 cell.

Results: We found a lower Sirt1, PGC-1a, Atgl and Plin2 expression and an higher pErk1/2 in C-PEP rats compared to the D-CTR group cardiac muscle. In addition, the C-PEP group showed improved contractile and vascular function by increasing protein levels of Troponin I (p<0.05) and Vimentin (p<0.05) compared with D-CTR group. Furthermore, we demonstrated that C-peptide reduces hypertrophy induced by high glucose levels (p<0.01) and increases protein expression of α -Actinin (p<0.001) in *in vitro* cardiomyopathy model.

Conclusion: Our results, for first time, suggest C-peptide therapy can improve cardiac function and reverse cardiac remodeling in diabetic rats.

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THE ROLE OF CX3CR1-T280M IN CHRONIC KIDNEY DISEASE: A LONG-TERM PROSPECTIVE STUDY

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Chronic kidney disease (CKD) is a leading cause of mortality worldwide, primarily due to population aging and an increased incidence of hypertension and type 2 diabetes mellitus. Lowgrade systemic inflammation and genetic factors play a key role in CKD pathogenesis. CX3CR1, the sole known receptor for the chemokine fractalkine, has garnered scientific interest for its involvement in various inflammatory processes across multiple tissues. Specifically, the single nucleotide polymorphisms (SNPs) T280M and V249I in the CX3CR1 gene have been shown to play roles in the progression of AIDS, cardiovascular disease, and glomerular and tubulointerstitial kidney diseases.

This prospective observational study included 121 patients with CKD of vascular or diabetic etiology, divided into two groups based on the CX3CR1-T280M genotype: group 1, wild-type T/T

(71.9% of the sample) and group 2, heterozygous T/M and homozygous M/M (28.1%). The primary outcome was initiation of renal replacement therapy (RRT) over an 18-year observation period. Figure 1 shows that the M allele is associated with higher baseline serum creatinine levels; these results are consistent with findings from previously published cross-sectional studies. The two groups considered showed no statistically significant differences in other baseline parameters. Survival analysis reveals a significant correlation between the CX3CR1-T280M polymorphism and CKD progression (Figure 2); group 2 patients exhibit a higher rate of RRT initiation, with a hazard ratio of 43.8% (p=0.039).

In conclusion, despite the limited sample size, this study confirms the role of the CX3CR1-T280M polymorphism in determining prognosis in CKD patients, particularly in predicting the risk of a critical outcome such as initiation of RRT.

Serum creatinine (a) and eGFR calculated with CKD-EPI 2021 formula (b) are compared between patients carrying the 280 T/T wild-type, heterozygous T/M and homozygous M/M. Significance have been taken for p values <0.05, using independent samples T-test; the difference results statistically significant for both variables only between wild-type and heterozygous, probably due to sample size (p=0.033 and p=0.029, respectively)

The figure shows that subjects carrying the M allele (Group 2) present a greater incidence of KRT that wild type subjects (Group 1). A total of 26 events is reported: 15 in T/T patients, 11 in T/M or M/M patients. The analysis was performed with Cox univariate (hazard ratio 43.8%, CI 0.200-0.958, Wald statistic 4.271, p 0.039).



Figure 1 - In-depth analysis of baseline renal function based on T280M genotype.



Figure 2 Renal survival of patients carrying -280 T/T and the M allele.

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LONG-TERM FOLLOW-UP OF CAROTID ATHEROSCLEROTIC DAMAGE IN NON-DIABETIC PATIENTS WITH METABOLIC SYNDROME

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Background and Aim: Metabolic syndrome (MetS), defined by excess visceral adiposity, high blood pressure, and altered glucose-lipid metabolism, is a highly prevalent condition in the general population and a risk factor for developing atherosclerotic cardiovascular disease (ASCVD). The study aims to evaluate atherosclerotic vascular damage in the carotid district by ultrasound in patients with a previous diagnosis of MetS that we recalled and re-evaluated after at least 15 years.

Methods: We performed a long-term follow-up study on 32 non-diabetic outpatients with a previous diagnosis of MetS and who received the indication to cardiovascular risk primary prevention treatments evaluated at the Clinical Medicine and Geriatrics Unit, IRCCS-INRCA Ancona, "Politecnica delle Marche" University. Participants were re-evaluated in a later outpatient visit after at least 15 years and underwent carotid bilateral ultrasonography. Based on the available demographic, anthropometric, metabolic, and therapeutic parameters, insulin resistance (IR) was calculated (triglyceride-glucose index or TyGi as a surrogate biomarker), and cardiovascular risk was assessed using the SCORE2-SCORE2OP score. A comparison within the study population was performed based on the presence of atheroscle-rotic plaque at the ultrasound assessment.

Results: The median follow-up was 18 years. 71% (n=23) of participants had carotid lesions compatible with atherosclerotic plaque. Patients with carotid plaque had a mean age of 72±8 years; 36% were male, with a mean body mass index (BMI) of 27±3 Kg/m², mean waist circumference of 101±12 cm, mean waist-to-height ratio of 0.60±0.06 cm, with a frequency of overweight and/or visceral obesity of 73%, mean systolic blood pressure (SBP) of 129±11 mmHg, mean diastolic blood pressure (DBP) of 81±7 mmHg, mean serum glucose of 103±14 mg/dL, mean total cholesterol (TC) of 163±23 mg/dL, mean HDL cholesterol (HDL-C) of 52±13 mg/dL, median triglycerides (TG) of 73 mg/dL (IQR 68-89), mean LDL cholesterol (LDL-C) of 90±17 mg/dL, and mean non-HDL cholesterol (non-HDL-C) of 113±22 mg/dL. The median TyGi was 8.6 (IQR 8.2-8.7). Seventy per cent of patients were on anti-hypertensive therapy, and 70% were on lipid-lowering therapy. Seventy per cent had office BP controlled. Almost all participants had a high or very high cardiovascular risk (29% and 67%, respectively), according to SCORE2/ SCORE2-OP. According to the individual cardiovascular risk, LDL-C and non-HDL-C therapeutic goals were achieved in 22% and 12%, respectively. There were no clinically significant differences between groups except for the prevalence of patients with LDL-C at a therapeutic goal based on cardiovascular risk, which was significantly lower in patients with carotid plaque than those without plaque (8% vs. 22%, p=0.013).

Conclusions: In non-diabetic patients with MetS at high and very high cardiovascular risk, failing to achieve the LDL-C therapeutic goal is associated with the presence of carotid atherosclerotic plaque in long-term follow-up. These findings strengthen the necessity to lower LDL and non-HDL cholesterol to protective goals in MetS patients.

A REAL-LIFE STUDY ON THE EFFICACY OF BEMPEDOIC ACID IN A POPULATION OF OUTPATIENTS AT HIGH AND VERY-HIGH CARDIOVASCULAR RISK

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Background and Aim: Bempedoic acid (BA) reduces serum low-density lipoprotein cholesterol (LDL-C) by selectively interfering with the endogenous production of cholesterol in the hepatocytes. Guidelines recommend BA alone or on top of the background lipid-lowering therapy (LLT) with the maximum tolerated statin +/- ezetimibe whenever LDL-C is not at goal according to individual cardiovascular risk and in patients who have no access to PCSK9-inhibitors. The study aimed to evaluate the impact of LLT with statin and BA +/- ezetimibe in a "real life" setting of outpatients, comparing the trend in their metabolic serum lipid profile.

Methods: An observational longitudinal study on 108 adult outpatients referred to the Clinical Medicine and Geriatrics Unit, IRCCS-INRCA Ancona, "Politecnica delle Marche" University for diagnosing and managing cardiovascular risk. All patients enrolled in the study had the clinical indication to add BA or BA plus ezetimibe to the maximum tolerated statin to reach the LDL-C goals based on the individual cardiovascular risk estimated using the SCORE2-SCORE2OP. The complete fasting lipid profile was assessed at the first visit (t0) and reassessed at the follow-up visit, scheduled after about three months, including total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), non-HDL-C, triglycerides (TG), lipoprotein (a) (Lp(a)), and ApoB.

Results: Mean age 67±13 years, 51% women, mean body mass index (BMI) 26.8±4.4 Kg/m2, 71% hypertensives, 59% smokers (previous and actual), 20% diabetics, 21% with a history of ischemic heart disease (IHD). All patients fell into the high or very high cardiovascular risk category according to SCORE2-SCORE2OP (20% and 80%, respectively). Ninety per cent of patients were already on statin at the enrolment. Forty-seven patients reached the follow-up visit at a median of 109 (IQR 90-152) days. At follow-up, 28 patients were on BA plus statin, and 19 were on BA plus ezetimibe and statin. In both groups, an improvement in the serum lipid profile was observed regarding atherogenic indices, with a mean LDL-C reduction of -20.2% and -25.1% from baseline, respectively. However, according to individual cardiovascular risk, only 38.3% of patients reached the LDL-C goal. In the remaining 61.7%, the LDL-C mean deviation from the goal was 21±18.6 mg/dL. Besides a slight and statistical/clinical non-significant increment of mean creatinine, AST, ALT, urea and uric acid, no side effects were reported during the follow-up.

Conclusions: In this "real-life" setting, despite the bias derived from potential suboptimal adherence to the prescribed therapies, our data confirmed the efficacy in reducing LDL-C of BA +/- ezetimibe on top of the maximum tolerated dose of statin, which was similar in terms of % reduction to what previous RCTs reported. However, after about three months of therapy, the LDL-C goal was not reached in most cases, eventually suggesting the need for a therapeutic indication broadening regarding PCK9-inhibition.

INDIVIDUAL CARDIOVASCULAR RISK ASSESSMENT BASED ON THE SCORE2 AND 2021 ESC GUIDELINES USING THE VALIDATED WEB-BASED APP WWW.HUMTELEMED.IT

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Background and Aim: In 2022, we introduced a user-friendly, free-to-use web application, www.humtelemed.it, based on the SCORE2/SCORE2-OP model and 2021 ESC Guidelines risk charts. This was meticulously designed to simplify cardiovascular risk (CVR) assessment, particularly for non-expert users or busy physicians, ensuring a comfortable and effortless experience in three languages. The application has recently been validated with a peer-reviewed published study, and we are reporting the first primary analyses of the dataset.

Methods: We performed a descriptive analysis based on the www.humtelemed.it data set. The web application reached over 17 thousand accesses. After checking for double inputs, complete data of 7317 40-year-old or older individuals were available. Results: The mean age was 59.4±11.8 years, with a male prevalence (55.3%). Ninety-eight per cent were from Italy, epidemiologically considered a moderate risk region. The mean body mass index (BMI) was 26.4±8.5 Kg/m², with 18% of individuals with obesity. The mean estimated glomerular filtration rate (eGFR, based on the CKD-EPI equation) was 81.6±20.9 mL/min, with 11.8% of likely chronic kidney disease (CKD) (eGRF<60 mL/min) and with 1.7% of stage 4 CKD (eGFR<30 mL/min). Ninety-six per cent of patients were in primary CV prevention. Actual smokers and past smokers were 20% and 17.3%, respectively. Nine per cent were diabetics, 16% reported peripheral artery disease (PAD, intending plaque detection and not as an obstructive disease), and 46.4% were hypertensive based on the use of blood pressure (BP) lowering treatments. About one-third (29.5%) of the population was on lipid-lowering treatment (LLT). The mean systolic BP (SBP) and diastolic BP (DBP), based on reported data and office measurements, were 127.8±14.1 mmHg and 78.2±9.5 mmHg, respectively. The mean total cholesterol (TC) was 199.3±42.7 mg/dL, and the mean high-density lipoprotein cholesterol (HDL-C) was 57.1±15.6 mg/dL. The median value of triglycerides was 100 mg/dL (IQR 75-138 mg/dL). The mean low-density lipoprotein cholesterol (LDL-C), calculated using the Friedewald formula as modified by Martin/Hopkins, was 118.7±51.7 mg/dL. The mean non-HDL-C was 142.1±41.6 mg/dL. The median SCORE2 (or SCO-RE2OP if age >70 years) was 6% (IQR 3-10%). The application classified 21.9% (n=1603), 38% (n=2779), 39.3% (n=2876), and 0.8% (n=59) patients into low-moderate, high, very high and extreme CV risk, respectively. Considering all BP reports as office measures, 75% (n=5537) were at goal. In the overall sample, a risk-based LDL-C at goal was found only in 11.2% (n=822). Similarly, a risk-based LDL-C at goal was found only in 15.9% (n=342) in the group on LLT (n=2156).

Conclusions: Our web application, www.humtelemed.it, was first designed to assist occasional users and busy clinicians in assessing individual CVR. On a larger scale, it may also be considered a reliable epidemiological population-based data source.

Analysing the trends of the demographic, anthropometric, metabolic and therapeutical patterns in the general population must be part of the fundamental strategy to identify and adequately manage the gaps in CVR assessment and prevention. Our data showed that most of the users had high and very high CVR and evidenced the unacceptable and discouraging reality of scarce CVR factor control, particularly LDL-C.

LOMITAPIDE AND EVINACUMAB COMBINATION IN A PATIENT WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLAEMIA

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Introduction: Treatment of homozygous familial hypercholesterolaemia (HoFH) involves high-intensity statins and ezetimibe as first-line therapy, to which PCSK9-directed therapy can subsequently be added. In many patients, however, this is still insufficient to reach low-density lipoprotein cholesterol (LDL-C) targets, making lipoprotein apheresis (LA) necessary. The introduction of new LDL-C receptor-independent therapies, such as Lomitapide and Evinacumab, and their combination may allow for the achievement of target LDL-C levels, potentially eliminating the need for LA.

Case Report: We present the case of a 56-year-old HoFH female patient. Medical history included previous statin-induced rhabdomyolysis and mild non-obstructive coronary artery disease. Due to statin and ezetimibe intolerance, her baseline LDL-C level was 434 mg/dL. PCSK9 inhibitor (Evolocumab 140 mg every 2 weeks) was then started, without a significant reduction in LDL-C levels, making the initiation of LA necessary. In 2022, treatment with Lomitapide was initiated at a dose of 5 mg and increased until 30 mg, enabling the discontinuation of LA. However, LDL-C target (<55 mg/dl) for this patient was not achieved, with mean concentration of 98 mg/dL, prompting the decision to add Evinacumab (15 mg/kg every 4 weeks). A baseline lipid profile was taken before starting Evinacumab and repeated before each subsequent infusion. LDL-C level decreased from 74 mg/ dL to 33 mg/dL (-55.4%) after the first infusion, further reducing to 28 mg/dL (-62.2%) after the second one. No gastrointestinal symptoms or elevated transaminase levels were reported; however, a slight increase in liver fat was observed during follow-up. No adverse effects were noted during any of the infusion sessions.

Conclusion: This report suggests that combination of Evinacumab and Lomitapide may have a significant effect on lipid levels in HoFH patients, with results comparable to those reported in clinical trials and a good tolerability, representing an effective and less burdensome alternative to LA.

PREGNANCY THREE MONTHS AFTER INCLISIRAN INJECTION: A UNIQUE CASE REPORT INCLUDING NEWBORN BABY MONITORING

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Background: Proprotein convertase subtilisin/kexin type 9 (PCSK9)-inhibition is pivotal in treating high low-density lipoprotein-cholesterol (LDL-C). The LDL metabolism is critical in placentation and early embryogenesis, and PCSK9 plays a crucial role in its regulation. Both monoclonal antibodies (mAbs) directed against PCSK9 and inclisiran, a small interfering RNA (siRNA) specifically designed to hamper hepatic PCSK9 production, have been shown to reduce LDL-C levels with a reassuring safety profile. PCSK9 inhibitors can cross the placenta. Despite lacking clinical data, manufacturers advise against PCSK9 inhibition during pregnancy.

Methods: We present a case report of a pregnancy in a young woman with basal LDL-C of 364 mg/dL secondary to heterozygous familial hypercholesterolemia (HeFH), genetically confirmed by the presence of loss-of-function variant affecting an allele of the gene coding for the LDL receptor [c.1374_375del AG (exon 10) p.Arg458Serfs*8 Null allele]. Clinical course: The patient developed a painful and relapsing injection site reaction to both available mAbs against PCSK9 (alirocumab and evolocumab) with a consequent treatment shift to the siRNA inclisiran. The patient had an unplanned pregnancy 13 weeks after the second administration of inclisiran. As soon as the pregnancy was diagnosed, the patient was advised to discontinue lipid-lowering treatment. To date, this is the first documented case of pregnancy initiated after an inclisiran injection. Consequently, the patient's gestation was closely monitored throughout its duration, and after delivery, the newborn's health was assessed in the first three months of life. The pregnancy proceeded without complications except for mild gestational hypothyroidism. Prenatal ultrasound was performed at the first, second and third trimester of pregnancy, always with normal findings. After a normal and full-term gestation, birth occurred in the 41st week. Delivery was vaginal, eutocic, and without complications. The Apgar score was 9 at 1 minute, 10 at 5 and 10 minutes. The newborn was healthy, with standard anthropometry for gestational age. Growth and development in the first months followed a regular course. At the last measurement we took at 3 months, length was 60.5 cm (50-75th percentile, with mother of short stature). weight 6.3 kg (75-90th percentile), body mass index (BMI) 17.21 kg/m^2 , and head circumference 40.7 cm (50-75th percentile).

Conclusions: Due to ethical concerns, clinical trials evaluating the safety of PCSK9 inhibition during pregnancy are not practicable. To the best of our knowledge, this is the first case of pregnancy initiated a few months after inclisiran administration. Inclisiran is no longer detectable in the circulation 48 hours after administration. Given its highly selective hepatic uptake, we can reasonably assume there was no circulating drug at conception and during gestation. More data on the pharmacodynamics and safety of siRNAs in this specific setting are needed.

TOWARDS A SUSTAINABLE NUTRACEUTICAL TREATMENT: CARDIOMETABOLIC EFFECTS OF AGRO-FOOD WASTE EXTRACTS ON SW872 ADIPOCYTE MODEL

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Introduction: Metabolic Syndrome (MetS) is a worldwide health concern associated with higher risk to develop cardiovascular and metabolic diseases. Inflammatory and oxidative signatures and other alterations of adipose tissue, including the perivascular one, are involved in pathological mechanisms leading to the development of MetS. While lifestyle changes and conventional therapies are considered standard approaches to counteract MetS patients, nutraceuticals represent a promising strategy for prevention, and management as add-on therapy. Recently, a Prunus domestica extract showed to exert promising anti-inflammatory and antioxidative effects, which could be exploited in the management of MetS.

Aim: This study aims at characterizing the SW872 cell model and exploring how the sustainable extract of Prunus domestica could revert the inflammatory and oxidative status of the adipose tissue.

Methods: A human-derived *in vitro* model of adipocytes, SW872, a dysfunctional model of SW872 induced with 100 mM oleic acid (OA), SW872-OA, and a spontaneously differentiated model of SW872 (SW872-AUTO), obtained after seventeen days in standard culture conditions, were selected. Each *in vitro* model was characterized by evaluating gene expression, lipid accumulation, oxidative stress, glucose internalization and NMR metabolomics. Then, cells were treated with Prunus domestica extract at different exposure time (6, 24 and 48 hours) and concentrations (0.01, 0.1, 0.5, 1 mg/mL).

Results: The characterization confirmed the dysfunctional metabolic phenotype in SW872-OA and SW872-AUTO. Prunus domestica extract showed no toxicity across all cell models. It stimulated glucose uptake, reduced reactive oxygen species and lipid accumulation, and positively modulated gene expression in a dose-dependent manner.

Conclusion: This study may contribute to evaluate the applicability of SW872-OA and SW872-AUTO in the study of MetS. Moreover, our findings suggest that Prunus domestica may positively modulate adipose tissue functions, which in turn affect the cardiometabolic health. Overall, these data value the effects of sustainable extracts aligned with the One Health approach.

CARDIOVASCULAR RISK IN SUBJECTS WITH METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE (MASLD) AND VARIABLE ADHERENCE TO MEDITERRANEAN DIET. THE INTERNIST'S PERSPECTIVE

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Background: The novel definition of MASLD underscores the close association of steatotic liver disease (SLD) with cardiovascular disease (CVD). We investigated the association between each subtype of SLD and CVD risk, including the adherence to Mediterranean diet (MD) and amount of abdominal fat.

Methods: A cohort of 405 consecutive patients (age 58.5±SEM0.9 yrs; BMI 28.9±0.3 Kg/m²; M/F=224/181) were enrolled at the outpatient clinic for cardiometabolic diseases. A validated questionnaire on MD adherence (score: low 0-6; intermediate 7-12; high 13-18) was used. SLD, subcutaneous (SAT), and visceral fat (VAT) were evaluated by ultrasonography. SLD patients were subclassified into No-MASLD (cryptogenic SLD, liver steatosis without metabolic disorders), MASLD, and MetALD (MASLD with increased alcohol intake). CVD risk was determined by the ASCVD (Atherosclerotic Cardiovascular Disease) Risk Calculator, determining 10-year risk of heart disease or stroke.Result: Among subjects, 34.1% had type 2 diabetes, 58.0% arterial hypertension, 43.8% metabolic syndrome, and 40.5% obesity. MD adherence score was intermediate in 83.1% of subjects. No-SLD were 140 subjects (34.6%), while SLD was detected in 265 (65.4%) subjects. The majority of patients with SLD were classified as MASLD (92.0%), while few subjects were no-MASLD (2.3%) or MetALD (5.7%). Subjects without SLD had comparable ASCVD score and higher adherence to MD than patients with SLD (MD score 10.5±0.2 vs. 9.6±0.1, P=0.002). Among SLD patients, AS-CVD score increased significantly from No-MASLD (3.3%), to MASLD (14.2%) to MetALD (29.7%, P=0.0007, ANOVA), while MD score and the extent of fat thickness (SAT/VAT) remained comparable.

Conclusion: Individuals with MASLD or MetALD have increased CVD risk. The risk was higher in subjects with MetALD, as compared to MASLD, pointing to an additive and harmful impact of alcohol consumption. In clinical practice, a comprehensive screening for CVD risk factors, alcohol consumption and adherence to MD in subjects with MASLD/MetALD is highly recommended.

MOLECULAR CHARACTERIZATION BY NEXT GENERATION SEQUENCING IN PEDIATRIC PATIENTS WITH DYSLIPIDEMIAS

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Introduction: Genetic dyslipidaemia includes inherited diseases caused by variants in the genes responsible for the processes of synthesis, transport and metabolism of plasma lipoproteins. The most well-known lipid disorders in children include familial hypercholesterolemia (FH) and familial chylomicronemia syndrome (FCS). Therefore, early diagnosis, especially in pediatric patients, plays a fundamental role for early treatment and prevention of clinical events in these patients.

Material and Methods: The study included twenty-four patients with age below 18 years, referred to the lipid clinic at the University of Palermo, Italy from 2015 to 2021. We performed targeted Next Generation Sequencing (NGS) analysis to study the coding regions and intron/exon boundaries of genes involved in the metabolism of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c).

Results and Conclusion: Our results have showed that ten were not carriers of pathogenetic variants in candidate genes, three were carrier of pathogenetic variants in heterozygosity in LDLR gene, two were carrier of pathogenetic variants in heterozygosity in APOB gene, two were carrier of pathogenetic variants in LPL (one in heterozygosity and the other in homozygosity) and one patient was carrier of biallelic pathogenetic variants in LIPA. The analysis also allowed us to identify variants with rare allelic frequency. Screening performed on patients below 18 years of age is useful for early diagnosis of genetic dyslipidemias and early intervention for preventing dyslipidemia-related comorbidities.

N-ACETYL THREONINE PREDICTS THE BURDEN OF CORONARY ATHEROSCLEROSIS IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Background and Aim: The SYNTAX score (SS) is a semi-quantitative screening tool calculated on angiograms to assess the extent and complexity of coronary atherosclerotic lesions. It is crucial to determine the most effective treatment method between coronary artery bypass grafting and percutaneous coronary intervention. This study investigates the relationship between the cardiometabolic characteristics, metabolomic profile, and the burden of coronary atherosclerosis in patients with acute coronary syndrome. The aim is to identify early detection and intervention biomarkers in high cardiovascular-risk conditions. Method: Thirty-nine patients admitted to the Cardiac Intensive Care Unit of the Policlinic Tor Vergata in 2014 for an acute coronary event were evaluated and divided into four groups using the interquartile ranges of SS: Q1 SYNTAX group (n=7), Q2 SYN-TAX group (n=11), Q3 SYNTAX group (n=8), and Q4 SYNTAX group (n=11). To assess the influence of metabolic comorbidities on the relationship between SS and circulating metabolites, we divided the cohort based on the presence of at least two comorbidities among obesity, diabetes mellitus, arterial hypertension, low HDL cholesterol values, and high triglyceride values, identifying two groups: MetCom NO (n=20) and MetCom YES (n=19). To reduce the dimensionality of the metabolomics data, weighted correlation network analysis (WGCNA) was done using the R WGCNA package. We identified three metabolite clusters highly associated with SS and arbitrarily named MEblue, MEbrown, and MEturquoise clusters. The eigenvalues in each sample of the resulting clusters were used for further analyses. Results: The four Q SYNTAX groups were comparable for clinical, anthropometric, and laboratory variables. Applying a linear regression model adjusted for major cardiovascular risk factors, the SS was positively associated only with stenosis >50% of the common trunk. Regarding metabolomic analysis, the SS was associated with the MEblue cluster comprising 63 metabolites even after linear regression model adjustment for eGFR (p<0.05). Metabolomic comparative analysis between Q1 SYN-TAX and Q4 SYNTAX groups was performed using the LIM-MA R package, identifying N-acetyl threonine (logFC 0.37826, p adj. 0.00302471), N-methyl pipecolate (log FC 1.24873, p adj. 0.00302471), O-sulfo-L-tyrosine (log FC 0.44222, p adj. 0.00302), and stachydrine (log FC 1.07538, p adj. 0.024574386) as significantly more abundant in the Q4 SYNTAX group and all belonged to the MEblue cluster. Furthermore, the concentrations of MEblue cluster metabolites were higher in the MetCom_YES group (p.0.048). Combining SS, metabolic comorbidities, and the MEblue cluster, we have drawn a Venn diagram highlighting 27 metabolites associated with SS and metabolic comorbidities. N-acetyl threonine was present among these.

Conclusion: Increased circulating levels of some metabolites have a clinically significant predictive value for major coronary atherosclerotic damage. N-acetyl threonine may directly contribute to vascular damage and predict the extent and complexity of coronary atherosclerosis in patients with acute coronary syndrome.

AN UNUSUAL COMBINATION OF AN UNKNOWN VARIANT OF FAMILIAL HYPOBETALIPOPROTEINEMIA TYPE 1 AND ALPHA1 ANTITRYPSIN DEFICIENCY

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A 47-year-old Hispanic woman presented with deficient plasma concentrations of low-density lipoprotein cholesterol (LDL-c) and apolipoprotein (apo) B since adolescence in the absence of typical symptoms and signs of hypobetalipoproteinemia such as growth failure, malabsorption, hepatomegaly, and neurologic and neuromuscular signs. The patient reported a previous diagnosis of alpha1 antitrypsin (AAT) deficiency and hepatic steatosis. No family history of these conditions was found. Laboratory tests showed plasma AAT levels at the low limits of routine (90.1 mg/dl) and a lipid profile characterized by total cholesterol 97 mg/dl, HDL-c 53 mg/dl, LDL-c 31 mg/dl, triglycerides 20 ml/ dl, lipoprotein A <3.1 mg/dl, apo A1 143 mg/dl, apo B 23 mg/ dl.Ultrasounds showed a liver of average size and mild-moderate steatosis. Non-invasive evaluation of hepatic fibrosis by transient elastography shows stiffness values equal to 5.3 kPa. Pulmonary function tests were regular.Genetic testing revealed a homozygous mutation of the S allele of the gene encoding AAT (p.E288V - E264V - c.863A >T rs17580), identifying the genotype PI*SS. Additionally, the heterozygous presence of the variant NM_000384.3:c.11404C>T,p. (Gln3802Ter) in the Apo B gene HGVS genotype N M_000384.3:c. [114040C>7]; [11404 =] was highlighted. This unique case shows an unusual combination of an unknown variant of Familial Hypobetalipoproteinemia Type 1 and AAT deficiency, both associated with a significantly increased risk of liver steatosis and impaired lipid secretion. The PI*SS genotype is consistent with plasma AAT levels measured on the sample, corresponding to an intermediate protein deficiency. The c. 11404C>T, p.(GIn3802Ter) in the APOB gene is not reported in the literature or reference databases. Based on the ACMG-AMP criteria, this variant can be classified as probable pathogenic (class 4) since truncating variants of the APOB gene are associated with Familial Hypobetalipoproteinemia Type 1 (FHBL1, OMIM:615558). The implications of these genetic findings are significant and warrant further investigation.

INVESTIGATING THE PHARMACOLOGICAL MODULATION OF LDL RECEPTOR BY siRNA ANTI-PCSK9: SYNERGISTIC EFFECT WITH SIMVASTATIN IN *IN VITRO* MODELS

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Aim: Small interfering RNA anti-PCSK9 (inclisiran) degrades PCSK9 mRNA in the liver, thus leading to a reduction of circulating PCSK9. In this study, we investigated the pharmacological modulation of LDL receptor (LDLR) by siRNA anti-PCSK9 alone and in combination with simvastatin in *in vitro* cultured cells.

Methods: Human hepatocarcinoma HepG2 overexpressing-PCSK9 (HepG2over-PCSK9) and Huh7 cells were treated with siRNA anti-PCSK9 (ON-TARGETplus-SMARTpool, Dharmacon) and Simvastatin (Merck). LDLR and PCSK9 protein expression were measured by western blot. From conditioned media PCSK9 was measured by using ELISA assay.

Results: In Huh7, siRNA anti-PCSK9 efficiently reduced PCSK9 protein expression, determined by western blot, after 24 and 48h (-46 and -67%, respectively), while the extracellular levels showed a reduction of 60% after 48 and 72h, as measured by ELISA. Despite the reduction in PCSK9, there was no modulation on LDLR. The combination of siRNA anti-PCSK9 with increasing concentration of simvastatin increased LDLR expression in a concentration-dependent manner compared to single treatments. Differently, in HepG2over-PCSK9, a 2-fold increase of LDLR expression was observed after transfection of siRNA anti-PCSK9. As expected, in this cell line, simvastatin (5µM) increased LDLR protein expression by 5.7-fold. However, the combination of siRNA anti-PCSK9 and simvastatin caused a synergistic 10-fold increase of LDLR. Under the same experimental conditions, extracellular levels of PCSK9 were reduced with siRNA anti-PCSK9 of 42%, while the combination led to a dose-dependent increase of PCSK9.Finally, short time-course experiments revealed that siRNA starts reducing intracellular PCSK9 from 4 to 8h (-30 and -35%, respectively), effect associated to lower extracellular PCSK9 starting from 8h post-treatment (-37%).

Consclusions: siRNA anti-PCSK9 reduced PCSK9 expression from 4 until 72h after treatment. Despite this, there is no modulation on LDLR in Huh7 cells, while HepG2over-PCSK9 seemed a more suitable experimental model. siRNA anti-PCSK9 in combination with simvastatin leads to a synergistic induction of LDLR especially in HepG2over-PCSK9.

COVID-19 INCREASES ARTERIAL STIFFNESS INDUCING GREATER DIFFICULTY IN BLOOD PRESSURE CONTROL

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Introduction: COVID-19 causes endotheliitis, leading to longterm cardiovascular complications. Pulse wave velocity (PWV), a key marker of arterial stiffness, has been associated with adverse outcomes in various cardiovascular conditions. However, the long-term effects of COVID-19 on vascular stiffness in hypertensive patients are still under investigation.

Aim: The aim of this study is to investigate the relationship between COVID-19 and vascular stiffness, as measured by PWV, in hypertensive patients.

Methods: We enrolled, in a multicenter study, 185 hypertensive patients with a positive nasopharyngeal swab for SARS-CoV-2 in the 6 months prior to the follow-up visit (Group1, 90F, age 63.38±10.29 years) and 97 hypertensive patients consistently negative for SARS-CoV-2 (Group2, 49F, age 64.20±10.79 years), comparable in terms of age, sex, weight, and BMI. We evaluated the follow-up visit (T1) and the one prior to the infection, within 12 months of the follow-up (T0).

Results: Group1 showed a significant increase in PWV at T1 compared to T0, indicating increased arterial stiffness post-COV-ID-19. Group2 had a higher percentage of patients with dyslipidemia and a significantly lower percentage of patients with hypertensive cardiomyopathy (HTN-CM) compared to Group1. In Group1, we observed a significant decrease in the number of smokers and an increase in the diagnosis of HTN-CM at T1, with a significant increase in systolic values (134.30±14.93 mmHg *vs.* 132.40±16.19 mmHg, p<0.05) and a decrease in diastolic values (78.30±8.77 mmHg *vs.* 79.63±8.41 mmHg, p<0.05) compared to T0. Moreover, there was a significant increase in the number of antihypertensive drugs taken at T1 in Group1, particularly angiotensin receptor blockers, compared to T0.

Conclusions: COVID-19 is associated with increased vascular stiffness in hypertensive patients, as evidenced by elevated PWV. This may contribute to poorer blood pressure control and a higher need for pharmacological intervention, highlighting the importance of monitoring arterial stiffness in post-COVID management strategies.

NOVEL ROLE OF INOSITOL IN REDUCING INFLAMMATORY SIGNALING IN HUMAN ADIPOCYTES

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Inositol plays a critical role as a secondary messenger in the insulin signaling pathway, regulating insulin secretion, mitochondrial function, and glycogen storage. These actions make it a promising treatment for metabolic disorders like obesity, PCOS, and menopause-related fat accumulation. Since chronic inflammation is a key factor in metabolic dysfunction linked to overweight and obesity (1), this study aims to assess inositol's anti-inflammatory effects and its potential to combat inflammatory dysfunction in hypertrophic adipocytes. To this aim we aimed to characterize the effects of myo-inositol in inflamed and dysfunctional human adipocytes. Human Simpson-Golabi-Behmel syndrome (SGBS) adipocytes were treated with myo-inositol for 5 h before stimulation with TNF- α . At a concentration of 100 µmol/L, myo-inositol markedly reduced the stimulated expression and secretion of pro-inflammatory mediators, such as CCL-2, CXCL-10, and IL-6, demonstrating its strong capability to regulate messenger and protein expression. These effects were functionally linked to a reduction in monocyte adhesion to inflamed adipocytes. Similarly, under the same conditions, myo-inositol reversed the TNF-a-induced expression of ICAM-1 on the cell surface, a crucial adhesion molecule that facilitates monocyte attachment to inflamed adipose tissue. Finally, to preliminarily explore the underlying mechanisms of action, we demonstrated that inositol mitigated the activation of NF-kB, the master regulator of adipocyte inflammation. In conclusion, myo-inositol reduces adipose dysfunction in pro-inflammatory activated adipocytes. Although further investigations are required, these data suggest that myo-inositol may improve the inflammatory and dysmetabolic milieu in obese patients.

Reference

 Quarta S., Massaro M., Carluccio M.A., Calabriso N., Bravo L., Sarria B., Garcia-Conesa M.-T. An Exploratory Critical Review on TNF-α as a Potential Inflammatory Biomarker Responsive to Dietary Intervention with Bioactive Foods and Derived Products. Foods. 2022; 11: 2524. https://doi.org/10.3390/foods11162524

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DIETARY FLAVAN-3-OL METABOLITES MODULATE PROINFLAMMATORY HUMAN FIBROBLAST ACTIVATION *IN VITRO*: POTENTIAL PERSPECTIVES IN THE PREVENTION OF CVD

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Aim: Consumption of food rich in flavan-3-ols, like tea, dark chocolate, red wine, grapes, has been associated with a protective effect against chronic diseases characterized by systemic inflammation, such as CVD. Dietary flavan-3-ols are vastly metabolized to hydoxyphenyl- γ -valerolactones (OH-PVL) by the gut microbiota. This study aims to evaluate the *in vitro* anti-inflammatory effect of colonic metabolites of flavan-3-ols as OH-PVL, previously identified in a metabolomics study as the most representative molecules present in plasma after the ingestion of flavan-3-ol-containing food.

Methods: We tested 10 colonic metabolites, belonging to the OH-PVL family in their sulphonated, methylated or enantiomerically pure forms. The cytotoxicity of all compounds and the efficacy in inhibiting lipopolysaccharide LPS-induced IL-6 secretion were evaluated in human fibroblasts by MTT assay and ELISA kit, respectively. Supernatant IL-6 concentrations were normalized against the cell extract protein content, assessed by BCA assay. Statistical analysis was done by two-way ANOVA (significant p-value≤0.05).

Results: LPS toxicity and effective concentration in increasing IL-6 secretion were preliminarily assessed. LPS resulted non-cytotoxic (IC50>50µM) and efficient at 1µM (10.000pg/mL) (p=0.0087 *vs.* untreated cells). All compounds were non-cytotoxic (IC50>50µM) nor induced IL-6 secretion at 1µM and 10µM concentrations, corresponding to their plasmatic levels after ingestion and metabolism of flavan-3-ols. Five of the tested compounds, i.e. R-C001, S-C003, R-C002 and R-C003 at 1µM significantly reduced LPS-induced IL-6 secretion by 69%(p=0.0067), 85%(p=0.0004), 87%(p=0.0003), 78%(p=0.0012) and 71%(p=0.0041), respectively. The remaining five compounds did not show a statistically significant effect.

Conclusions: Five OH-PVL compounds have shown anti-inflammatory activity in human fibroblasts *in vitro*, with no differences between enantiomers. Our data, pointing to the biological activity of specific colonic metabolites of flavan-3-ols, pave the way to further research on the mechanism of action of these molecules and support the usefulness of *in vivo* studies for the prevention and modulation of CVD.

REFRACTORY HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA TO MAXIMAL HYPOLIPIDEMIC THERAPY IN A PATIENT WITH CUSHING'S DISEASE

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Familial hypercholesterolemia (FH) is an autosomal dominant disorder, the frequency of the heterozygous FH (HeFH) has been estimated at 1 in 500 and of the homozygous FH (HoFH) at 1 in 1,000,000 individuals globally. However, in a recent meta-analysis estimated prevalence of FH was 1:250 and it could be classified as the most frequent genetic disease globally. Although several therapeutic options are available FH seems to be underestimated and in turn under treated. A 22-year-old patient referred to the "Federico II" University Hospital Lipid Clinic because of refractory hypercholesterolemia, 190 mg/dl LDL-C. Patient were on maximally tolerated lipid-lowering therapy: Rosuvastatin 20 mg, Ezetimibe 10 mg, Evolocumab 140 mg. The patient reported history of spontaneous gastric perforation in 2019 and type 2 diabetes mellitus. We decide to perform a genetic test for FH causative mutation resulted in heterozygous mutations of LDLr c.465 C>A and PCSK9 c.523+9C>T of uncertain pathological significance. On physical examination, we observed the presence of purple or pink stretch marks (striae) on abdomen and hips and I° central obesity (BMI 32 kg/m²), blood pressure of 135/90 mmHg. That are findings compatible with the suspicion of Cushing's syndrome. Blood Cell count reveled a mild neutrophilic leukocytosis (WBC 13.8 x 103 uL: lymphocytes 16% neutrophils 83% eosinophils 0.5%, basophils 0.3%, monocytes 0.2%). CRP <0.3 mg/dl in absence of fever. Then we proceed with diagnostic iter for Cushing syndrome.We found urinary cortisol 1221µg (21-292) on diuresis of 1800 mL24, ACTH 70 pg/ ml and serum cortisol 22 µg.The dexamethasone suppression test (with dose of 2 mg) is performed to evaluate the possible suppression of ACTH production by the pituitary gland. The test shows a minimal variation in ACTH values. On cerebral MRI, the adenohypophysis presents a convex upper profile and a non-homogeneous signal due to the presence of a small nodular formation (approximately 3 mm), referable to a microadenoma. To integrate and confirm the diagnosis, has been performed the sampling of the pituitary vein with catheterization of the petrous sinuses (BIPSS). In the presence of hypercortisolism, BIPSS is used to differentiate pituitary ACTH production from ectopic ACTH production. ACTH should be present in higher blood concentrations adjacent to the pituitary gland than in more distant peripheral areas, where levels are generally lowered by dilution with peripheral blood. The suppression of normal corticotropic cells by persistent hypercortisolism is critical to the diagnostic accuracy of BIPSS because it ensures that the ACTH dosed is exclusively from the tumor source, pituitary or ectopic: Our patient presents ACTH in the periphery 51 pg/ml, ACTH right sinus 590 pg/ml and ACTH left sinus 481 pg/ml. The concentration of ACTH in the right and left petrous sinus relative to that in the periphery determines the C/P ratio: a C/P ratio ≥ 2 under basal conditions, as in this case, deposes hypercortisolism of pituitary origin. Then is possible to obtain a Cushing's disease from pituitary microadenoma diagnosis. Is the pathophysiological mechanism involving cortisol and LDLr would explain the high cholesterol levels despite maximal therapy. Cortisol acts at different levels to reduce LDL uptake in the liver, either by

decreasing the expression of LDL receptors or by reducing their functional activity. This could lead to an accumulation of LDL in the blood and contribute to the hypercholesterolemia associated with Cushing's disease. Furthermore, cortisol may regulate LDL receptors gene in hepatocytes. In our patient heterozygous FH combined by Cushing syndrome could be associated to low response to lipid lowering therapy. FH combines with the metabolic complications of Cushing's disease, increase even more the risk of cardiovascular events. Therapies aimed at inhibiting cortisol production and managing hypercholesterolemia are crucial for reducing cardiovascular risk. The complexity of the management of patients with familial hypercholesterolemia associated with Cushing's syndrome necessitates of early diagnosis, due to a multidisciplinary therapeutic approach that is essential to improve clinical outcome and reduce the risk of complications.

EFFICACY AND ADVERSE EVENTS OF THE USE OF BEMPEDOIC ACID IN A REAL-LIFE POPULATION

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Bempedoic acid is an inhibitor molecule of adenosine triphosphate citrate lyase, an enzyme upstream of HMGCoA reductase, used as a lipid-lowering agent in patients with hypercholesterolemia in association or not with other lipid-lowering agents. Unlike statins, bempedoic acid is not metabolised at the muscular level and has proven to be a key molecule in patients with muscular intolerance to statins. The present study aims to observe dyslipidemic patients, in primary and secondary prevention, who have started bempedoic acid, in association or not with ezetimibe, and evaluate the response rate, any impacts on uric acid and creatinine levels and any intolerances in real life. Our study confirmed that there is a statistically significant decrease in total cholesterol levels and LDL cholesterol levels (p<0.05; 95% CI) as shown by studies performed in main clinical trials.Uric acid levels did not increase statistically significantly (p>0.05, 95% CI). As regards creatinine levels, a slight statistically significant increase can be observed (p<0.05; 95% CI). The study we conducted highlighted how the use of bempedoic acid, in association or not with other lipid-lowering therapies, is effective in reducing total cholesterol and LDL cholesterol, in agreement with the main clinical trials. This drug is safe and is not associated with a statistically significant increase in uric acid levels. Creatinine levels were significantly increased with the use of bempedoic acid in line with the increases described in the registration trials. Ultimately our study, with the limitations of the sample size, confirms the effectiveness of bempedoic acid therapy, confirming the proven benefits that emerged from the main studies.

DIRECT ORAL ANTICOAGULANTS, ALL-CAUSE MORTALITY AND CARDIOVASCULAR EVENTS IN PATIENTS WITH ATRIAL FIBRILLATION WITH AND WITHOUT OBESITY: INSIGHT THE NATIONWIDE START REGISTRY

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Introduction: Atrial fibrillation (AF) is often associated with cardiovascular comorbidities, such as obesity. Direct oral anticoagulants (DOACs) use in patients with AF and obesity is still uncertain for the low number of obese patients enrolled in clinical trial and for peculiar pharmacokinetic characteristics of obese patients. We compared the all-cause mortality and cardiovascular events (CVEs) risk in obese patients with AF treated with DOAC or warfarin.

Methods: 10.369 AF patients on oral anticoagulants were enrolled in the multicenter nationwide START registry. Obesity was defined as body mass index (BMI) \geq 30 Kg/m². 1st degree obesity was defined by a BMI between 30 and 34.9 Kg/m², while \geq ^{2nd} degree was defined if BMI was \geq 35 Kg/m². The association between DOACs use, all-cause mortality and CVEs was investigated with Cox regression analysis to estimate hazard ratio (HR) and 95% confidence interval (95%CI).

Results: Mean age was 76.3±9.4 and 45.3% were women. 3,725 (35.9%) patients were overweight and 2,240 (21.6%) were obese. Obese patients were more frequently affected by arterial hypertension, diabetes, anemia, heart failure, and chronic lung disease. During a mean follow up was 23.9±19 months, 704 deaths and 821 CVEs occurred. In obese patients, elderly (HR 2.460, 95% 1.690-3.583, p<0.001), anemia (HR 1.514, 95%CI 1.045-2.192, p=0.028), cerebrovascular disease history (HR 1.614, 95%CI 1.056-2.467, p=0.027), peripheral artery disease (PAD) (HR 2.204, 95%CI 1.278-3.800, p=0.004) and heart failure (HF) (HR 1.769, 95%CI 1.220-2.563, p=0.003) were associated with higher risk of all-cause mortality, while, among treatment, only DOAC use was associated with a lower mortality risk (HR 0.637, 95%CI 0.445-0.912, p=0.014). Elderly (HR 2.102, 95%CI 1.501-2.943, p<0.001), anemia (HR 1.440, 95%CI 1.019-2.036, p=0.039), cerebrovascular disease history (HR 1.707, 95%CI 1.160-2.512, p=0.007), PAD (HR 1.931, 95%CI 1.147-3.250, p=0.013) and HF (HR 1.562, 95%CI 1.105-2.208, p=0.012) were also associated with higher risk of CVEs, while DOAC treatment was only associated with a trend towards a reduction of CVE in obese patients (HR 0.751, 95%CI 0.542-1.041, p=0.086).

Conclusion: In conclusion, DOAC use seems to be associated with a lower risk of all cause-mortality but not so effective in the reduction of CVEs in obese AF patients. Further study, especially on severe obesity are needed.

WAIST-TO-HIP RATIO VERSUS BODY MASS INDEX FOR THE PREDICTION OF CARDIOVASCULAR EVENTS IN ATRIAL FIBRILLATION

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Introduction: Some previous studies described an inverse association between obesity and cardiovascular risk in patients with atrial fibrillation (AF). However, in these studies obesity was defined by a body mass index (BMI) \geq 30 Kg/m², without an evaluation of visceral adiposity. For this reason, we investigated the predictive value of waist-to-hip ratio (WHr) on cardiovascular events (CVEs) comparing it to BMI.

Methods: We included patients from the ATHERO-AF study, a monocentric observational prospective ongoing study including AF patients treated with direct oral anticoagulants (DOACs). Obesity was defined as BMI \geq 30 Kg/m² and a pathological WHr was defined \geq 0.9 in male and \geq 0.85 In female. We used Receiver operating characteristic (ROC) to estimate the predictive value of WHr and BMI against CVEs.

Results: We enrolled 704 AF patients, of which 44.2% were female. Mean age was 75.2±16.6. Patients with pathological WHr were more frequently affected by hypertension, diabetes and had higher mean BMI and lower serum concentration of high-density lipoprotein (HDL). 43 CVEs were collected. The ROC analysis showed that WHr had an area under curve (AUC) of 0.659 (95% confidence interval [CI] 0.621-0.695, p<0.001) while BMI had a AUC of 0.580 (0.541-0.618, p=0.061).

Conclusion: In our study we found that WHr seems to have a better predictive value of CVEs compared to BMI, probably due to a better characterization of visceral adiposity. Further studies are needed to confirm this finding.

IMPACT OF THE MEDITERRANEAN DIET ON INFLAMMATORY AND LIPID PROFILES AMONG SUBJECTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Aim of the Study: Familial hypercholesterolemia (FH) is a genetic autosomal dominant disorder characterized by high LDL-C levels leading to early atherosclerotic cardiovascular disease (ASCVD) onset. FH management focused on decreasing LDL-C concentrations through pharmacological therapy, while lifestyle-based therapies have historically been considered as secondary approaches. The objective of this study was to evaluate the impact of different levels of adherence to the Mediterranean diet (MD) on dyslipidemia and inflammatory biomarkers in genetically confirmed FH subjects. Moreover, we aim to detect how pharmacological treatments influence the level of adherence to healthy lifestyle habits such as the MD.

Methods: This was a cross-sectional study involving 253 subjects aged over 18 years with genetically confirmed FH. Nutritional data were surveyed by administering a previously validated form of the Mediterranean Diet, the Pyr-Mediterranean Diet Score (PyrMDS). Moreover, we collected data on pharmacological treatment, cardiovascular adverse events, intima-media thickness (IMT), carotid and femoral atherosclerosis, subendo-cardial viability ratio (SEVR) and blood samples to analyze lipid profile and high sensitivity C-reactive protein (hs- CRP) concentrations. Our population was stratified into tertiles based on the values obtained with the PyrMDS score, thus obtaining three groups with different levels of adherence to the Mediterrane-an diet (Group 1= High PyrMDS, Group 2 = medium PyrMDS, Group 3= low PyrMDS).

Results: Group 1 showed a higher age as well as an older age at diagnosis of familial hypercholesterolemia compared to Group 3 (p value for all <0.01). The majority of subjects affected by arterial hypertension were in Group 1 than Group 3 (p<0.01) while the opposite was observed in smoker prevalence; finally, BMI was homogeneous across the three groups. Group 1 showed a significant lower levels of total cholesterol (p<0.01) and LDL cholesterol (p<0.001). Furthermore, a decreasing level of C-reactive protein was found in the Group 1 than Group 3 (p<0.01). Group 1 exhibited a higher value of SEVR than Group 3 (p<0.01). Compared to Group 3, subjects in Group 1 were more frequently treated with statins (p<0.01), ezetimibe (p<0.01], PCS-K9 inhibitors (p<0.01).

Conclusions: Our results confirm that a greater adherence to a healthy dietary pattern such as the Mediterranean diet improves the lipid and inflammatory profiles in FH patients. Therefore, the Mediterranean diet should be recommended as a standard dietary regimen over an intensive lipid lowering therapy in FH patients in order to prevent major adverse cardiovascular events.

CHARACTERIZATION OF microRNAs PROFILE AND THEIR ASSOCIATION WITH ANGPTLS IN PATIENTS WITH SARCOPENIC OBESITY: PRELIMINARY RESULTS

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Background: Sarcopenic obesity (SO) is characterized by the loss of skeletal muscle mass and increased ectopic lipid deposition, posing significant health risks, particularly in the development of cardiovascular diseases. The pathogenesis of SO is complex and involves factors such as insulin resistance, lipotoxicity, glucose imbalances and pro-inflammatory cytokines. Recent evidence suggests that the differential expression of certain microRNAs (miRNAs) may influence muscle atrophy and lipid infiltration. Although Angiopoietin-like proteins, key proteins in lipid metabolism and energy regulation, may be implicated in the development of SO.

Aim: The study aimed to:

1) identify dysregulated miRNAs,

2) analyze the levels of ANGPTLs,

3) their relationship in patients with SO.

Methods: After obtaining informed consent, participants were consecutively enrolled from those under follow-up at the Department of Nutrition. Body composition was assessed using DEXA, and muscle function was evaluated with a handgrip strength test. Then we classified patients as SO or obese (Ob), the latter serving as controls. Fasting blood samples were collected to

measure circulating mRNA levels using RT-PCR and serum ANGPTL3 and ANGPTL4 levels using ELISA. **Results:** Sixteen patients with sarcopenic obesity (SO) and 16

Results: Sixteen patients with sarcopenic obesity (SO) and To obese (Ob) patients were included in this preliminary analysis. Our findings revealed an upregulation of miR-27b and miR-206 in the SO group compared to the Ob group (P=0.044 and P=0.043, respectively), while miR-133 levels were significantly lower (P=0.037). We observed an upregulation of ANGPTL4 in the SO group (p=0.021) and a trend toward increased ANGPTL3 levels compared to Ob patients. Interestingly, in SO patients miR-206 levels were negatively correlated to ANGPTL3 (p=0.023, r= -0.570).

Conclusion: These preliminary data suggest that levels of circulating miRNAs and ANGPTL3 and 4 are modulated in SO. The biological role of this observation and wheatear miRNAs pattern and ANGPTLs can contribute to the pathogenesis of SO sill remain to be investigated.

THE POTENTIAL ROLE OF CIRCULATING AND IN-SITU PRO-INFLAMMATORY BIOMARKERS IN CAROTID PLAQUE PROGRESSION

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To date, many studies reported chronic inflammation as one important factor of carotid plaques progression, suggesting a possible connection between the occurrence of cardiovascular risk factors and inflammation-related plaque instability. More specifically, this study aims to evaluate the possible role of both the high sensitivity C-Reactive Protein (hs-CRP) and the in-situ presence of some pro-inflammatory markers on carotid plaque instability. One hundred and fifty-six carotid plaques from symptomatic and asymptomatic patients were collected and histologically classified as unstable and stable plaques. Also, anamnestic and hematochemical data, such as age, gender, hypertension, diabetes mellitus, smoking habit, therapy, low-density lipoprotein (LDL)-C, kidney failure and hs-CRP, have been registered. Moreover, the most representative area of each plaque was included to build a Tissue Microarray, in order to test some pro-inflammatory markers, such as CD3, CD4a, CD8, CD20, CD86, CD163 and interleukin (IL)-2, IL-6, IL-17, by immunohistochemical stainings. The results revealed a 67% increased risk of plaque instability in patients with high levels of hs-CRP. Precisely, the risk of carotid plaque instability was enhanced in dyslipidemic males and in aged female patients. In addition, plaque destabilization appeared associated with high in-situ expression of some pro-inflammatory cytokines, such as IL-2, IL-6, IL-17. As concern the association with the analysed cardiovascular risk factors, a significant increase of IL-6-positive and IL-17-positive cells was detected in unstable carotid plaques of female patients as compared with unstable plaques of males. In conclusion, our data highlighted an important role of CD8+ T-cells in plaque progression, with no correlation with the analysed risk factors. Nonetheless, a significative correlation between the presence of some in-situ pro-inflammatory cytokines and biological sex were registered. Furthermore, hs-CRP levels may be considered a potential screening factor to be of use for prevention and management of carotid atherosclerotic disease.

THE STEROL ELEMENT BINDING PROTEIN 1C (SREBP1c) CONTROLS OXIDATIVE METABOLISM AND SUPPRESSIVE FUNCTION OF REGULATORY T CELLS

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Aim: Immunosuppressive T regulatory cells limits inflammation in different pathological settings, including atherosclerosis. Several studies have demonstrated that Treg lipid metabolism is critical for their function. Previous studies of our group showed that deficiency of SREBP1c, a key protein regulating intracellular fatty acid (FA) metabolism, worsened Tregs immunosuppressive function by derailing energetic metabolism toward glycolysis and affecting phospholipid metabolism. Therefore, aim of this study was to investigate how SREBP1c controls Treg function by modulating lipid metabolism.

Methods: T cells isolated from WT and SREBP-1c KO mice and *in vitro* induced Treg (iTreg) were analyzed through RT-qPCR, flow-cytometry and lipidomics. iTreg metabolism was studied using the Seahorse XF technology. iTreg suppressor and migratory phenotype were assessed by *in vitro* assay.

Results: Deficiency of Srebp1c resulted in a reduced in vitro generation of iTreg (-6,76%, p<0,0001), inhibition of Tell proliferation (-5%, p<0,01), increased migration (+80% vs. CCL19/21, p<0,05) and glycolytic metabolism (+18,8%, p<0,05) compared to WT iTreg. Inhibition of fatty acid oxidation by Etomoxir (5µM) impaired suppressive phenotype of WT but not Srebplc KO iTreg (-3,93%, p<0,05 for WT), suggesting a defective FA oxidation of the latter. Addition of palmitate improved suppressive phenotype of WT iTreg (+5,6%, p<0,01), but did not revert the functional and metabolic phenotype of Srebp1c KO iTreg. Protein and mRNA expression of CD36 were reduced in Srebp1c KO vs. WT iTreg, confirming missed compensation of impaired lipid metabolism by extracellular fatty acids uptake. By lipidomic analysis Srebp1c deficiency resulted in an altered phospholipid composition, showing an accumulation of LPC over PC, which is associated to an impaired Lands cycle. Moreover, LPCAT3 inhibition showed a reduced expression of specific Treg proteins (CD73, AKT) required for their immunosuppressive activity.

Conclusion: SREBP1c plays a key role in the immune-metabolic response of Tregs: by modulating lipid cellular metabolism SREBP1c controls energetic and functional phenotype of Treg.

NEONATAL SCREENING FOR LYSOSOMAL ACID LIPASE DEFICIENCY IN THE SARDINIA REGION, ITALY

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Cholesterol Ester Storage disease (CESD) is a term used to group lysosomal acid lipase deficiencies associated with a less severe clinical phenotype than that of Wolman disease, a fatal disease within one year of the newborn's life. This is because in CESD the enzymatic defect is subtotal, while in Wolman the enzymatic deficiency is practically totally. In both cases the transmission is autosomal recessive. The enzyme deficiency is due to mutations in the acid lipase gene (LIPA). This enzyme plays a fundamental role in the hydrolysis of cholesterol esters and triglycerides at the endosomal and lysosomal levels. In CESD, lipid storage abnormalities mainly concern the accumulation of esterized cholesterol, with abnormalities in the accumulation of triglycerides less evident than in Wolman's disease. The age of onset and progression of CESD are extremely heterogeneous, with a continuous spectrum of the disease between almost asymptomatic forms, early-onset and severe forms which may be related to a change in the level of residual acid lipase activity. The estimated prevalence of the enzyme deficiency in the Caucasian population causing Wolman is not known, while the estimate varied between 1/40,000 and 1/300,000 in CESD. In the latter pathology, the main clinical signs may be a significant elevation of LDL-c and hepatomegaly. The age of onset varies but is usually early (generally before the age of 10-12), although the appearance of first symptoms in adulthood has been described. Splenomegaly is also present in a third of cases. Liver disease may remain asymptomatic for a long period, but tends to progress to steatosis, liver failure, fibrosis and even micronodular cirrhosis in severe cases. In the majority of patients, plasma hyperlipidaemia with hypercholesterolemia is present, associated with an increase in LDL cholesterol levels, a reduction in HDL cholesterol levels with variable hypertriglyceridemia (Phenotype IIB). Paediatric and adult patients have a significant risk of atherosclerosis. Radiographs can detect adrenal calcifications in Wolman disease. In both forms the diagnosis can be made by measuring the enzymatic activity of LAL on Dry Blood Spot samples (DBS). Genetic analysis can be used to confirm the diagnosis. The differential diagnosis of CESD must be made with IIB related dyslipidaemias (Combined Hyperlipidaemia) and with liver diseases that lead to micronodular cirrhosis. For both disorders, replacement therapy of acid lipase (Sebelipase alpha) is available intravenously once every week for patients with rapidly progressive LAL-D symptomatic in infancy or one every other week for patients initially symptomatic at paediatric or adult age. The aim of the present study, financed by Alexion and approved by local ethics committee, was the early identification in Sardinian newborns, by means of a drop of blood obtained with a heel stick and normally used for neonatal screening for congenital hypothyroidism and phenylketonuria, of Wolman disease and CESD, respectively, due to a total or subtotal acid lipase enzyme deficiency. Every year approximately 6,000 babies are born in Sardinia. In spite of a discontinuously activity due to the Covid 19 pandemia, from February 2020 until October 2023, 4,889 enzymatic assays on DBS have been performed. Until now, no samples with zero activity and no sex differences were found; 14 samples with very low activity have been obtained (<0.20). The considered normal range of LAL activity is 0.20 – 0.80 nmol/ spot/h; the mean normal LAL activity is 0.44 nmol/spot/h. The genetic analysis of the 14 samples with low enzymatic activity revealed only already known SNPs variants for all 10 exons. At the present time, neonatal LAL screening analysis is still in progress. The goal of an extension of the study would be to screen more than 40,000 newborns.

SLEEP QUALITY IN ELDERLY PATIENTS WITH TYPE 2 DIABETES: FROM GLYCEMIC CONTROL TO ANXIETY-DEPRESSIVE DISORDERS

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Introduction: Type 2 Diabetes Mellitus (T2DM) is a complex condition associated with numerous comorbidities. Recently, factors previously ignored, such as sleep disturbances, have been shown to play a much more significant role. Indeed, growing evidence has demonstrated that both qualitative and quantitative sleep alterations are associated with an increased risk of cardiovascular events, obesity, anxiety-depressive disorders, and poorer glycemic control in diabetic patients. The aim of our study was to evaluate the relationship between sleep disturbancees, anxiety-depressive disorders, and quality of life in elderly patients with T2DM.

Material and Methods: Elderly patients with T2DM aged over 65 years attending the Internal Medicine division of ARNAS Garibaldi-Nesima Hospital in Catania were recruited. Patients with obstructive sleep apnea syndrome, those receiving treatment for anxiety-depressive disorders, or those with known sleep disorders were excluded. Each patient underwent collection of anthropometric, laboratory, medical history data and completion of three questionnaires: the Pittsburgh Sleep Quality Index (PSQI), the Geriatric Depression Scale (GDS), and the WHO-QoL-BREF to investigate sleep quality, depression, and quality of life, respectively.

Results: A total of 67 patients were recruited and divided based on their PSQI score into a PSQI≥8 group (n=24) and Controls (PSQI<8, n=43). Patients in the PSQI≥8 group showed a higher prevalence of depressive disorders (4.71±3.8 *vs.* 2.65±2.10, p=0.021) and poorer quality of life. Patients in the PSQI≥8 group also had a longer duration of diabetes (15.5±10.7 *vs.* 12.6±9.44 years, p=0.277), were more frequently undergoing insulin therapy (33.3% *vs.* 21.4%, p=0.26), and had a higher prevalence of retinopathy compared to controls (26.7% *vs.* 6.9%, p=0.07).

Conclusion: Sleep disturbances, being associated with greater use of insulin therapy, diabetic retinopathy, anxiety-depressive disorders, and poorer quality of life, should be an important factor to consider in the management of diabetic patients.

GENETIC CHARACTERIZATION OF THE KIV2 LPA POLYMORPHISM IN SUBJECTS WITH BICUSPID AORTIC VALVE: COMPARISON BETWEEN qPCR AND DDPCR METHODS

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The bicuspid aortic valve (BAV) is a congenital cardiac malformation with an incidence ranging from 0.5 and 2.0% in the general population. Beyond hemodynamic valvular impairment, a frequent determinant of BAV natural history, dyslipidemia and elevated lipoprotein (a) [Lp(a)] levels also favor progression and complications of aortic valve disease. Lp(a) levels are known to be under a strict genetic control and are largely influenced by LPA Kringle IV type 2 (KIV-2) size polymorphism affecting apolipoprotein (a) isoform dimensions. In the present study, we evaluated the relationship between LPA KIV-2 repeat number, measured through digital droplet PCR (ddPCR) and real-time PCR method (qPCR). The genetic characterization of the LPA KIV2 polymorphism was carried out through ddPCR (QX200 Droplet Digital PCR System) and qPCR [7900HT Fast Real-Time PCR System (Applied Biosystem). The cohort under analysis is represented by 64 subjects of Caucasian origin [79.7% male; median age 45.50 year] referred to the Center for Cardiovascular Diagnosis, AOU Careggi. As expected from literature, for both methods, an inverse correlation is observed between Lp(a) levels and the number of LPA KIV2 repeats, although statistical significance is not reached (ddPCR: R=-0.144, p=0.256; qPCR: R=-0.114, p=0.371). However, subgrouping the cohort according to Lp(a) 500 mg/L cut-off value, the ddPCR shows significantly lower values of repeats in the group of subjects with higher levels of Lp(a). As concerns KIV2 repeat evaluation according to BAV complications, in subjects with severe calcification, in whom significantly higher Lp(a) levels were reported (p=0.016), a more marked decrease in repeat values with ddPCR compared to qPCR was found. Data obtained highlight a greater potential of the ddPCR approach vs. qPCR in identifying subjects with bicuspid aortic valve complications, probably due to the greater stability and less variability in the identification of KIV2 repeats.

THE IMPACT OF DIFFERENT FACTORS ON LDL-C TARGET ACHIEVEMENT AMONG PATIENTS WITH DYSLIPIDEMIA TREATED WITH BEMPEDOIC ACID THERAPY: A PRELIMINARY RETROSPECTIVE ANALYSIS

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Introduction: Atherosclerotic cardiovascular disease remains a leading cause of death globally, with dyslipidemia being a major risk factor. Bempedoic Acid, a prodrug which activated at the liver level acts by selectively inhibiting ATP citrate lyase, have enhanced the management of hypercholesterolemia, however some patients fail to reach low-density lipoprotein cholesterol (LDL-C) targets. Thus, we aim to evaluate different factors that could influence the achievement of LDL-C target in patients treated with Bempedoic Acid.

Methods: A retrospective cohort study was conducted on patients treated with Bempedoic Acid at the Lipid Clinic of Policlinic San Martino Hospital, Genoa, between May 2023-September 2024. Demographic, clinical, andanthropometric data were collected. Lipid profile was measured at baseline and after 3 months. The primary outcome was LDL-C target achievement according to ESC/EAS guidelines.

Results: Among the 68 patients (40 females, 28 males, median age 65 years), approximately 54% achieved therecommended LDL-C target. After 3 months we observed a significant reduction of LDL-C (p<0.0001), TC (p<0.0001), TG (p=0.015) and a significant increase of HDL-C (p=0.041) and uric acid (p=0.001). Logistic regression analysis highlighted that heterozygous familial hypercholesterolemia (He-FH) was associated with lower likelihood of achieving LDL-C targets (OR 0.07, 95%CI 0.01-0.30, p<0.0001) and concomitant oral therapy with Ezetimibe plus High intensity statin was related to a significantly lower probability of reaching LDL-C target (OR 0.05, 95%CI 0.03-0.71, p=0.027). Moreover, increasing body mass index (BMI) value is related to a significant lower probability of reaching the LDL-C target (OR 0.73, 95%CI 0.59-0.91, p=0.006).

Conclusions: Bempedoic Acid has been shown to significantly improve the lipid profile, however causing an increase in uric acid. It is essential to consider factors such as diagnosis of He-FH, a high BMI and use of concomitant oral lipid-lowering therapy to optimize the achievement of LDL-C targets.

USE OF BEMPEDOIC ACID TO TREAT HYPERCHOLESTEROLEMIA IN PATIENTS AFFECTED BY NEUROMUSCULAR DISEASES

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Background: Statins have been used for decades in the primary and secondary prevention of cardiovascular diseases. These drugs are known to be relatively safe yet associated with side effects, of which the most reported are related to the muscle such as myalgia, myositis or rhabdomyolysis. Lipid lowering treatment could be challenging in patients affected by neuro-muscular diseases. Statins have been classified in the uncertain/minor risk group in these patients. Bempedoic acid (BA) was recently introduced as lipid lowering agents. BA is an ATP citrate lyase inhibitor that targets cholesterol synthesis upstream of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the enzyme inhibited by statins but it is activated in the liver and not in most peripheral tissues, including skeletal muscle.

Methods: We evaluated efficacy and safety of BA in patients with rare neuromuscular disease clinical to assess efficacy and safety of BA. We evaluated lipid profile, liver and muscular enzyme at baseline and after 12 weeks of bempedoic acid treatment.

Results: We enrolled 6 patients (3 carriers of Charcot-Marie-Tooth disease. 1 affected by mitochondrial myopathy with cox deficiency, 1 affected myoadenylate deaminase deficiency myopathy and 1 carrier Myasthenia gravis), the mean age was 53,66±18,32 years,1/2 patients had nonalcoholic steatohepatitis, 1/2 were hypertensive, 1/6 was diabetic, no patient reported cardiovascular event or carotid atherosclerosis. Although patients were on maximally tolerated hypolipidemic therapy with Ezetimibe, they have not achieved the desired LDL-C target. After 12 weeks treatment with BA, we observed a significant reduction in LDL-C levels (from 153,8±56,87 mg/dl to 99,46±35,18 mg/dl p<0,001), with an average reduction of 35,34%. We observed also significant changes in lipid profile (TC from 223,33±65,13 mg/dl to 172,33±44,56 mg/dl, TG from 140,5±51,6 mg/dl to 146,33±51,01, HDL from 43±14,39 mg/dl to 43±14,3. None reported any adverse event such us elevation of liver and muscular enzymes (creatinine from 0,78±0,56 to 0,77±0,43, uric acid from 5,7±2,43 to 6,49±2,15, AST from 27,33±5,12 to 31,33± 8,14 U/l, ALT from 32,16±18,01 to 32,5±13,47 U/l, CPK from 275,33±103,91 to 258±93,44).

Conclusion: Hypercholesterolemia is often reported in patients with neuromuscular disease. For example, Charcot-Marie-Tooth disease is associated with a decreased uptake of LDL. Although, we reported data in a small population, BA has been shown to be promising for patients affected by neuromuscular disease in which statin treatment could be difficult to manage.

CEREBROSPINAL FLUID AND PLASMA HDL (dys)FUNCTION IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an inflammatory and immune-mediated neurodegenerative disease in which cholesterol plays a key role. Dysregulation of cholesterol homeostasis in the central nervous system (CNS) has been associated with neurodegenerative disorders. In particular, cholesterol transport in the brain is fundamental for the maintenance of physiological functions, and it is mediated by lipoproteins similar to plasma high-density lipoproteins (HDL) found in human cerebrospinal fluid (CSF), which mediate the transport of cholesterol from astrocytes to neurons, through the major efflux transporters ABCA1 and ABCG1. However, the precise involvement of HDL in the pathogenesis of MS is still not clear. This study aimed to investigate the relationship between cholesterol metabolism and MS, focusing on cerebral and serum HDL function to promote cerebral and systemic cellular cholesterol efflux (HDL-CEC). This aim was pursued in an observational study including CSF and serum from MS patients (n=25) and age- and sex-matched controls (n=12). Cerebral and serum HDL cholesterol transport capacity was evaluated by radioisotope assay using standardized central and peripheral cell models. The results of this study showed that the CSF HDL-CEC from astrocytes cell models was significantly lower in MS subjects compared to controls through ABCA1 (p=0.002631) and ABCG1 (p=0.02192). No significant differences were observed between the groups for the serum HDL-CEC ABCA1- and ABCG1-mediated. However, stratification of MS population by severity of pathology based on the prognostic parameter Oligoclonal bands (OCB), CSF HDL-CEC from astrocytes (p=0.009179) and serum HDL-CEC ABCA1-mediated (p=0.02467) were significantly lower in subjects OCB+.MS is associated with a defect in CSF HDL capacity to promote the first step of cerebral cholesterol transport. In addition, the observation that also serum HDL-CEC ABCA1-mediated is lower in MS subjects OCB+ may put the premises to study serum HDL-CEC as a potential less invasive biomarker of the disease.

IMPACT OF THE METABOLIC ADAPTATIONS TO SHORT-TERM HIGH FAT FEEDING ON NEUTROPHIL BEHAVIOR

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Background and Aims: Neutrophils participate to the chronic metabolic consequences of High Fat Diet (HFD). Nevertheless, neutrophils are characterized by short half-life which is determined by a fine tuning in expression and function of CXCR4, which dictates the egress from the bone marrow (BM), and CXCR2, which facilitates their mobilization from BM and the patrolling activity in the periphery. In the quest to study the behavior of the neutrophil with the short-term consequences of HFD feeding, we investigated whether the metabolic adaptations affect blood neutrophil count and their membrane expression of indirect markers of function.

Methods: To assess the gluco-metabolic impact of a short-term HFD feeding, indirect calorimetry and plasma glucose dosage were performed on mice previously fed a HFD (60% Kcal from fat) for seven days, followed by immunophenotyping of blood over 24 hours. To test whether changes in circulating neutrophil count during short-term HFD feeding were related to a differential egress from the BM, we repeated the same experimental design in mice harboring a conditional deletion of CXCR4 (CX-CR4fl/flMrp8Cre+).

Results: Short-term HFD feeding was sufficient to induce a profound metabolic impact (e.g. reduced respiratory exchange ratio, increased energy expenditure, and insulin levels), and to induce an increase of circulating neutrophils (p=0,052), without impacting other leukocytic fractions over 24 hours, compared to chow diet feeding mice (20% Kcal from fat). HFD feeding significantly altered the expression pattern of multiple membrane markers of neutrophil function (CD11b, CD62L, CXCR2) over 24 hours, driving neutrophils toward a phenotype featuring increased migration and activation. Finally, the CXCR4fl/flMrp-8Cre+ mice, which present significantly higher circulating neutrophilia, showed lower insulin sensitivity upon HFD compared to WT.

Conclusions: We suggest that the metabolic adaptations induced by a short-term exposure to HFD affect neutrophil behavior, surmising it as appealing target for cardio-metabolic diseases.

THE DIAGNOSIS OF HYPERTENSION DURING PREGNANCY CORRELATES WITH EARLY MENARCHE AND WORSENED CARDIOVASCULAR DAMAGE

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Introduction: During pregnancy multiple factors cause maternal and neonatal morbidity and mortality, with an increased longterm risk of cardiovascular disease. One potential mechanism underlying this risk is increased vascular stiffness, a marker of endothelial dysfunction and arterial damage. Early identification of factors contributing to vascular stiffness in women could improve cardiovascular prevention strategies especially in those whose history of gestational hypertension occurs.

Aim: The aim of this study is to evaluate the relationship between gestational hypertension, age at menarche, and vascular stiffness in women with hypertension, to identify potential early predictors of arterial damage.

Methods: We retrospectively evaluated 224 Caucasian women(59.31±11.02 years)affected by hypertension. History of gestational hypertension (group1=9) was used to differentiate who not (group2=215). All patients underwent a comprehensive physical examination, blood tests, echocardiogram, tonometry (PWV) and ankle-brachial-index (ABI).

Results: Collected data shows that patients with gestational hypertension (group1) have a significantly higher body mass index (BMI) (p<0,0001) and abdomen circumference (p=0,006) compared to group2. In group1, a significantly lower age at menarche (p<0,001) and diagnosis of hypertension (p<0,0001) is observed compared to group2. Furthermore, group1 shows reduced levels of total cholesterol (p=0,003), LDL (p=0,006), and non-LDL (p=0,007), but an increase in glomerular filtration rate (p<0,0001), proteinuria (p=0,033), and albuminuria (p<0,0001). Regarding vascular stiffness, Group 1 showed a significant reduction in cfPWV (p=0.012), despite displaying increased aortic root diameter (p=0.001) and enhanced right ventricular systolic function assessed by TAPSE (p<0.0001). These alterations were accompanied by elevated proteinuria (p=0.033) and albuminuria (p<0.0001), suggesting renal involvement.

Conclusions: Our findings indicate that women with a history of gestational hypertension may develop distinct patterns of vascular and cardiac remodeling. The observed reduction in cfPWV could reflect adaptive vascular changes, potentially linked to hormonal factors. Long-term monitoring of vascular stiffness in these women is essential to detect early signs of cardiovascular damage.

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SECONDARY DYSLIPIDAEMIA IN PEDIATRIC AGE: TWO CASE REPORTS

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Introduction: Dyslipidemias are disorders characterized by elevated plasma cholesterol and/or triglycerides and/or low HDL-cholesterol (HDL-C) levels, which contribute to the early development of cardiovascular disease. They can have either primary or secondary etiologies and may sometimes underlie complex diseases characterized by heterogeneous complications that, if not treated promptly, can reduce quality of life and life expectancy. Therefore, a careful diagnostic approach, including genetic analysis in selected cases, is essential.

Case 1: Firstborn (13 years old), Moroccan origin, born at term (adequate weight and length), poor weight gain. Poor visual acuity from birth associated with progressive retinal degeneration from homozygous mutation of INPP5E gene (c.1861C>T). Diagnosed with nonautoimmune diabetes mellitus at 12 years old, on multi-injection insulin therapy. Referred to our Centre at 13 years and 3 months old for mixed dyslipidemia (total cholesterol 325 mg/dl, triglycerides 2056 mg/dl) and decompensated diabetes (glycated haemoglobin 10.6% on insulin therapy 1.6 U/kg/day). At clinical evaluation: height + 0.60 SDS, weight -1.10 SDS, body mass index (BMI) -1.83 SDS, puberty in progress. Generalized loss of subcutaneous adipose tissue, muscle hypertrophy, mammary gland atrophy, venomegaly, acromegalic features, umbilical hernia, acanthosis nigricans. Reported hyperphagia. Additional diagnostic exams: aspartate aminotransferase (AST) 127 U/L, alanine aminotransferase (ALT) 131 U/L, proteinuria (0.36 g/24H), undosable leptin and adiponectin. Abdominal ultrasound: hepatomegaly with steatosis; densitometry: fat mass -1.7 Z-score, total body less head -1.7 Z-score. In the suspect of lipodystrophy carried out genetic investigation: variant in homozygosity c.194G>A (p.Trp65Ter) of AGPAT2 gene, inherited from parents (heterozygous), causative of generalized congenital lipodystrophy type 1 (BSCL1), later also found in younger sister with similar phenotype. After 14 months of treatment with metreleptin, there was an improvement in metabolic parameters: triglycerides -90%, glycated haemoglobin -30%, significant reduction in insulin requirement (currently about 0.6 U/kg/day), transaminases within normal limits, resolution of hyperphagia. Mild proteinuria persists in 24hour assay and mild fibrosis on fibroscan evaluation (5.46 kPa).

Case 2: First-born, born at term (adequate weight and length), perinatality normal. At 3 years old, during hospitalisation for epiphyseal osteomyelitis of the femur, evidence of hypertransaminasemia (AST 120 U/L, ALT 148 U/L, gamma glutamyl transpeptidase 71 U/L) and hepatomegaly on abdominal ultrasound. Subsequent laboratory investigations revealed mixed dyslipidaemia (total cholesterol 318 mg/dL, LDL-cholesterol 228 mg/dL, HDL-cholesterol 21.7 mg/dL, triglycerides 207 mg/dL). Diagnostic investigations for the main causes of hypertransaminasemia were negative: markers of inflammation, antinuclear antibodies (ANA), hepato-renal antimicrosome antibodies (LKM), anti-smooth muscle antibodies (ASMA), serologies for hepatitis B and C viruses, ceruloplasmin, screening for coeliac disease, immunoglobulins, creatine phosphokinase, alpha 1 antirypsin. She was referred to us for mixed

dyslipidaemia at 4 years and 3 months old. Considering the negativity of the first and second level examinations, on suspect of lysosomal acid lipase (LAL) deficiency, a genetic investigation was carried out: two mutations in heterozygosity in the LIPA gene: c.894G>A and c.455T>C, classifiable as pathogenic and probably pathogenic respectively. Diagnosis confirmed by enzyme activity assay with a 97.4% reduction found. Currently waiting for approval for compassionate use treatment with sebelipase alpha.

Conclusions: These cases emphasise the importance of the differential diagnosis of secondary dyslipidaemia in two patients with a complex clinical framework in paediatric age and the usefulness of genetic investigation, where indicated, in order to make early diagnosis of rare diseases and to undertake effective treatment paths quickly.

REDOX STATE OF HDL AND TYPE II DIABETES: PILOT STUDY ON A FEMALE POPULATION

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The decline in estrogen related to menopause increases the risk of developing Type II Diabetes Mellitus (T2DM) in women compared to age-matched men. In turn, the occurrence of T2DM can trigger a wide range of metabolic alterations, such as dyslipidaemia and oxidative stress. Although Low-Density Lipoproteins (LDLs) are the main lipoproteins undergoing oxidation, High-Density Lipoproteins (HDLs) are also highly susceptible to oxidative modifications, making them pro-inflammatory and pro-atherogenic. The objective of this study was to examine if T2DM was linked to changes in the redox state of HDL in a cohort of healthy pre- and post-menopausal women (n=64 and n= 65, respectively), and diabetic postmenopausal women (n=29). To address this aim, the contributors to HDL antioxidant activity, such as Paraoxonase-1 (PON-1) arylesterase and lactonase activities, lipoprotein phospholipase-A2 (Lp-PLA2) and levels of oxidized HDL were assessed in the serum of study participants. Postmenopausal T2DM women had approximately 20% higher levels of oxidized HDL (oxHDL) compared to healthy postmenopausal women (p<0.05). Notably, body and abdominal fat accumulation and menopausal transition, which may influence systemic redox state and T2DM development, were associated with changes in oxHDL. Small HDL particles seemed more prone to oxidation, since oxHDL levels were positively correlated with these subspecies (r=0.224, p<0.05), and negatively with medium or large HDLs (r=-0.483, p<0.001 and r=-0.439, p<0.001, respectively). Finally, consistent with the role of PON-1 in the antioxidant protection of HDL, oxHDL was inversely correlated with arylesterase (r=-0.193, p<0.01) and lactonase (r=-0.215, p<0.01) activities of this enzyme. In conclusion, our preliminary results suggest that in women, the occurrence of T2DM may be associated with increased oxidation of HDL, leading to a decrease in the athero-protective function of these lipoproteins. This could be one of the factors contributing to the heightened risk of cardiovascular disease in diabetic postmenopausal women.

LYSOSOMAL ACID LIPASE DEFICIENCY (LAL-d) A UNDERDIAGNOSED CAUSE OF MIXED HYPERLIPIDEMIA

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We report a case of a 57 years old woman who came to our attention for hypercholesterolemia and hypertransaminasemia since he was 22 years old. Total cholesterol was (CT) 315 mg/ dl, LDL cholesterol 255 mg/dl, HDL cholesterol 39 mg/dl, triglycerides (TG) 105 mg/dl, AST 64 U/l, ALT 125 U/l. She started simvastatin 20 mg in 2000 with no benefits and she has been shifted to 10 mg of rosuvastatin. At the beginning her elevation of transaminases were thought to be correlated with her hypercholesterolemia but since no improvement with the therapy and the discovery of liver fibrosis (in 2018 8.1 Kpa at hepatic elastography) a liver biopsy was performed. Her biopsy showed: portal fibrous enlargement, accumulation of foamy histiocytes, hepatocytes with foamy and enlarged cytoplasm, absence of inflammation. Screening for hepatitis viruses was negative. In 2019 a genetic exam was performed and a compound heterozygous mutation c894G>A (pSer275 Gln298del)in exon 8 and c.260G>A (pGly87Asp) in exon 4 was found in the LIPA gene. A diagnosis of Lysosomal Acid Lipase (LAL) Deficiency has been made. In 2019 he started, in association with rosuvastatin, ezetimibe. Lysosomal Acid Lipase (LAL) Deficiency, also known as Wolman disease in newborns and Cholesteryl Ester Storage Disease (CESD) in other patients, is a rare autosomal recessive disorder characterized by impaired lysosomal acid lipase activity, leading to the accumulation of cholesterol esters and triglycerides in various tissues. LAL Deficiency arises due to mutations in the LIPA gene, which encodes the lysosomal acid lipase enzyme. These mutations result in reduced or absent enzymatic activity, leading to the accumulation of cholesteryl esters and triglycerides primarily in the liver, spleen, gastrointestinal tract, and other tissues. The lipid accumulation triggers inflammation, tissue damage, and organ dysfunction, contributing to the clinical manifestations of the disease. The clinical presentation of LAL deficiency varies depending on the age of onset and the severity of the disease. In infants with Wolman disease, symptoms typically appear within the first few months of life and include hepatosplenomegaly, failure to thrive, malabsorption, and adrenal calcifications. These infants often experience rapid disease progression and have a poor prognosis. In contrast, patients with CESD may present later in childhood or adulthood with hepatomegaly, hyperlipidemia, liver fibrosis, and cardiovascular complications. Currently, the management of LAL Deficiency focuses on supportive care and symptomatic treatment. Enzyme replacement therapy (ERT) with recombinant human lysosomal acid lipase has shown promising results in clinical trials, leading to improvements in liver function, lipid profiles, and disease-related symptoms. Additionally, lipid-lowering medications, such as statins and ezetimibe, may be used to manage hyperlipidemia and reduce cardiovascular risk in affected individuals Despite adequate lipid-lowering therapy, the patient's liver function deteriorated with worsening fibrosis (Liver stiffness in 2023 11.6 Kpa). Her last exams were: CT 162 mg/dl, HDL cholesterol 49 mg/dl, LDL cholesterol 99 mg/dl, TG 65 mg/dl, AST 61 U/l, ALT 65 U/l, GGT 34 U/l ALP 110 mg/dl, albumina 42 mg/dl. Our patient is now waiting for ERT.

ATP CITRATE LYASE GENE SILENCING: IMPACT ON LIPID METABOLISM OF HEPATOCYTES

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Background and Aim: ATP Citrate Lyase (ACLY) is a step limiting enzyme of fatty acids and cholesterol biosynthesis. An ACLY inhibitor, bempedoic acid, has been recently approved for the treatment of hypercholesterolaemia, expecting it would affect different metabolic pattern within the liver. Aim of this project is to evaluate the efficacy and the liver metabolic consequences of hepatic selective gene silencing approach directed toward ACLY (ACLY-GS).

Methods: Efficacy of several ACLY siRNAs was tested in HepG2 cells by profiling energetic metabolism. Western Blot Analysis, SeaHorse analysis and Flow cytometry were used. HepG2 were treated with Palmitate (200uM), Oleate (400uM) and a mixture of both, respectively for 24 h and 7 days to mimic different induced-steatosis models.

Results: Among tested siRNA sequences, was identified the one that reduced ACLY expression of more than 80%. To characterize the impact of ACLY over cellular metabolism, changes in mitochondrial mass and lipid accumulation were investigated with specific staining (Mito Tracker green; Nile Red). After 24h, ACLY gene silencing resulted in a significant increase in mitochondrial mass in oleate treated cells, while after 1 week incubation this change is visible only in the basal condition. In parallel, at both time points, ACLY GS increased lipid accumulation only in oleate/palmitate treated cells. Mito Stress assay shows how ACLY GS with oleate determines a significant reduction in all mitochondrial parameters, especially in maximal respiration and ATP production, suggesting important adaptations in mitochondrial metabolism with the possibility to redirect oleate towards different metabolic pathways.

Conclusion: These results show that ACLY-GS deeply influences cellular functions, by affecting and reprograming cellular energetic metabolism depending on the type and time of exposure of different nutrient substrates. These findings set the stage for investigating, *in vivo*, the impact of ACLY-GS on metabolic profile.

LOW CONCENTRATION SIMVASTATIN IMPROVES SENESCENCE-INDUCED MITOCHONDRIAL DYSFUNCTION IN VASCULAR SMOOTH MUSCLE CELLS

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Statins are well-known lipid-lowering drugs, but they also have other beneficial effects, such as reduction of oxidative stress and inflammation and improvement of mitochondrial function. Mitochondrial alteration and senescence are main indicators of aging and are associated with multiple age-related diseases. Evidence indicates that senescent cells exhibit mitochondrial dysfunction. Moreover, senescent vascular smooth muscle cells (VSMCs) are present in atherosclerotic plaques and contribute to their instability. In our study, we characterized mitochondrial dysfunction in replicative (RS) and doxorubicin-induced senescence (DIS) in VSMCs and tested simvastatin as a therapeutic intervention. We established and characterized mitochondrial dysfunction in DIS VSMCs or VSMCs serially passaged to induce RS. We measured mitochondrial respiration by Seahorse, reactive oxygen species (ROS) production and mitochondrial membrane potential (MMP) by flow cytometry, mitochondrial morphology by confocal microscopy, and mitochondrial transcription factor A (TFAM) gene expression by RT-qPCR.In both senescent models, we observed an accumulation of dysfunctional mitochondria, but down-regulation of TFAM gene expression was observed only in RS cells. Next, we investigated whether simvastatin could ameliorate age-associated phenotypes in senescent VSMCs. Sinvastatin 0.1 µM reduced senescence-associated secretory phenotype (SASP) and improved mitochondrial respiration in DIS and RS VSMCs. Interestingly, the effects of simvastatin on mitochondrial respiration were abolished by adding mevalonic acid (MVA) (100 µM). In addition, the low concentration of simvastatin (0.1 µM) significantly reduced ROS production, while a higher concentration (0.5 µM) increased oxidative stress in senescent VSMCs. In conclusion, both models of senescent VSMCs accumulate dysfunctional mitochondria. The down-regulation of TFAM expression suggests a reduction in mitochondria number only in RS. Simvastatin 0.1 µM seems to be a potentially beneficial therapeutic intervention for improving senescence-induced mitochondrial dysfunction, while a higher amount causes oxidative stress. MVA supplementation inhibited the statin-mediated improvement of mitochondrial respiration, indicating that effects are mediated by inhibiting HMG-CoA reductase rather than an off-target effect.

CREB3L3 NONSENSE MUTATION: LOOKING FOR A THERAPY THAT MAKES SENSE

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In 2016 we visited a family at our Lipid Clinic (mother: M1, 49 vo, and two children, a female: D2, 22 yo, and a male: S3, 18 yo), affected by severe hypertriglyceridemia [M1=Total Cholesterol (TC) 481, HDL-C 30, Triglycerides (TG) 1290 mg/dL; D2=TC 267, HDL-C 19, TG 1355; S3=TC 276, HDL-C 20, TG 2697]. M1 had 5 out of 12 brothers with early MACE. They all were genotyped as carriers of heterozygous nonsense mutation (c.718 G>A) in the CREB3L3 gene with a different and variable pattern of penetrance. M1 (but not D2 and S3) was also carrier of $\varepsilon 2/\varepsilon 2$ genotype, maybe responsible for the phenotypic expression like the type III FHL, differently from her sons. Interestingly, both M1 and D2 did not respond/tolerate fenofibrate and showed a partial response to n-PUFA, thus they did not reach the target. At that time, we were able to optimize the lipid profile (M1=TC 213, HDL 29, TG 301 mg/dL; D2=TC 119, HDL 29, TG 241) with the addition of atorvastatin (results partially expected for M1, ε2/ ϵ 2+, less for D2, ϵ 2/ ϵ 2-). S3, despite his more severe hypertriglyceridemia, as expected responded better to the fibrate than to the statin, with optimization of lipid profile (S3=TC 150, HDL 31, TG 391). During the years of the SARS-CoV-2 pandemic we lost contact with the family who, in the meantime, was followed by the general practitioner. About one year ago they were referred to another Lipid Center in the neighboring area where the therapy of all three family members was modified: MI was suspended from Atorvastatin and prescribed Inclisiran alone. D2 had already stopped Atorvastatin due to pregnancy and was maintained on therapy only with n-PUFA 1250 mg, showing a worsening lipid profile up to TG values >4600 mg/dl and TC 690 mg/dL without complications till delivery but without changes in treatment even after stopping breastfeeding. Recently, the family returned to our observation. D2 had remained on occasional treatment with n-PUFA (and currently has values of TC 166, HDL 34 and TG 511 mg/dL). S3 was on ineffective treatment with n-PUFA 2 g and with lipid profile TC 198, HDL 26, TG 907. M1 was on treatment with Inclisiran and n-PUFA 2 g but has TC 541, HDL 24, TG 915 mg/dL. M1 was not renewed on Inclisiran treatment, and the previous therapy with Atorvastatin was reinstituted; D2 was advised to gradually resume treatment with Atorvastatin 10 mg + n-PUFA 2 g; S3 didn't tolerate Fenofibrate anymore and was advised to undergo therapy with Atorvastatin 20 mg and n-PUFA 2 g.

Conclusion: the nonsense variant of CREB3L3 confirmed only partial response to hypotriglyceridemic drugs. M1 ($\epsilon 2/\epsilon 2+$ and with severe hypercholesterolemia) responded to statins, as expected, and not to inclisiran (probably due to a lack of substrate on which to act?). Anyway, in this rare form of hypertriglyceridemia the response to therapy remains unexpected and partially inexplicable and the effect on the progression of atherosclerosis remains still uncertain.

AN ACUTE RESPONSE OF NEUTROPHILS TO METABOLIC ADAPTATIONS DUE TO FEEDING

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Background: Diets impacts the long-term expansion and the activation of immune responses, with mechanisms that have been well described so far. However, it is less clear if the immune system can promptly respond to an acute exposure to diet, nor which is the principal immune cell, either adaptive or innate, in charge of this response.

Methods: C57Bl/6j mice were characterized for the metabolic profile (inverse calorimetry, glycemia and triglycerides levels) at five time points, counted from when day light was switched on ("zeitgeber times" (ZT)), both in fasting and upon feeding with a chow diet. We also immunophenotyped blood by flow-cytometry. The metabolic and immune profile was also studied in mice in fasting upon re-feeding for up to two hours.

Results: Fed animals ate more during the night (ZT17-22). Glycaemia raised more during feeding, remaining elevated in the night, while it persisted constantly lower upon fasting. Similarly, triglycerides levels remained constantly higher during feeding, while it reduced by half over all the timepoints compared to ZT1 in fasting. No significant changes were observed in the number of circulating leukocytes, which persisted stable during the day, both during fed and fasting. Conversely, when fasting mice were then refed, glycemia increased twice as high, and triglycerides levels raised by three times two hours later. Besides, circulating levels of Ly6G+ neutrophils increased by two times versus fasting, while the levels of other leukocytes (CD3+T, CD19+ B cells and Ly6c+ monocytes) did not change. We also studied the membrane expression of activation markers of neutrophils during these same experimental conditions.

Conclusions: Our data suggest an innate, but acute, control of neutrophils in response to feeding. Further studies are needed to better understand the underlying mechanisms and the effect of more caloric diets.

ADVANCED GLYCATION END-PRODUCT INTAKE PREDICTS INSULIN RESISTANCE IN A SEX-SPECIFIC MANNER

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The ability of dietary advanced glycation end products (AGEs) to promote insulin resistance in a mixed population made up of males and females remains controversial. Thus, this study aims at evaluating if the relationship between dietary AGEs an insulin resistance may be sex-dependent. This question was address in a study cohort made up of four hundred and thirty four individuals were included into this study (195 males and 239 females). Study participants underwent anthropometric and metabolic characterization. Their AGE intake was estimated using food frequencies questionnaires and validated databases reporting AGE content in individual food items. The relationship between AGE intake and insulin resistance (HOMA-IR) was assessed in the whole study cohort as well as independently in males and females using Spearman correlation test. Stepwise linear regression was employed to evaluate the predictive power of dietary AGEs towards HOMA-IR in both male and female study participants. The intake of AGEs correlated positively with HO-MA-IR in the whole study cohort (p<0.05), in female (p<0.01) but not in male study participants (p>0.05). In agreement with this, AGE intake was able to increase the predictive power of BMI towards insulin resistance in females but not males. On the contrary, anthropometric variables were the only discriminants able to predict insulin resistance in males. Dietary AGEs exert a sex-dysmorphic effect on insulin resistance with females appearing to be more susceptible to the deleterious impact of these glycotoxins on insulin sensitivity. Nevertheless, direct evidence from clinical trials are warranted in order to confirm the present findings.

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PEDIATRICS CASCADE SCREENING FOR INHERITED DYSLIPIDEMIAS: A LIPOPROTEIN APHERESIS CENTER EXPERIENCE

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Aim: Familial hypercholesterolemia (FH) is less rare than one might think and despite highly effective lipid-lowering therapies (LLT) over half of the patients treated do not reach the lipid target indicated by the guidelines. In these patients, lipoprotein apheresis (LA) is the most effective means to lowering the apo-B containing atherogenic lipoproteins.

Methods: In own center, since 1994, thanks to routinely cascade testing performed in patients who start LA, we have identified a pediatric population (30 subjects) which we evaluated retrospectively.

Results: At the baseline, 60% of patients were aged between 16 to 18 years old (18/30) and LDL cholesterol level \geq 190 mg/ dl was present in 80% of subjects (24/30). Furthermore, 57% (17/30) of subjects inherited a heterozygous mutation in the LDL receptor gene like that of relatives treated with LA, while in subjects with tendon xanthoma (2/30), a double heterozygotes mutation for LDL-receptor were identified. No diabetes mellitus, arterial hypertension, or renal failure was present in any patients, while a worrying number (9/30) of subjects are current smoking. During the follow-up we recorded an improvement in patient lipid profile (LDL cholesterol baseline 255±90 mg/dl vs. follow-up 121±44, g/dl; p<0.001), but also a low compliance in LLT. Only a percutaneous coronary revascularization was recorded in a male patient with double heterozygotes mutation for LDL-receptor. Carotid ultrasound studies have been performed in a subgroup of 19/30 subjects (63%), no c-IMT variation have been recordered while a significant development of carotid atherosclerosis, defined as new onset of carotid plaque and/or high bulb IMT, was shown (baseline 0/19 vs. follow-up 9/19; p<0.001). Conclusion: Cascade screening, performed in subject with premature cardiovascular event or inherited dyslipidemias, is an effective approach to identified paediatric FH, a condition for which community paediatricians should also be made aware. A dedicate network is required to investigate the involved gene mutation and to set up a management program including lipoprotein (a) measurement and subclinical atherosclerosis evaluation. Moreover, it is important that medical staff have therapeutic pathways to help patients overcome discomfort from the disease and chronic LLT, as well as improve adherence to lipid-lowering drugs.

LIPOPROTEIN APHERESIS REDUCE MACE INCIDENCE IN HIGH-Lp(a) SUBJECTS ON PCSK9 INHIBITORS THERAPY

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Aim: Lipoprotein (a) [Lp(a)] is a prevalent genetic risk factor for coronary artery disease, awaiting specific antisense oligonucleotide directed to apo(a), the 2022 EAS consensus recommends an intensive management of the other risk factors. The proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i), although effective in the LDL-cholesterol reduction, have demonstrated a variable Lp(a)-lowering effects that could lead to a reduction in Lp(a) up to 25-30%, while lipoprotein apheresis (LA) is the only therapy that reduces lipoprotein (a) values by 70-80%.

Methods: The aim of this study is to evaluate the impact of LA in reducing major cardiovascular events (MACE) in patients with familial hypercholesterolemia, Lp(a)-hyperlipidemia (plasma level >50 mg/dl) and coronary heart disease on PCSK9i therapy. To evaluate the LA effect, we retrospective reviewed the incidence of 4-point major cardiovascular events (MACE - definite as acute myocardial infraction, CABG/PCI, stroke, cardiovascular death) in 35 patients (mean age 61±9 years, male 91%) affected by familial hypercholesterolemia, Lp(a)-hyperlipidemia and coronary heart disease, who archived on PCSK9i therapy an LDL-cholesterol below 55 mg/dl, with or without chronic LA (8 and 27 subjects respectively). Moreover, previous multiple MACEs, concomitant peripheral vascular disease or previous cerebrovascular accident are reported respectively in 20, 9 and 2 patients. The PCSK9i treatment was administered every 2 weeks (Evolocumab 140 mg s.c. or Alirocumab 150 mg s.c. on 23 and 12 subjects respectively) on top of lipid-lowering-therapy.

Results: The two study groups had not significant difference in lipids profile (LDL cholesterol PCSK9i group 43±17 mg/dl *vs.* PCSK9i+LA group 51±23 mg/dl; p=0.280 - Lipoprotein (a) PCSK9i group 76 [59-101] mg/dl *vs.* PCSK9i+LA group 92 [71-164] mg/dl; p=0.719) but, during the follow-up (45±31 months), a significant higher MACE incidence was reported on subjects without LA treatment (PCSK9i group 6/27 mg/dl *vs.* PCSK9i+ LA group 1/8; p<0.010).

Conclusion: There is a general agreement on the reduction of major adverse cardiovascular events by LA. In our clinical practice, patients referred to LA had previous multiple MACE and/ or associate peripheral vascular disease. These aspects may explain the observed additive effect on the MACE reduction. With the aim of improving the personalized therapy, individual risk assessment and specific treatment to reduce Lp(a) can represent a very interesting and useful approach, although prospective and randomized multicenter clinical trials are needed.

A THERAPEUTIC COOPERATION BY BEMPEDOIC ACID AND PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 INHIBITORS IN STATIN INTOLERANCE INHERITED DYSLIPIDEMIAS

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Aim: Data from clinical practice have highlighted that up to 60% of patients with familial hypercholesterolemia (FH) do not reach the recommended lipid targets despite treatment with PCSK9i and receiving the maximally tolerated lipid-lowering therapies. Nowadays, treatment with bempedoic acid can, therefore, be considered a useful additional therapeutic option.

Methods: The study aim was to evaluate the achievement of the LDL-C target (goal for LDL-C ≤55 mg/dl) in familial hypercholesterolemia subjects on PCSK9 inhibitors therapy with statin intolerance history, atherosclerotic cardiovascular disease (AS-CVD) or with another major cardiovascular risk factor adding bempedoic acid to their backbone lipid lowering therapies.

Results: We enrolled 40 patients (mean age 65±9 years, male 58%) assigned to bempedoic acid (180 mg/day) for six months of treatment. Chronic PCSK9i injectable drugs (alirocumab 150 mg every 2 week in 22 subjects, evolocumab 140 mg every 2 week in 14 subjects or inclisiran 286 mg in 4 subject) were administered just on top of backbone lipid lowering therapy. Associated Lp(a)-hyperlipoproteinemia (defined as >50 mg/ dl) was observed in 13/40 (33%) subjects, 5/40 (13%) patients was also on chronic lipoprotein apheresis treatment. At the follow-up visit, 34/40 patients adhered to the treatment, while 6/40 (15%) discontinued bempedoic acid therapy due to adverse events: gastrointestinal disorders (2 cases), increase in AST/ ALT (2 cases), gouty attack (1 case) and onset of renal failure (1 case). During the follow-up no major cardiovascular events were recorded. The addition of bempedoic acid results in a further reduction in LDL-C (114±36 vs. 68±30 mg/dl; p<0.001) as well as the increase of plasma uric acid concentrations (5.0±1.1 vs. 5.8±1.4 mg/dl; p<0.001) and loss of renal function (estimated glomerular filtration rate - eGFR - 76±16 vs. 70±20 mg/dl; p<0.05). The LDL-C target has been reached in 15/40 (38%) patients as recommended by international guidelines, 4/40 (10%) showed LDL-C values between 56 and 70 mg/dl, 8/40 (20%) have LDL-C concentrations between 71 and 100 mg/dl and 7/40 (18%) still had LDL-C levels above 100 mg/dl.

Conclusion: Patients with inherited dyslipidaemias and statins intolerance represent a very difficult therapeutic challenge, PCSK9 inhibitors can help to achieve the goal of plasma lipid levels recommended to guideline, however their use is often limited by an LDL-C cut-off threshold too low. Nowadays, bempedoic acid, an inhibitor of the cholesterol biosynthesis pathway significantly reduces LDL-C and appears to be safe and well tolerated, even in patients with statin intolerance.

PORTRAIT SURVEY: PATIENT-CENTERED OUTCOMES RELATED TO TREATMENT PRACTICES IN LIPOPROTEIN APHERESIS: ITALIAN INVESTIGATING TRAJECTORIES

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To date, despite the new lipid-lowering drugs, some subjects do not reach LDL-cholesterol and/or lipoprotein (a) [Lp(a)] goals indicated by guidelines. In these patients the lipoprotein apheresis (LA) plays a role in atherosclerosis prevention. The aim of this study is to paint a portrait of the current LA activity in Italy. collecting data via an electronic survey based on a structured questionnaire, uploaded on a dedicate website. Forty-seven centers were contacted, data from 140 patients (male 66%) were obtained from 14 sites with a homogeneous geographical distribution. Two sites had discontinued LA treatment during the COVID-19 pandemic while, in the active sites, a median of 18 [14-19] LA treatment/patient per year was performed, 7/12 sites used more than one LA systems, the most common technique being the heparin-induced LDL precipitation apheresis (10/12 sites) whit venous vascular access used in 88% of cases. High Lp(a) plasma concentrations (>60 mg/dL or \geq 145 nmol/L) was recorded in 73/140 patients; 13/34 homozygous familial hypercholesterolemia patients were on lomitapide or evinacumab therapy. The PORTRAIT (Patient-centred Outcomes Related to Treatment practices in lipopRotein Apheresis: Italian investigating Trajectories) survey would like to promote network to better manage the patients on chronic LA.List of GILA (Gruppo Interdisciplinare Aferesi Lipoproteica) study collaborators:

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THE IMPACT OF GENETIC MUTATIONS ON THE LIPID PROFILE OF INDIVIDUALS AFFECTED BY FAMILIAL HYPERCHOLESTEROLEMIA: INSIGHTS FROM THE FERRARA LIPIGEN CENTER

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Background: Familial Hypercholesterolemia (FH) is the most common genetic disease associated with premature atherosclerotic cardiovascular. The most frequently identified mutations concern genes coding for LDLR, APOB and PCSK9. Currently, genetic mutations detected in patients screened for FH are classified as follows: I (pathogenic), II (likely pathogenic), III (variant of uncertain significance), IV (likely benign), V (benign). Despite the pathogenicity of genetic variants potentially associated with FH is continuously updated, their role in dictating disease outcomes remains to be fully elucidated.

Objective: The aim of this study was to assess the impact of different genetic mutations on the lipid profile of a population screened for FH in the LIPIGEN Centre in Ferrara.METHOD-SThe lipid profile (total cholesterol, LDL-cholesterol, triglycerides and HDL-cholesterol) of 209 patients screened for FH was characterised at baseline and at the most recent follow-up (MRFU). Patients were divided into three groups: no mutations, non-causative mutations and causative mutations. Differences between the groups were assessed by one-way ANOVA followed by Tukey or Tamhane test.

Results: Patients with non-causative mutations displayed a greater reduction in LDL-C at MRFU compared to patients without mutations (-53.6% vs. -40.2%); additionally, the delta of LDL-C levels between baseline and MRFU did not significantly differ when comparing patients with and without causative mutations. This also held true in the comparison between patients with non-causative and causative mutations. Finally, no difference between groups were detected in terms of total cholesterol, triglycerides and HDL-cholesterol.

Conclusion: The absence of significant differences in terms of LDL-C reduction between patients without mutation and those with causative mutations may be due to opposite causes. Patients without mutation may be undertreated. In patients with causative mutation there may be greater difficulties in lowering LDL-C despite maximum lipid-lowering therapy.

STRATEGIES TO IMPROVE THERAPEUTIC ADHERENCE IN ELDERLY PATIENTS WITH CARDIOVASCULAR DISEASES: A SYSTEMATIC REVIEW ACROSS DIFFERENT HEALTHCARE SETTINGS

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Background and Objectiven: On-adherence to prescribed therapies is a significant public health issue, especially among the elderly, where the presence of cardiovascular (CV) conditions often requires the chronic use of multiple medications. Identifying effective strategies to improve adherence is crucial. As part of the Eldercare project, a systematic literature review was conducted to map strategies aimed at improving therapeutic adherence in elderly patients (>60 years) with CV conditions.

Methods: A systematic literature search was performed on MEDLINE, Embase, and Web of Science until December 31, 2023. Studies reporting the outcomes of interventions to improve adherence to therapies in elderly patients with CV conditions were included.

Results: Twenty-eight studies were included, of which 21 were randomized controlled trials and 7 were observational studies. The conditions studied included hypertension (n=13), type 2 diabetes (n=8), heart failure or myocardial infarction (n=7), hyperlipidemia (n=4), cardiovascular disease (n=4), or atrial fibrillation (n=2). Interventions occurred mostly in hospital or specialized outpatient settings (n=9), primary care (n=8), local healthcare services (n=7), and community pharmacies or nursing homes (n=4). In 13 studies, interventions focused on health education, reminders, and cognitive-behavioral support with feedback, while 7 used mHealth tools such as smartphone/tablet apps or electronic pill dispensers. Four studies were based on pharmacist-led counseling; only 2 studies involved multidisciplinary teams. Six studies monitored the effectiveness of interventions for 12 months or more. Of the 28 included studies, 25 reported improvements in adherence post-intervention, and 3 observed fewer CV-related hospitalizations.

Conclusion: Interventions to improve adherence in the elderly have shown benefits, albeit often marginal and short term. The heterogeneity of approaches highlights the need for personalized strategies, adaptable to available resources in different healthcare settings and to specific CV conditions.

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HIGH-GLUCOSE CONCENTRATIONS ARE INSTRUMENTAL FOR PALMITIC ACID TO INDUCE METABOLIC INFLEXIBILITY TO LIPIDS AND DEFECTIVE INSULIN SIGNALLING IN HUMAN MYOTUBES

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Long-chain saturated fatty acids play a pivotal role in fostering skeletal muscle insulin resistance. However, in vivo and in response to overnutrition, myotubes are cyclically exposed to elevated concentrations of saturated fatty acids and glucose. This study aims at evaluating whether high glucose concentrations are dispensable for long-chain saturated fatty to induce insulin resistance in human myotubes. Human immortalised myotubes were exposed to 200 µM palmitic acid in the presence (PA+G-LU) or absence (PA) of high glucose concentrations (13 mM glucose) for 24 hours. Thereafter, mitochondrial membrane potential was assessed using the JC-10 fluorescent dye and oxygen consumption rate (OCR) evaluated by employing the MitoXpress Xtra oxygen consumption assay in the presence of PA as a metabolic substrate. Insulin-induced phosphorylation of protein kinase B (Akt) and insulin receptor substrate (IRS) was investigated by Western blot. This technique was also applied to assess the activation of the NFkB signalling pathway, complemented by the evaluation of interleukin 6 (IL-6) expression by qPCR.PA did not impair insulin signal transduction both at IRS1 and AKT level (both p>0.05), whereas it increased mitochondrial membrane potential (p<0.05) and oxygen consumption rate (p<0.001) relative to controls. Additionally, PA did not elicit the activation of the NFkB signalling pathway (p>0.05). On the contrary, PA+-GLUC hampered insulin-induced IRS1 phosphorylation (p<0.05) and tended to decrease insulin signalling at AKT level (p=0.054). Moreover, PA+GLU did not improve mitochondrial membrane potential compared to controls and displayed a lower ORC compared to PA alone-treated cells (p<0.05). Finally, PA+GLU induced a downregulation of IKBa which is synonymous with the activation of the NFkB pathway. Thus, nutrient overload, in the form of PA and glucose, impaired myotube metabolic flexibility to lipids which, in turn, potentially contributed to the onset of inflammatory responses and impaired insulin signalling.

EFFECTIVENESS AND TOLERABILITY OF BEMPEDOIC ACID AND DISTANCE TO LDL-TARGET: REAL-LIFE DATA

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Background: Lipid-lowering therapy is a fundamental pillar in the prevention of cardiovascular events. Background therapy with statins plus ezetimibe often fails to bring patients to target, partly due to poor tolerability.

Objective: To evaluate effectiveness and tolerability of bempedoic acid (BA) in achieving LDL-target levels.

Methods: We included 151 patients (39% male, 61% female, mean age 70±10 years, mean BMI 28±5, waist circumference 100±11 cm) who started treatment with BA in March 2023. 89% were in primary prevention, with very high cardiovascular risk (CVR) in 26%, high in 46%, moderate in 20% and low CVR in the remaining 8%.82% of patients reported intolerance to statins or a previous lipid-lowering therapy (ezetimibe, nutraceuticals containing monacolin K). About 25% of patients were not on therapy at the time of the baseline visit, mostly at high and very high CVR (73%). 90% of patients were not at target for LDL-C levels.122 out of 151 patients underwent an initial evaluation after 1-3 months and 32 patients had already a follow-up at 8-12 months; 26/122 patients (21%) discontinued treatment before three months for reasons unrelated to the drug's efficacy/tolerability, but due to bureaucratic issues and only a small portion of patients, just 6/122, stopped BA due to adverse events (myalgia, headache, gastrointestinal disorders). The mean baseline LDL-C level was 132 ± 42 mg/dl, with an average distance from the target of 40%.74% of patients continued BA therapy at 3 months, reaching a mean LDL-C level of 88±27 mg/dl, with a mean reduction of 35% from baseline and a remaining average distance from LDL-target of 28%. 36% of patients fully reached the LDL-C target. Subgroup analyses were conducted based on pharmacological therapy (BA alone, BA + ezetimibe, BA + ezetimibe + low-dose high-intensity statin) and CVR class. It has been found that BA as monotherapy reduces LDL-C by about 34.5% and up to about 50% when combined with ezetimibe. BA alone did not allow the target to be achieved, except for those with low to moderate cardiovascular risk who require a smaller percentage reduction in LDL-C (3/27 patients on BA, 11%), whereas combination therapy with ezetimibe allowed to be at target in 47% of patients, most of whom also had low and moderate CVR. In patients treated with triple therapy, the percentage of patients reaching target levels is about 42%, with an average LDL-C reduction of 31%.

Conclusions: Our study confirms that BA is effective and well-tolerated leading at LDL-target a part of dyslipidemic patients, especially those with low to moderate cardiovascular risk. Although the tolerability profile, even compared to statins, appears optimal, the drug in real-life settings seems to be hindered by issues related to prescriptability and market availability which can add some difficulties to therapeutic continuity and in reducing LDL-target distance.

SEVERE HYPERCHOLESTEROLAEMIA DUE TO AUTOIMMUNE THYROIDITIS WITH LONGSTANDING HYPOTHYROIDISM

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Among the secondary causes of severe hypercholesterolaemia, hypothyroidism is not common. Often, hypothyroidism can be associated with symptoms that may lead to diagnosis, which can, however, be confirmed through blood tests. At other times, the symptoms are less obvious, which can delay diagnosis. We report the case of severe hypercholesterolaemia related to longstanding hypothyroidism due to autoimmune thyroiditis. A 39-year-old male was referred to our dyslipidaemia clinic by a nephrologist after the detection of severe hypercholesterolaemia (total cholesterol 453 mg/dl, HDL 34 mg/dl, triglycerides 218 mg/dl, LDL 375 mg/dl). The patient had consulted the nephrologist for a slight increase in serum creatinine (1.1 mg/dl) during a routine examination. The patient reported having been aware of his severe hyperlipidaemia for at least five years, and during the same period, he complained of progressive fatigue and a feeling of heaviness in his legs, which had been swollen for the past few years. On physical examination, his body mass index was 29.32 kg/m², his skin appeared waxy and dry, with the presence of pitting oedema in the lower limbs, and his hair and eyebrows were thin and sparse. During the visit, the patient underwent a carotid colour-Doppler ultrasound, which was negative, and an electrocardiogram, which showed low voltage QRS complexes. Given this instrumental finding, an echocardiogram was performed, revealing the presence of pericardial effusion (6 mm circumferential). The patient returned for follow-up with test results showing TSH>100 µIU/ml, fT4 of 0.04 pmol/l, TPO antibodies 536 IU/ml, and TG antibodies 56.8 IU/ml. Treatment with levothyroxine was initiated with gradually increasing doses, achieving euthyroidism in two months. With the correction of hypothyroidism, total cholesterol levels decreased to 238 mg/ dl, HDL to 19 mg/dl, triglycerides to 210 mg/dl, and LDL to 178 mg/dl. After two months, the symptoms disappeared, and the oedema in the legs improved. At the subsequent follow-up, there was no longer any evidence of pericarditis. The clinical case we present is of interest due to the striking symptomatology shown by the patient and the fact that the symptoms had been present for five years. Treatment of the hypothyroidism caused by autoimmune thyroiditis resolved the symptoms and corrected the severe hypercholesterolaemia.

REAL-LIFE MULTICENTRIC STUDY ON EFFICACY AND SAFETY OF BEMPEDOIC ACID IN THE OLDEST OLD PATIENTS: PRELIMINARY DATA

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Background: Real-world data on older, multimorbid patients regarding bempedoic acid (BA) are scarce.

Aim: Our study was aimed to evaluate the efficacy and safety of BA (which is reimbursable by the Italian National Health System unlike PCSK9-Is) in this older population.

Methods: Prospective, multicenter study on 22 consecutive patients referred to cardiovascular (CV) prevention centers of Ancona, Chieti, L'Aquila and Terni to diagnose and/or manage non-target hypercholesterolemia. Inclusion criteria: age ≥ 80 years, clinical indication for BA based on baseline LDL-C values and CV risk, assessed with SCORE2-OP charts and ESC 2021 guidelines. Patients were assessed at baseline (TO) and after 1-3 months (T1) from the beginning of BA therapy.

Results: Our 22 patients (M=7, F=15, 68.2%; mean age 83.0±2.4 years), had mean values of body mass index (BMI), and eG-FR-EPI respectively =29.1±5.0 kg/m² and =62.9±21.4 ml/ min/1.73 m². Overall, 59.1% (n=13) patients were in primary and 40.9% (n=9) in secondary prevention, but all were classified as very high CV risk. At baseline, 59.1% (n=13) of patients were taking statin therapy (mainly atorvastatin and rosuvastatin), while 36.1% (n=8) of patients reported previous statin intolerance. Other ongoing lipid-lowering therapies included ezetimibe (94.4%) and anti-PCSK9 (15.4%). After the addition of BA. at T1. LDL-C decreased from 98.0±27.7 mg/dl to 62.9±26.1 mg/dl (-35.1 mg/ dl, p<0.001), with a median of -33.9% (IQ range -42.1% - -22.2%). Based on individual CV risk, the LDL-C target was achieved in 36.4% of patients. No patient discontinued the drug due to adverse events. No statistically significant increase was found both in creatinine (from 1.24±0.44 mg/dl to 1.37±0.54 mg/dl, p=0.616) and in uric acid values (from 6.2±1.1 mg/dl to 6.6±0.8 mg/dl, p=0.477).

Conclusion: Our data show that BA has rapid efficacy and good tolerability even in the older population (over 80 yo). Therefore, this drug could be very useful to optimize lipid-lowering therapy in this high-risk population of CV events, when statin and eze-timibe are not tolerated or not sufficient.

POST-MARKETING EVALUATION OF THE EFFICACY ON LDL OF A NEW NUTRACEUTICAL

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Background: In certain types of patients, lipid-lowering therapy with nutraceuticals can be an alternative to pharmacological treatment (B. Napolitano, SISA 2021-23). The most commonly used active ingredient in various nutraceuticals is monacolin K (MK). Following a European Union directive, the reduction of the dosage to <3 mg/day has necessitated combining it with other molecules to maintain a significant reduction in LDL levels.

Patients and Methods: We aimed to evaluate the efficacy on LDL cholesterol levels [percentage reduction in LDL and percentage of achieving the therapeutic goal (<100 mg/dl)] after three months of therapy with a nutraceutical composed of a combination of 2.9 mg MK, 25 mg of tocopherols and tocotrienols, 10 mg of coenzyme Q10, and 10 mg of fenugreek in one hundred patients with common polygenic hypercholesterolemia, who had a moderate cardiovascular risk (SCORE >0.9 <5% at 10 years) and were consecutively referred to our clinic, and who were unwilling to take statins. Of the one hundred patients, data for one were incomplete and were excluded from the evaluation. Adherence to therapy was assessed using the Morisky scale. Fifty-four patients were female and forty-five male. Table 1 shows the data on age and body mass index (BMI) in Kg/m² before and three months after the start of therapy with the nutraceutical.

Table 1

Age (year)	BMI before	BMI after	BMI difference (%)
56.33±10.46	26.54±3.78	25.87±2.8	-2.52 º
55.33±10.82	26.01±3.94	25.73±3.65	-1.08 ď
57.53±10.25	27.17±3.61	26.03±1.27	-4.20 All (# 99)

Results: Table 2 presents the lipid values in mg/dl, along with the percentage changes, before and three months after the start of therapy with the nutraceutical.

Table 2

	Before therapy	At three months	Difference (%)
Total cholesterol	199.97±21.44	187.91±22.99	- 6.03 ♀
	196.94±22.57	183.44±21.19	- 6.85 °
	203.60±20.16	193.27±24.63	- 5.07 All
HDL cholesterol	54.79±14.96	6 56.10±11.87 + 2.3	
	55.94±16.38	58.39±13.09	+ 4.38 ~
	53.4±13.48	55.33 ± 9.95	- 0.13 All
Triglycerides	116.45±44.50	109.67±50.70	-5.62 º
	107.17±39.57	98.11±37.84	- 8.45 ď
	127.6±48.80	123.53±61.31	- 3.19 All
LDL cholesterol	124.97±22.78	112.33±22.18	- 10.11 º
	122.28±21.36	107.22±19.72	- 12.32 ď
	128.20±14.73	11847±24.05	- 7.59 All

Results: Of all the patients, 30 (30%) achieved the LDL goal of <100 mg/dl. The results of our study showed efficacy indices lower than those reported in the literature (-10.11 *vs.* -17.4% on LDL). Although no specific dietary recommendations were provided, there was a reduction in body weight, which could have also influenced lipid levels.

Conclusions: Our study demonstrated a modest reduction in LDL levels and a mediocre achievement of the therapeutic goal with this combination of nutraceuticals. A better strategy for lipid-lowering nutraceuticals, based on the combination of 2.9 mg of MK with other more potent lipid-lowering molecules, would be desirable to achieve a good therapeutic response.

THE PREVALENCE OF HYPERHOMOCYSTEINAEMIA IS HIGH IN PATIENTS IN SECONDARY CARDIO AND CEREBRO VASCULAR PREVENTION

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Hyperhomocysteinaemia (HHcy) is a pathological condition characterised by elevated plasma homocysteine (PH) levels (>11.1 μ mol/L in females and >13 μ mol/L in males). HHcy is implicated in the onset of various pathological conditions, including cardiovascular and cerebrovascular diseases (CVD). Although the relationship between HHcy and CVD is well known, HHcy

often remains undetected and untreated. We aimed to assess the prevalence of HHcv in a consecutive series of 130 patients undergoing secondary prevention (one or more CVD events) at a clinic dedicated to cardiovascular prevention. Table 1 presents the characteristics of the patients and their associated risk factors. Table 2 shows the vascular events of the patients under study. Of the 130 patients with vascular disease (VD) who consecutively attended our clinic, a high percentage presented with (HHcy) (86.1%); none of them were aware of their condition, and none were receiving therapeutic treatment for HHcy. Patients with HHcy had a higher average age and a lower prevalence of other associated risk factors for VD, except for a higher frequency of chronic kidney disease (CKD). Patients with HHcy exhibited a higher prevalence of myocardial infarction (MI) or revascularisation compared to those without HHcy (78.6% vs. 70%) and a higher prevalence of stroke or revascularisation (37.5% vs. 30%). Despite the relatively small sample size, we demonstrated a high and entirely unrecognised prevalence of HHcy among our patients with VD. Patients with HHcv had a lower frequency of association with classic cardiovascular risk factors and a higher prevalence of cardiac and cerebrovascular disease compared to those without HHcy.

Table 1

	All patients (%)	Woman (%)	Man (%)	All HHey (%)	HHey woman (%)	HHey man (%)	All not HHey (%)
	130	42	88	112 (86.1)	38 (90.5)	74 (84.1)	18 (13.9)
Age (year)	71.77±10.34	73.09±9.71	71.14±10.68	72.9±10.28	73.42±10.04	72.67±10.53	65.4±8.59
Smoke	24 (18.5)	4 (9.5)	20 (22.7)	14 (12.5)	2 (5.3)	12 (16.2)	9 (50)
Hypertens.	104 (80)	30 (71)	30 (34.1)	86 (76.8)	28 (73.7)	58 (74.4)	16 (88.9)
Diabetes	48 (36.9)	6 (14.3)	6 (6.8)	38 (33.9)	4 (10.5)	36 (48.6)	9 (50)
Hypercolest.	112 (86)	34 (80.9)	34 (38.6)	86 (76.8)	32 (78.9)	64 (86.5)	15 (83)
Chronic Kidney disease (CKD)	32 (24.6)	8 (19)	8 (9.1)	28 (25)	8 (21)	20 (27)	3 (17)

Table 2

	All patients	Woman	Man	All HHey	HHey woman	HHey man	All not HHey
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
MI and/or revasc.	104 (80)	20 (47.6)	84 (95.4)	88 (78.6)	18 (47.4)	70 (94.6)	14 (70)
Stroke and/or revasc.	48 (36.9)	26 (61.9)	74 (84.1)	42 (37.5)	22 (57.9)	20 (27)	6 (30)

MUTATIONS IN SMAD3 GENE: RELATIONSHIP WITH CARDIOVASCULAR FEATURES

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SMAD3 gene is linked to Loeys-Dietz syndrome (LDS) and aortic dissection. Our study investigated connection between rare genetic variants in SMAD3 gene and clinical features in individuals evaluated for Marfan syndrome and Related Disorders at Careggi Hospital in Florence. Targeted Next Generation Sequencing (NGS) approach of at least 17 genes associated with aortopathy has been performed through Illumina technology. Among 327 analyzed, seven SMAD3 rare variants have been identified in 6 patients (4 males and 2 females). Among these, two adjacent missense variants in the same individual led to a premature stop codon. Four variants identified in 4 out of the 6 subjects are located in exons 6 and 8, both encoding MH2 domain. Individuals with dominant negative SMAD3 variant in MH2 domain experience more major events at a younger age than those with haploinsufficient variants. Among these subjects, 2 had type A aortic dissection, one had open surgery for abdominal aortic aneurysm and ascending aortic replacement surgery, and the last spontaneous coronary dissection following a coronary angioplasty intervention. One patient had a splicing variant in intron 7 and underwent mitral valve surgery for mitral cord rupture; he also had root ectasia, ascending aorta replacement, and various aneurysms. In a further subject, clinically presenting aortic dissection, dilated cardiomyopathy, and heart failure, a SMAD3 missense variant located in MH1 domain was identified. The segregation analysis in family members has evidenced that SMAD3 variant has been transmitted to daughters who have mitral valve prolapse with mitral ring disjunction (MAD) associated with arrhythmia and cardiomyopathy, supporting the segregation of SMAD3 variant with the clinical phenotype. Correlation between pathogenic variants in SMAD3 and heart failure are already described in the literature. The results emphasize the importance of molecular characterization in individuals with aortopathy for diagnosis, risk stratification, and treatment planning.

TELEMEDICINE IN OUTPATIENT SETTING IN LIPID CLINIC: GENOA EXPERIENCE

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Background: Telemedicine is the remote diagnosis and treatment of patients by means of telecommunications technology. Main purpose of implementing telemedicine in Lipid Clinic of Policlinic San Martino Hospital in Genoa is to facilitate access to care for patients who live far from the hospital and have difficulty traveling, or for workers or busy patients.

Methods: Using the platform integrated into the hospital's OneSys management software, in collaboration with LIGURIA DIGITALE team, we offer effective and secure virtual consultations. On average, about 40 telemedicine visits are conducted each month. The patients involved are primarily individuals undergoing treatment with PCSK9-inhibiting monoclonal antibodies, which require renewal of the therapeutic plan every 6 months or annually. The age of the patients ranges from 20 to 80 of age. It is essential that each patient has been seen at least once in person, allowing the physician to perform an initial physical examination. The methodology involves patients uploading their recently performed blood tests onto the platform, even in the days preceding the visit. During the teleconsultation, the physician analyzes these tests and takes any necessary measures based on the lipid targets that need to be achieved. All visits are regularly codified.

Results: The observed benefits include a high level of patient satisfaction, as they can usually complete the visit in less than half an hour without facing travel or city traffic, enjoying the comfort of their own home or working place or even during vacation periods. For the medical staff, the management of telematic appointments is simplified, with a significant reduction in patient delays. Time of work is the same of a visit in presence. Furthermore, the absence of physical contact reduces the risk of airborne disease transmission to the most vulnerable patients. The main challenge encountered concerns the difficulty of some patients, especially the elderly, in correctly using the platform, including the management of webcams and microphones. Other limit is the quality of connection sometimes not good, the impossibility to directly measure blood pression and anthropometric variations or to perform an objective exam in case of necessity.

Conclusions: In the future, the goal is to increase the number of services provided through telemedicine, while ensuring the adequacy of clinical practice.

LIPID CHARACTERIZATION OF EXTRACELLULAR VESICLES' MEMBRANES SECRETED BY A METASTATIC MELANOMA CELL LINE: NEW INSIGHTS FOR A POSSIBLE USE AS DRUG-DELIVERY SYSTEM

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Extracellular vesicles (EVs) are to small entities released by cells mostly in pathological conditions (e.g. tumors). EVs transfer their biological cargo (lipids, proteins, genetics materials) from parental to target cells and may be utilized as pathology biomarkers or drug-delivery systems. Despite the broad increase in EVs knowledge, their role is not fully understood yet, due to intrinsic heterogeneity, biological contaminations and to the lack of proper separation methods. To overcome this problem, we proposed a sequential ultracentrifugation method based on the algorithm developed by Livshits et al. 2015, by which we obtained five size- separated EVs subfractions secreted from the human lymph-node metastatic melanoma cell line LM-16. Subfractions' dimensions ranged between >200 nm and <50 nm, with the smallest compehending "non-vesicular extracellular par-ticles (NVEPs)" or "exomeres", following MISEV 2023 Guidelines. This method has been validated by dimensional- (zetasizer, TEM, AFM), lipidomics and proteomics analysis (GLC, LC-MS, WB).Lipidomics document an increase of saturated fatty acid concomitant with a decrease of monounsaturated ones, ranging from parental cells to exomeres, together with a different distribution of free cholesterol/phospholipid. These data, merged with those from AFM suggest different membrane structure and fluidity among fractions. Intriguingly, the exomeres fraction is the smallest, most saturated and stiffest, thus conferring increased cargo protection and differential membrane exchanges. New series of experiments confirmed that the smaller the particles are, the less prone are to opening with chemical tools. Finally, preliminary data suggest that EVs subfractions after opening display a decrease of saturated fatty acid and a relative increase in unsaturated ones, suggesting membrane changes and calling for more in vitro proofs to validate the quality of vesicles for a future drug-delivery system. We are already setting functional assays to evaluate membrane properties before and after opening and/or pharmacological treatment of parental cells.

PRELIMINARY DATA OF INCLISIRAN TREATMENT IN PATIENTS WITH HYPERLIPIDEMIA: DATA FROM TWO ITALIAN CENTERS, CHIERI & CUNEO (C&C STUDY)

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Objective: This study presents preliminary data from a clinical evaluation of inclisiran, a small interfering RNA that targets PCSK9 (proprotein convertase subtilisin/kexin type 9), in ten patients with hyperlipidemia across two centers in Italy. The primary aim was to assess the safety and efficacy of inclisiran in reducing low-density lipoprotein cholesterol (LDL-C) levels in this cohort.

Methods: A total of ten patients with a history of elevated LDL-C levels were enrolled (9 in Chieri Hospital) and 1 in Cuneo Hospital). Baseline LDL-C levels were measured, and participants received an initial dose of inclisiran, followed by a second dose at three months. Lipid profiles were assessed at baseline and three months post-treatment initiation. Safety evaluations included monitoring for adverse events and laboratory parameters.

Results: Preliminary findings indicate an apparent worsening in LDL-C levels at three months in the whole group of patients (from 117.3±66.8 mg/dl to 134.6±82.0 mg/d). On the contrary, four individuals not previously treated with any prior lipid-lowering therapies showed a significant LDL reduction at three months (from 181.9±39.5 mg/dl to 130.7±50.4 mg/d).The treatment was generally well-tolerated.

Conclusion: These findings suggest that inclisiran may offer significant benefits in lowering cholesterol levels among individuals with hyperlipidemia, particularly those who have not previously been exposed to lipid-lowering medications. Further investigation with a larger sample size and longer follow-up is warranted to confirm these early results and to establish the long-term safety and efficacy of inclisiran in this population.

CLINICAL AND INSTRUMENTAL ASSESSMENT OF CARDIOVASCULAR RISK IN A COHORT OF PATIENTS AFFECTED BY COMMON VARIABLE IMMUNODEFICIENCY

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Background: The immune system plays a central role in atherosclerosis. Common Variable Immunodeficiency (CVID) is a rare immunological disorder characterized by a deficiency in antibody production due to altered functionality of B-Lymphocytes. The impact of common cardiovascular risk factors on the development of early vascular damage and atherosclerosis progression in these patients remains unclear.

AIM of the Study: To assess the impact of common cardiovascular risk factors and immunological risk factors on early vascular damage in patients affected by CVID.

Materials and Methods: We conducted an observational study on 84 CVID patients followed at the Treviso Hospital. For each patient, we collected medical history, anthropometric data, biochemical parameters, and immunological characteristics. Vascular damage was estimated by determining Pulse-Wave Velocity (PWV) and measuring the intima-media thickness (IMT) of common carotid artery.

Results: The average age of our population was 53.9±13.0 years, with a higher prevalence of females (63.1%). To estimate the impact of cardiovascular risk factors on vascular damage, patients were stratified by the presence or absence of the following conditions: overweight (BMI>25 kg/m²), hypertension (BP>140/90 mmHg), dyslipidemia (LDL>115 mg/dl), diabetes (HbA1c≥48 mmol/mol), smoking habits, and immunological phenotype according to the Chapel classification ("infection only" vs. "complicated"). Only overweight (p=0.004) and hypertensive (p=0.024) patients showed a significant increase in Pulse-Wave Velocity compared to patients without these characteristics; however, this significance was lost in multivariate regression analysis. No statistically significant difference was observed among the different subgroups regarding carotid IMT values. Stratification by Chapel phenotype showed no differences between the two clinical phenotypes in terms of either PWV or carotid IMT values.

Conclusions: In patients with Common Variable Immunodeficiency, common cardiovascular risk factors seem to contribute to an increase in Pulse-Wave Velocity without significant differences in early atherosclerotic damage. Further studies are needed to determine whether immune system dysfunction may be associated with a reduced progression of atherosclerosis.

CARDIOVASCULAR RISK STRATIFICATION IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE: THE ROLE OF NON-INVASIVE IMAGING AND TRADITIONAL RISK SCORES

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Patients with chronic inflammatory diseases, including inflammatory bowel diseases (IBD), have a 20% increased risk for atherosclerotic cardiovascular disease (ASCVD) as compared to non-inflamed subjects. The current validated cardiovascular(CV) risk stratification algorithms are based on traditional risk factors, not taking into account the contribution of chronic inflammation. Therefore, these algorithms could underestimate the actual CVrisk in patients with IBD. Our study aimed to stratify the CV risk of IBD patients using validated scores (SCORE2/ SCORE2-OP/SCORE-2Diabetes) and performing carotid ultrasonography to identify subclinical atherosclerosis. Data from 80 in-/outpatients with Crohn's disease (CD) and ulcerative colitis (UC) aged ≥40 years under care in the Gastroenterology and IBD Unit of the AOU Policlinico G. Martino-Messina (April-to-July 2024) were collected. We recorded data on age, gender, region of origin, body mass index, smoking history, family, personal and pharmacological history, blood pressure values, biochemistry (creatinine, fasting glucose, glycated hemoglobin, total cholesterol, HDL-cholesterol, triglycerides); LDL-C and non-HDL-C were thus calculated. The clinical disease activity in IBD patients was assessed using the validated scores. Based on their medical history, 10% of patients were already classified at very-high CVrisk; 30% of patients had high CVrisk, and 60% had moderate CVrisk. After performing carotid ultra sonography, 30% of the moderate-risk individuals were re-classified as high risk because of evidence of asymptomatic atherosclerosis. Our study demonstrates that carotid-US contributed to re-classification of the CVrisk in patients who were previously considered to be in lower risk categories confirming that the use of traditional risk scores underestimates CVrisk in a significant number of IBD patients. Despite the limited data in the literature and the lack of guidelines on screening and managing patients with risk factors, CVrisk stratification before starting biotechnological or small molecule therapies could effectively guide clinicians in choosing the most appropriate therapy with fewer side effects (including CV side effects) for the patient.

HIGH TRIGLYCERIDES AND **RENAL DAMAGE: INSIGHTS FROM** FAMILIAL AND MULTIFACTORIAL HYPERTRIGLYCERIDEMIA IN ITALIAN AND CANADIAN COHORTS

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Background and Aim: The occurrence and consequences of severe hypertriglyceridemia (SHTG, TGs≥500mg/dl) on kidney function are poorly understood. Patients with SHTG presenting proteinuria or kidney disease are generally excluded from clinical trials. The objective of this study was to evaluate kidney function in patients with SHTG and chylomicronemia (TG>890 mg/dl) due to familial (FCS) or multifactorial (MCS) chylomicronemia syndrome.

Methods: The study population comprises 84 molecularly characterized patients with SHTG (38 FCS and 46 MCS) from Europe (Rome, Italy N=60) and French Canada (N=24). Data were retrospectively collected. Abnormalities of kidney function was defined by the presence, at any time during follow-up, of proteinuria (defined as any of the following: protein in the urine spot ≥30 mg/dl, urinary protein $\geq 150 mg/24$ -hour, urine albumin >3 mg/24-hour, urine albumin >mmol or albumin creatinine ratio (ACR) >30 mg/g) or decline of eGFR calculated by CKD-EPI formula. Two kidney biopsies were conducted on two patients with nephrotic syndrome and in one case histological examination was carried out on renal tissue from autopsy material to provide a translational understanding. Histological examination and electron microscopy were performed.

Results: At entry, the median (IQR) TG levels was 1675 mg/ dl (1016-2667 mg/dl). As expected FCS patients exhibited very high levels of TGs with a high prevalence of AP episodes compared to MCS patients in both cohorts. Overall, proteinuria was observed in more than 50% of FCS patients. Specifically, 52.6% of FCS patients had a history of proteinuria compared to 34.8% of MCS patients, while 52.2% of MCS patients experienced a decline in eGFR (<90 ml/min) compared to 44.7% of FCS patients (P=NS). Proteinuria tended to importantly fluctuate over time in both groups. During the observational period, four patients developed nephrotic syndrome. Of these, two underwent kidney biopsies and histological examinations revealed characteristic features consistent with lipoprotein-related glomerulopathy, including lipid deposits and mesangial expansion. Additionally, one patient's renal tissue, obtained post-mortem following an acute pancreatitis crisis, exhibited similar pathological findings

with numerous brown stained foam cells within the glomeruli, indicating significant macrophage infiltration and underscoring the potential role of lipid accumulation in glomerular injury. Conclusions: These findings suggest that in SHTG, the occurrence of kidney disease may be more common than expected, with a tendency towards a higher rate in FCS than MCS. Kidney abnormalities may be due to lipoprotein accumulation in the glomerular structure. The mechanisms explaining renal abnormalities in SHTG remain to be elucidated.

CONTINUOUS GLUCOSE MONITORING (CGM) AND LIPID PROFILE IN A **POPULATION OF YOUTH WITH TYPE 1** DIABETES

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The aim of the study was to evaluate the association between the lipid profile and glycemic control metrics obtained through Continuous Glucose Monitoring (CGM). Studies on type 1 diabetes (T1D) in pediatric patients are scarce and have highlighted a positive association between the coefficient of variation (CV) and HDL cholesterol levels, as well as between the time spent in hyperglycemia (TAR) and triglyceride levels. Seventy-seven patients with T1D were studied (age: 16.9±3.1 years; disease duration: 7.2±3.4 years; HbA1c: 7.1±1.1%). All patients were using a CGM (13 were using an insulin pump and 63 were on multiple daily injections therapy). The results show a significant correlation between HDL-C and TIR (Time in Range, 70-180 mg/dL), Pearson's r: +0.285, p<0.02. Additionally, patients with target HbA1c (<7.0%) had lower LDL-C levels (88.9±26.4 vs. 102±27.6, P<0.04), and patients with target HDL-C levels showed reduced Glucose Management Indicator (GMI) levels (7.1±0.9% vs. 7.8±1.0%; p<0.04). Finally, target triglyceride levels were associated with a significantly lower CV (38.7±6.0 vs. 42.8±7.9%, p<0.05). The use of CGM metrics to optimize the management of T1D can help improve not only glycemic control but also provide insights into the improvement of the lipid profile in patients with T1D. Further studies will be needed to understand how these parameters relate to long-term cardiovascular risk.

THE EFFICACY OF BEMPEDOIC ACID THERAPY IN NO-TARGET PATIENTS TREATED WITH PCSK9 INHIBITORS

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Introduction: PCSK9 inhibitors revolutionized the approach to patient with cardiovascular risk, in primary and secondary prevention, allowing the achievement of their therapeutic objectives in almost all cases. Some patients, despite complete lipid-lowering therapy fail to reach the target recommended for their risk class. Bempedoic acid, recently introduced, could represent a

support to therapy with PCSK9 inhibitors in achieving therapeutic objectives. **Purpose:** The aim was to evaluate efficacy of the addition of bempedoic acid to patients followed by the University of Naples Federico II and treated for at least 6 months exclusively with PCSK9 inhibitors, because intolerant to statins and ezetimibe,

who had not reached the target according to the ESC 2019 guidelines. Sample: We enrolled 26 patients, 11 Male (42.3%) and 15 female

(57.7%), with mean age of 56.1 ± 11.4 years. **Methods:** For each patient at the initial visit (Visit 1), the clin-

Methods: For each patient at the linual visit (visit 1), the chirical and pharmacological history was collected, as well as the presence of modifiable cardiovascular risk factors to calculate their SCORE risk. At the first follow-up (Visit 2) after prescribing bempedoic acid, total cholesterol (C-TOT), HDL, triglycerides (TGL) and LDL-c were assessed and compared with Visit 1. In both visits, uric acid (RH), creatinemia (CREA) and glomerular filtration rate (eGFR) were evaluated to monitor the safety of therapy.

Results: At Visit 1, C-TOT values were 195.1±51.3 mg/dl, with a mean SCORE risk of 5.7±3.5 % and LDL-c values of 118.1±50.7 mg/dl; at Visit 2, C-TOT values were 153.3±61 mg/dl, while LDL-c values were 81.1±54.8 mg/dl; there was a reduction of 41.0±56.1mg/dl of LDL-c, statistically significant (p value<0.05), without changes in RH and CREA.

Conclusions: bempedoic acid added to PCSK9 inhibitor represents a safe tool for achieving the therapeutic target, reducing LDL-c of 20% and patients'SCORE risk.

CLINICAL MANAGEMENT OF AUTOSOMAL RECESSIVE HYPERCHOLESTEROLEMIA (ARH): A REAL LIFE EXPERIENCE

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Homozygous familial hypercholesterolemia (HoFH) is a rare disorder of lipid metabolism characterized by increased plasma LDL-cholesterol levels, accelerated atherosclerotic process, and early cardiovascular events. The underlying genetic defects involve loss-of-function mutations in the gene encoding for the LDL -LDLR receptor (most frequent); mutations in the APOB gene; and gain-of-function mutations in the PCSK9 gene. Another rare form with recessive transmission is caused by mutations in the LDLRAP1 gene, coding for the LDL receptor adaptor protein. Individuals with this condition therefore require standard hypolipidemic therapy, based on the use of statins, ezetimibe, combined with low lipid diet therapy and LDL apheresis procedures. We report the management of a 51-year-old patient with homozygous recessive familial hypercholesterolemia. She was born from consanguineous parents, and her family history is positive for severe hypercholesterolemia and acute cardiovascular events at an early age. On clinical examination, she presented with xanthelasmas, mild obesity (BMI: 35.7) and very high levels of LDL-C (400mg/dl). Molecular genetic analysis confirmed the ins432A mutation in the LDLRAP1 gene in homozygosity. The patient therefore started standard hypolipidemic treatment with a highly effective statin and ezetimibe with little benefit. She was started on treatment with Lomitapide in combination with her existing conventional hypolipidemic treatment. This treatment was worth a significant reduction in LDL-C values of more than 70% from baseline with a good safety profile at each quarterly follow-up checkup performed. This case report describes the efficacy, safety and tolerability of Lomitapide in real life as primary prevention in a patient with homozygous recessive familial hypercholesterolemia (ARH).

COMBINATION OF BEMPEDOIC ACID, PCSK9 INHIBITOR AND EZETIMIBE LEAD TO ACHIEVE THE LDL-C TARGET IN TWO PATIENTS WITH BOTH HE-FH AND LIMB-GIRDLE MUSCULAR DYSTROPHY TYPE 1: A CASE REPORT

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Introduction: Current guidelines recommend that high-risk patients, such as those with heterozygous familial hypercholesterolemia (HeFH), be treated with maximally tolerated statins. For patients with persistent elevated LDL-C or statin intolerance, non-statin therapies like ezetimibe and proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) are recommended. Recently we can consider BA as a possible non statin therapy. Ezetimibe reduces intestinal cholesterol absorption, PCSK9i increase the number and durability of hepatic LDL receptors (LDLR), and BA decreases cholesterol synthesis in the liver. Investigation: A now 66-year-old man referred to us in 2002, 44 years old, was diagnosed with HeFH due to a mutation in LDLR gene (c.1415_1418dupACAT, p.(Q474Hfs*63)). In 2017, following neurological evaluations for persistently elevated CPK levels (up to 2000 U/L), even after discontinuing statin therapy, he was diagnosed with limb-girdle muscular dystrophy type 1, caused by mutations in the calpain 3 gene. In 2024, the patient showed mild stenosis (around 20-25%) on color Doppler ultrasound, and remains under primary prevention.

Results: At baseline in 1994, before starting lipid-lowering therapy, the patient's LDL-C was 299 mg/dL with Ezetimibe 10 mg alone. Since starting Alirocumab 150 mg plus Ezetimibe in 2016, the LDL-C has reached 155 mg/dL (-48.1% from baseline). The addition of BA in 2024 further reduced the LDL-C to 69.4 mg/dL (-76.8% from baseline), within 3 months, with no reported adverse effects.

Conclusions: Patients with HeFH who are intolerant to statins, treated with only PCSK9i plus Ezetimibe frequently do not meet LDL-C threshold. The addition of BA should be considered for patients who remain far from their LDL-C target, especially if they are already on PCSK9i therapy. These lipid-lowering therapies work synergistically to lower LDL-C and have shown reductions in the risk of major adverse cardiovascular events; when combined, they should provide an even greater reduction in cardiovascular disease risk.

COMPARING THE RISK OF SPECIFIC ADVERSE EVENTS BETWEEN STATINS, EZETIMIBE, BEMPEDOIC ACID, PCSK9 INHIBITORS, AND THEIR COMBINATIONS IN PATIENTS WITH DYSLIPIDEMIA: A NETWORK META-ANALYSIS

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Statins, ezetimibe, bempedoic acid, and PCSK9 inhibitors (PCSK9i) are widely used treatments for hypercholesterolemia. Although their safety profile is known, there are no comprehensive comparative assessments. We aimed to compare the risk of muscle-related symptoms, diabetes, liver dysfunction, and neurocognitive disorders between these treatments. We conducted a network meta-analysis according to PRISMA guidelines; databases were searched from inception to November 2023. Inclusion criteria were:

- 1) randomized controlled trials (RCTs) in adults (≥18 years), parallel design, phase II, III or IV;
- 2) English language;
- using statins, ezetimibe, PCSK9i, bempedoic acid, or their combinations as intervention;
- 4) including patients with dyslipidemia;
- reporting the information about any of the selected adverse events;
- 6) a total sample size of ≥ 200 subjects;
- 7) with intervention duration of more than 3 weeks.

Pooled estimates were assessed by both fixed-effect and random-effects models within a frequentist setting, assuming equal heterogeneity across all comparisons. Data were shown in risk ratios (RRs) and their 95% confidence interval or p-score (possibility of having the lowest risk). A total of 310,407 subjects from 143 RCTs were included. Bempedoic acid ranked the lowest risk of myalgia (p-score=0.94). Statins were associated with higher incidence of diabetes (RR 1.12 [1.03, 1.22]), but lower risk of neurocognitive disorders (RR 0.70 [0.60, 0.80]) compared to PCSK9i. Statins plus ezetimibe (RR 1.49 [1.21, 1.84]), statins alone (RR 1.35 [1.14, 1.58]), and bempedoic acid (RR 1.69 [1.20, 2.38]) had a higher risk of liver dysfunction than PCSK9i.In conclusion, PCSK9i appears to have a more favorable safety profile regarding the risk of diabetes and liver dysfunction. Bempedoic acid or statins seem to be a better choice for subjects with high risk of myalgia or neurocognitive disorders, respectively. This information can be valuable when selecting therapy for specific patient subgroups at higher risk of certain adverse events.

HIGH RCV PATIENTS IN PRIMARY PREVENTION, STEPWISE STRATEGY IN REAL LIFE: ECONOMIC IMPACT OF BEMPEDOIC ACID THERAPY IN REACHING THE TARGET

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Background: Lipid-lowering therapy remains one of the fundamental milestones for prevention of cardiovascular events. The availability of new therapeutic approaches (PCSK9 inhibitors and Bempedoic Acid) has allowed us to reach increasingly ambitious LDL-C levels. The guidelines recommend a stepwise approach to improve statin tolerability and reach the target.

Purpose: Evaluate Bempedoic Acid (BA) impact on target achievement and on costs for the Italian National Health System. Methods: We evaluated 151 patients (M 39%, F 61%, mean age 70+10 years, mean BMI 28+5, waist circumference 100+11 cm) who started treatment with BA from March 2023; 89% were in primary prevention, with very high cardiovascular risk (CVR) in 26%, high in 46%, moderate in 20% and low in the remaining 8%.Among these, we selected only patients with high and very high CVR in primary prevention (85 patients, 61% of evaluated patients). 100% (of 85) reported intolerance to statins or to a previous lipid-lowering therapy (ezetimibe, nutraceuticals based on monacolinK). About 1/3 of patients (28.2%) were not on therapy at the time of the baseline visit. 96.5% of patients had LDL-C values not at target (100% of patients with very high CVR), with an average LDL-C value at baseline of 131±44 mg/dl and an average distance to target (DTT) of 45%. These patients were not eligible according to the prescription form, but not at target according to the guidelines and therefore eligible for antiPCSK9. The choice to treat with BA was aimed to demonstrate its economic advantage. 54 patients (of 85) performed a first evaluation after 1-3 months and 21 patients already have an evaluation at 8-12 months; during the study period 20 patients stopped treatment before three months, 14 (16.5%) for reasons not related to the efficacy/tolerability of the drug; and 6 (7.1%) for adverse events (myalgia, headache, gastrointestinal disorders).63.4% of patients continued BA therapy at 3 months reaching a mean LDL-C value of 88±26 mg/dl, with a mean reduction of 37% compared to baseline, a mean DTT of 31%. 26% of patients fully reached the LDL-C target. Sub-analyses were performed distinguishing patients based on pharmacological therapy (28% BA alone, 35% BA+ezetimibe, 32% BA + ezetimibe + low dose high intensity statin). From this it was found that BA alone is not able to bring the patient with high and very high CVR to the target (nobody achieved the target). Combination therapy with ezetimibe (FDC) allowed to reach the target in 9.3% of patients (patients with high CVR and with an average reduction of LDL-C of 40% compared to baseline and 54% compared to naïve LDL-C values). Triple therapy instead brought 16.7% to the target (3.7% of patients with very high CVR) with a further LDL-C reduction of 31% with BA compared to background (effect of BA alone). Patients with

very high CVR had a baseline DTT of 49% and the addition of a PCSK9i would have brought them to target. We performed a cost analysis of lipid-lowering therapies and estimated that BA alone costs on average 45-53 euros per year for each percentage point of reduction in LDL-C. BA+ezetimibe costs on average 29-39 euros per year for each percentage point, while therapy with iPCSK9 costs 168-187 euros per year for each percentage point. **Conclusions:** Our study demonstrates that in patients with high CVR, in primary prevention, intolerant to statins, and therefore potentially eligible for therapy with anti-PCSK9, the use of the stepwise strategy allows to reach the objectives set with an effective cost-effective therapy.

A VAGAL-DEPENDENT PERIPRANDIAL INCREASE OF NEUTROPHILS IN BLOOD PATROLS METABOLIC ADAPTATION TO HIGH FAT DIET FEEDING

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Background: Everyone spends most of the time in a "periprandial" state, consuming each meal results into a "post-prandial" situation that both involves the intestinal absorption and the hepatic metabolism of nutrients and associates with a prominent, acute increase of neutrophils in blood. The vagus nerve connects the brain with the intestine to regulate the absorption of nutrients and we now ask ourselves whether it could be also involved in the periprandial increase of neutrophils in blood.

Methods: We studied the metabolic and the immunophenotypic periprandial profile of mice models lacking the α 7 isoform of the Acetylcholine receptor (α 7nAChR KO, n=5), compared to that of wild-type (WT, n=5), fed either a standard fat diet (STD) or a high-fat diet (60% energy from fats; HFD). After one week of feeding, the periprandial profile was studied by inverse calorimetry and by collecting blood from tail vein at "zeitgeber times" (ZT): ZT1, ZT5 and ZT10 (day), ZT17 and ZT22 (night). We measured blood glucose, triglycerides, cholesterol and flow-cytometry-based analyses was performed.

Results: α 7nAChR KO consumed less food over the periprandial condition compared to WT mice, despite a comparable energy consumption, both upon chow and HFD feeding. However, periprandial glucose levels were persistently higher in α 7nAChR KO and triglycerides increased more in nocturnal stages. Of note, the periprandial blood levels of Ly6G+ neutrophils increased upon STD feeding both in WT and α 7nAChR KO while, upon HFD feeding, they remained stable in α 7nAChR KO. Also, while the periprandial profile of Ly6C+ monocytes were comparable between both groups, the blood levels of CD19+ B cells reduced in WT upon HFD feeding, while they increased in α 7nAChR KO, suggesting a myeloid skewing of the immune profile starting from the bone marrow.

Conclusions: Our data suggest a vagal-dependent, immune skewing towards an increase of neutrophils during the periprandial metabolic adaptations to high fat diet feeding.

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