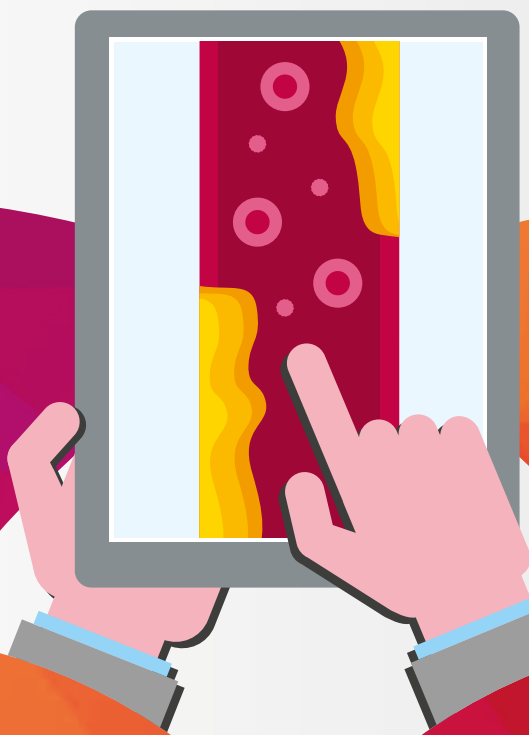
34° CONGRESSO NAZIONALE S.I.S.A.

VIRTUAL EDITION

22-23-24 Novembre 2020
FAD SINCRONA IN LIVE STREAMING

22 Novembre 2020 > 22 Febbraio 2021
FAD ON DEMAND - PERCORSI 1 / 2



SOCIETA' ITALIANA
PER LO STUDIO
DELL'ATEROSCLEROSI

Il 34° Congresso Nazionale della Società Italiana per lo Studio dell'Aterosclerosi (SISA) quest'anno si svolgerà dal 22 novembre in modalità Virtuale durante la quale si alterneranno sessioni live streaming ed altre on demand. Come consuetudine, il Congresso affronterà diversi argomenti di interesse nell'ambito della cura delle patologie cardiovascolari aterosclerotiche.

Per rimanere nella attualità del momento, si prevede di organizzare una Tavola Rotonda che affronterà la gestione del rischio cardiovascolare in un momento di pandemia infettiva prendendo in considerazione sia gli aspetti clinici sia soprattutto quelli organizzativi.

Altre tematiche saranno legate ai rapporti tra metabolismo lipidico e decadimento cognitivo che si configura come una grande emergenza epidemiologica. Inoltre, una specifica sessione live streaming sarà dedicata ad esaminare i vantaggi e i limiti degli interventi terapeutici nella prevenzione delle malattie cardiovascolari nel paziente anziano. Infine, saranno affrontati, grazie alla presenza live di esperti, anche alcuni temi controversi relativi alle scelte nutrizionali orientate alla prevenzione cardiometabolica, come quelle inerenti alla riduzione del consumo di sale, al controllo dell'uso dell'alcool o degli zuccheri.

Altre sessioni live saranno dedicate all'esame delle evidenze che la ricerca cellulare e molecolare sta offrendo per l'individuazione di futuri possibili bersagli terapeutici della aterosclerosi e delle sue complicanze d'organo (miRNA, metaboloma, vescicole extracellulari etc.).

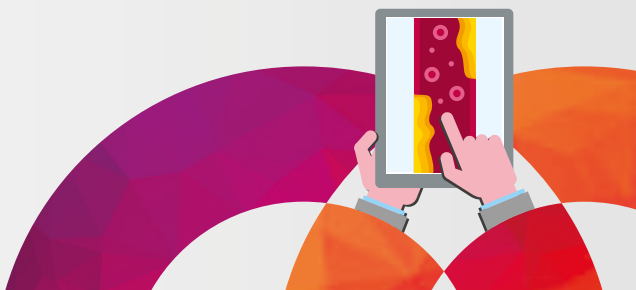
Ampio spazio sarà inoltre dedicato a discutere i risultati più recenti dell'uso degli inibitori della proteina PCSK9 nella prevenzione delle complicanze ischemiche e ad esaminare quali nuove strategie farmacologiche PRESENTAZIONE si stanno affacciano all'orizzonte della terapia dell'ipercolesterolemia (acido bempedoico, inclisiran, inibitori ANGPTL3).

Attraverso sessioni on demand, ampio spazio sarà riservato alla descrizione di due importanti dislipidemie rare, l'ipercolesterolemia familiare omozigote (HoFH) e la sindrome iperchilomicronemica familiare (FCS), per le quali finalmente abbiamo la disponibilità di farmaci efficaci. Il capitolo delle malattie rare del metabolismo sarà ulteriormente approfondito attraverso un minicorso che si svolgerà in formato on demand, nel quale saranno esaminate le patologie del metabolismo intermedio (lipodistrofie, glicogenosi, Niemann-Pick etc.). Un secondo minicorso sarà anche dedicato a fare il punto sulla ricerca che la SISA sta dedicando all'ipercolesterolemia familiare (Progetto LIPIGEN).

Un'altra sessione on demand affronterà temi emergenti nelle strategie di prevenzione della aterosclerosi, quali l'uso dei test genetici, l'imaging cardiovascolare o l'impiego in nuove formulazioni o indicazioni dei farmaci convenzionali (ad esempio l'impiego degli SLGT2 inibitori nella prevenzione dello scompenso). Infine, il Congresso vuole gettare uno sguardo nel futuro affrontando le potenzialità e prospettive dell'impiego dell'Intelligenza Artificiale nello studio delle malattie cardiovascolari.

Il Congresso sarà inoltra la vetrina per l'attività dei ricercatori italiani più giovani che studiano i numerosi aspetti della fisiopatologia del danno vascolare che anche quest'anno si realizzerà attraverso lo svolgimento live degli Spazio Giovani e delle Comunicazioni Orali e dei Poster alle quali abbiamo potuto garantire ampi spazi di interattività on demand.

Prof. Marcello Arca
Presidente SISA



RIASSUNTO DELLE COMUNICAZIONI PRESENTATE AL 34° CONGRESSO NAZIONALE S.I.S.A.

HORMONE TREATMENT IMPACTS ON HIGH DENSITY LIPOPROTEIN FUNCTION IN TRANSGENDER PEOPLE

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Introduction. Previous studies have shown a decrease in HDL cholesterol concentration during transgender hormone therapy. However, the ability of HDL to remove cholesterol from macrophages, termed cholesterol efflux capacity (CEC), has proven to be a better predictor of cardiovascular disease. As transgender individuals are exposed to lifelong exogenous hormone administration, it becomes of interest to study whether HDL-CEC is affected by hormone therapy.
Aim. To evaluate HDL-CEC in 15 trans men and 15 trans women at baseline and after one year of hormone therapy. Methods. Trans men were treated with testosterone gel in a daily dose of 50 mg or a mix of testosterone esters in 250 mg injections once per three weeks or 1000 mg injections of testosterone undecanoate once per twelve weeks. Trans women were treated with twice daily oral estradiol valerate 2 mg tablets or a transdermal preparation in a twice weekly dose of 100 µg/day. Cyproterone acetate (daily dose of 50 mg), was prescribed to all transwomen. HDL CEC was evaluated by using a cell-based radioisotopic technique. We evaluated total HDL-CEC from macrophages and its major contributors, the ATP-binding cassette transporters (ABC) A1 and ABCG1 HDL-CEC and HDL-CEC by aqueous diffusion.
Results. In trans women, total HDL-CEC decreased by 10.8% ($p<0.001$), ABCA1 HDL-CEC decreased by 23.8% ($p<0.001$) and aqueous diffusion HDL-CEC by 4.8% ($p<0.01$). In trans men, non-significant changes were found in total and ABCA1 HDL-CEC: -6.7% and -0.7%, respectively. Conversely, aqueous diffusion HDL-CEC significantly decreased, with a 9.8% ($p<0.01$) reduction. No differences in ABCG1 HDL-CEC were observed in both groups.
Conclusion. Total HDL-CEC decreased during hormone therapy in trans women, through a specific reduction in ABCA1 HDL-CEC. This finding might contribute to a higher cardiovascular disease risk observed in these subjects, despite its lowering effect on other risk factors.

SIZE AND CONTENT CHARACTERIZATION OF DIFFERENT EV POPULATIONS HARVESTED FROM TUMOR CELL LINES FOR A NOVEL PHARMACOLOGICAL APPROACH IN TREATING PROLIFERATIVE DISEASES

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Introduction. Extracellular vesicles (EVs) are physiologically secreted by cells (particularly during inflammation and in cancer), which deliver nucleic acids, bioactive lipids, proteins and enzymes to recipient distant cells. Latest guidelines classify EVs as small- (50-80 nm), large- (80-120 nm) exosomes, microvesicles (120-1000 nm). A new interesting population of nanoparticles with unknown functions (exomeres; <50 nm) also emerged.Aims Since lack of characterization and separation methods impairs the comprehension of EVs origins and functions, we:
a) set up a method to separate, quantify and characterize (dimensions, composition) the abovementioned EVs subpopulations.
b) pharmacologically modulate EVs synthesis/secretion, by interfering with parental cell lipid metabolism.

Materials and Methods. EVs harvested from lymph-node melanoma- and prostatic metastatic cell lines are then separated by differential ultracentrifugation and dimensionally analyzed by Transmission Electron Microscopy, Z-sizer, nanocyte. The content in total fatty acids is calculated after GLC.
Results. Differences in size of the five EV populations previously assessed by nanocyte have been further confirmed both by TEM and Z-sizer. Moreover, from a lipidomic point of view, a strong correlation exists between decrease in EVs size and increase in saturated fatty acids, ranging from microvesicles (35.9%), whose fatty acids profile resembles that of parental cells, to exomeres (65.3%). We also found differences in cholesterol and phospholipid mass in each fraction but lipidomics is now undergoing. On the same specimens we also run detailed proteomics. Preliminary data suggest the existence of various unique proteins in each fraction (of among >4000 found in EVs), that we are validating as potential biomarkers. Based on these findings we are running gene ontology to unravel the function(s) of each fraction, based on their gene and protein content.We also started the pharmacological modulations of parental cells with simvastatin and KT182, respectively involved in cholesterol and phospholipid metabolism.
Future Aims. The goal will be to administer different EVs populations harvested from naïve- or pharmacologically-treated cells to prove their effects in functional tests (e.g. proliferation, apoptosis, migration, invasion), to shed light on their pathophysiological roles, for a possible new pharmacological approach or for the discovery of novel tumor biomarkers.

ALTERATION IN GUT MICROBIOTA COMPOSITION AND FUNCTIONAL RELEVANCE IN SUBCLINICAL CAROTID ATHEROSCLEROSIS

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Aims. Variations in Gut Microbiota (GM) composition and function associate with advanced cardiovascular diseases (CVDs); whether this also holds true at early stages of CVD is not known. We profiled GM in subjects without diabetes, metabolic syndrome and in primary prevention for CVD, to investigate the association between GM profile and markers of Subclinical Carotid Atherosclerosis (SCA).
Methods. Fecal GM composition was assessed (16S rRNA) in 345 subjects from the PLIC population-based study, already analyzed for personal clinical, history, biochemical profile, lifestyle and alimentary habits. Gut Metagenome shotgun sequencing was performed on 23 subjects with advanced ultrasound-based SCA vs 23 age- and gender-matched subjects without SCA.
Results. In presence of SCA, the relative abundance of Bacteroides was reduced, while Escherichia, Coriobacteriaceae and Streptococcaceae were increased. Specifically, Escherichia coli was the metagenomic most abundant marker in samples from subjects with advanced SCA vs those without. At the species level, we observed increased abundance of Fecalibacterium prausnitzii in subjects without SCA, in which pathways related to the synthesis of anti-inflammatory short chain fatty acids (like starch degradation for the synthesis of butyrate) were overrepresented. Vice versa, in presence of advanced SCA, E.Coli was the hit metagenomic marker and pathways involved in virulent activation (alcohols and sugars degradation, palmitate biosynthesis and amino acids metabolism) and in the metabolism of choline, carnitines and substrates of pro-atherogenic molecules (including Trimethylamine N-oxide) were overrepresented. No association between GM and ongoing pharmacological treatments while interaction with lifestyle and alimentary habits was outlined in presence of SCA.
Conclusions. We have identified a unique signature of GM dysbiosis in subjects with SCA among the general population and we have focused attention on bacterial species related to specific metabolic pathways to be considered in primary CVD prevention setting.

THE PROGNOSTIC VALUE OF ULTRASOUND-BASED EVALUATION OF ACHILLES TENDON XANTHOMAS IN FAMILIAL HYPERCHOLESTEROLEMIA: RESULTS FROM THE ACTUS-FH SUB-STUDY OF THE LIPIGEN NETWORK

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Introduction. Achilles tendon xanthomas (ATX) are a pathognomonic feature of familial hypercholesterolemia (FH), included in the Dutch Lipid Clinic Network (DLCN) diagnostic algorithm. We aim to evaluate the value of ATX by ultrasound (US-ATX) as a prognostic tool, in addition to physical examination (PE-ATX), in the management of FH.
Methods. The ACTUS-FH (AChilles Tendon UltraSonography in Familial Hypercholesterolemia) Group was built up within the LIPIGEN network. Beyond the clinical, biochemical, and genetic data collected in the LIPIGEN database, each Center provided information on PE-ATX and US-ATX (focal hypoechoic lesions) and measurement of the maximum thickness bilaterally on each tendon.
Results. A total of 769 adult clinical FH patients (49.5% males, mean age[SD] 47.4[14.8] years, 578 [75.2%] positive at genetic test) were included in this analysis. As compared to PE-ATX, US-ATX improved the detection of ATX, from 9.75% to 33.16%, and increased the proportion of patients classified as definite FH according to DLCN, from 32.5% to 43.2%. Patients with US-ATX were more likely to be mutation-positive than patients without ATX (94.9% vs. 65.4%). Of note, maximum tendon thickness correlated with untreated LDL-C levels only in mutation-positive patients (rho 0.39, p<0.001), and it was not able to discriminate between genetically positive or negative patients (ROC curve: sensitivity 0.34, specificity 0.87). By dividing the ACTUS-FH cohort according to absence of any xanthomas vs. presence of only US-ATX vs. presence of both, we found a gradient of increased severity across the three groups for age (45.5[15.3] vs. 50.2[12.7] vs. 53.5[13.2] years; p<.0001), untreated LDL-C (254.4[63.4] vs. 300.5[78.5] vs. 347.6[85.3] mg/dl; p<.0001, and prevalence of cardiovascular disease (9.53% vs. 16.67% vs. 29.33%; p<.0001).
Conclusions. The ACTUS-FH sub-study showed that the ultrasound-based detection of tendon xanthomas might serve as a tool to improve the recognition of the most severe FH phenotypes.

A REDUCED ACTIVITY OF PLASMA SUPEROXIDE DISMUTASE IS ASSOCIATED WITH HIGH ON-ASPIRIN PLATELET REACTIVITY IN TYPE 2 DIABETES MELLITUS AND PRIMARY HYPERCHOLESTEROLEMIA

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Several lines of evidence suggest a strong correlation between metabolic disorders and cardiovascular diseases (CVD). Oxidative stress may be considered a "common soil" able to create a feed-forward cycle that can deeply influence the development of a prothrombotic tendency also due to platelet hyperreactivity. Aspirin, a suppressor of thromboxane (TX)A2 synthesis by irreversibly inhibiting cyclooxygenase-1 (COX-1), is extensively used as medication in CVD prevention. However, some patients on aspirin show a higher than expected platelet reactivity. Aim of this study was to investigate platelet function in type 2 diabetes mellitus (T2DM) or primary hypercholesterolemia (HC) and its relationships between a pattern of pro- and anti-oxidant, inflammation and endothelial dysfunction biomarkers. To this end, in T2DM (n=103) and HC (n=61) patients treated with 100 mg/die aspirin we evaluated:

1) light transmission aggregometry (LTA) to arachidonic acid (AA), collagen and ADP; response to platelet function analyser (PFA)-100 with collagen/epinephrine (CEPI) cartridges; serum TXB2 and urinary 11-dehydro-(11-dh)TXB2;
2) plasma superoxide dismutase (SOD) activity, and urinary 8-iso-prostaglandin F2alpha (8-iso-PGF2alpha) as markers of redox status and a pattern of markers of inflammation, endothelial dysfunction and platelet activation.

As results, in T2DM and HC subjects, respectively, a comparable prevalence of high on-aspirin platelet reactivity (HPR) was found for LTA-AA (12% and 11%, p=ns), LTA-collagen (9% and 13%, p=ns), 11-dhTXB2 (26% vs 25%, p=ns) or CEPI PFA-100 assays (23% vs 25%, p=ns). Based on response to CEPI PFA-100, in comparison with HPR-, HPR+ showed lower SOD activity in both T2DM ($p<0.0001$) and HC ($p=0.02$) subjects. In a stepwise linear regression, SOD was the only predictor of platelet reactivity. To conclude, in T2DM and HC, similarly, the impairment of redox equilibrium associated with a decrease of SOD activity could contribute to a suboptimal response to aspirin.

ANTIPLATELETS EFFECT OF ALBUMIN INFUSION IN PATIENTS WITH CONGENITAL ANALBUMINEMIA

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Background. Congenital analbuminemia is a rare autosomic recessive inherited disorder characterized by strongly decreased concentration, or complete absence, of serum albumin (SA). Several lines of evidence indicate that SA has an anti-coagulant and anti-thrombotic effect as supported by inhibitory effect of albumin on platelet aggregation. However, in vivo platelet function and the role of oxidative stress as mechanism promoting platelet activation have never been studied in analbuminaemic patients.
Methods. We report two cases of congenital analbuminemia (1.0 g/dL) in a 29-year-old male and in in a 67-year-old woman. Human albumin (40 g) was infused over a period of 30 min'. Blood was collected immediately before infusion (T0) and 2 hours later (T2h) to evaluate platelet function by aggregation, sCD40L and surface αIIbβ3 integrin and P-selectin expression. Platelet oxidative stress was evaluated by NOX2-derived peptide (sNOX2-dp) and 8-iso-PGF2α-III. In the male subject, using flow cytometry, we measured the integrin activation and the α-granule secretion, before and 2h after albumin infusion in naïve platelets and in platelets treated with a P2Y12 inhibitor or with aspirin and stimulated with or without PAR1p, or ADP or convulxin.
Results. On admission (T0), patients displayed higher platelet aggregation and plasma levels of sCD40L and increased level of sNOX2dp, and 8-iso-PGF2α compared to healthy subjects. A significant and positive association was found between platelet aggregation and 8-iso-PGF2α ($p=0.01$, $r2=0.972$), platelet aggregation and sNOX2dp ($p=0.02$, $r2=0.953$), sCD40L and 8-iso-PGF2α ($p=0.02$, $r2=0.919$), sCD40L and sNOX2dp ($p=0.03$, $r2=0.939$). Two hours after infusion (T2), serum albumin levels rose from 20 to 26 g/dL coincidentally with a significant reduction of platelet aggregation, sCD40L levels and of oxidative stress biomarkers. Moreover, we found a decreased expression of markers of platelet activation and platelet-leukocyte aggregates after albumin infusion compared to T0. Finally, treatment with either aspirin or a P2Y12 inhibitor reduced integrin activation, but not α-granule secretion, more effectively after infusion with albumin.
Conclusion. Analbuminaemic patients show higher platelet activation and oxidative stress. Infusion of albumin reduces platelet activation by decreasing oxidative stress. Albumin could also help reduce the high prothrombotic tendency of these patients by increasing the anti-thrombotic effect of antiplatelet drugs.

EFFECT OF STATINS ON LIVER FUNCTION TESTS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE. A SYSTEMATIC REVIEW AND METANALYSIS

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Background. Patients with non alcoholic fatty liver disease have increase chance to develop cardiovascular disease due to coexistence of cardiometabolic disorders. In particular, the presence of an atherogenic dyslipidemia is a strong risk factor for cardiovascular events. For this reason, NAFLD patients are often prescribed on statins. However, statin liver safety in non-alcoholic fatty liver disease (NAFLD) patients is not well defined. Aim of the study is to summarize data from studies reporting differences in liver function tests (LFTs) in patients treated or not with statins.
Methods. We performed a systematic review of MEDLINE via PubMed and Cochrane (CENTRAL) databases and metanalysis of clinical studies investigating levels of aspartate transaminase (AST), alanine transaminase aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) in NAFLD patients treated or not with statins.
Results. We included 22 studies with 3,473 NAFLD patients. The mean age of patients ranged from 43 to 73 years, and the proportion of women ranged from 20.5% to 60%. All patients included in the interventional studies had raised LFTs at baseline. The mean difference between patients treated and untreated patients was -20.33 U/l (95% Confidence Interval (CI) -28.26/-12.39) for ALT, -13.17 U/l (95%CI -19.55/-6.79) for AST and -15.15 U/l (95%CI -22.62/-7.69) for GGT. These differences were most evident in interventional studies (ALT -27.48 U/l [95%CI -36.89/-18.08]; AST -17.56 U/l 95%CI -25.18/-9.94]; GGT -20.48 U/l [95%CI -28.02/-12.94]).
Conclusion. In interventional studies with statins, ALT, AST and GGT were reduced by 34.8%, 30.6% and 26.1%, respectively, while observational studies showed a null effect suggesting a favorable liver safety in NAFLD patients.

HYPERCHOLESTEROLAEMIA IMPACTS GLYCANS PROFILE ON CIRCULATING IMMUNE CELLS

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Aim. Leukocytes infiltration and cellular extravasation in the vessel wall result from the interaction of endothelial cells with specific receptors on immune cells surface. Most of these receptors present several glycans on their surface, among which Sialic acid (Sia) normally represents the last glycan of the chain. Changes in the composition of glycan branches on immune cells surface impact their function and therefore the immune response. Aim of our study was to profile terminal N-glycan signature on immune cells in hypercholesterolemic conditions.
Methods. Glycan residues expression on circulating T cells and monocytes was investigated by flow cytometry in LDL-R KO mice fed with standard or high cholesterol diet (WTD) for 8 weeks and in patients with heterozygous familial hypercholesterolemia (HeFH). The glycosylation landscape of immune cells was profiled by specific lectins staining, including MAL and SNA to detect α2,3-linked and α2,6-linked Sia respectively, RCA for Galactose (Gal) and WGA for both Sia and N-acetylglucosamine (GlcNAc).
Results. Overall, the total amount of Sia and GlcNAc expressed on circulating immune cells was similar between WTD-fed LDL-R KO mice and control animals; however a significant decrease in α2,3-linked Sia expression was observed in T cells (0,68±0,040 fold* for CD4+, 0,49±0,02 fold* for CD8+), whereas a significant increase in α2,6-linked Sia was observed in monocytes (2,12±0,15 fold*) of hypercholesterolemic LDL-R KO mice compared to their control counterparts. Moreover, Gal expression increases significantly only on circulating monocytes (1,27±0,05 fold*) and, particularly, in Ly6C-high monocytes (1,27±0,06 fold*). (*p<0.05). In line with these observations, T lymphocytes from FH patients presented decreased expression of α2,3 sialylation (0,75±0,03 fold** for CD4+, 0,87±0,04 fold* for CD8+) and the same was true for CD16+ neutrophils (0,68±0,11 fold*) compared to age- and sex-matched controls. (*p<0.05; **p<0.01).
Conclusions. These results highlight that hypercholesterolaemia affects glycans signature of immune cells both in humans and in experimental models. Whether the impact of increased α2,6/α2,3-linked Sia and Gal/Sia ratios, observed in hypercholesterolemic conditions, impact the function of immune cells during atherosclerosis development remains to be addressed.

MODIFICATIONS OF THE CONCENTRATIONS OF PCSK9 IN SERUM AND CEREBROSPINAL FLUID IN PATIENTS WITH COGNITIVE DISORDERS

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Background and Aim. The relationships between alterations in cholesterol metabolism and neurodegenerative disorders/dementia are poorly defined. Interest is growing on Proprotein convertase subtilisin/kexin type 9 (PCSK9), and on PCSK9 inhibitors as cholesterol-lowering drugs. Preliminary data have shown increased cerebrospinal fluid (CSF) concentrations of PCSK9 in Alzheimer's disease (AD) compared to control subjects. This study is aimed to investigate the relationships between sterol metabolism and AD by analysis of PCSK9 in serum and CSF.
Patients and Methods. We recruited subjects followed by the Neurology Unit of Modena City Hospital, with a diagnosis of:-mild cognitive impairment with a stable clinical behavior at follow up (MCI); - MCI and a subsequent diagnosis of AD (MCI-AD); - AD upon initial evaluation (AD). Analysis on serum and CSF included PCSK9 determination, performed by ELISA.
Results. We studied 28 patients with MCI, 24 MCI-AD and 28 patients with overt AD.No difference was observed in CSF levels of PCSK9 among all groups; serum PCSK9 concentrations were lower in AD patients, compared with MCI and MCI-AD subjects (respectively, $p<0.01$ and $p<0.05$, one-way ANOVA). In AD patients, apoE4 allele carriers presented lower CSF PCSK9 levels compared to non-carriers (-38%; $p=0.04$). No correlation was detected between plasma and CSF concentrations of PCSK9 in the whole cohort ($r=0.110$; $p=0.331$). However, a correlation was present in AD subjects ($r=0.521$; $p=0.004$).
Discussion. No significant differences in CSF levels of PCSK9 were detected among the different groups; however, CSF concentrations of PCSK9 might be influenced by apoE4 isoform, as recognized. Plasma PCSK9 was lower in AD patients. Interestingly, we found a significant correlation between CSF and plasma PCSK9 in AD patients. We speculate that PCSK9 may reach the SNC in AD due to increased permeability of the blood-brain barrier.

GENDER DIFFERENCES IN LIPOPROTEIN(A) CONCENTRATION AS PREDICTORS OF CORONARY REVASCULARIZATION IN PATIENTS WITH CORONARY ARTERY DISEASE

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Background and Aims. The use of Lp(a) levels as a screening determinant in secondary prevention, particularly in coronary patients, have been receiving increasing attention, although discordant findings are reported with limited evidence on women. Thus, in a follow-up study of 2 years, we evaluated the association between Lp(a) levels and the occurrence of major adverse coronary events in a large series of coronary patients (32% of women).
Methods. This single Center prospective cohort study investigated 3,034 consecutive patients admitted to the Coronary Care Unit with a diagnosis of coronary ischemia. According to the inclusion criteria, 2,374 patients were monitored every 3-6 months. The endpoints were non-fatal myocardial infarction, revascularization and coronary deaths
Results. Lp(a) levels associate only with revascularitazion. According to Lp(a) stratification (<30 mg/dL, >30-50 mg/dL and ≥**50** mg/dL) there was a significant rise of revascularization events in the whole sample of participants with a trend hazard ratio (HR) of 1.23 and a 6% rise for every 10 mg/dL increment in Lp(a) levels. This effect was mainly driven by women (HR 4.15) who showed a 14% incremental risk for every 10 mg/dL rise in Lp(a) levels.
Conclusions. In an Italian cohort of patients with coronary artery disease, these findings support the predictivity of Lp(a) levels on coronary revascularizations, especially in female patients.

EFFECTS OF BARIATRIC SURGERY ON PLASMA LEVELS OF ANGPTL3 AND ANGPTL4: ASSOCIATION WITH PARAMETERS OF GLUCOSE AND LIPID METABOLISM

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Introduction. ANGPTL3 and ANGPTL4 are main regulators of lipid metabolism due to their main action on LPL inhibition. Adipose tissue metabolism as well as insulin sensitivity might be also affected by ANGPTLs function. Aim of this research is to identify relations between changes of ANGPTLs levels in obese patients undergoing bariatric surgery and their relations with parameters of glucose and lipid metabolism.
Methods. Baseline and 1-year after bariatric surgery serum levels of ANGPTL3 and ANGPTL4 were measured in 42 patients submitted to Roux-en-Y gastric bypass (RYGB n=27) or bilio-pancreatic diversion (BPD n=15). Patients were analysed according to type of surgery and clinical condition (obese normal glucose tolerant (Ob NGT) vs Ob with type 2 diabetes (Ob T2D) in the RYGB group vs non-obese T2DM in the BPD group. Routine laboratory tests, markers of adipose tissue metabolic inflammation and dynamic insulin resistance tests were also determined.
Results. All patients achieved significant weight loss at 1-year after surgery [p<0,001]. Total Cholesterol (TC) did not show a significant reduction after surgery in RYGB group, while important TC reduction was achieved in BPD group [p=0.009]. However, HDL-C raised significantly in T2DM treated by RYGB [p=0,02]. All patients showed a significant improvement in insulin sensitivity by euglycemic clamp after surgery. Adiponectin levels, measured in RYGB patients only, nearly doubled 1-year after surgery either in NGT and T2DM patients.ANGPTL3 levels in non-obese patients submitted to BPD showed, surprisingly, a significant rise 1-year after surgery [from 225±20 to 300±15; p=0.003]. No change was observed in both ObNGT and ObT2D after RYGB. In the all data set. As expected, serum levels of ANGPTL3 were found to be associated with insulin sensitivity and, in T2D-BPD, with NEFA reduction and bile acid increase (p<0.02). ANGPTL4 levels, on the other hand, were reduced in all groups after surgery [p<0,05]. ANGPTL4 was inversely related to adiponectin and insulin sensitivity.
Discussion. ANGPTL3 increase was not expected in study design and may relate to chronic nutrient loss typical of BPD, which led to enhanced cholesterol synthesis. On the other hand, a drop in circulating ANGPTL4 levels was indeed expected. ANGPTL4 might exert a function in regulating adipokine production in adipose tissue and therefore may be crucial in indirect regulation of lipid metabolism and insulin resistance.

SUBCUTANEOUS ADIPOSE TISSUE ELASTOSONOGRAPHY IN OBESE PATIENTS: A PRELIMINARY REPORT

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Introduction. Elastosonography is a diagnostic method measuring the rigidity or "stiffness" of the tissues. Its main application has been primarly the measurement of hepatic stiffness in patients with chronic liver disease. Recently, the use of new software and technologies has allowed its application to several organs (thyroid, muscle, prostate). The main objective of our study was to test the application of elastosonography to subcutaneous adipose tissue.
Materials and Methods. we recruited 12 obese non-diabetic subjects referred to the obesity clinic of the SS. Annunziata Hospital of Chieti. A clinical evaluation was performed with measurement of anthropometric parameters and laboratory tests (blood count, creatinine, uric acid, AST, ALT, GGT); then the patients underwent abdominal ultrasound with measurement of SAT, preperitoneal and visceral fat (VAT), hepatic elastosonography and SAT. The elastosonography of the SAT was performed by centering the roi in the subcutaneous adipose tissue, positioning the probe 2 cm above the navel along the xiphoumbilical line, between the external surface of the rectus abdomen and the skin; the final value was derived from an average of 10 measurements.
Results. The average age of the subjects examined was 54 years; median BMI was 40, waist circumference 117.5 cm and hip circumference 125.5 cm. The median hepatic stiffness was 4.65 kPa. The median stiffness value of the SAT was 9.9 kPa. On statistical analysis, E-SAT correlated with preperitoneal fat thickness (p=0.001; rho =0.786), with VAT thickness (p=0.011; rho=0.676), with body weight (p=0.039; rho=0.601) and with the waist circumference (p=0.034; rho=0.613). E-SAT did not correlate with the thickness of the SAT itself (p=0.448; rho=0.231), did not correlate with E-liver (p=0.183; rho= 413) and did not correlate with age of the patients (p=0.795).
Conclusions. The elastosonography of the SAT seems to be a feasible and repeatable method. Comparative studies and with a larger sample size are needed to create a standardized method. The fact that the stiffness value does not correlate with the size of the SAT itself, but with the weight and waist circumference, suggests that the stiffness of the tissue is linked to a histological-tissue remodeling due to obesity itself, rather than to its actual measurable size.

METABOLIC REGULATION OF CD8+ T LYMPHOCYTES ACTIVATION BY THE LOW-DENSITY LIPOPROTEIN RECEPTOR (LDLR)

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Aim. Activation of T lymphocytes combines functional to metabolic rewiring of cell machinery, including cholesterol homeostasis. Here, we evaluated the role cellular cholesterol acquired via the LDLR pathway on Tcell biology.
Methods. Immunophenotypic characterization of T cells from WT and LDLR KO mice was performed in vitro (anti-CD3/CD28) and in vivo (vaccination, homeostatic proliferation in Rag2 KO mice adoptively transferred with Tcells from LDL-R KO and WT mice) coupled to proteomic and seahorse analysis on isolated T cells. In parallel, T cells from FH (familial hypercholesterolemia) patients, carrying mutations in the LDLR gene, were tested.
Results. LDLR mRNA and protein expression increased after in vitro activation of CD8, suggesting a peculiar regulation of cholesterol homeostasis in CD8 T cell subset. Functionally, LDLR deficiency mainly dampened CD8+ proliferation (-35%, $p<0.01$) paralleled by a reduction in INFγ production (-39.6%, $p<0.01$). In vivo antigen-specific activation by ovalbumin vaccination, but not homeostatic proliferation, resulted in a decreased proliferation and cytokines production (↓IFNγ $p<0.001$, ↓IL13 $p<0.01$, ↓perforin $p<0.05$) in CD8+ of KO mice. In addition, markers of rapid activation, such as CD69 (-32%, $p<0.01$), and AKT phosphorylation, a downstream molecule of the TCR, were decreased in KO CD8+ compared to WT. Finally, proteomic and seahorse analysis pointed out metabolic defects with reduction of both glycolytic and OXPHOS metabolism and impaired lysosomal-derived mTORC1 activation, thus linking functional to metabolic alteration in CD8+ T cells from LDLR KO compared to WT mice.When tested in humans, CD8+ T cells from FH patients proliferated less (-36%, $p>0.05$) compared to sex- and age-matched controls; in addition, when CD8+T cells from FH vaccinated for seasonal influenza were tested in vitro with virus-derived peptides, presented a decreased granzyme production (-60.3%, $p<0.01$) compared to CD8+T cells from vaccinated controls, indicating a reduced CD8 effector response to virus infection.
Conclusions. LDLR plays a critical role in regulating the immunometabolic responses in CD8+ Tcells, and thus might represent a checkpoint linking cellular cholesterol metabolism to adaptive immune response.

ATTAINMENT OF 2019 ESC/EAS LIPID TARGETS IN A COHORT OF VERY-HIGH RISK SUBJECTS IDENTIFIED THROUGH CARDIOLOGY AND LABORATORY DATABASES

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Introduction. In 2019, ESC/EAS released new guideline for the management of dyslipidemias, further reducing LDL-C targets as compared to 2016 guideline. The new LDL-C targets appear to be particularly challenging and literature data suggest that their attainment is largely suboptimal in clinical practice. We evaluated lipid goal attainment in a cohort of very-high risk patients.
Methods. Data of patients (≤65 years) subjected to percutaneous transluminal coronary angioplasty (PTCA) in 2014 and 2015 were extracted from interventional cardiology database (657) and those of subjects with LDL-C ≥250 mg/dl in the same period were extracted from clinical laboratory database (284). Dutch Score (DS) was calculated on the basis of pre-treatment LDL-C values, family and clinical history of pCAD. Three groups were identified:
1) PTCA with DS<3 (PTCA_<3, 462);
2) PTCA with DS≥3 (PTCA_≥3, 195);
3) LDL-C subjects all having DS≥5 (LDL_≥5, 284).

Lipid profiles of all subjects were retrieved until March 2019 and comparison among 3 groups was performed.
Results. During the follow up period LDL-C values were significantly different among three groups: LDL-C median (IQR) mg/dl were 81(68-97) for PTCA_<3, 94(78-122) for PTCA_≥3 and 186(143-220) for LDL_≥5. Percentage of patients reaching the LDL-C goals recommended by 2016 and 2019 guidelines (≤70 mg/dl, ≤55 mg/dl and 50% reduction) were 29%, 11% and 19% for PTCA_<3 and 15%, 3% and 35% for PTCA_≥3. In the LDL_≥5 group, 20% of subjects achieved 50% LDL-C reduction. Among PTCA patients, pre-treatment LDL-C values were significantly inversely associated with the probability to reach LDL-C≤55 mg/dl target and directly associated with the attainment of the 50% reduction goal.
Conclusions. Our study demonstrated that achievement of lipid goals was far to be reached in our cohort patients. Among PTCA patients pre-treatment LDL-C values significantly influence the response to the established lipid lowering therapy and the percentage of patients reaching the recommended targets.

MOLECULAR CHARACTERIZATION OF PATIENTS WITH AND WITHOUT CORONARY ARTERY DISEASE WITH "EXTREME" LEVELS OF APOC-III AND TRIGLYCERIDES

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Introduction. Epidemiological and GWAS studies suggest a correlation between plasma triglyceride (TG) levels and coronary artery disease (CAD). Among genetic determinants involved in the metabolism of TG-rich lipoproteins, variants of genes encoding for ApoC-III and ApoA-V, seem to play a major role. Heterozygous carriers of loss of function (LOF) variants APOC3 gene have lower plasma levels of TG and apoC-III and a 40% risk reduction of developing CAD. More, rare loss of function variants of the APOA5 gene contribute to higher plasma TG levels and an increased risk of developing CAD. The NGS analysis allows to identify rare variants in candidate genes and their association to risk of developing CAD.
Materials and Methods. The study sample includes patients of Verona Heart Study (VHS) with angiographically documented CAD (positive CAD) and subjects without CAD who underwent coronary angiography for other clinical indication (negative CAD). We selected patients with extreme ApoC-III phenotype (<5° percentile - 6.1 mg/dl and >95° percentile - 17.3 mg/dl) and with TG <2° percentile (57 mg/dl) and >98° percentile (298 mg/dl). For genetic analysis, a large-scale sequencing system based on Ion Torrent technology has been developed. For each patient we selected variants in candidate genes known to be associated to regulate TG levels.
Results. Among the patients with ApoC-III levels <5° percentile, the NGS analysis revealed the presence of a pathogenetic mutation in APOC3 gene in one CAD negative subject. No other pathogenic variants have been identified in patients with low/high levels of TG. 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.

ABNORMAL ANKLE-BRACHIAL INDEX IN A COHORT OF HIGH CV RISK PATIENTS WITH OR WITHOUT TYPE 2 DIABETES: DATA FROM THE MULTICENTER RELIVE STUDY

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Background. Ankle-brachial index (ABI) is a known risk factor for cardiovascular disease in general population and a reproducible tool for the early detection of peripheral artery disease (PAD). Our study aim was to evaluate the ABI distribution in high CV risk patients visited by Lipid clinics and Diabetological centers in Italy and its association with other CV risk factors and patients characteristics.
Methods. For this multicenter study, we consecutively enrolled 768 patients (F: 47.4%), with age included between 40 and 80 years old, high CV risk patients (Lipid clinics) and/or diabetics (Diabetological centers) at their first visit, without known PAD.
Results. The 30% (N. 198) of the enrolled patients with valid measurement had an abnormal ABI. Lipid-lowering therapies were equally distributed among subjects with normal and abnormal ABI. Abnormal ABI was significantly more prevalent in patients in secondary prevention than in those in primary prevention (41.4% vs. 25.5%, p<0.001). ABI distribution was similar in men and women, in smokers and non-smokers, hypertensives and normotensives, diabetics and non-diabetics.
Conclusion. The main result of this multicenter transversal investigation is that abnormal ABI is highly represented in high CV risk patients with or without type 2 diabetes. Further investigation are needed to understand if the implementation of ABI measurement could help to identify subjects with a worse CV risk profile, even in a high risk setting.

THE DIAGNOSTIC PERFORMANCE OF THE ITALIAN SOCIETY FOR THE STUDY OF ATHEROSCLEROSIS (SISA)-MODIFIED DUTCH LIPID CLINIC NETWORK (DLCN) CRITERIA IN A PEDIATRIC COHORT FROM LIPIGEN STUDY POPULATION: PRELIMINARY DATA

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Background. Familial hypercholesterolemia (FH) is a common and under-diagnosed autosomal dominant disorder associated with premature atherosclerotic cardiovascular disease (ASCVD). A strong association exists between elevated LDL-c levels and atherosclerosis, even in children. Early diagnosis and optimal FH-treatment is essential. The first step for FH diagnosis is the clinical valuation, although no international recommended clinical criteria exist yet. One of the main diagnostic tools includes the Dutch Lipid Clinic Network (DLCN) criteria. However, it is not validated in the pediatric population. Recently, the Italian Society for the Study of Atherosclerosis (SISA) proposed modified DLCN criteria adapted for patients younger than 18 years. Our aim was to compare the diagnostic performance of SISA-modified DLCN criteria with the European Society of Cardiology (ESC)-validated DLCNs in a pediatric cohort of patients derived from the LIPIGEN study.
Methods. We enrolled 82 consecutive children and adolescents [mean age 11±3 years old, 58% of male (n=48)], referred to the Lipigen Center of Chieti and Rome, for clinical suspicion of FH. All the patients enrolled underwent the FH DNA-test. SISA-modified and ESC-validated DLCNs criteria were assessed prior the genetic analysis. The diagnostic performance of both scores was assessed and compared utilizing the receiver operating characteristics (ROC) curves.
Results. The 65% of patients (n=53) were positive at the FH DNA-test for genetic mutations. The SISA-modified DLCNs showed better sensitivity than the ESC-validated DLCNs (53% vs. 21%, respectively), but less specificity (73% vs. 93%, respectively). When compared, no-statistical significance (P=0.68) was found between the two methods [AUC 0.65, 95% CI (0.52, 0.77) and AUC 0.63, 95% CI (0.52, 0.75), respectively].
Conclusions. The modified pediatric cut-offs proposed by SISA did not improve the diagnostic performance of DLCNs when compared with the ESC-validated DLCNs. Even if the SISA-modified DLCNs showed better sensitivity, the DLCNs by ESC showed higher specificity.

EZETIMIBE TREATMENT IN THREE PEDIATRIC PATIENTS WITH CHOLESTEROL ESTER STORAGE DISORDER (CESD)

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Introduction. Lysosomal Acid Lipase deficiency (LAL-D) is a rare metabolic storage disease characterized by cholesteryl esters and triglycerides accumulation in lysosomes. The variant cholesterol ester storage disorder (CESD) usually presents with dyslipidemia and liver involvement. No formal treatment guidelines exist so far and therapeutic options are still questionable. The aim of the study was to evaluate the ezetimibe therapy effects.
Methods. LAL-D was confirmed by analysis of LIPA gene in 3 children (SA, SF and PR, diagnosed at age 3, 4 and 7 years) showing dyslipidemia and slight elevation of transaminases. Liver ultrasonography, magnetic resonance (MR) and elastosonography by FibroScan were performed and lipid profile and transaminases monitored by classical methods, before and after 2 years of Ezetimibe 10 mg/day treatment.
Results. Molecular analysis of LIPA gene found in SA and SR the homozygosity for the classic mutation c.894G>A in exon 8 and in PR compound heterozygosity for c.894G>A and c.883C>T(p.H295Y) in exon 8. Liver ultrasonography and MR showed very mild steatosis and elastosonography were normal. Baseline lipid profile mean values in patient SA-SF-PR were: TC 241-223-340 mg/dl, LDL-C 176-159-269 mg/dl, HDL-C 29-34-37 mg/dl, TG 152-145-176 mg/dl. Ezetimibe treatment led to a TC reduction of 17-19-15% and LDL-C reduction of 22-27-18%, while HDL-C and TG showed mild discordant modifications. Baseline transaminases mean values were: ALT 84-79-55 UI/l, AST 75-59-44 UI/l. During Ezetimibe, ALT decreased by 32 and 29% in SA and SF, while increased by 2.5% in PR. AST decreased by 44 and 22% in SA and SF, while increased by 3% in PR.
Conclusions. Short- and medium- term ezetimibe therapy improved significantly TC and LDL-C in three children CESD affected, while the transaminase effect was variable. In LAL-D pediatric patients with mild phenotype Ezetimibe should be considered before enzyme replacement therapy, under a mandatory strict control.

ATORVASTATIN IN PEDIATRIC FAMILIAL HYPERCHOLESTEROLEMIA: TOWARDS A PERSONALIZED APPROACH

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Familial Hypercholesterolemia (FH) is the most common inherited cholesterol disorder. People with FH usually develop early signs of atherosclerosis (artery thickening and blockage) by school age and have a higher likelihood of a heart attack before they reach middle age. If detected in childhood, treatment is available to delay or perhaps even prevent premature cardiovascular disease (CVD). Atorvastatin (ATV) is a lipid-lowering agent, approved in Italy for treatment of FH once daily at 10-20 mg doses in children aged 10 years or older. Although ATV is suggested as lipid-lowering therapy in children, the pharmacokinetics (PK) of ATV has not been clearly characterized in pediatric patients. Statins are highly effective prophylactics against arteriosclerosis. Nevertheless, a significant proportion of children consuming statins are not able to reduce LDL-C to a protective level for preventing cardiovascular disease. These outcomes suggest that genetic factors may influence patient responses to treatment with statins. It may be argued that a combination of data on Therapeutic Drug Monitoring (TDM) and pharmacogenetics for each patient are useful to personalize the therapeutic approach. The study was approved by the local Ethical Committee and is ongoing to date. We are enrolling and collecting data on 21 pediatric patients, divided into 3 subgroups: Naïve (N, they have just begun therapy), Low dose responders (LD-R, they assume therapy at the lowest dose, 10 mg/day, since 6 months or more), Low dose non-responders (LD-NR, they increased atorvastatin up to 20 mg/die because unresponsive). These findings, if confirmed, may have an impact on modulating and personalizing therapies selected for each patient.

CHANGES IN MARKERS OF SUBCLINICAL ATHEROSCLEROSIS IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA TREATED WITH EVOLOCUMAB: A PROSPECTIVE COHORT STUDY

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Background. PCSK9 inhibitors demonstrated efficacy in cholesterol reduction and in the prevention of cardiovascular events. We evaluated changes in lipid profile, oxidation markers and markers of subclinical atherosclerosis in patients with familial hypercholesterolemia (FH) during 12 weeks of treatment with a PCSK9 inhibitor, Evolocumab.
Methods. Patients with FH starting a treatment with Evolocumab were included. Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), lipoprotein(a) (Lp(a)), small dense LDL (assessed by LDL score), 11-dehydro-thromboxane (11-TXB2), 8-iso-prostaglandin-2alpha (8-iso-PGF2α), flow-mediated dilation (FMD), reactive hyperaemia index (RHI) and carotid stiffness were evaluated before starting treatment and after 12 weeks of treatment.
Results. Twenty-five subjects were enrolled (52% males, mean age 51.5 years). At 12-week assessment, the median reduction was 38% for TC, 52% for LDL-C, 7% for Lp(a) and 46% for LDL score. In parallel, 11-TXB2 and 8-iso-PGF2α were reduced of 18% and 17%, respectively. FMD changed from 4.78%±2.27 at baseline to 10.6%±5.89 ($p<0.001$) at 12 weeks, with RHI changing from 2.37±1.23 to 3.76±1.36 ($p<0.001$). Carotid stiffness changed from 8.8 (IQR: 7.0-10.4) m/sec to 6.6 (IQR: 5.4-7.5) m/sec, corresponding to a change of 21.4% ($p<0.001$), with a significant increase in carotid distensibility (from 12.1, IQR: 8.73-19.3 kPA–1×10–3 at T0 to 21.8, IQR: 16.6-31.8 kPA–1×10–3 at T12w) corresponding to a median change of 62.8% ($p<0.001$). Change in LDL score was an independent predictor of changes in FMD (β=-0.846, p=0.015), carotid stiffness (β=0.429, p=0.041), and in 8-iso-PGF2α (β = 0.778, p = 0.012).
Conclusions. Small dense LDL reduction is related to changes in oxidation markers, endothelial function and carotid stiffness in patients with FH treated with Evolocumab.

IMPROVEMENT OF QUALITY OF LIFE IN A PATIENT WITH FAMILIAL CHYLOMICRONEMIA SYNDROME TREATED WITH VOLANESORSEN

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Background. Familial chylomicronemia syndrome (FCS) is a rare autosomal recessive disease due to alteration in clearance of chylomicrons, large lipoprotein rich in triglycerides (TG). This clinical condition is characterized by the presence of very high levels of TG (>885 mg/dl), multiple recurrent acute pancreatitis, abdominal pain, eruptive xanthomas, hepathosplenomegaly, lipemia retinalis, neurologic symptoms. FCS patient are usually refractory to standard therapies. Symptoms related to high TG levels and consequences of recurrent pancreatitis have a strong impact on patients' quality of life (QoL). The antisense oligonucleotide against APOC3 mRNA volanesorsen showed efficacy in treatment of FCS patients.
Case report. A 42-years old man with FCS and severely high TGs and history of necrotizing pancreatitis (2017) was admitted to our dyslipidemia-center to treat hypertriglyceridemia. The patient reported constant abdominal pain, fatigue and anxiety related to the disease burden. An initial management included a very restricted low caloric diet (reduced intake of carbohydrates, fats, no alcohol) and therapy with fenofibrate 145 mg, maximum doses of omega-3 fatty acids, atorvastatin 20 mg, medium chain TG oil. Despite full adherence to therapy and diet for 18 month fasting TG value was 1763 mg/dl associated to symptoms persistence. In June 2020, we added Volanesorsen 300 mg subcutaneously once a week to background lipid lowering therapy. After 12 weeks of treatment TG value was 472 mg/dl. The marked reduction of TG was associated to total disappearance of abdominal pain, fatigue and in turn of anxiety. No adverse events or blood test abnormalities were reported during treatment. In our patient, treatment with volanesorsen lead to significant TG and symptoms reduction having a positive impact on QoL improving patient activities of daily living.

TREATMENT WITH EVOLOCUMAB IN A PATIENT WITH PREVIOUS STATIN-INDUCED NECROTIZING MYOPATHY: A CASE REPORT

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Background. Immune-mediated necrotizing myopathy (IMNM) is a rare and dangerous side effect due to statin therapy. IMNM is related to antibodies against the catalytic domain of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (anti-HMG-CoA). Clinical picture of IMNM is characterized by myalgia with symmetrical and proximal weakness and severe elevation of creatinine kinase (CK) levels. PCSK9 inhibitors demonstrated efficacy and safety in patients with statin intolerance.
Case Report. A 56-years-old woman with polygenic hypercholesterolemia was treated for 10 years with simvastatin 10 mg. In 2017, her general practitioner increased the dose of simvastatin from 10 to 20 mg to reach therapeutic target. After two weeks patient reported muscle pain, weakness and elevation of CK value (14,000 U/L). She was admitted to emergency department with diagnosis of rhabdomyolysis. Subsequently, because of symptoms persistence in the frame of a second level diagnostic workflow she was diagnosed with "IMNM with high title anti-HMGCoAR antibodies". Corticosteroid and immunosuppressive therapy was set up, with a recovery of muscle function, and anti-HMGCoAR antibodies negativization. In 2018 the patient was admitted to our dyslipidemia center to treat hypercholesterolemia. Her LDL-C was 183 mg/dl. CK was 180 U/L. We suggested life style changes and evaluated the patient after six months. Lipid parameters were unchanged. We started treatment with Evolocumab 140 mg biweekly. After 12 weeks LDL-C value was 94 mg/dl and no adverse events were reported. During follow up, patient reported worsening asthenia associated with CPK elevation (maximum of 730 U/L). Thus, we decided to stop therapy with Evolocumab. A musculoskeletal RM was performed and confirmed an exacerbation of IMNM that lead to a strengthening of immunosuppressive therapy. In our experience, PCSK9 inhibitors should be considered with great caution in patients with IMNM. Dedicated studies are needed to address this issue.

EFFECT OF ANTI-PCSK9 MONOCLONAL ANTIBODY THERAPY ON PLATELET ACTIVATION IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Background. High circulating levels of proprotein convertase subtilisin/kexin 9 (PCSK9) are associated with an increased risk of cardiovascular events (CVEs) and with platelet activation. Furthermore, hypercholesterolemia (HC), a risk factor for CV disease, is characterized by platelet overactivity. Thus, the aim of this study is to demonstrate that PCSK9 inhibitors can influence directly platelet activation (PA) and elucidate the underlying mechanism.
Methods. We conducted a prospective, multi-center cohort study of 80 patients with confirmed genetic diagnosis of familial HC undergone treatment with anti-PCSK9 mAbs. We selected and balanced patient for age, sex and cardiovascular risk factors and evaluate platelet function at baseline and after 6 months of treatments. PA was assessed by evaluation of plasma TxB2 formation. Nox2 activation as assessed by soluble NOX2-derived peptide (sNox2-dp), plasma H2O2 and oxLDL and isoprostanes were used as markers of oxidative stress (OS).
Results. The results showed that treatment with anti-PCSK9 mAbs, induce an improvement of platelet function and oxidative stress parameters. In particular, we found that patients after 6 months of treatment with anti-PCSK9 mAbs showed lower levels of serum TxB2 compared to baseline. Similarly, OS parameters like sNox2-dp release, plasma H2O2 productio, oxLDL formation and isoprostanes levels were significantly decreased after 6 months of treatment.
Conclusions. In this study, we provide evidence that PCSK9 inhibitor could play a role in the reduction of CV risk in HC patients by acting on PA and involving the mechanisms of oxidative stress.

PROPROTEIN CONVERTASE SUBTILISIN-KEXIN TYPE 9 BINDING TO SERUM LIPOPROTEINS: A METODOLOGICAL COMPARISON

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Introduction. Elevated low-density lipoprotein cholesterol (LDL-C) is a well-established cardiovascular risk factor. Proprotein convertase subtilisin-kexin type 9 (PCSK9) increases LDL-C levels in plasma by enhancing the degradation of hepatic LDL receptor. PCSK9 seems to travel in circulation associated to lipoproteins (LPs), but data in literature are discordant. By comparing different protocols for LPs isolation, we aimed at finding the best one for confirming the existence of this association and building up future clinical studies.
Material and Methods. Fresh serum was collected from healthy volunteers and lipoproteins were obtained using different methods, including precipitation with phosphotungstic acid, fast protein liquid chromatography (FPLC), ultracentrifugation using KBr or iodixanol gradient (IGr). The PCSK9 content of the lipoprotein fractions obtained was quantified with ELISA, while lipoprotein(a), apolipoprotein B, apolipoprotein A1 and cholesterol were measured using clinical-grade reactives.
Results and Conclusions. The precipitation-mediated assay resulted in more than 80% of PCSK9 found associated with the apoB precipitate; conversely, negligible amount of PCSK9 was detectable in lipoprotein fractions isolated by KBr ultracentrifugation. With FPLC, around 10% of recovered PCSK9 co-eluted with LDL, whereas around 20% of PCSK9 was found in the LDL fractions obtained with the IGr ultracentrifugation.Our results suggest that the association of PCSK9 and LDL is sensitive to high salt concentrations and the extent of the estimated association depends on the methodology used. IGr ultracentrifugation and FPLC appear to be both suitable for further biological and clinical studies.

LPA VARIANTS ARE ASSOCIATED WITH INCREASED LIPOPROTEIN(A) CONCENTRATIONS AND ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction and Aims. The rs10455872 and rs3798220 variants in LPA gene are associated with increased plasma concentrations of lipoprotein(a) [Lp(a)] and atherosclerotic cardiovascular disease (ASCVD) in the general population. We aimed to determine if this holds true also in familial hypercholesterolemia (FH).
Methods. 148 consecutive patients (men/women 75/73, age 48[12-82] years) with clinical suspicion of FH from the Lipid Clinic in Modena underwent comprehensive evaluation, including Lp(a) measurement and genotyping of rs10455872 and rs3798220 LPA variants, within the LIPIGEN Project. HyperLp(a) was defined as Lp(a) concentrations higher than 50 mg/dl.
Results. Median Lp(a) levels were 23.8[1.3-340] mg/dl; 43 patients (29.1%) presented hyperLp(a). 23 patients (15.5%) had a history of ASCVD. A mutation in FH-causing genes and at least one LPA variant were found in 105 (70.9%) and 15 (10.1%) patients, respectively. 10 patients were heterozygous for the rs10455872 LPA variant; 3 patients were heterozygous and 2 were homozygous for the rs3798220 LPA variant. Carriers of at least one LPA variant had significantly higher Lp(a) levels (130.3[3.9-340] vs. 19.3[1.3-226.9] mg/dl, p:<.001) and were more likely to have hyperLp(a) (80% vs. 23.3%, p:<.001) than non-carriers. Patients without mutations in FH-causing genes more frequently presented hyperLp(a) (41.9% vs. 23.8%, p:.028) and at least one LPA variant (18.6% vs. 6.7%, p:.029) than FH mutation-positive patients.FH patients with a history of ASCVD showed significantly higher levels of Lp(a) (p:.031) and were more likely to be carriers of at least one LPA variant (26.1% vs. 7.2%, p:.006) than patients in primary CV prevention. Of note, carrying at least one LPA variant was significantly associated with a history of ASCVD independently of age, gender, FH mutational status and presence of hyperLp(a) (adjusted OR:4.7, 95%CI:1.1-19.6;p=0.035).
Conclusions. Our study confirms that rs10455872 and rs3798220 LPA variants are associated with increased Lp(a) concentrations and ASCVD also in FH patients.

EFFICACY AND SAFETY OF INCLISIRAN: A SYSTEMATIC REVIEW AND POOLED ANALYSIS OF PHASE 2 AND PHASE 3 CLINICAL STUDIES

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Aim. To assess the efficacy and safety of inclisiran treatment with a systematic review of the literature and a meta-analysis of the available clinical studies.
Methods. A systematic literature search in several databases was conducted up to August 13th, 2020, in order to identify clinical trials assessing the effect on lipoproteins and the safety profile of inclisiran. The magnitude of the effect for lipid changes was expressed as percentage mean differences (MD) and 95% confidence intervals (CI). For the safety analysis, odd ratios (OR) and 95% CI were calculated using the Mantel-Haenszel method.
Results. Data were pooled from 4 clinical studies comprising 24 arms, which included 4226 subjects overall, with 2254 in the active-treated arm and 1972 in the control arm. Meta-analyses of data suggested that the multiple-dose regimen of inclisiran yielded a significant reduction in serum levels of proprotein convertase subtilisin/kexin type 9 (MD=-78.23, 95%CI: -86.74,-69.71) and low-density lipoprotein cholesterol (MD=-45.48%, 95%CI: -50.36%,-40.61%) throughout the studies. Furthermore, treatment with inclisiran significantly affected total cholesterol (MD=-13.67%, 95%CI: -20.78%,-6.57%), high-density cholesterol (MD= 8.29%, 95%CI: 4.66%,11.93%), non-HDL cholesterol (MD= -39.45%, 95%CI: -43.6%,-35.31%), apolipoprotein B (MD=-34.58%, 95%CI: -38.78%, -30.78%) and lipoprotein(a) (MD=-20.9%, 95%CI: -25.8%, -15.99%). Multiple-dose regimens of inclisiran were associated with increased risk of injection-site reactions (any reaction: OR=5.86, 95%CI: 3.44,9.98; mild reactions: OR=5.19, 95%CI: 1.68,16.07; moderate reactions: OR=13.37, 95%CI: 3.17,56.46), and bronchitis (OR=1.58, 95%CI: 1.10,2.26), while the incidence of the pre-specified exploratory CV endpoint significantly decreased at 18 months (OR=0.74, 95%CI: 0.58,0.94).
Conclusions. Inclisiran has favourable effects on lipids serum levels and an acceptable safety profile. Further well-designed studies are needed to explore its longer-term safety.

PREVALENT COOKING FATS, HAEMODYNAMIC AND METABOLIC FEATURES IN A RURAL POPULATION SAMPLE: DATA FROM THE BRISIGHELLA HEART STUDY

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Background. Dietary fats have been variably associated with cardiovascular risk factors levels. Our study aim was to evaluate the association of different prevalent everyday use of cooking fats with a large number of parameters.
Methods. For this study, we selected from the Brisighella Heart Study cohort subjects not assuming antihypertensive drugs and who reported their mean daily use of cooking fats. Based on the prevalent cooking fat source the subjects were classified as prevalent extra-virgin olive oil (EVO) users, prevalent corn oil users, prevalent users of different vegetable oils, and prevalent animal fat users, and we compared their characteristics.
Results. Overall everyday consumption of EVO as a main cooking fat source was associated to the healthier anthropometric, metabolic and haemodynamic profile, especially when compared with animal fats as main cooking fat sources. In particular, in an age and SBP adjusted model, cfPVW value was significantly predicted by the prevalent use of EVO (RR=0.84, 95%CI 0.67-0.94 vs. other prevalent fat sources), LDL-C (RR=1.12, 95% CI 1.02-1.42), SUA (RR=1.21, 95%CI 1.09-1.54) and eGFR (RR=0.77, 95%CI 0.59-0.99).
Conclusion. Prevalent EVO use as everyday cooking fat is associated with an overall healthier metabolic and haemodynamic pattern in a large rural population sample.

COMPARISON BETWEEN DUTCH LIPID CLINIC NETWORK (DLCN) CRITERIA, SIMON BROOME REGISTER GROUP (SB) AND US MEDPED PROGRAM DIAGNOSTIC PERFORMANCE IN A PEDIATRIC COHORT FROM LIPIGEN STUDY POPULATION: PRELIMINARY DATA

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Background. Familial hypercholesterolemia (FH) is a common, under-diagnosed, genetic cause of premature atherosclerotic cardiovascular disease (ASCVD). So far, no international recommended clinical criteria for FH exist and the Dutch Lipid Clinic Network (DLCN) criteria are not validated in pediatric population. On the other hand, both UK Simon Broome Register Group (SB) and US MedPed Program criteria have specific LDL-c levels cut-off for pediatrics. Our aim was to compare the diagnostic performance of DLCN criteria to the SB and the US MedPed Program ones, in a pediatric cohort derived from the LIPIGEN study.
Methods. We enrolled 52 consecutive children and adolescents [mean age 10.8±3.2 years old, 65% of male (n=34)], referred to the LIPIGEN Centers of Chieti and Rome, for clinical suspicion of FH. All the patients enrolled underwent the FH DNA-testing. As DLNCs is a point score, the clinical diagnosis of FH was considered "likely" for patients who achieved a DLCN score ≥6 and "unlikely" for who achieved a DLCN score <6. Participants with FH-likely diagnosis as for DLCNs were compared to the ones who showed a high probability of carrying a FH mutation with SB and MedPed criteria. The diagnostic performance of the three scores was assessed and compared utilizing the receiver operating characteristics (ROC) curves.
Results. The 63% of patients (n=33) were positive at the FH DNA-test for genetic mutations. The SB score showed the best diagnostic performance [sensitivity 51.51%, specificity 94.74%; AUC 0.73, 95% CI (0.63, 0.83), p=0.0017)] as compared to both DLCN [sensitivity 24.24%, specificity 94.74%, AUC 0.59, 95% CI (0.50, 0.68)] and MedPed scores [sensitivity 48.48%, specificity 89.47%, AUC 0.69, 95% CI (0.57, 0.80)].
Conclusions. In our cohort, the SB criteria demonstrated the best performance for FH diagnosis; however, no one of the three scores showed to improve the clinical detection of FH in pediatrics.

DEVELOPMENT OF LIPUTILS, A MODULE TO EXTRACT FATTY ACID MOIETIES FROM LIPIDOMIC ANALYTES

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Lipidomic analyses address the problem of characterizing the lipid components of given cells, tissues and organisms by means of chromatographic separations coupled to high-resolution, tandem mass spectrometry analyses. A number of software tools have been developed to help in the daunting task of mass spectrometry signal processing and cleaning, peak analysis and compound identification, and a typical finished lipidomic dataset contains hundreds to thousands of individual molecular lipid species. To provide researchers without a specific technical expertise in mass spectrometry the possibility of broadening the exploration of lipidomic datasets, we have developed liputils, a Python module that specializes in the extraction of fatty acid moieties from individual molecular lipids. There is no prerequisite data format, as liputils extracts residues from RefMet-compliant textual identifiers and from annotations of other commercially available services. We provide an overview of liputils capabilities and three examples of real-world data processing with this newly developed module.

CARDIOMETABOLIC PROFILE IN PLIC-MI AND PLIC-CHIESA COHORTS: COMPARISON OF TWO DIFFERENT GEOGRAPHIC SETTINGS

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Background. It is widely recognised that environmental factors and exercise affect cardiovascular health. We characterised two cohorts of subjects enrolled in two projects: the PLIC-MI study (conducted in the urban area of Milan) and the PLIC-CHIESA study (performed in Chiesa in Valmalenco [Sondrio], a clustered village in the Italian Alps). Because of their distinct geographical localisation, these two populations have different nutritional and socio-cultural habits, and lifestyles. Considering these differences, we aimed at comparing the cardiometabolic profiles between these two cohorts.
Methods. PLIC-MI and PLIC-CHIESA volunteers underwent fasting blood sampling, clinical examination, and carotid ultrasonography. For this analysis, subjects of the two cohorts were matched 1:1 by gender and age. Clinical and biochemical parameters and history of cardiovascular events were compared. To compare lifestyle behaviours, we calculated a score based on physical activity, alcohol consumption, smoke, and adherence to Mediterranean diet (ranging 0-4; score ≥3 was considered as 'healthy lifestyle').
Results. A total of 406 subjects (42.8% males; mean age 55 years) from each cohort were selected. PLIC-MI subjects exhibited higher mean values of weight (72.1 vs 68.5 kg, $p<0.001$), body mass index (26.5 vs 25.8 Kg/m^2, $p<0.05$), and waist circumference (91.0 vs 88.2 cm, $p=0.001$), and were more prone to metabolic syndrome (25.1% vs 19.3%, $p<0.05$), despite no differences in lipid parameters were observed. They showed also greater prevalence of sub-clinical atherosclerosis (31.0% vs 24.9%, $p<0.05$) and cardiovascular disease (5.8% vs 3.4%, $p<0.01$). The PLIC-CHIESA individuals were less adherent to Mediterranean diet (mean PREDIMED score 7.38 vs 8.22, $p<0.001$), but had higher prevalence of physical activity (35.4% vs 22.2%, $p<0.001$) and 'healthy lifestyle' (61.6% vs 53.5%, $p<0.05$).
Conclusions. Despite a lower adherence to Mediterranean diet, PLIC-CHIESA subjects showed an overall healthier cardiometabolic profile. The identification of the determinants of such differences deserves further insights.

PREVALENCE OF VASCULAR INVOLVEMENT AND LIVER FIBROSIS IN A COHORT OF NON-INSULIN RESISTANT MIXED HYPERLIPEMIC PATIENTS

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Aim. Familial combined hyperlipidemia (FCHL) is the most prevalent primary dyslipidemia. Frequently it remains undiagnosed. It is characterized by fluctuations in serum lipid concentrations and may present as mixed hyperlipidemia, isolated hypercholesterolemia, hypertriglyceridemia, or as a normal serum lipid profile in combination with abnormally elevated levels of apolipoprotein B. An increased risk of hepatic steatosis has been observed in FCHL, with consistent associations for both NAFLD and non-alcoholic steatohepatitis (NASH) and up to 20-37% of the variability in intrahepatic fat content attributable to genetic factors in FCHL. The aim of our study was to evaluate the prevalence and incidence of NAFLD and/or NASH in FCHL' patients.
Methods. In collaboration with general practitioners, we enrolled 69 patients (mean age 53 years; 35 male, 34 female) whose satisfied inclusion and exclusion criteria (HOMA-IR <2.4; ApoB >124 mg/dl and LDL-C >160 mg/dl, or HDL-C <50M and <60F mg/dl or Tg >180 mg/dl). Clinical and instrumental examination consisted of carotid ecocolorDoppler with PWV estimation, liver ultrasonography with Fibroscan.
Results. At baseline we found a mean CT level of 247.94±30.24, HDL-C level 56.26±16.29 in men and ± in women, Tg 124.14±65.91 mg/dl. ApoB was 131.38±29.53 on the average. HOMA-IR was 1.53±0.49. We found a carotid artery involvement, as cIMT >0.9 mm, in 28 pts (38%), and pts 24 (33%) had already carotid plaque. Any grade of liver steatosis was found in pts 35 (50%). Mean liver stiffness value was 5.38±1.76. We found a significant correlation between PWV and LS: r=0.83, p<0.001.
Conclusions. We found that about one third of included patients presented with carotid atherosclerosis already at diagnosis; moreover, mean LS was; PWV was also higher than age-related expected values. Moreover, we found a strong correlation between PWV and LS as measured by Fibroscan, also in our cohort of insulin-sensitive subjects.

ROLE OF PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) IN HEART METABOLISM

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Background and Aim. Patients with diabetes are more prone to develop congestive heart failure which could also be the direct consequence of underlying dyslipidemia which could drive cellular lipotoxicity and cardiac damage independently of ischemic cardiomyopathy. Plasma lipoproteins contribute to delivering cholesterol to cardiomyocytes by interacting with specific cell surface receptors including the low-density lipoprotein receptor and CD36. Under diabetic conditions, increased expression of lipoprotein receptors in the heart has been associated with lipid accumulation in cardiomyocytes through augmented uptake of cholesterol esters in the heart. Lipid overload in diabetic cardiomyocytes, in turn, associates with impaired mitochondrial function, decreased ATP and energy production, thus supporting heart failure in animal models and humans under dysmetabolic and diabetic conditions. This study aimed was to test the impact of PCSK9 deficiency which regulates lipoprotein and Fatty acids receptors on cardiac lipotoxicity and heart metabolic rewiring.
Methods. 2-months old WT and PCSK9 KO male mice were fed for 20 weeks with SFD (Standard Fat Diet - 10% Kcal fat). Echocardiographic analysis has been performed on these mice and the hearts have been collected. Mitochondrial respiration was investigated under resting conditions and following maximal coupling and uncoupling conditions in all mice models. A metabolomic analysis has been performed and changes in the profile of mitochondrial proteins were tested by western blotting, metabolomic and proteomic analysis.
Results. PCSK9 deficiency result in morphological alteration in the heart of PCSK9 KO mice that has an increased thickness of the left ventricular wall with preserved Ejection Fraction compare to WT. PCSK9 KO mice show a reduced running distance and time compared to WT while no difference was observed in muscular strength. The lack of PCSK9 is further associated with reduced Oxygen consumption in the heart and WB analysis of electron transport chain subunits displayed a reduced expression of key proteins of complex 1, 2 and 3 in PCSK9 KO mice compared to controls. The lack of PCSK9 in the heart is affecting the expression of the major receptor involved in fatty acids and cholesterol uptake leading to intracardiac cholesterol accumulation. The accumulation of intermediates of fatty acid oxidation, including carnitine-conjugated fatty acid (C8; 0,066±0,047 Vs0,195±0,035 and C12; 0,021±0,018Vs0,11±0,069 pg/ug of prot, p<0,05) which was paralleled by a reduction of glucose 6-P, ribose-5P and erythrose-4P levels in the heart, suggesting a shift from fatty acid oxidation toward glycolysis. As a net effect, intermediates of Krebs cycle were reduced in PCSK9 KO hearts compared to WT samples suggesting an impaired activity.
Conclusion and Discussion. PCSK9 deficiency results in a morphological and metabolic alteration in the heart that develops Heart Failure with preserved ejection Fraction.

PROGNOSTIC MEANING OF CORONARY FLOW RESERVE ON THE LEFT ANTERIOR DESCENDING ARTERY IN HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: A CASE REPORT

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Aim. Homozygous familial hypercholesterolemia (HoFH) is a rare disease that complicates with severe cardiovascular disease since childhood. HoFH patients need to be followed by cardiovascular imaging through their entire life span facing the risk of excessive radiological/ nuclear exposure.
Case description. In a 36-years old female, compound heterozygote for LDL-Receptor gene mutations, coronary flow reserve (CFR) was evaluated on left descending artery (LAD) during transthoracic dipyridamole stress echocardiography (DSE), before and after two years of lipid lowering therapy. The patient was asymptomatic for angina and, during two years treatment obtained a satisfactory lipid profile control by adding evolocumab to high-intensity statin therapy (LDL-C from 320 to 75 mg/dl). At baseline, besides normal wall motion, CFR was slightly reduced, sign compatible with coronary microvascular disease. After 2 years the DSE revealed inverted coronary flow on LAD (compatible with vessel occlusion or sub-occlusion) and a severe CFR reduction in absence of wall motion alterations. At coronary angiography chronic LAD occlusion in presence of valid (Rentrop 3) intracoronary collateral flow was confirmed and treated with percutaneous coronary revascularization (PTCA/stenting). Seriate carotid artery Doppler examination revealed stable intimal medial thickness <1 mm in presence of <30% stenosis at the carotid bulb bilaterally.
Conclusion. DSE with measure of flow velocity in HoFH patients, even with negative wall motion, is a valid tool to evaluate the inducible ischemia and to assess microvascular function so avoiding radiation and nuclear exposure.

EFFECTS OF NEXT-GENERATION TOBACCO AND NICOTINE PRODUCTS ON PHENOTYPIC MODULATION AND INFLAMMATORY RESPONSE OF AORTIC VASCULAR SMOOTH MUSCLE CELLS

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Background and Aims. Hypercholesterolemia and cigarette smoke (CS) are the main risk factors for cardiovascular disease. Whole CS is made up of over 7000 chemical components distributed between two phases: particulate and gas. Alternative next-generation tobacco products (such as E-cigarettes (E-cig) and tobacco heating products (THP)) are being developed as less toxic cigarettes. Cholesterol-loading of smooth muscle cells (SMCs) causes a phenotypic switch (less-differentiated cells that lack SMC markers but have an increased inflammatory profile), by downregulating the myocardin axis in a Kruppel-like factor 4 (Klf4)-dependent manner. To characterize the role of CS on SMC behavior, we studied the effects of CSC (lipophilic components of particulate) and AEs (CS aqueous extract containing water-soluble components present in both phases), obtained from a conventional cigarette, E-cig, and THP, on SMC phenotypic modulation and inflammatory pathways.
Methods. Human aortic SMCs (HSMCs) were incubated for 48 hours with both CSC and AEs and gene/protein expression analyzed by real-time PCR, western blot analysis, and confocal microscopy.
Results. CSC incubation promoted SMC phenotypic switch (reduced α-actin and calponin and increased Mac-2 and CD68 expression). On the contrary, all the AEs showed an opposite effect by inducing the expression of contractile genes (α-actin, calponin, and SM22). Unlike CSC, AEs treatment increased the expression of myocardin and reduced KLF4 expression, both at gene and protein levels. Interestingly, the expression of pro-inflammatory markers such as IL-1β, IL-8, and IL-6 were increased by AE from the conventional cigarette (2-fold) and halved by the E-cig and THP AEs. Similar effects were observed on matrix metalloproteinases expression.
Conclusions. Our data suggest that, differently from CSC and cholesterol, AEs promote a contractile state in HSMC. Interestingly, the expression of pro-inflammatory markers is upregulated by the AE of a conventional cigarette and reduced by the E-cigarette and THP AEs. Based on these results, next-generation tobacco products have a different effect on HSMC behavior. New studies are needed ascertain if this will lead to a reduced toxicity.

SANGER SEQUENCING DATA VS HIGH QUALITY NGS DATA: A COMPARISON OF RELIABILITY

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Background. Next-generation sequencing(NGS) has a crucial role in genetic diagnosis of polygenic diseases (familial hypercholesterolemia and other cardiovascular disorders with atherosclerosis as dominant cause). Their wide application led to Guidelines for Diagnostic NGS by the European Society of Human Genetics. Potential false-positive/-negative NGS data and paucity of internationally accepted guidelines providing quality metrics for diagnostics, made Sanger validation (costly, time consuming, error-susceptible) mandatory. We reported the analysis of 3 cases of discrepancy between NGS and Sanger validation in 218 patients admitted to the Advanced Molecular Genetics Laboratory, University of Florence.
Methods. NGS was performed by Illumina MiSeq and Haloplex/SureSelect protocols targeting 97 connectivopathies and 55 familial hypercholesterolemia genes. Variants called following Broad Institute guidelines and identified according to MAF<0.01 and allele balance >0.2 were Sanger validated.
Results. 3 out of 945 variants showed a discrepancy between NGS and Sanger: n=2 in LTBP2, n=1 in TGFB1. The first LTPB2 was Sanger validated with new primers as the previous were designed in a DNA sequence carrying polymorphisms, confirming the NGS datum. Concerning the other LTBP2 variant, the second primers pair in a region not interested by the presence of polymorphisms, didn't solve the discrepancy. Only the choice of a third further primer combination confirmed the NGS call. Regarding TGFB1, the discrepancy was solved by PCR followed by restriction fragment length polymorphism approach (AluI enzyme), demonstrating the problem was Sanger exclusively.
Conclusions. Our data suggest the urgent need to reconsider Sanger validation of NGS as mandatory in genetic diagnostics. High quality NGS data could not benefit from Sanger validation for its susceptibility to failures. Sanger's criticisms reduce its utility determining higher costs, delayed analysis conclusion, potential misdiagnosis. Our data indicate the necessity to determine standardized quality thresholds for different platforms and aims as object of International Consensus and Guidelines for clinical genetic testing limiting Sanger confirmation to variants with quality parameters under optimal cutoffs.

RISCHIO CARDIOVASCOLARE NEL SOGGETTO ANZIANO ULTRASESSANTENNE CON CARENZA DI GLUCOSIO-6-FOSFATO DEIDROGENASI

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Obiettivo. Studi recenti sembrano suggerire che la carenza dell'enzima glucosio-6-fosfato deidrogenasi (G6PD), condizione ereditaria legata al sesso che può causare crisi emolitiche in seguito all'ingestione di certi farmaci o vegetali, costituisce un fattore di rischio cardiovascolare. Per testare tale associazione è stato effettuato uno studio retrospettivo caso-controllo esaminando le cartelle cliniche di pazienti con attività G6PD nota.
Pazienti e Metodi. Sono stati reclutati 9.604 pazienti sottoposti ad esofagogastro-duodenoscopia tra il 2002 e il 2017 nell'Istituto di Clinica Medica di Sassari. È stata determinata l'attività G6PD nel sangue ed è stata raccolta un'anamnesi clinica completa, che includeva anche la presenza di eventuali episodi di cardiopatia ischemica nonché i principali fattori di rischio cardiovascolare, tra cui l'infezione da Helicobacter pylori.
Risultati. L'analisi multivariata ha confermato un aumento del rischio cardiovascolare nei pazienti con carenza di G6PD (OR: 3,24; intervallo di confidenza al 95% 2,44-4,30) dopo correzione per potenziali fattori confondenti e modificatori di effetto, inclusa l'infezione da Helicobacter pylori. Prima dei 60 anni il rischio cardiovascolare è risultato simile nei soggetti con e senza deficit di G6PD (OR, 1,26; IC 95% 0,78-2.04, P<0.562), mentre risultava aumentato dopo i 60 anni nel primo gruppo (OR, 3.05; 95% CI 2.22-4.19, P<0.0001) soprattutto nel sesso maschile (OR 3.67; 95% CI 2.19-6.14) rispetto a quello femminile (OR, 2,96; 95% CI 1,89-4,64) mediante analisi di regressione logistica specifica per sesso.
Conclusione. Il rischio di episodi cardiovascolari era significativamente superiore nei pazienti con carenza di G6PD dopo i 60 anni, sia nei maschi che nelle femmine, rispetto a quelli con attività enzimatica normale, dopo correzione per fattori di rischio CVD convenzionali e infezione da Helicobacter pylori. La disfunzione di importanti meccanismi protettivi contro lo stress ossidativo nei pazienti anziani potrebbe spiegare i risultati ottenuti nello studio.

RS629301 CELSR2 POLYMORPHISM CONFERS A TEN YEARS EQUIVALENT RISK OF CRITICAL STENOSIS ASSESSED BY CORONARY ANGIOGRAPHY

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Background and Aims. Novel genetic determinants associated with coronary artery disease (CAD) have been discovered by genome wide association studies. Variants encompassing the CELSR2, PSRC1 and SORT1 genes have been associated with CAD. This study is aimed to investigate the rs629301 polymorphism association with the extent of CAD evaluated by coronary angiography (CAG), and to evaluate its effects on an extensive panel of lipid and lipoprotein measurements in a large Italian cohort of 2429 patients.

Methods and Results. The patients were collected by four Intensive Care Units located in Palermo and Verona. Clinical Records were filed, blood samples were collected, lipids and apolipoproteins (apo) were measured in separate laboratories. CAD was defined by the presence of stenotic arteries (>50% lumen diameter) by CAG. The presence of CAD was associated with the rs629301 genotype. Patients with CAD were 78% and 73% (p=0.007) of the T/T vs. T/G+G/G genotype carriers respectively. T/T genotype was also correlated with the number of stenotic arteries, with a 1.29 (1.04-1.61) risk to have a three-arteries disease. T/T genotype correlated with higher levels of LDL-, non-HDL cholesterol, apoB, apoE and apoCIII, and lower HDL-cholesterol. Logistic Regression confirmed that rs629301was associated with CAD independently from the common risk factors, with a risk similar to that conferred by ten years of age [odds ratios were 1.43 (1.04-1.96) and 1.39 (1.22-1.58) respectively].

Conclusions. rs629301 risk allele was independently associated with the extension and severity of CAD and positively with apoE and apoB containing lipoproteins.

BERGAMOT CITRUS AND CYNARA CARDUNCULUS IMPROVES ENDOTHELIAL FUNCTION IN NON-DIABETIC INDIVIDUALS WITH LIVER STEATOSIS

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Background. Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world. Evidences suggest that NAFLD is associated with endothelial dysfunction and increases the risk of cardiovascular events. Endothelial dysfunction marks an early stage of atherosclerosis and is an important prognostic marker for cardiovascular disease (CVD). Reactive hyperemia peripheral arterial tonometry (RH-PAT) is a non-invasive, quantitative method useful to measure endothelial dysfunction. This method offers a digital measurement of hyperemic response (reactive hyperemia index, RHI). RHI score is inversely correlated with CVD. In a recent randomized clinical trial, we found that bergamot polyphenolic fraction (BPF) in combination with Cynara cardunculus (CyC) is able to reduce hepatic fat content in non-diabetic patients with NAFLD. The purpose of this study was to evaluate the effect of this nutraceutical on endothelial function in individuals with NAFLD.

Materials and Methods. We examined the data from 32 non-diabetic patients with NAFLD and endothelial dysfunction (RHI score ≤1.67) enrolled in a clinical trial (ID ISRCTN12833814) carried out at the University of Catanzaro from February to June 2019. Sixteen individuals received one capsules daily of a nutraceutical containing 150 mg of BPF, 150 mg of CyC and 300 mg of albedo fibers micronized for 12 weeks. The intervention group was matched for age, gender and body weight to 16 individuals that received one capsule daily of placebo (600 mg maltodextrin). RHI score, by EndoPAT 2000 technique, liver fat content, by transient elastography, serum transaminases, lipids, glucose and insulin were measured at the baseline and the end of the study.

Results. A total of 69% were male. The mean age was 52±9 years, mean BMI was 29.3±3 kg/m^2 and RHI score was 1.15±0.4. The overall frequency of dyslipidemia was 44% and hypertension was 34%. After 12 weeks, we found a greater RHI score increase in the participants taking the nutraceutical rather than placebo (0.58±0.5 vs 0.13±0.5; p=0.02; +95% vs +30%). In intervention group, we also found a greater reduction in liver fat content than placebo group (69% vs 31%, p=0.03). There were no significant differences in the change of body weight, blood pressure, glucose, insulin, lipids and serum transaminases values between two groups after 12 weeks. A relationship between the liver steatosis reduction and improvement of endothelial function was not found.

Conclusions. Current data show that a nutraceutical containing BPF and CyC, in addition to reducing hepatic fat content, improves endothelial function in non-diabetic subjects with NAFLD and, thus, could become an effective strategy for the prevention of CVD risk in subjects with liver steatosis.

EVOLOCUMAB IMPROVES VASCULAR OXIDATIVE STRESS AND ARTERIAL STIFFNESS OF HYPERCHOLESTEROLEMIC SUBJECTS WITH HIGH CARDIOVASCULAR RISK

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Background and Aim. Cardiovascular diseases (CVD) are the main causes of death worldwide with several conditions being affected by oxidative stress. Atherosclerosis and other correlated disorders such as coronary heart disease are characterized by high serum levels of low-density lipoprotein cholesterol (LDL-C) that can promote the generation of reactive oxygen species (ROS). To answer the need of a better LDL-C control in subjects at high and very high risk for CVD, new injectable lipid-lowering drugs with the innovative protein convertase subtilisin/kexin type 9 (PCSK9) inhibition as mechanisms of action have been developed. However, the effect of these drugs on vascular function, such as ROS generation and arterial stiffness, has not been extensively described yet.
Methods. In this study, we enrolled 15 male subjects at high or very high risk for cardiovascular disease. Patients were evaluated at baseline and after two months of treatment with 140 mg Evolocumab every 2 weeks.
Results. Enrolled patients experienced, after 2-month treatment with Evolocumab, an improvement in blood pressure-adjusted carotid-femoral pulse wave velocity (cfPWV) (P-value =0.044), which was significantly associated with a decrease of H2O2 production in freshly isolated leukocytes (PBMCS) (P-value=0.007).
Conclusions. Our data support the view of systemic oxidative stress involvement in hypercholesterolemic subjects and give further rationale for using Evolocumab independently of its lipid-lowering effect.

SAFETY EVALUATION OF α-LIPOIC ACID SUPPLEMENTATION VERSUS PLACEBO: A SYSTEMATIC REVIEW AND META-ANALYSIS

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A-lipoic acid (ALA) is a natural short-chain fatty acid that attracted great attention in the last years as antioxidant molecule. However, some concerns have been recently raised regarding its safety profile. To address the issue, we aimed to assess ALA safety profile through a systematic review of the literature and a meta-analysis of the available randomized placebo-controlled clinical studies. Literature search included PubMed Medline, SCOPUS, Google Scholar and ISI Web of Science by Clarivate databases up to August 15th 2020. Data were pooled from 70 clinical studies, comprising 153 treatment arms, which included 4679 subjects, with 2523 treated with ALA and 2259 subjects assigned to placebo. Meta-analysis of extracted data suggested that supplementation with ALA was not associated with an incresed risk of developing adverse events (AEs), including hypoglycaemic episodes, gastrointestinal AEs (e.g. heartburn, gastric complaints, nausea, gastrointestinal complications, duodenitis and abdominal bloating), neurological AEs (e.g. headache, foggy thinking, drowsiness, leg weakness, legs periodic numbness and tingling, tingling in toe and fingers and intermittent bilateral toe numbness), psychiatric disorders (e.g. bipolar disorders), musculoskeletal AEs (e.g. neck pain, lower back pain and spasms), skin AEs (e.g. skin rash, disseminated maculopapular rash, itching sensation and urticaria), infections (e.g. laryngitis, pneumonia and yeast infections), cardiovascular system AEs (e.g. increase in arterial blood pressure, palpitations, myocardial infarction, heart rate and rhythm disorders and heart valve disorders), hospitalization and death.

EVALUATION OF ENDOCAN LEVELS IN CLINICAL SUBSETS CHARACTERIZED BY CHRONIC INFLAMMATORY STATUS: A POSSIBLE LINK TO HIGH CARDIOVASCULAR RISK

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Background and Aim. Endocan is a proteoglycan secerned by several cell type, including endothelial cells, involved in the onset and progression of atherosclerosis. Recent researches suggest a role of Endocan in inflammation and neovascularization; thus, it has been proposed as a marker of vascular stress. It was found elevated in multiple conditions, including sepsis, oncology, autoimmune or infectious diseases. In the light of these findings, we aimed to assess Endocan levels in other clinical settings: Familial Hypercholesterolemia (FH), Systemic Sclerosis (SSc), IBD.
Materials and Methods. We tested adult patients affected by FH (30), IBD (28) or SCL (37). All patients and healthy controls (18) underwent blood sampling on which we tested an Elisa immunoassay to assess circulating Endocan levels. FH and IBD patients underwent bloody sampling and the Elisa assay both when on conventional treatment either after the therapy with biologics (i-PSCK9 or anti-TNFα Ab, respectively).
Results. Highest levels of Endocan were detected in patients affected by SSc (2.1 fold, p<0.001 vs controls) vs controls). IBD patients refractory to conventional therapy showed higher Endocan levels when compared with control group (1.66 fold, p=0.02 vs controls), however the same patients showed a reduction of Endocan levels after the induction treatment with Biologics (1.18 fold, p=0.50 vs controls). FH population on conventional therapy (statin plus Ezetimibe) showed Endocan levels lower than healthy controls (0.7 fold, p<0.001). i-PCSK9 therapy seemed to slightly but not significantly reduce Endocan plasma levels (0.55 fold, p=0.32).
Conclusions. High circulating levels of Endocan were detected in high cardiovascular risk conditions as FH, IBD, SSc. Our data on FH seem to show that statin treatment could improve endothelial function even better if taken in association with i-PCSK9. Poor controlled IBD correlates with higher Endocan levels, while optimizing therapy could reduce Endocan plasma levels.

ASSOCIATION BETWEEN ADIPOSITY-RELATED TRAITS AND CORONARY ARTERY DISEASE: A MENDELIAN RANDOMIZATION ANALYSIS

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The causal role of adiposity in coronary artery disease (CAD) is still unclear. Therefore, we applied a Mendelian randomization (MR) approach to estimate the effect of body mass index (BMI), as a measure of adiposity, on CAD.As instrumental variables we used a polygenic score made by 97 single-nucleotide polymorphisms (SNPs) associated with BMI. The study included individual participant data from subjects enrolled in the UK Biobank study (N=459,322), and summary-level data from participants enrolled in observational cohorts included in the CARDIoGRAMplusC4D Consortium. We explored causal associations with a two-sample MR method. The 97-point estimates derived from the individual participant data (beta-coefficients for the SNP–BMI association, with variants aligned to higher BMI) and the summary data (beta-coefficients for the SNP–outcome association) were then combined across studies in a fixed-effect inverse variance-weighted meta-analysis to produce an overall summary point estimate. We also assessed what proportion of the association between BMI and CAD was mediated by systolic blood pressure (SBP), lipids and glycated haemoglobin using a regression-based multivariable MR model.Genetic predisposition to higher BMI was significantly associated with CAD. The odds ratio (OR) per 1kg/m2 increase in BMI was 1.10 (95% confidence interval (95%CI) 1.08-1.12), confirmed in a sensitivity analysis where beta-coefficients for the SNP–BMI association were adjusted for waist-to-hip ratio (OR 1.12, 95%CI 1.09-1.15). The effect of BMI on CAD was slightly attenuated in the multivariable MR analysis (OR 1.064, p-value <0.001), when the association was evaluated net of the effect of increasing adiposity on SBP, glycated haemoglobin, and lipids.These results suggest a casual association between lifetime exposure to high BMI and higher CAD risk. However, further analyses are necessary, to understand if losing weight will be useful to reduce cardiovascular risk in addition to the clinical benefit achieved by monitoring well-established cardiovascular risk factors.

HOW TO IMPLEMENT CLINICAL DIAGNOSIS OF FH IN CHILDREN AND ADOLESCENTS? PRELIMINARY EVIDENCE FROM THE LIPIGEN PAEDIATRIC GROUP

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Background. The early identification of familial hypercholesterolemia (FH) in childhood is made challenging by the lack of validated diagnostic criteria and by the usually less severe phenotype in first decades of life. We aimed to compare the Dutch Lipid Clinic Network (DLCN) criteria in genetically-confirmed FH adult and paediatric patients.

Methods. From the LIPIGEN study, an observational study collecting data about FH patients in Italy, we selected 1204 (≥18 years) and 798 (<18 years) FH patients with a positive genetic test. DLCN parameters and proportion of missing data were compared between the two groups. Based on the percentile distribution of LDL-c classes among adults, we identified specific LDL-c cut-off for children/adolescents, and collected data about premature CHD also in second-degree family members.

Results. Among these genetically-confirmed FH patients, according to the original DLCN score, probable/definite FH (score ≥6) was found in 67.1% of adults, but only in 26.9% of children/adolescents. In this latter group, the percentage increased to 52.1% using the new LDL-c classes (142-162, 163-214, 215-261, ≥262 mg/dL). The lower prevalence of typical FH features in children/adolescents vs adults was confirmed: tendon xanthoma 2.9% vs 17.5%, arcus cornealis 1.7% vs 17.5%, respectively. No children presented clinical history of premature CHD or cerebral/peripheral vascular disease (in adults 12.9% and 4.5%, respectively). Data about premature CHD in first-degree family members were missing for 4.7% of adults and 14.2% of children/adolescents. On valid data, this prevalence was 43.9% in adults and only 22.0% in children, increasing to more than half of subjects (51.8%) when considered also second-degree relatives (data available for 229 individuals <18y), but without relevant changes in the proportion of probable/definite FH.

Conclusion. In children the DLCN score is clearly less informative than in adults. A re-modulation and validation of new score for children/adolescents is necessary to improve the detection rate.

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APPLYING TARGETED NEXT-GENERATION-SEQUENCING TO DRIED BLOOD SPOT SPECIMENS FOR UNIVERSAL MOLECULAR SCREENING OF FAMILIAL HYPERCHOLESTEROLEMIA IN PEDIATRIC PATIENTS

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Introduction. Familial Hypercholesterolemia (FH) is the most common genetic dyslipidemia, with an estimated frequency of 1/200- 250 at heterozygous state, associated to premature cardiovascular disease. FH remains mainly underdiagnosed and undertreated globally. We evaluated the use of a dried blood spot (DBS) sample as a simple method to obtain a DNA sample from children to be used for next-generation-sequencing, to encourage the genetic diagnosis of FH since young age.

Materials and Methods. An NGS panel targeting the entire coding sequence of the six genes mainly involved in FH were used to analyze DNA extracted from venous blood (VB) and from DBS, from 46 patients with suspicious of FH. The sequencing was performed using Illumina MiSeq Reagent Micro Kit V2 and data analysis was performed by Amplicon Suite software. Identified pathogenic or uncertain significant single nucleotide variants (SNVs) were confirmed by Sanger Sequencing, while the pathogenic copy number variations (CNVs) were confirmed by MLPA analysis.

Results. A depth sequencing of 30X was considered as our minimum threshold; coverage of different amplicon highly fluctuated; an average read depth of 30X were obtained in 100% of the amplicons of genes in VB sample (80X-4838X), while in 3/46 DBS sample at least one amplicon was below 30X (3X-6046X). We also evaluated the concordance of variant calling between sequencing from VB DNA and DBS DNA, which was 100% for the SNVs. No concordance was observed for CNVs, which were not always identified in the DBS-samples.

Conclusions. Targeted NGS can be performed on DBS-samples to reliably detect SNVs which represent 80-90% of FH-causative variants. Identification of CNVs should be performed separately. This genetic screening could be used as a workflow useful also to set-up an universal screening for FH in children.

EFFECTIVENESS AND SAFETY OF LOMITAPIDE IN A PATIENT WITH FAMILIAL CHYLOMICRONEMIA SYNDROME

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Background. Familial chylomicronemia syndrome (FCS) is characterized by severe fasting hypertriglyceridemia, abdominal pain and recurrent acute pancreatitis. Available triglyceride-lowering drugs are insufficient to avoid pancreatitis. Therefore, there is a significant unmet medical need for effective triglyceride-lowering drugs for patients with FCS.
Materials and Methods. We report the second case of a patient with FCS and recurrent pancreatitis treated with lomitapide. Results: Lomitapide treatment resulted in a reduction of fasting TG levels from 2897 mg/dl (32.71 mmol/l) to an average of 954 mg/dl (10.77 mmol/L) on the 30 mg lomitapide equating to a 67% reduction from baseline. After 26 months of lomitapide treatment, histological activity score for hepatic fibrosis was stable although liver biopsy showed a marked increase of liver steatosis and mild perivenular and perisinusoidal fibrosis.
Conclusion. Lomitapide is effective in reducing triglycerides in FCS and preventing the recurrence of acute pancreatitis. A longer follow-up is necessary to evaluate long term risk of progression towards severe stages of liver fibrosis. A prospective clinical trial may identify which subgroup of FCS patients would benefit from lomitapide treatment in the absence of significant liver adverse effects.

THE METABOLIC SYNDROME IN A MEDITERRANEAN POPULATION: RESULTS OF A 25-YEARS FOLLOW-UP OF THE "VENTIMIGLIA HEART STUDY"

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Introduction. Metabolic syndrome consists of a cluster of conditions that increase the risk of cardiovascular disease, diabetes and stroke. The "Ventimiglia Heart Study" is an epidemiological study started in 1989 in a rural Mediterranean community and is currently in progress to evaluate the cardiovascular (CV) risk factors and their role on prediction of CV events and mortality. The aim of this study was to evaluate the metabolic features changes of this community after 25-years follow-up.
Materials and Methods. Among the subjects enrolled in the "Ventimiglia Heart Study" project in the years 1989 and 2014, we selected, respectively, 841 subjects (M=378, F=463) and 589 subjects (M=257, F=332) between 25 and 75 years of age. Anthropometric measures, clinical and biochemical parameters, life style and CV outcomes were evaluated. The metabolic syndrome was defined according to the 2004 Adult Treatment Panel III (ATPIII)/NCEP criteria.
Results. Metabolic syndrome prevalence was not significantly changed in 25-years follow-up, although in 2014 it was increased in males (30.4% vs 16.9%, p<.0001) while in females it was slightly decreased (27.1% vs 32.4%, NS). A high prevalence of visceral obesity, already highlighted in 1989, was confirmed, with a marked worsening of metabolic parameters in males and a partial improvement in females. Compared to 1989, an improvement in blood pressure, triglyceridemia and glucose plasma levels was observed in 2014, probably due to the increased pharmacological interventions in the population.
Conclusions. The 25-years follow-up "Ventimiglia Heart Study" highlights the great importance of metabolic syndrome as a public health issue in a rural population and indicates that the metabolic parameters have profoundly changed due to the socio-economic and cultural modifications of this rural community.

STATINS THERAPY AND SARS-CoV-2 INFECTION: WHAT'S UP WITH THIS?

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Background. Some studies put their attention on the antinflammatory activity by statins, trying to understand if such drugs could effectively be valuable in COVID-19 therapy. The aim of this study is to understand whether a relation between statin therapy and SARS-CoV-2 infection exists and whether this therapy could be useful in COVID-19 management. Secondary goal was to deepen the relations of statin therapy with the main disease outcomes: the need for an intensification of care, inhospital mortality, 30-days mortality.
Patients and Methods. We enrolled 502 patients, admitted to our Internal Medicine Department from the 15th of March to the 15th of June, 2020. All patients got a laboratory diagnosis of SARS-CoV-2 infection. Clinical, demographic and laboratory data were collected from the informatics' system of our Hospital. Anamnestic informations were collected from patients and/or from their relatives. Of these 502 patients, 60 had a clinical reason to a statin home therapy prescription. Following current guidelines we did not interrupt statin therapy while treating them for SARS-CoV-2 infection, according to their clinical needs. Statistical analysis were performed using SPSS software, v.26.0 with the execution of univariate and multivariate analysis.
Results. We searched for statistical relations between statin therapy and patients' need for an intensification of care, with the inhospital mortality rate and with 30-days mortality rate. At the time of writing we missed clinical data of 15 out of 60 patients: preliminary data did not show any significant relation between the chosen variables. We also tried to understand whether a co-administration of statins and the other administered therapies could improve patients' immune response to SARS-CoV-2 infection, finding no preliminary differences in the three disease outcomes chosen.
Conclusions. Compatibly with the missing data, we found no statistically significant relation between patients with a statin therapy and patients without.

ATHEROSCLEROSIS, PCSK9 AND EXTRACELLULAR VESICLES - EVIDENCE FROM IN VITRO MODELS

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Background. The function of extracellular vesicles (EVs) is being investigated in the context of several pathological conditions, including cardiovascular disease (CVD). EVs have been proposed to contribute to the pathogenesis of atherosclerotic cardiovascular diseases (ASCVD) by promoting inflammation and thrombosis via different mechanisms. Endothelial injury, a key step in the development of atherosclerotic lesions, triggers the release of EVs that may play a role in regulating endothelial dysfunction and vascular wall inflammation by increasing the expression of surface adhesion molecules (VCAM-1, ICAM-1), promoting smooth muscle cells differentiation and determining the release of pro-inflammatory cytokines (Deng 2019). In line with these findings, circulating levels of EVs associate with the occurrence of major adverse events and are raised in patients with CV risk factors, such as hypercholesterolemia, hypertension or metabolic syndrome (Jansen 2017). EVs are extracellular structures enclosed by a lipid bilayer and are secreted by all known cell types. EVs carry proteins, RNAs and/or microRNAs among other molecules and act as vehicles in cell-to-cell communication. AIM: To unravel the impact of EVs derived from human smooth muscle cells (hSMC) overexpressing or not PCSK9 on inflammation, migratory capacity and mitochondrial activity of human monocytes THP-1, human derived-THP-1 macrophages and hSMC.
Methods
a) cell lines: hSMC overexpressing or not PCSK9, human THP-1 monocytes and human THP-1 derived macrophages;
b) EVs isolation (by ultracentrifugation) and characterization (by nanoparticle tracking analysis);
c) gene and protein expression (qPCR and Western blot);
d) cell migration (by using a Transwell chamber with polycarbonate membrane).

Overall, cells have been treated for 24 hours with EVs.
Results. Compared to EVs derived from hSMC, over expression of PCSK9 led to a reduction in concentrations (17.1*10^9 EVs/ml vs 6.2*10^9 EVs/ml, respectively). Conversely, no size differences were detected (202 nm vs 210 nm, respectively). In THP-1 monocytes and THP-1 derived macrophages, 24-h exposure to EVs derived from hSMC overexpressing PCSK9 activated the gene expression of MCP-1/chemokine (C-C motif) ligand 2 (+27 fold), interleukin (IL)-1 α (+28 fold), IL1beta (+25 fold), IL-6 (+94 fold), and IL-8 (+4 fold). In accordance to changes in mRNA levels, in all cell models, protein expression of inflammatory pathways of signal transducer and activator of transcript 3 (STAT3) and suppressor of cytokine signaling (SOCS3) were also raised upon exposure to EVs isolated from hSMC overexpressing PCSK9. Conversely, no differences have been found when the inflammasome (NLRP3)

cascade was evaluated. EVs isolated from hSMC overexpressing PCSK9 negatively affected monocyte migration. Out of 400,000 THP-1 cells per well seeded, 2.83% migrated when exposed to EVs isolated from hSMC and 0.21% when exposed to EVs isolated from hSMC overexpressing PCSK9.The mitochondrial OXPHOS protein expression (complex -I, -II, -III, -IV and -V) was not altered in any of the cell lines evaluated.
Conclusions. In the context of atheroma formation, these findings are in line with our previous evidence reporting a feed-forward inflammatory loop exerted by PCSK9.(Ruscica 2016, Ricci 2018, Macchi 2020).

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MANAGEMENT OF HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: GENOTYPING AS DRIVER OF APPROPRIATE THERAPEUTIC APPROACH

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Introduction. Homozygous Familial Hypercholesterolemia (HoFH) is a rare genetic disorder characterized by very high levels of LDL-C and premature atherosclerotic cardiovascular disease (ASCVD). HoFH due to LDLR gene mutations are classified into two groups based on the residual functional activity of LDL-R: patients < 5% of normal LDLR activity are classified as receptor negative and patients with 5 to 25% of normal LDLR activity as receptor-defective. We describe the clinical and molecular characterization of a subject with severe hypercholesterolemia.
Materials and Methods. The proband is a 52-year-old man. Severe hypercholesterolemia (about 400 mg/dL) was first noted when he was 23 and treatment with simvastatin was prescribed. He suffered an episode of acute coronary syndrome at 38 years of age and he underwent to PTCA procedure. The coronary angiography revealed a diffuse non stenotic atherosclerosis of the coronary tree beside the treated culprit lesion. Treatment with high efficacy statin at the maximum tolerated dose (rosuvastatin 20 mg/d) in association to ezetimibe did not allow to reach the suggested LDL-C goal (LDL-C 150 mg/dl). Genetic analysis was performed by Next Generation Sequencing (NGS) using Ion Torrent PGM in order to analyze candidate genes involved in FH.
Results. We identified an homozygous LDLR gene mutation (c.2359G/A; p.Val797Met) previously described and classified as receptor-defective. We decide to start PCSK9 monoclonal antibody therapy. Administration of evolocumab 420 mg monthly, on top of standard care, effectively reduced LDL-c levels (from 150 mg/dL to 22 mg/dL). Residual LDLR expression in HoFH is a major determinant of LDL-C levels and it drives the individual response to anti PCSK9 MAb evolocumab.

BIOHUMORAL MARKERS OF ACUTE ISCHEMIC STROKE: THE RISK STUDY

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Background. Ischemic stroke is a heterogeneous disorder and a biomarker panel may be able to better reflect the diverse pathophysiology involved in stroke. Literature data suggested the important role of MMP: in particular, MMP9 correlates with Hemorrhagic Transformation and Death, as well as some inflammatory molecules are associated with three-month functional outcome. The reperfusion therapies for acute ischaemic stroke are represented by the rapid restoration of cerebral blood supply by intravenous thrombolysis (IVT) with recombinant tissue plasminogen activator (rt-PA) and by mechanical thrombectomy. This study has been conducted in a consecutive series of patients with AIS candidates to IVT and/or to endovascular treatment with the purposes of: 1) to evaluate the role of circulating biomarkers, namely metalloproteinase, interleukins, adhesion molecules and growth factor in relation to clinical outcomes.
Methods. We investigated 110 AIS patients treated with systemic or endovascular t-PA thrombolysis, enrolled between October 2015 and October 2018. A blood withdrawal for assessment of MMPs, was also performed at baseline and at 24 hours. Circulating biomarkers were determined in serum by using a multiplex assays.
Results. In this large series of patients we found that:
1) pre-tPA EMMPRIN and MMP-2 circulating levels were associated with death;
2) pre-tPA VCAM-1 and CXCL-10 circulating levels were associated with worse clinical outcome (mRS>2);
3) ICH occurred more frequently in patients with high levels of pre-tPA, IL-6 and CXCL-8;
4) the occurrence of edema at 24 hours after thrombolysis was slightly associated with low EMMPRIN and VCAM-1 pre-tPA levels, whereas pre-/post-tPA-variations of MMP-3, VCAM-1 and EMMPRIN were positively and significantly associated with edema at 24 hours.

Conclusions. Our results obtained in ischaemic stroke patients provided interesting insights into the mechanisms underlying the ischaemic stroke and the response to thrombolysis and discover new biohumoral markers.

TALMUD POLYGENIC SCORE: CONTRIBUTION OF COMMON GENETIC VARIANTS IN THE MOLECULAR CHARACTERIZATION OF FAMILIAL HYPERCHOLESTEROLEMIA

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Background. Familial Hypercholesterolemia (FH) represents an autosomal disorder due to pathogenic variants in LDLR or APOB or PCSK9 (dominant form), and in LDLRAP1 (recessive form). Previous data showed that in about 60% of patients who are mutation-negative the clinical phenotype can be associated with an accumulation of common small-effect LDL-C-raising alleles using a 12- Single nucleotide polymorphisms (SNPs) score. Aim of this study was to evaluate Talmud (2013) genetic score in patients with FH with and without variants in LDLR gene.
Method. We analysed 47 patients with clinically possible/probable or definite FH using the most common diagnostic algorithm, Dutch Lipid Clinic Network Score. SNPs included in Talmud score were detected through high-throughput (HTS) or Sanger sequencing. HTS was performed using Illumina MiSeq Reagent Kit. Sequencing results were analyzed using a pipeline optimized by the Florentine bioinformatics group. The possible pathogenicity of variants was evaluated using five different in silico tools. For statistical analysis we used SPSS v.25 software.
Results. Among 47 patients analyzed, 20 had a pathogenetic variant in LDLR and 27 did not carry mutations in this gene. Significantly higher Talmud score mean value in patients without LDLR mutation with respect to those with LDLR variant was observed [mean±SD: 1.031±0.146 vs 0.894±0.196, p=0.008]. Mean Talmud score value found in LDLR mutation negative group relates to a risk ratio (95% CI) of LDL-C>4.9 mmol/L 2.73 (1.93-3.17) (8th LDL-C gene score decile). Conversely, in patients carrying LDLR mutations mean score value relates to a risk ratio (95% CI) 2.21 (1.55-3.17) (5th LDL-C gene score decile). Accordingly, a higher percentage of patients within a LDL-C gene score decile ≥ 8th was present in the LDLR mutation negative group (48%) in comparison to that observed in the LDLR mutation positive group (30%). As concerns Dutch score, significantly higher values were observed in the LDLR positive group than in patients negative for LDLR pathogenetic variant [median (IQR): 7.0 (5.8-8.0) vs 5.0 (4.0-6.0), p=0.002].
Conclusion. The results suggest a possible contribution of common variants in modulating the lipid profile in FH patients. Further expansion of present data will allow to confirm the hypothesis that FH can also be caused by an accumulation of common small-effect LDL-C-raising alleles. At present, the relationship between Talmud score and both pharmacological response to statins treatment and cardiovascular events is also under evaluation.

TRANSCRIPTOMICS STUDY IN CEREBRAL THROMBI AND PERIPHERAL VENOUS BLOOD OF ACUTE ISCHEMIC STROKE PATIENTS

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Background. Acute ischemic stroke (AIS) therapies consist of cerebral blood restoration by i.v. rt-PA thrombolysis and/or mechanical thrombectomy (MT). MT has created the opportunity to evaluate morphology and biomolecular characteristics of cerebral thrombi (CT). Few previous studies showed that thrombus composition can correlate with both AIS pathophysiology and outcomes.
Aims of the study were:
1) to investigate the global gene expression profile of CT and venous peripheral blood (PB) in order to identify markers of different pathophysiological mechanisms of AIS;
2) to investigate whether information obtained by studying gene expression profiles in CT are different from those of PB.
Methods. We performed gene expression profile of 52 AIS patients. CT obtained during MT were collected in RNA later. Blood samples were collected before and 24 hours after MT. RNA was extracted by PAX gene blood miRNA kit. The global gene expression was assessed by Affymetrix technology using GeneChip Human Transcriptome Array 2.0. Data analysis was performed in R environment with dedicated pipelines.
Results. After data processing, the average of analyzable probe sets numbered 440,085 in CT and 602,874 in PB samples. The Gene Ontology enrichment analysis in CT and PB indicating that peripheral and local mechanisms of damage and response to damage are present in CT and PB. In particular, in CT, we observed 221 significant biological processes associated with poor outcome according to mRS, and in PB, we observed 27 terms associated with 24 h edema. Among significant terms in CT, those associated with regulation of neutrophil mediated immunity and activation play a crucial role.
Conclusions. Our data provided interesting insights into the mechanisms underlying the AIS and the response to thrombolysis. Transcriptomics analysis according to stroke outcomes, confirmed and extended several known pathophysiological mechanisms and suggested novel pathways to be explored to enlarge knowledge on AIS.

INDEPENDENT PREDICTORS OF CHOLESTEROL REVERSE EFFLUX IN PATIENTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction. Reverse cholesterol transport (RCT) is a process in which excess of cholesterol accumulated in peripheral tissues, is removed and transported by HDL to the liver and successively, excreted into the feces via the bile. Cholesterol efflux capacity (CEC) from peripheral cells is thought to be a critical metric of HDL functionality. The evaluation of serum cholesterol efflux capacity (CEC) may be a predictor of atherosclerosis extent and is considered to be better target for the prevention of CVD. Many studies showed that CEC could be inversely associated with incident CVD. Nevertheless, few studies have analyzed CEC in patients with familial hypercholesterolemia, the most common autosomal dominant genetic disease, caused by mutations in the genes associated to LDL clearance. Familial hypercholesterolemia (FH) is a genetic disease associated with a high cardiovascular risk, despite lipid-lowering therapy, compared to controls.
Scope. The aim of this study is to evaluate differences of CEC between FH patients and no-FH subjects and analyze its independent predictors.
Patients and Methods. A total of 183 patients (31-80 years old) were enrolled: 61 heterozygous FH patients and 122 no-FH (matched by gender, age and BMI). The three most important RCT pathways were measured by using specific cell models: total CEC, Basal-CEC and ATPbinding cassette A1 (ABCA1)-mediated CEC. Briefly, cells were labeled with [1,2–3H]-cholesterol and exposed to HDL, isolated from subjects serum by polyethylene glycol. Serum HDL CEC was expressed as a percentage of the radioactivity released to the medium in 4 h (6 h for ABCG1-CEC) after over the total radioactivity incorporated by cells. LDL and HDL subfraction serum were separated and quantified using the Lipoprint System in large-buoyant LDL (lbLDL) and small-dense LDL (sdLDL) and large, medium and small HDL, respectively. VLDL, LDL and HDL were isolated from serum by Ultracentrifugation.
Results. Results showed higher CEC levels in FH patients compared to controls. There were not significate differences of CEC between FH patients with and without CVD and ever lipid-lowering treatment did not provoke significate variations. CEC levels were not correlated with age; instead, it had a positive correlation with total cholesterol, HDL-cholesterol, triglycerides, VLDL-cholesterol, lbLDL-cholesterol sdLDL-cholesterol and LDL size. Linear regression analysis shown that independent predictors of CEC were to have the familial hypercholesterolemia, VLDL-cholesterol concentration, levels of medium HDL-cholesterol, HDL-cholesterol concentration and total cholesterol.
Conclusion. Higher incidence of CVD does not seem be associated with lower CEC levels. Lipid-lowering therapy not influence CEC levels. Independent predictors of CEC levels are cholesterol levels included in different lipoproteins.

LIVER STEATOSIS AND FIBROSIS ARE ASSOCIATED WITH METABOLIC COMORBIDITIES BUT ARE NOT INDEPENDENTLY ASSOCIATED WITH ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction and Aims. Atherosclerotic cardiovascular disease (ASCVD) risk stratification in familial hypercholesterolemia (FH) is challenging due to high heterogeneity. Liver steatosis and fibrosis are associated with an increased ASCVD risk. We aim at determining the prevalence and the predictors of liver steatosis and fibrosis, and at evaluating whether they are associated with increased prevalence of ASCVD in adult FH patients.
Methods. 180 adult patients with a clinical (DLCN ≥6) and/or molecular diagnosis of FH consecutively enrolled within the LIPIGEN Project underwent comprehensive evaluation, including assessment of liver steatosis by abdominal ultrasound. In 154 patients the Fibrosis-4 (Fib-4) index was also measured (as a marker of liver fibrosis), and values higher than 1.30 were considered at risk of advanced liver fibrosis.
Results. Men and women were 92 and 88, respectively; median age was 48[18-83] years. Among 165 patients with available genetic test, 84.2% were carriers of a "pathogenic" or "likely pathogenic" mutation in FH-causing genes. Median body mass index (BMI) was 25.2[17.9-44.1] kg/m2 and the prevalence of dysglycemia, high blood pressure and metabolic syndrome (MetS) was 22.2%, 47.8% and 17.9%, respectively. 17.2% of patients had a positive history of ASCVD.The prevalence of liver steatosis was 31.7% (n=57). Patients with liver steatosis were significantly more likely to have visceral adiposity (p:<.001), dysglycemia (p:.005), high blood pressure (p:.043) and MetS (p:<.001) than those without liver steatosis. They also showed significantly higher age (p:.037), BMI (p:<.001), waist circumference (p:<.001), systolic (p:.033) and diastolic (p:.029) blood pressure, triglycerides (p:<.001), glucose (p:.001), HbA1c (p:<.001), uric acid (p:<.001), aspartate (p:.007) and alanine (p:.001) aminotransferases and gamma-glutamyltransferase (p:<.001) levels and lower HDL cholesterol levels (p:.012) than their counterpart without liver steatosis. Median Fib-4 values were 1.04[0.25-3.28] and 52 patients (33.8%) were at risk of advanced liver fibrosis. Fib-4 was significantly and positively correlated with age (rho:.803, p:<.001), waist circumference (rho:.177, p:.031), systolic (rho:.170, p:.037) and diastolic (rho:.204, p:.012) blood pressure, glucose (rho:.377, p:<.001), HbA1c (rho:.307, p:<.001), number of factors of the MetS (rho:.311, p:<.001), aspartate aminotransferase (rho:.327, p:<.001) and gamma-glutamyltransferase (rho:.171, p:.036). Accordingly, patients at risk of advanced liver fibrosis were significantly more likely to have dysglycemia (p:.001), high blood pressure (p:.002) and MetS (p:.046) than patients not at risk.The prevalence of liver steatosis and the risk of advanced liver fibrosis both increased across the number of factors of the MetS (steatosis: 7.3% for 0 factors vs. 100% for 5 factors, p:<.001; fibrosis: 12.5% for 0 factors vs. 50% for 5 factors, p:.001).Patients with liver steatosis (26.3% vs. 13.0%, p:.028) and those at risk of advanced liver fibrosis (38.5% vs. 10.8%, p:<.001) were both significantly more likely to have a positive history of ASCVD than their counterpart. Moreover, Fib-4 was also significantly associated with carotid artery plaques (p:<.001) and carotid intima-media thickness (rho:.486, p:<.001). At univariate logistic regression analysis, liver steatosis (OR:2.4, 95%CI:1.1-5.3; p:.031) and the risk of advanced liver fibrosis (OR:5.2, 95%CI:2.2-11.9; p:<.001) were both significantly associated with ASCVD. However, after adjustments for sex, age, smoking status, untreated LDL-cholesterol and metabolic comorbidities, the significant associations between liver steatosis, liver fibrosis and ASCVD went lost.
Conclusions. Liver steatosis and liver fibrosis, mainly attributable to metabolic dysfunction-associated fatty liver disease (MAFLD), are common in adult FH patients and are associated with metabolic comorbidities and ASCVD. However, the associations between liver steatosis, liver fibrosis and ASCVD seem to be largely mediated by metabolic comorbidities.

IN VITRO, IN VIVO AND CLINICAL INVESIGATIONS TO UNRAVEL THE INVOLVEMENT OF PROPROTEIN CONVERTASE SUBTILISIN/KEXINE TYPE 9 IN VASCULAR CALCIFICATION SECONDARY TO CHRONIC KIDNEY DISEASE

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Background and Aim. Chronic kidney disease (CKD) lead to a higher rate of cardiovascular death (CVD) due to severe alterations in lipidic profile and a massive vascular calcification (VC) of the tunica media in the vessels' wall. Indeed, due to the hyperphosphatemia triggered by the renal damage, vascular smooth muscle cells (VSMCs) in the tunica media trans-differentiate in osteoblastic-like cells. Proprotein convertase subtilisin/kexin type 9 (PCSK9) turned out as a cornerstone pharmacological target for familial hypercholesterolemia (FH), with two anti-PCSK9 monoclonal antibodies approved so far. Recently, PCSK9 plasma levels were associated with a higher calcification rate in the general, FH, CKD, and diabetic populations. Starting from these premises, the present study aims to study the putative causal role of PCSK9 in VC under uremic conditions.
Methods. Plasma PCSK9 has been measured in a cohort of 594 patients with renal impairment (RI) divided according to their GFR and the presence of atherosclerotic cardiovascular disease (ASCVD). Sprague-Dawley rats were fed a standard diet (n=11) or uremic diet containing 0.5% adenine (n=11) for 6 weeks. Urine volumes have been measured every two weeks by housing rats in metabolic cages for 24h. At sacrifice, abdominal aortas, plasma, livers, and kidneys have been collected. Calcium crystals in tunica media of aortas have been visualized by von Kossa staining and quantified by a colorimetric assay. Plasma creatinine and phosphate levels have been evaluated by clinical standardized methods. PCSK9 expression in kidneys and liver has been evaluated by Western Blotting (WB). PCSK9 expression in tunica media was visualized by immunohistochemistry (IHC). The overexpression of PCSK9 in vascular smooth muscle cells (VSMCs) has been realized through retroviral infection. The cells have been cultured with low-FCS/high-phosphate media (2.5% FCS plus 2.0 mM or 2.4 mM of Pi solution) for 7 days, changing media every two days. Hydroxyapatite deposition by cells has been measured by a calcium colorimetric assay. Calcification markers, such as tissue-nonspecific alkaline phosphatase (ALP), osteopontin (OPN), matrix GLA protein (MGP) and bone morphogenetic protein 2 (BMP2) expressions were evaluated through WB and real time-qPCR (RT-qPCR). Extracellular vesicles (EVs) have been evaluated by flow cytometry (FC) and were tested for their calcium and ALP content.
Results. In a cohort of 594 patients with RI, the lower the GFR the higher plasma PCSK9, as previously reported. ASCVD as co-morbidity worsens this outcome. In our uremic model, the uremic condition was documented by increased urine volume (26 mL/day vs 58 mL/day), plasma creatinine (25.7 µM vs 208 µM) and phosphate levels (2.64µM vs 6.11 µM). The proved renal damage triggered extensive aortic calcifications, determined by measuring aorta calcium concentrations (0.34 mg/g tissue vs 2.48 mg/g tissue) and by Von Kossa staining. Moreover, the model showed a significant increase in TC (from 75.3 mg/dL to 107.6 mg/dL) and PCSK9 levels (from 40.1 ng/mL to 109.7 ng/mL). Higher expression of PCSK9 was also observed in kidney (+4.8-fold) and liver (+1.5-fold). The overexpression of PCSK9 in VSMCs induced a significant increase of extracellular calcification in response to 7 days exposure to 2.4 mM Pi (+39% compared to control hSMCs). This increase in calcification rate is mediated by a significant reduction in VC inhibitors (OPN and MGP) and by a significant increase in VC stimulators, such as ALP and BMP2. We also observed an increase in Ca2+ and ALP-loaded EVs budding.
Conclusion. The present study indicates a direct role of PCSK9 on VC associated to a CKD condition. Further analysis on PCSK9 knockout models for CKD-mediated vascular calcification will attempt to prove the PCSK9 causality in VC process.

PCSK9 AFFECTS BRAIN CHOLESTEROL METABOLISM AND NEUROINFLAMMATION IN HUMAN CELL MODELS OF ASTROCYTES AND NEURONS

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Background. Altered lipid metabolism and increased neuroinflammation are associated with Alzheimer's disease (AD) pathogenesis. In the brain, PCSK9 is able to degrade the neuronal receptors that, by interacting with HDL-like particles containing apoE (apoE-HDL), are able to promote the uptake cholesterol derived from astrocytes. In addition, PCSK9 has shown a pro-inflammatory effect in macrophages, microglial-like cells. This study aims to investigate the molecular mechanisms by which PCSK9 may exert a pathogenetic role and a deleterious effect on brain cells by negatively modulating cholesterol homeostasis or neuroinflammation in astrocytes and neurons.
Methods. The following cell models have been utilized: human astrocytoma cells (U-373) exposed to exogenous human recombinant PCSK9; human neuroblastoma cells (SH-SY5Y) retrovirally transduced to overexpress human PCSK9. The effect of PCSK9 has been evaluated on: cholesterol synthesis, efflux and uptake by radioisotopic assays; gene and protein expression by qRT-PCR and Western Blot analyses, respectively; the interaction between fluorescent apoE and living cells, by confocal microscopy.
Results. In U-373 cells, incubation with PCSK9 significantly increased endogenous cholesterol synthesis in the presence of 10% FCS (+2 folds; $p<0.001$). In U-373 stimulated with LXR/RXR agonists, treatment with human recombinant PCSK9 significantly reduced the efflux to apoE-HDL (-25%; $p=0.008$), while it did not affect cholesterol efflux to isolated apoE. In SH-SY5Y overexpressing PCSK9, the uptake of 3[H]-cholesterol-labeled apoE-HDL was significantly reduced compared to control SH-SY5Y (-30%; $p<0.001$). PCSK9 overexpression also reduced the interaction between fluorescein labelled-apoE and neuroblastoma cells. Moreover, in the same cellular model, apoER2 and LDLr expression was significantly reduced ($p<0.05$). Regarding neuroinflammation, PCSK9 worsened the inflammatory response induced by beta-amyloid aggregates in U-373, further increasing the gene expression of IL-1beta and TNF-alfa ($p<0.05$). Altogether, these results suggest an influence of PCSK9 on cerebral cholesterol homeostasis and neuroinflammation, indicating a possible role in AD pathogenesis.

PRIMARY DYSLIPIDAEMIAS IN THE PEDIATRIC AGE: CLINICAL AND BIOCHEMICAL IMPACT OF NUTRITIONAL COUNSELLING

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Background. Children affected by primary dyslipidaemia are exposed to cardiovascular risk, so they have to be treated early to avoid premature coronary heart disease. Dietary counselling is the first approach but its effectiveness questioned. The aim of this study was to investigate the dietary impact on lipid profile of pediatric patients affected by different form of dyslipidaemia. The second purpose was to check any relationship with different diagnosis of dyslipidaemia.
Methods. n. 225 familial hypercholesterolemia (FH), n.229 familial combined hypercholesterolemia (FCHL) and n.331 polygenic hypercholesterolemia (HyperCT), not undergoing phytosterols or statin therapy, were selected. Subjects underwent two consecutive medical examinations to assess their plasma lipid levels and daily diet. Throughout the first examination, they received dietary counselling, according to the CHILD-1 diet protocol. Week diaries checked compliance and LDL-c reduction was used as parameter of responsivity to the diet.
Results. 66.4% of patients showed adherence to the dietary therapy with a lipid profile improvement in the 81.7% of them. CT, LDL-c and non-HDL-c decrease was observed in FH -7.5%, -10.6%, -9.9% respectively, FCHL -6.4%, -6.4%, -5.6% respectively and HyperCT -9.4%, -11.8%, -10.7% respectively. No significant reduction in triglycerides. Concerning nutritional changes: all groups reduced total lipids and saturated acid fat (-20%), increased complex carbohydrates (+6%) and dietary fiber (+20%) intakes. A significant difference in LDL-c reduction was detected comparing HyperCT and FCHL: the former had an average response greater of -6.7 mg/dl compared to the latter. The FH-FCHL comparison showed a mean difference in LDL-c and non-HDL-c of -11 mg/dl in favour of the FH group.
Conclusions. The study confirms the efficacy of dietary counselling as first approach in childhood, since the improvement of dietary habits has led to a significant decrease in plasma lipid values, regardless of diagnosis. HyperCT patients have recorded the greatest decrease followed by FH and FCHL patients; this could indicate that plasma lipid reduction may be influenced by the phenotype of dyslipidaemia besides the different dietary adherence.

ASSOCIATION BETWEEN DIETARY INFLAMMATORY INDEX AND SUBCLINICAL CAROTID ATHEROSCLEROSIS IN THE GENERAL POPULATION

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Introduction. Unhealthy dietary habits account for both the occurrence of chronic metabolic conditions, like type 2 diabetes (T2D) and metabolic syndrome (MetS) and Atherosclerotic Cardiovascular Diseases (ACVDs).So far, this association has been attributed to the metabolic effects of highly-caloric dietary intakes and majorly to saturated fats, carbohydrates and sugars. At the same time, macro- and micro-nutrients can exert anti- or pro-inflammatory effects beyond their metabolic impact. The question is whether the balance between the intake of pro- and anti-inflammatory effects of diet might associate with Subclinical Carotid Atherosclerosis (SCA), even in absence of T2D and MetS.
Materials and Methods. We collected information about clinical history, anthropometric measurements, and dietary habits (7-days dietary record) of 339 subjects (PLIC cohort) without T2D and MetS and in primary prevention for ACVD. We hereby were able determine the balance between the intake of pro- and anti-inflammatory micro- and macro-nutrients, by calculating the Energy Adjusted- Dietary Inflammatory Index (EA-DII). Lipid profile, glucose-metabolic parameters, haematocrit, and high-sensitivity C-Reactive Protein (hs-CRP) were measured.A detailed ultrasound-based vascular characterization identified subjects with SCA and to cluster four different vascular phenotypes, combining the information about presence/absence of SCA (-/+ SCA) and the parietal intima media thickening (IMT>0.9 mm) (-/+ IMT).
Results. The balance in the intake of pro- and anti-inflammatory nutrients in the first EA-DII quartile was significantly different between subjects with SCA (41.9% of the population) and subjects without SCA (-2.19±0.10 vs -1.87±0.10 p=0.030). Differences between subjects with SCA and those without SCA were also observed in the fourth EA-DII quartile (2.21±0.14 vs 1.81±0.12, p=0.042). It is finally of note that the identification of subjects with different vascular phenotypes, unmasked the association between hs-CRP and EA-DII. In fact, higher hs-CRP was significantly associated with higher EA-DII in subjects with –SCA/-IMT and was numerically (but not statistically) associated with higher EA-DII in subjects with +SCA/+IMT.
Conclusion. Our results suggest an association between SCA and unbalance between the intake of pro- and anti-inflammatory nutrients. The association found between EA-DII and hs-CRP calls for further investigation on larger numbers.

EFFECTS OF A SYNERGISTIC FOOD SUPPLEMENT BASED ON PROBIOTIC MICROORGANISMS ON "LIPID ACCUMULATION PRODUCT" IN OVERWEIGHT-OBESE ADULTS

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Background. Obesity is a major public health problem because of its association with non-communicable diseases and all-cause mortality. Lipid accumulation product (LAP), which is calculated as a combination of waist circumference (WC) and fasting plasma triglyceride (TG) levels, has been proposed as an alternative measure of excessive lipid accumulation. Recently, a growing number of studies have shown that LAP is a powerful marker for insulin resistance, metabolic syndrome, diabetes, and hepatic steatosis in the general population and associated with risk of cardiovascular diseases (CVDs). Currently, gut dysbiosis has been identified as a remarkable factor to be considered in the pathogenesis of CVDs. The importance of gut microbiota in health and disease is being highlighted by numerous research groups worldwide. Some research has revealed that glycaemic, lipid, blood pressure and inflammation indicators are significantly improved by supplementing probiotics. The aim of this study was to evaluate the effects of a food supplement based on probiotic microorganisms on lipid accumulation product in overweight/obese subjects.
Methods. We examined the data from 46 overweight/obese adults of both genders with high levels of LAP, enrolled in a trial (prot. CE.58/2019) carried out at the Clinical Nutrition Unit of the University of Catanzaro. LAP was calculated using the formula [WC (cm) – 65] × TG concentration (mmol/L) for men, and [WC (cm) – 58] × TG concentration (mmol/L) for women. In our analyses, the cut-off value for men was 56.7 and 30.4 for women. The intervention group (n=21) was given a synergistic food supplement based on probiotic microorganisms: Bifidobacterium lactis BS05 (DSM 23032) and Lactobacillus plantarum LP01 (LMG P-21021), capable of promoting the balance of the intestinal bacterial flora. The placebo group (n=25) received food supplement containing maltodextrins. All participants received intensive oral and written recommendations to increase adherence to the Mediterranean diet with energy restriction (-500kcal/day). All subjects underwent fasting blood samples to evaluate lipid, hepatic and glycaemic profiles; they were also subjected to anthropometric measurements and body composition assessment. All measurements were performed at baseline and at 1, 3 and 6 months of follow-up.
Results. A total of 69% were females. The mean age was 55±9 yrs, and BMI was 30.5±4 kg/h2. In probiotic group, we found a reduction statistically different in LAP index between 1, 3 and 6 months (LAP= -8.8±17, p=0.03; -9.2±17, P=0.02; -17±20, P=0.001 respectively). In the placebo group we did not found statistically significant change in LAP index during the study. In addition, in the probiotic group we found a statistically significant reduction of serum glucose at 3 and 6 months (glucose: -4±8 mg/dl, p=0.03; -5±9 mg/dl, p=0.01) and triglycerides after 6 months (-26 48 mg/dl, p=0.02). No significant differences in glucose and lipid levels were found in the placebo group. Both groups had a statistically significant reduction of body weight at each follow-up.
Conclusions. The present study suggests that a food supplement based on probiotic microorganisms reduces lipid accumulation product, and improves serum glucose and triglycerides in overweight/obese individuals. If confirmed in randomized clinical trials, the food supplement based on probiotic microorganisms could represent an effective strategy in the prevention of cardiometabolic risk for these patients.

NON-ALCOHOLIC FATTY LIVER DISEASE AND METABOLIC SYNDROME IN ATRIAL FIBRILLATION. A MULTICENTER PROSPECTIVE COHORT STUDY

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Background. Metabolic syndrome (MetS) is highly prevalent in patients with atrial fibrillation (AF), and frequently associated with non-alcoholic fatty liver disease (NAFLD). The risk of cardiovascular events (CVEs) in AF patients according to the presence of MetS and NAFLD is unknown.
Methods. Prospective observational multicenter study including 1,735 patients with non-valvular AF treated with vitamin K antagonists (VKAs) or non-VKAs oral anticoagulants (NOACs). NAFLD was defined by a fatty liver index ≥60. We categorized patients in 4 groups: 0= neither MetS or NAFLD (38.6%), 1= NAFLD alone (12.4%), 2= MetS alone (19.3%), 3= both MetS and NAFLD (29.7%). Primary endpoint was a composite of CVEs.
Results. Mean age was 75.4±9.4 years, and 41.4% of patients were women. During a mean follow-up of 34.1±22.8 months (4,926.8 patient-years), 155 CVEs were recorded (incidence rate of 3.1%/year): 55 occurred in Group 0 (2.92%/year), 12 in Group 1 (2.17%/year), 45 in Group 2 (4.58%/year) and 43 in Group 3 (2.85%/year). Multivariable Cox regression analysis showed that use of NOACs, and female sex were inversely associated with CVEs, whilst age, heart failure, previous cardiac and cerebrovascular events, and group 2 (Group 2, Hazard Ratio 1.517, 95% Confidence Interval, 1.010-2.280) were directly associated with CVEs.
Conclusions. In patients with AF, cardiovascular risk seems to be related to the presence of MetS rather than NAFLD. The coexistence of NAFLD and MetS does not seem to increase the risk of CVEs.

EFFECTS OF PCSK9 INHIBITORS ON GLUCOSE METABOLISM AND β CELL FUNCTION IN HUMANS

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PCSK9 inhibitors are monoclonal antibodies effective in reducing LDL-C and cardiovascular events by neutralizing circulating PCSK9. Since PCSK9 is also expressed in other organs, including pancreas, studies on PCSK9 KO mice, have shown impaired insulin secretion. Other murine models in which PCSK9 was silenced only in the liver (mimicking the action of PCSK9i) had normal glucose metabolism.The aim of our study was to evaluate the effect of PCSK9 inhibitors on glucose metabolism and beta cell function in humans.12 non-diabetic subjects candidate for PCSK9i therapy were enrolled in the study. All subjects underwent an OGTT at baseline and after 6 months of therapy, at which point surrogate insulin sensitivity indices were obtained (Matsuda; OGIS). During OGTT, insulin secretion parameters were derived from C-peptide levels by deconvolution; we evaluated basal insulin secretion rate, total insulin secretion rate and β-cell glucose sensitivity, for all of the 12 enrolled subjects.All patients showed a 58.5% reduction in LDL cholesterol after 6 months of therapy; the mean LDL-C baseline level (B) was 205.6±58 mg/dl, while mean LDL-C post therapy (PT) value was 85±31 mg/dl. Glycemic and insulinemic curves showed no significant differences between baseline and follow-up. Matsuda index (B 3.8±1.6, PT 3.6±1.4 mL min-1kg-1) and OGIS (B 379.3±63, PT 400.4±49 ml min-1m-2), showed no differences between baseline and post-therapy evaluation. There were no significant differences in insulin secretion parameters: basal insulin secretion rate (B 65.6±29, PT 70.7±31 pmol min-1m-2), total insulin secretion rate (B 49.8±14, PT 47.7±13, nmol m-2) and β-cell glucose sensitivity (B 105.7±54, PT 123.3±57 pmol min-1m-2mM-1) obtained from c-peptide deconvolution. Our study, although preliminary and in a small sample of patients, suggests that PCSK9is does not cause alterations in glucose metabolism and beta cell function. Further studies and a longer follow-up may provide us with further data to confirm our observations.

MCT1 LACTATE TRANSPORTER DELETION IN T LYMPHOCYTES AFFECTS VISCERAL ADIPOSE TISSUE HOMEOSTASIS DURING OBESITY

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Introduction. Recent studies have shed light on the interconnection between metabolism and immunity in multicellular organisms and their functional coordination for an effective establishment and resolution of immune responses. Imbalance of this delicate signaling network might lead to non-resolving inflammation and consequently to the development of obesity associated chronic inflammation (ObCI). T lymphocytes (T cells) accumulate in the adipose tissue during obesity and their activation leads to a switch in their metabolism from oxidative phosphorylation to aerobic glycolysis which involves the production of elevated amount of lactate. MCT1 is a lactate transporter expressed in different type of cells including T lymphocytes. Aim of this project is to investigate the relevance of T cells lactate transport by MCT1, in the context of adipose tissue inflammation during obesity.
Materials and Methods. MCT1f/f CD4-cre mice, with specific deletion of MCT1 in both CD4+ and CD8+ T lymphocytes, and MCT1f/f littermates were generated and fed with an high-fat diet (HFD; 45% Kcal from fat) for 20 weeks. Body weight was measured weekly; glucose metabolism (glucose-tolerance test (GTT) and insulin-tolerance test (ITT)) was checked at 10 and 20 weeks. Immunophenotyping of different tissues (blood, lymph nodes, adipose tissue, thymus) was performed at 20 weeks by flowcytometry. qPCR analysis was performed on visceral adipose tissue.
Results. T cells activation results in the increase of MCT1 expression in both human and mouse T lymphocytes. Following high fat diet feeding, MCT1f/f CD4-cre mice in spite of a similar weight gain and insulin response compared to MCT1f/f, present a decreased visceral (VAT) and subcutaneous (SCAT) fat accumulation. Moreover, MCT1 deficiency in T cells results in a reduction of CD8+ T lymphocytes number in visceral and subcutaneous adipose tissue (VAT MCT1f/f mice 48098cells/g±36587, MCT1f/f CD4-cre 18497cells/g±14508, $p<0.05$; SCAT MCT1f/f 2738cells/g±1189, MCT1f/f CD4-cre 1669cells/g±684, $p<0.05$); this profile was associated with a different T cell subsets distribution (T effector memory (Tem) CD8+ VAT: MCT1f/f 84.20%±5.72, MCT1f/f CD4-cre 57.29±8, $p<0.001$; Tem CD8+ SCAT: MCT1f/f 72.2%±14.16, MCT1f/f CD4-cre 44.93%±14.25, $p<0.001$), but a similar number of innate immune cells (monocyte and macrophages) infiltrating adipose depots. This lower number of T cells in MCT1f/f CD4-cre mice is furthermore associated with a reduction in the adipocytes area in the adipose tissues and to a lower expression of preadipogenic markers in the visceral adipose tissue (e.g. PPARγ, PPARδ, cEBPδ).
Conclusions. Our data suggest that MCT1 transporter supports T lymphocytes activation and proliferation during obesity. MCT1 deficient CD8+ T lymphocytes accumulate less in VAT and this reflects into reduced adipose tissue hypertrophy under HFD conditions.

UNMET NEEDS FOR CARDIOVASCULAR PREVENTION IN A LARGE POPULATION OF PATIENTS WITH IN TYPE 2 DIABETES. AN ITALIAN SURVEY ON LIPID LOWERING THERAPIES AND ACHIEVEMENT OF LDL-CHOLESTEROL TARGETS

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Background. The well-known benefit of lowering LDL-c has led clinical practice guidelines to lower the LDL-c targets for cardiovascular prevention. Despite this, there is a surprising lack of real-world studies (RWS) evaluating whether these information are transferred into clinical practice. We, therefore evaluated, in a large RWS, the pattern of Lipid-Lowering Treatments (LLTs) use and the achievement of LDL-c targets in patients with type 2 diabetes (T2D) in Italian specialist clinics.
Methods and Results. We collected data from 46 diabetes outpatient clinics, following a total of 281,381 patients. We extracted information from 104,726 T2D patients for whom use of LLT between 2015-2016 was ascertained. 63,861 (61.0%) patients were on statin therapy, 9.2% of whom were also on ezetimibe. According to EAS/ESC-2019 guidelines, almost all subjects were at very-high (70.4%) or high (29.3%) cardiovascular risk. Among very-high-risk patients, 35% were not on statin despite half of them had LDL-c >2.6 mmol/l (100 mg/dl), and among the 65% taking statins, only 15% had LDL-c <1.4 mmol/l (55 mg/dl). 83% of subjects in secondary prevention were on a statin, but half of them had LDL-c >1.8 mmol/l (70 mg/dl). Based on anticipated response to treatment, we estimated that 38% of the entire population would require initiation or shift towards high-intensity (HI) statin, 27% a combination of HI-statin+ezetimibe, and 27% the addition of PCSK9-inhibitors. These intensifications of LLT would reduce the incidence of cardiovascular events by 32%, from 23.511 events per 100.000/10 years to 16.022 per 100.000/10 year (IRR 0.68; 95% C.I. 0.67-0.70, $P<0.001$).
Conclusions. The use of LLT in T2D is increased over the last decades. However, the majority of subjects with T2D did not achieve their LDL-c targets. Given the very-high cardiovascular risk of these patients, improving LLT is expected to have a dramatic impact on cardiovascular prevention.

GUT PERMEABILITY-RELATED TOLL-LIKE RECEPTOR 4-PRIMED PLATELETS IN MYOCARDIAL INFARCTION

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Background. Lipopolysaccharides (LPS) from Gram-negative bacteria are implicated in coronary thrombosis but the underlying mechanism is unclear. Objectives. We hypothesized that circulating platelets from myocardial infarction (MI) patients are primed by LPS upon interaction with its receptor Toll-like receptor 4 (TLR4).
Methods. Serum levels of LPS and zonulin, and platelet aggregation (PA) by collagen alone or in combination with a TLR4 inhibitor (TLR4i) were studied ex vivo in platelets from 40 MI patients and 40 controls matched for age, sex and atherosclerotic risk factors; platelet TIR domain-containing adaptor protein (TIRAP) and TIRAP-MyD88 interaction were also investigated by western blot and co-immunoprecipitation, respectively.
Results. MI patients showed higher levels of serum LPS and zonulin compared to controls; serum zonulin and LPS significantly correlated. Platelets from MI patients incubated with TLR4i showed a significant reduction of PA while no effect was detected in controls. LPS serum levels significantly correlated with the rate of PA lowering induced by TLR4i in MI patients. TIRAP was over-expressed in platelets from MI compared to controls and co-immunoprecipitated with MyD88; conversely, co-immunoprecipitation was not detected in platelets from controls. In vitro study showed that LPS, at concentrations similar to those found in MI, dose-dependently activated TIRAP and amplified the platelet response to the agonist, an effect blunted by TLR4i.
Conclusions. The study provides the first evidence that in MI patients LPS primes platelets via TLR4 activation; enhanced gut permeability is suggested as mechanism accounting for LPS-mediated TLR4 priming of platelets.

ADOPTIVE CELL TRANSFER OF ENGINEERED REGULATORY T CELLS IMPROVES ATHEROSCLEROSIS IN EXPERIMENTAL MODELS

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Aim. Regulatory T cells exert a pivotal immunosuppressive function during atherosclerosis by dampening the inflammatory response associated with the disease. Established that Tregs engineering improves their homing to atherosclerotic plaques in experimental models, aim of this study was to investigate the effect of adoptive cell transfer (ACT) with Tregs previously engineered to home to the atherosclerotic plaque, on atherosclerosis progression.
Methods. LDLR-KO mice fed with cholesterol-enriched diet (WTD) for 8 weeks were treated with engineered Tregs which have been retrovirally transfected with CX3CR1. After four weeks we evaluated atherosclerosis progression at the level of the aorta by immunohistochemistry and profiled aortic proteomic signature by shotgun proteomics.
Results. The gene expression analysis of a set of chemokines in LDLR-KO mice fed a cholesterol-rich diet revealed that CX3CL1 expression is selectively increased during atherosclerosis in the aorta but not in other tissues, as compared to control mice. We, therefore, engineered Tregs to overexpress CX3CR1, the receptor matching CX3CL1, to force homing preferentially in the aorta of LDLR-KO mice and tested this approach on atherosclerosis progression. Adoptive cell transfer with CX3CR1+/Tregs reduced atherosclerotic plaque area in the aortic sinus of LDLR-KO mice (65016±9446 μm^2 for CX3CR1+/Tregs treated mice compared to 99658±11782 μm^2 for controls). The lesions in CX3CR1+/Tregs treated mice presented also increased extracellular matrix (+25%) and α-SMA+ content (+29%), and a lower lipid content (-12%). Proteomics analyses highlighted a different activation of several correlated biological pathways, including increased fibroblasts mobility (z-score=2,727) and integrin signaling (z-score=1,732), and decreased apoptosis (z-score=-0,888), necrosis (z-score=-1,498), and cell infiltration (z-score=-2,206) in the aorta of CX3CR1+/Tregs treated mice compared to controls. Moreover, the first group presented also increased expression of proteins related to endocytosis, actin cytoskeleton organization, LXR activation, and even efferocytosis (such as MFGE8, LRP1 and calreticulin). The detection of macrophages surrounding apoptotic cells in the lesion of CX3CR1+/Tregs treated LDLR-KO mice confirmed the upregulation of efferocytosis. These data overall support an improved atherosclerotic plaque phenotype.
Conclusions. ACT with aorta-targeting regulatory T cells improves atherosclerosis by promoting plaque stability and reducing inflammatory burden.

COMPARING THE DISTRIBUTION OF A 12 LDL-C RAISING SINGLE NUCLEOTIDE POLYMORPHISMS SCORE IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA ENROLLED IN THE LIPIGEN STUDY

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Background. A significant proportion of individuals clinically diagnosed with familial hypercholesterolemia (FH), where a disease-causing mutation was not found, are likely to have a polygenic cause for their hypercholesterolemia, due to the inheritance of common LDL-cholesterol (LDL-C) raising single nucleotide polymorphisms. This study aimed at comparing the distribution of a polygenic risk score, comprised of 12 LDL-C raising variants (LDLc-score), in subjects clinically diagnosed with FH.
Methods. The analysis was performed in patients enrolled in the LIPIGEN study, an observational, multicenter study collecting data on FH patients. Adult subjects were divided in mutation-positive FH patients (with at least one causative mutation in one of the candidate genes) and mutation-negative FH patients (without a causative mutation, and pre-treatment LDL-C levels >190 mg/dL).
Results. A total of 823 mutation-positive FH patients (females 53.6%, mean age 42.4±15.1 years) and 457 mutation-negative FH patients (females 55.0%, mean age 51.5±12.3 years) were identified. Mutation-negative individuals were characterized by a higher mean LDLc-score compared to mutation-positive patients (1.01±0.17 vs 0.93±0.20, p-value <.0001), even if the latter had higher mean pre-treatment LDL-C level (237.1±42.0 vs 266.6±67.7 mg/dL, p-values <.0001). A higher mean LDLc-score in the mutation-negative cohort was also detected when the two groups were matched by pre-treatment LDL-C levels (1.01±0.18 vs 0.93±0.20, p-value <.0001). Finally, we performed a ROC analysis to determine if the LDLc-score could be used in clinical practice to distinguish between individuals with a causative mutation and mutation-negative subjects. The area under the curve for recognizing subjects characterized by a polygenic hypercholesterolemia was 0.623 (95% CI, 0.590-0.653), with a sensitivity and specificity being 52% and 65% at 1.02 as a cut-off value.
Conclusions. Despite FH mutation-negative individuals are characterized by higher values of LDLc-score, the ability of the score to distinguish monogenic or polygenic FH appears to be limited.

CER-001 AMELIORATES LIPID PROFILE AND KIDNEY DISEASE IN A MOUSE MODEL OF FAMILIAL LCAT DEFICIENCY

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Background and Purpose. CER-001 is an HDL mimetic previously tested in different pathological conditions, but never in LCAT deficiency. This study was designed to investigate whether the absence of LCAT affects the catabolic fate of CER-001, and to evaluate the effects of CER-001 on kidney disease associated with LCAT deficiency.
Experimental. Approach: Lcat-/- and wild-type mice received intravenously CER-001 (2.5, 5, 10 mg/kg) for 2 weeks. Plasma lipid/lipoprotein profile and HDL subclasses were analyzed. In a second set of experiments, Lcat-/- mice were injected with LpX to induce renal disease and treated with CER-001. Plasma lipid profile, lipid accumulation in the kidney, albuminuria and glomerular podocyte markers were evaluated.
Results. In Lcat-/- mice a decrease in total cholesterol and triglycerides, and an increase in HDL-c was observed after CER-001. While in wild-type mice CER-001 enters the classical HDL remodeling pathway, in the absence of LCAT it disappeared from plasma shortly after injection and ended-up in the kidney. In a mouse model of renal disease in LCAT deficiency, CER-001 at 10 mg/kg had beneficial effects not only on lipid profile by reducing plasma triglycerides and increasing HDL-c levels, but also on renal disease by limiting albuminuria and podocyte dysfunction.
Conclusion. Treatment with CER-001 ameliorates the dyslipidemia typically associated with LCAT deficiency and more importantly, limits renal disease in a mouse model of renal disease in LCAT deficiency. The present results set the basis for the potential use of CER-001 in FLD patients.

IDENTIFICATION OF A RARE VARIANT IN ABCG5 GENE IN A PATIENT WITH FAMILIAL HYPERCHOELSTEROLEMIA

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Introduction. Familial hypercholesterolemia (FH) is one of the most common monogenic metabolic diseases and is characterized by very high plasma concentrations of total cholesterol (TC) due to increased low-density lipoprotein cholesterol (LDL-C). Approximately 20% to 40% of clinically defined FH patients do not show a causative variant in causative genes (LDLR, APOB and PCSK9). Mutation in other lipid-related genes can contribute to mimicking FH phenotype (FH phenocopies). Genetic diagnosis allows to perform cascade screening, increasing the disease diagnosis.
Patient, Materials and Method. We report a 11-years-old female child with a clinical suspect of FH (total cholesterol 214 mg/dL, LDL-c 140 mg/dL, triglycerides 59 mg/dL and HDL-c 61 mg/dL). The family history was positive for hypercholesterolemia and acute myocardial infarction. Patient was screened by next-generation sequencing (NGS) to detect variants in a large panel of lipid related-genes. Rare variants were confirmed by Sanger sequencing. Pathogenicity assessment was performed by ACMG guidelines.
Results. No clearly pathogenic variants in FH-causative genes were identified, although a rare variant was identified at heterozygous state in ABCG5 gene (causative of sitosterolemia): c.592C>T p.(Arg198Trp). This variant is absent from databases of gene variants (GnomAD; EVS; 1kG) and mutations (HGMD professional); according to the ACMG guidelines can be classified as a likely pathogenic variant. Phytosterol measurement revealed increased levels of campesterol 0.62 mg/dL (normal <0.33 mg/dL) and sitosterol 0.53 (normal <0.39 mg/dL).
Conclusion. The presence of the rare variant c.592C>T p.(Arg198Trp) in ABCG5 at heterozygous state is associated with lightly increased phytosterols in the FH pediatric patient. This variant could be a possible cause of hypercholesterolemia in the patient. The use of extended gene panels analyzed by NGS can help to identify variants in additional lipid-related genes with a potential role in FH.

A NEW MACROPHAGE-SPECIFIC LONG NON-CODING RNA REGULATES CELL CHOLESTEROL METABOLISM AND PROMOTES ATHEROSCLEROSIS

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Background. Long non-coding (lnc) RNAs are potent regulators of many pathophysiological processes including atherosclerosis. Using RNAseq profiling of the intima lesions of LDLR-/- mice, our group identified for the first time a macrophage-specific lncRNA MAARS (Macrophage Associated Atherosclerosis lncRNA Sequence), whose expression was strongly increased during atherosclerosis progression. Moreover, its in vivo silencing reduced atherosclerotic lesion formation. As macrophage cholesterol metabolism is strongly involved in atherogenesis, we explored the possibility of a direct effect of MAARS on cholesterol handling.
Material and Methods. Bone marrow was isolated from C57bl/6 mice and differentiated to BMDM (bone marrow-derived macrophages) with mMCSF (mouse macrophage-colony stimulating factor). BMDM were silenced using Lipofectamine and customized GapmeRs for MAARS or negative control. MAARS knockdown efficacy was verified through RT-qPCR. Cholesterol synthesis and efflux were evaluated though a radioisotopic technique, while macrophage cholesterol uptake was evaluated though a fluorimetric assay.
Results and Conclusions. MAARS expression in BMDM was significantly reduced after the silencing with MAARS GapmeRS compared to those transfected with control GapmeRs (- 72%). MAARS silencing in acLDL-loaded BMDM showed an increased cholesterol efflux to HDL ($p<0,01$), ApoA-I ($p<0,001$) and serum from normolipidemic subjects (NS; $p<0,05$) compared to control BMDM. MAARS knockdown BMDM exposed to acLDL and hypercholesterolemic human serum (HCS) showed a reduced cholesterol uptake as compared to control BMDM ($p<0,05$). Finally, MAARS knockdown BMDM displayed a reduced cholesterol synthesis compared to control BMDM ($p<0,01$). Altogether, these results suggest a potential regulation of MAARS on total cell and membrane cholesterol content, as demonstrated by an increased cholesterol efflux, decreased cholesterol uptake and cholesterol synthesis following the in vitro MAARS silencing. Thus, the impact of MAARS on macrophage cholesterol handling may be one of the mechanisms of its proaterogenicity; ongoing studies will explore the underlying molecular mechanisms.

STATIN USE AND MORTALITY IN ATRIAL FIBRILLATION: A SYSTEMATIC REVIEW AND METANALYSIS OF 100,287 PATIENTS

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Statins are effective for reducing cardiovascular disease in patients at risk or with cardiovascular disease. The benefit of statin therapy on adverse cardiovascular outcomes in patients with non-valvular atrial fibrillation (AF) is not clear. We performed a systematic review and metanalysis of studies retrieved from MEDLINE via PubMed and Cochrane (CENTRAL) database of studies investigating the efficacy of statins in AF patients. The principal endpoint was all-cause mortality. Other endpoints were cardiovascular mortality, ischemic stroke, composite endpoints and any bleeding. We included 14 observational studies (2 post-hoc analysis of randomized clinical trials, 8 prospective and 4 retrospective) with 100,287 AF patients, of whom 23,228 were on statins. The pooled hazard ratio (HR) for all-cause mortality was 0.59 (95% Confidence Interval [CI] 0.54-0.65). This association was consistent by aging, sex and prevalent cardiovascular or cerebrovascular disease. and the beneficial effect was evident already after 12 months of therapy. The pooled HR for statins against cardiovascular mortality was 0.75 (95% CI 0.58-0.96). No association was found with other secondary endpoints. Regarding bleeding events, the pooled HR for statin use was 0.60 (95%CI 0.48-0.76). Our metanalysis shows that in AF patients, statin therapy was associated with a reduction in all-cause and cardiovascular mortality are reduced by 41% and 25%, respectively. Randomized clinical trials in AF patients are necessary, as well as clarity on AF-specific LDL cholesterol targets.

MANAGEMENT OF FAMILIAL CHYLOMICRONEMIA SYNDROME IN REAL-WORLD PRACTICE: A REPORT OF TWO ITALIAN PATIENTS TREATED WITH VOLANESORSEN

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Familial Chylomicronemia Syndrome (FCS) is a rare genetic disease characterized by severe hypertriglyceridemia and consequent risk of recurrent and acute pancreatitis (AP), which entails a reduced quality of life. Traditional TG-lowering therapies (fibrates) are largely ineffective in FCS patients. Volanesorsen is a second-generation antisense oligonucleotide inhibiting apoC-III transcription that has been recently approved in Europe for FCS. We examined the efficacy and safety of volanesorsen in two FCS patients treated in Lipid Clinic in Milan, outside a clinical trial setting. Two female patients (aged 47 and 54) with genetically determined FCS and recurrent AP has been treated with volanesorsen on top of standard TG-lowering therapies since February 2020. Baseline TG levels were 1580 mg/dL and 913 mg/dL, respectively. Volanesorsen was administered according to the approved European Union prescribing information. Lipid profile and complete blood count were monitored. After only one dose, TG dropped by 32% in one patient and 90% in the other one. Although response fluctuations could be appreciated, TG levels have been always maintained below 1000 mg/dL. TG elevation was observed when dietary fat compliance was reduced. Volanesorsen was generally well-tolerated. Moderate thrombocytopenia was a common adverse event but required dose adjustment only in one case. Any specific intervention was needed. No AP occurred during the observation period. In conclusion volanesorsen is an effective and safe adjunct to standard therapies in patients with FCS.

PROGRESSION OF CHRONIC KIDNEY DISEASE IN FAMILIAL LECITHIN CHOLESTEROL ACYLTRANSFERASE DEFICIENCY: FOLLOW-UP OF THE ITALIAN COHORT

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Familial LCAT deficiency (FLD) is a rare genetic disorder of HDL metabolism, caused by loss-of-function mutations in the LCAT gene. Renal disease represents the first cause of morbidity and mortality in FLD cases. However, the prognosis is not known and the rate of deterioration of kidney function is variable and unpredictable. Here we report the follow-up of the Italian cohort of FLD patients, one of the largest cohorts described, followed for up to 24 years. The follow-up of the large Italian cohort of FLD patients showed that renal failure occurs at the median age of 46 years, with a median time to a second recurrence of 10 years. Survival curves categorized for sex showed that event-free survival time was extended in females compared to males (median time 62 vs 43 years in females and males, respectively), although not significantly (log-rank test: p=0.308). High plasma unesterified cholesterol level was a predicting factor for rapid deterioration of kidney function. In conclusion, this study highlights the severe consequences of FLD, and underlines the need of correct early diagnosis and referral of patients to specialized centres, and the urgency for effective treatments to prevent or slow the renal disease in patients with LCAT deficiency.

OMERO - OBSERVATIONAL MULTICENTER STUDY ON EFFECTIVENESS AND TOLERABILITY OF ALIROCUMAB IN REAL WORLD

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Background. Despite the extensive information collected in the ODYSSEY Phase 3 trial, long-term experience with alirocumab in real life setting is still limited. Thus, few evidences about its use in real life clinical practice are available in Italy since the launch.
Objectives. The OMERO study is aimed to assess the long term effectiveness (proportion of patients reaching the recommended LDL-C goals), tolerability and safety of the proprotein convertase subtilisin/kexin type 9 inhibitor (PCSK9-i) alirocumab in the real life in Italy, in patients with hypercholesterolemia at high and very high risk of cardiovascular (CV) events who are unable to achieve their LDL-C goal despite therapy with high intensity statin and ezetimibe.This study will also assess the use of electronic Informed Consent as a pilot project and the patient perception of use and acceptance of subcutaneous self- administration, in order to support clinicians in the daily use of this new drug (I-TAQ questionnaire).
Design. OMERO is a national, multicenter, observational study planned to include 800 patients, in 40 Italian sites, treated with alirocumab (Praluent®) on top of standard lipid lowering therapy and fully according to indications for reimbursement provided by the Agenzia Italiana del Farmaco (AIFA). Patients will be stratified by the type of hypercholesterolemia (familial or not familial) and on the basis of being in primary or secondary prevention, since it is related to the recommended LDL-C goals according to the EAS/ESC guideline 2016. In patients in secondary prevention, further stratification will be performed based on the date of the index event.The whole duration of the study is assumed to be approximately 5 years in order to ensure an adequate observation period (at least 2.5 years for all patients).
Conclusion. The OMERO study is expected to collect a huge clinical dataset of information on the use of alirocumab in daily clinical practice. Relevant insights on the use of long-term background therapies (statins, ezetimibe and other LLTs), related patients adherence and acceptance to PCSK9-i self-injection will be gathered. Moreover, the long-term follow-up will better clarify the journey in real life condition of hypercholesterolemic patients at high and very high CV risk.

MARKERS OF KIDNEY FUNCTION AND DAMAGE ARE NOT ASSOCIATED WITH LDL CHOLESTEROL BURDEN BUT WITH METABOLIC COMORBIDITIES IN ADULT PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction and Aims. Familial hypercholesterolemia (FH) is associated with increased risk of atherosclerotic cardiovascular disease (ASCVD), mainly attributable to coronary artery disease (CAD), owing to life-long exposure to high LDL cholesterol (LDLc) levels. However, the risk of chronic kidney disease (CKD), that could be a manifestation of peripheral ASCVD, is largely unknown. We evaluated the prevalence of and the factors associated with decreased kidney function and kidney damage in adult FH patients.

Methods. 270 consecutive patients with clinical suspicion of FH within the LIPIGEN Project underwent comprehensive evaluation, including glomerular filtration rate estimation (eGFR) with the CKD-EPI equation (as a marker of kidney function). eGFR values lower than 90 or 60 ml/min/1.73 m^2 were considered as mildly or moderately decreased, respectively. In 180 patients urine albumin-to-creatinine ratio (ACR) was also measured (as a marker of kidney damage), and values higher than 30 mg/g were considered as moderately increased.

Results. Men and women were 132 and 138, respectively; median age was 49[18-91] years. Median untreated LDLc levels were 260[156-640] mg/dl. 15.6% of patients had a positive history of ASCVD and 79.6% were treated with statins. Among 244 patients with available genetic test, 73.8% were carriers of a "pathogenic" or "likely pathogenic" mutation in FH-causing genes. Median body mass index (BMI) was 25.1[16.6-44.1] kg/m^2 and the prevalence of treated arterial hypertension and type 2 diabetes mellitus was 23.3% and 3.0%, respectively. Median eGFR values were 98[27-146] ml/min/1.73 m^2. 81 patients (30%) presented at least a mild decrease in eGFR, but only 5 (1.9%) had at least a moderate decline in kidney function. eGFR values were significantly and negatively correlated with female sex, age (rho:-.685, p:<.001), BMI (rho:-.201, p:.001), waist circumference (rho:-.260, p:<.001), systolic (rho:-.214, p:<.001) and diastolic (rho:-.148, p:.016) blood pressure, triglycerides (rho:-.171, p:.005), glucose (rho:-.270, p:<.001) and HbA1c (rho:-.390, p:<.001) levels. Moreover, we found a significant correlation between eGFR and Lp(a) values (rho:-.177, p:.005). However, there were not associations between eGFR values and untreated LDLc levels, DLCN and statin therapy. Of note, FH mutation-negative patients had lower eGFR levels and higher age, metabolic comorbidities and Lp(a) values than FH mutation-positive patients. Median ACR values were 5[1-190] mg/g. 11 patients (6.1%) showed moderately increased ACR levels. ACR was significantly and positively associated with female sex, systolic blood pressure (rho:.236, p:.001) and metabolic syndrome. We did not find any association between ACR and FH-related variables.We found significant and negative associations between eGFR, carotid intima-media thickness (rho:-.369, p:<.001) and prevalence of carotid artery plaques (p:<.001). Moreover, FH patients with a positive history of ASCVD had significantly lower eGFR values (p:.001) and a higher prevalence of moderately increased ACR (p:.048) than those without ASCVD.

Conclusions. Kidney function and damage, as assessed with eGFR and ACR, respectively, are normal or mildly altered in the majority of our cohort of adult FH patients, and are not associated with FH-related variables but with cardio-metabolic comorbidities. The pathophysiological mechanisms of CKD in FH may extend beyond LDLc burden.

OPTIMIZING THE START TIME OF STATIN THERAPY: LIPID PROFILE IN CHILDREN WITH TYPE 1 DIABETES MELLITUS

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Background. Large bodies of evidence have shown a strong association between Type 1 diabetes mellitus (T1DM) and an adverse lipid profile. Furthermore, high levels of HbA1c in the untreated newly diagnosed children were associated with significantly higher serum triglycerides level in comparison with treated patients with good glycemic control. However, limited data are available on the difference in the lipid profile among children affected by T1DM according to gender and age categories, thus, this study aimed to evaluate these unexplored features in children with T1DM and assess the start time of statin therapy.
Materials and Methods. This study included 89 children (≤19 years of age) with T1DM who had referred to the outpatient clinics of several Hospitals in Calabria region (Castrovillari, Cetraro, Cosenza, Crotone, Soveria Mannelli and Lamezia Terme) from November 2018 to March 2019. Based on gender, patients were divided into 2 groups and, then, according with age categories (5-9 ys; 10-14 ys; 15-19 ys), patients were divided into 3 groups. Mean lipid levels in the groups were compared with the reference values of the Italian child (Committee of Nutrition, Pediatrics 2008). We also assessed the atherogenic index of plasma (AIP) calculated as logarithm [log10] of the ratio of plasma concentration of TG to HDL-C. All patients had normal thyroid, renal and hepatic function.
Results. Mean age of the studied population was 14±3 years and 45 (51%) were boys. Hypercholesterolemia was most common in female in the 15-19 years category (178.4±35; +21 mg/dl). Furthermore, hypertriglyceridemia was found in the same group (88.9±49 mg/dl; +24.9 mg/dl). In male, a mild hypercholesterolemia was found in the 15-19 years category (163±33; +11 mg/dl). Glycemic control (serum glucose under replacement therapy) was associated with total cholesterol abnormality (p=0.003). Serum HbA1c was also associated with total cholesterol (p=0.002), while body weight did not associate with serum glucose.
Conclusion. A poor glycemic control is associated with hypercholesterolemia in young individuals with T1DM. Hypercholesterolemia was common 15-19 years category. These results need to be taken into account for planning preventative strategies for reducing T1DM-related cardiovascular disease in adolescents affecting with T1DM and in optimizing the start time of statin therapy.

CONTROL OF CARDIOVASCULAR RISK FACTORS IN A POPULATION OF HYPERTENSIVE PATIENTS ACCORDING TO EUROPEAN SOCIETY OF CARDIOLOGY/ EUROPEAN ATHEROSCLEROSIS SOCIETY GUIDELINES

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Aim. Prevalence and control of dyslipidemia, hypertension and diabetes mellitus in patients referred to our ESH "Hypertension Excellence Centre" for high blood pressure (BP) and evaluated by ambulatory blood pressure monitoring (ABPM).
Methods. Observational study on 1219 consecutive outpatients referred for high BP with valid ABPM. Low-density lipoprotein cholesterol (LDLc) targets and the cardiovascular risk stratification (SCORE) were defined according to 2016 and 2019 ESC/EAS Dyslipidemia Guidelines. Diabetics with glycated hemoglobin (HbA1c) <7% were considered controlled.
Results. Mean age: 56.5±13.7 years. Male prevalence: 55.6%. Diabetics: 10.8%. Lipid lowering drugs were taken by 23.1% of patients. LDLc control according to 2019 ESC/EAS dyslipidemia guidelines are lower then observed with 2016 ESC/EAS Guidelines (19.2% vs 28.5%, p<0.001). The higher the cardiovascular (CV) risk, the lower was the prevalence of LDLc control (p<0.001). Only 19.2% of treated patients took high-intensity statins and only 25.9% within patients in secondary prevention. BP was controlled in 41.5% and only in 22.3% of high risk patients (p<0.001). Median Hb1Ac: 6.0% (5.6-7.0%) with 27.1% of uncontrolled patients. The prevalence of diabetics increased with CV risk, while their HbA1c control worsened (40.7% of uncontrolled in very high risk).
Conclusion. Cardiovascular risk factors, especially dyslipidemia and diabetes mellitus, are still often neglected in hypertensives, in particular in patients at higher CV risk. After the new lower LDLc targets proposed by the 2019 guidelines, physicians should pay further attention recommending statins at the right dosages and/ or high-intensity statins, in order to control this important CV risk factor.

THE DIAGNOSTIC AND PROGNOSTIC IMPORTANCE OF THE ANKLE-BRACHIAL INDEX IN PRIMARY CARE: A PROSPECTIVE, SINGLE-CENTRE STUDY

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Introduction. Peripheral Arterial Disease (PAD) is a manifestation of systemic atherosclerosis that is common in the general population and that is associated with an increased risk of death and ischemic events. The Ankle-Brachial Index (ABI) is used for the diagnosis of lower extremity PAD. This study aims to propose a primary care PAD screening strategy in view of a subsequent implementation of risk modification strategies to minimise disease progression and reduce overall risk of cardiovascular mortality.
Methods. A prospective, single-centre study design was adopted. Participants were patients receiving care from a general practice between November 2018 and April 2019. Participants underwent ABI measurement, and their 10-year cardiovascular disease (CVD) risk was calculated using the American Heart Association ASCVD Risk Calculator. Measures of diagnostic accuracy of the ASCVD risk score for the detection of patients with a positive ABI were calculated. Sensitivity, specificity and accuracy were expressed as percentages.
Results. A total of 207 patients participated in the study. Among patients with a calculable 10-year CVD risk (n=181) PAD prevalence was 21% (n=38). The sensitivity, specificity and diagnostic accuracy rates for a positive ABI of a borderline or higher 10-year ASCVD risk were respectively 92,1%, 38,5% and 49,7%. For an intermediate or higher 10-year ASCVD risk, the sensitivity, specificity and diagnostic accuracy rates were respectively 89,5%, 48,3% and 56,9%. For high 10-year ASCVD risk patients only, the sensitivity, specificity and diagnostic accuracy rates for a positive ABI were respectively 60,5%, 72,7%, 70,2%.
Conclusion. Our study suggests that ABI measurement can detect asymptomatic PAD among primary care patients not already known to have atherosclerotic cardiovascular disease. To identify those patients who would mostly benefit from having their ABI measured, we recommend screening patients that have an intermediate or high 10-year ASCVD risk, as this cut-off minimises the number of false negatives.

POSITIVE EFFECTS OF MEDITERRANEAN DIET ON ANTHROPOMETRIC, METABOLIC PARAMETERS, AND INFLAMMATORY STATE

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Introduction. The Mediterranean diet is a nutritional model that endorse foods of plant origin (fruits, vegetables, olive oil) while limiting the consumption of red meat and sweets. Fruits and vegetable foods are characterized by a high content of antioxidants and anti-inflammatory compounds and can play an important role in the prevention of cardio-metabolic diseases, neurodegenerative disorders, some types of cancer and improvement of psycho-physical conditions.Aim. To evaluate the correlation between adherence to the Mediterranean diet (Mediterranean Diet score - MDS) and anthropometricand biochemical parameters in a group of elderly people.
Methods. Participants (456, age 54-81 years) agreed to anthropometricmeasurements, blood sampling collection and filled out a questionnaire to evaluate the frequency of intake of 90 foods or food groups. Based on habits and its importance in the food pyramid, a score (0, 1 or 2) was assigned to 13 macro categories: olive oil; vegetables; fresh fruit; dried fruit; cereals, bread and pasta; legumes; milk and dairy products; fish; eggs; white meat; red meat and processed meat; wine and sweets. Finally, MDS is obtained from the sum of the 13 macro categories.
Results. Statistical analysis showed a negative correlation between MDS and some parameters such as: BMI (-0.098; p=0.036), fat mass (-0.104; p=0.027), triglycerides (-0.083; p=0.082), insulin (-0.110; p=0.022), HOMA-IR (-0.103; p=0.032) and interleukin-18 (-0.114; p=0.016). Instead, a positive correlation is observed with HDL (0.109; p=0.022) and vitamin D (0.296; p=0.002).
Conclusions. Mediterranean diet adherence is associated with higher Vitamin D levels, a better metabolic profile, insulin sensitivity and a lower inflammatory state, reducing the risk of the onset of type 2 diabetes and other chronic diseases.

ACUTE EFFECT OF LIPOPROTEIN APHERESIS ON CORONARY FLOW VELOCITY RESERVE EVALUATED BY THE COLD PRESSURE TEST

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Background. Familial Hypercholesterolemia (FH), the major coronary risk factor, is in turn the best clinical model to study endothelial dysfunction with special attention to the regulation of vascular endothelium dependent tone. In FH high plasma cholesterol per se may be a sufficient stimulus to upregulate endothelial adhesiveness, phenomenon that can be acutely "restored", by Lipoprotein Apheresis (LA).
Methods. The Cold Pressor Test (CPT) is a validated test, which induces systemic stress involving immersion of an individual's hand in ice water (normally temperature between 0-5 °C for a period of 4 minutes), is used to measure cardiac microvascular function because modifies vascular tone and increased blood flow in the epicardial coronary arteries via sympathetic activation mainly through endothelial release of nitroxide in normal endothelium. The aim of the present work is to evaluate the behavior of coronary flow velocity reserve (CFR) during sympathetic stimulus induced by CPT before and after LA.
Results. We enrolled 12 patients (mean age 57±6 years, male 75%) with FH and ischemic heart disease on maximally tolerated lipid lowering therapy and chronic LA (median inter-apheresis interval of 14 [10-16] days). No relevant comorbidity was present in any patient. CPT was performed immediately before and within 48 hours to LA procedure.CFR showed a significant increase (from 1.34±0.22 to 1.59±0.33; $p<0.05$) after LA treatment. No adverse event was reported during the test.
Conclusion. This study, as performed in a small number of patients, show that a single LA procedure can increase a CFR measured by CPT. This diagnostic exam stands as a non-invasive, cheapest method that mimic a physiological effect, like cold exposure, is validated test to measure cardiac microvascular function. Have more methods to measure CFR has importance because myocardial blood flow should represent the ultimate target of any lipid lowering therapy.

EVOLOCUMAB IMPROVES INTIMA MEDIA THICKNESS REGRESSION IN HEFH SUBJECTS ON LIPOPROTEIN APHERESIS

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Background. The long-term effects of lipoprotein apheresis (LA) on carotid atherosclerosis in Heterozygous Familial Hypercholesterolemia (HeFH) are well known since 1999. The aim of the study was to verify if evolocumab, added to LA, improve intima media thickness regression respect to LA alone.
Methods. We retrospectively evaluated 14 HeFH patients (mean age 61±8 years, 12 male) enrolled in the TAUSSIG trial [NCT01624142] with known cardiovascular disease and on chronic LA.The TAUSSIG study was conducted in subjects affected by Heterozygous Familial hypercholesterolemia (HeFH); its aim was to assess the effects of long-term use of PCSK9 inhibition. During the 42 months of the TAUSSIG trial, evolocumab 420 mg was administered every 14 days at the end of each apheresis procedure in addition to conventional medical therapy. LA procedures performed in agreement to manufacturer's instructions, with a median inter-apheresis interval of 14 [10-16] days.
Results. During the study period a cumulative LDL-C reduction of -51% was reported and 7/14 patients discontinued LA because they were able to maintain LDL-C level below 70 mg/dl.During the chronic LA, the carotid intima-media thickness (IMT) variation rate resulted to be -0.72 [-1.43-0] x 10-3 mm/month) in absence of relevant changes in the echogenicity and size of the coexisting atheromatous plaques. After evolocumab addition a significant decrease in IMT variation rate was recorded (-3.90 [-5. 30-2.68] x 10-3 mm/month; $p<0.0001$). All patients had carotid plaque with <50% of diameter reduction which stable throughout the study period.
Conclusion. This study, although in a small number of patients, confirms that chronic LA procedure reduces IMT and that the association of LA and evolocumab further improves the beneficial effect on the atherosclerotic regression process, possibly acting on the common pathogenetic mechanisms underlying the carotid stiffness.

FTO RS9939609 VARIANT MAY PREDICT THE METABOLIC RISK IN OBESE SUBJECTS

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Introduction. Obesity, characterized by an abnormal or excessive fat accumulation, represents a risk for health worldwide. Life-style, especially in the western countries, and genetic factors are mainly involved in the etiology of obesity. Recently, Genome-Wide-Association-Studies (GWAS) have analyzed the genes associated to obesity and, among these, FTO (Fat mass and Obesity-associated) gene and its rs9939609 variant has been associated to BMI increase and the risk to develop type 2 mellitus diabetes (DM2) and metabolic syndrome.
Materials and Methods. The aim of this study was to evaluate the clinical and biochemical features, the metabolic risk of a cohort of 81 severe obese subjects (BMI >27 kg/m^2) and the correlation between these parameters and the effects of the FTO allelic variant SNP rs9939609. Anthropometric measures, clinical and biochemical parameters were evaluated. A total of 90 non-obese subjects from the "Ventimiglia di Sicilia Epidemiological Project" with a BMI <27 kg/m^2 were selected as controls.
Results. Obese subjects have shown a significant increase of anthropometric parameters (BMI and waist circumference), insulin-resistance (HOMA-IR index) and metabolic risk compared to controls; moreover the genotype A/A of the FTO SNP rs9939609 variant was strongly correlated to the BMI and waist circumference increase in both genders. We have confirmed a correlation between the anthropometric parameters (BMI and waist circumference), the metabolic state (insulin-resistance status) and the genetic FTO profile in our cohort of subjects. The genotype A/A of the FTO rs9939609 variant A/A is significantly associated to a metabolic risk increase in severe obese patients in both genders.

ENDOGENOUS PCSK9 MAY INFLUENCE CIRCULATING CD45NEG/CD34BRIGHT AND CD45NEG/CD34BRIGHT/CD146NEG CELLS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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To evaluate whether the effect of protease proprotein convertase subtilisin/kexin type 9 (PCSK9) on vascular homeostasis may be mediated by in vivo circulating endothelial progenitor cells (EPCs) in patients with or without type 2 diabetes mellitus (T2DM). Eighty-two patients (45 with, 37 without T2DM) at high cardiovascular risk were enrolled in this observational study. Plasma PCSK9 was measured by ELISA and EPCs with phenotype CD45neg/CD34bright and CD34bright/CD146neg were analyzed by a standardized flow cytometry method. Statin treatment was associated with higher circulating levels of PCSK9 in patients with and without T2DM ($p<0.001$ and $p=0.036$) and with reduced CD45neg/CD34bright ($p=0.016$) and CD45neg/CD34bright/CD146neg ($p=0.040$) only among patients with T2DM. PCSK9 correlated inversely with both CD45neg/CD34bright ($p=0.006$) and CD45neg/CD34bright/CD146neg ($p=0.002$) only in patients with T2DM. Dividing patients according to statin treatment, PCSK9 correlated inversely with CD45neg/CD34bright ($p=0.022$) and CD45neg/CD34bright/CD146neg ($p=0.004$) only in patients with diabetes on statin treatment. In high-risk T2DM patients, high endogenous levels of PCSK9 may have a detrimental effect on EPCs by reducing the endothelial repair and worsening the progression of atherothrombosis.

FAMILIAL MEDITERRANEAN FEVER AND EARLY CARDIOVASCULAR DAMAGE: A PRELIMINARY DATA

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Aims. Familial Mediterranean fever (FMF) is the most common monogenic periodic fever syndrome, characterized by recurrent episodes of fever, serositis, arthritis, dermal manifestations, and long-term renal complications. The MEFV gene responsible of FMF encodes for mutated protein pyrin, an important player in the innate immune system and the component of inflammasome which leads to exaggerated inflammatory response through uncontrolled production of interleukin-1. Inflammatory diseases are known to be associated with increased cardiovascular risk. Few studies have investigated the association between FMF and cardiovascular disease. To evaluate early cardiovascular damage in subjects suffering from FMF on the basis of the estimate of the Pulse Wave Velocity, the cIMT, liver stiffness, the biohumoral indices and the circulating hematopoietic cells.
Results. Preliminary data from patients with FMF were compared to normal values and divided according to age strata: ≤20 (PWV 5.94 vs 6.1 m/s, cIMT 0.47 vs 0.44 mm, liver stiffness 3.8 vs 4.7 kPa), 20-50 (PWV 7.5 vs 7 m/s; cIMT 0.62 vs 0.55 mm; liver stiffness 7.8 vs 3.9 kPa, p<0.001), 50-70 (PWV 10.7 vs 8.8 m/s, cIMT 1.1 vs 0.85 mm, liver stiffness 7.8 vs 3.9 kPa, all p<0.001)
Conclusions. In this explorative study, we found that FMF patients could present higher risk to develop cardiovascular/cardiometabolic disease; in fact, they presented with a time-dependent acceleration of vascular - and also liver - involvement, as suggested by the increased cIMT and PWV values in the highest age stratum (50-70) with respect to controls; moreover, we found a significant increase of liver stiffness values, likely as consequence of chronic inflammatory status (the link between serum amyloid A precursor levels in FMF subjects and liver fibrosis should be deepened). These data need to be replicated on larger scale.

ENDOTHELIAL DYSFUNCTION, THROMBIN GENERATION AND FIBRINOLYSIS IN COVID-19 PATIENTS

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Background. In patients affected by COVID-19, an hyper-inflammatory state and a coagulopathy have been documented. Aim of our study was to evaluate the role of thrombin generation, fibrinolytic alterations and endothelial dysfunction in 115 consecutive COVID-19 patients [67M/48F; median age 78(range 62-85).
Methods. 115 patients with confirmed Sars-Cov-2 infection, admitted to Careggi Hospital, from March 2nd to April 7th 2020 were enrolled. The clinical outcomes were monitored up to April 7th 2020 (median follow-up:11 days[interquartile range[IQR]:7–14]).
Results. Sixteen of 115 patients died (13.9 %). Dead patients had a higher median age [81(70-89)] with respect to alive patients [76(60-85)]. Seventy-two patients have been discharged (62.6%). Twenty-seven patients (23.5%) have been transferred to an ICU. Non-survivors had significantly (p<0.01) higher Von Willebrand Factor (VW) antigen [389(314-629%)] and D-Dimer [1609(860-4584)ng/ml] plasma levels than survivors [VW: 274(154-352)%; D-dimer 932(487-1805)]. VW antigen levels significantly and positively correlated with C Reactive Protein (CRP) levels (r=0.39,p=0.002) and White Blood Count (r=0.30,p=0.015). D-dimer plasma levels significantly correlated with CRP (r=0.20,p=0.033) and lymphocyte count (r=-0.30,p=0.001). ETP values were significantly lower (p<0.05) in dead patients with respect to those observed in survivors [931(360-1115) vs 1218(755-1500)]. Lag time, time to peak, peak and ETP ratio (ETP with thrombomodulin/ETP without thrombomodulin) values did not significantly differ between nonsurvivors and survivors. CLT values did not differ between survivors and nonsurvivors [83(76-91) min vs 82(77-86) min] as well as the percentage of patients with CLT above the normal range (82 min) [9/16(56%) in dead patients vs 39/72(54%) alive patients].
Conclusions. Our data demonstrate that in COVID patients an endothelial dysfunction, associated with a marked pro-inflammatory state, is present in COVID patients. Moreover, in dead patients endogenous thrombin potential was significantly reduced, suggesting the presence of a consumption coagulopathy. The coexistence of an endothelial dysfunction and a hypocoagulability is associated with a poor prognosis.

INSIGHT INTO FAMILIAL HYPERCHOLESTEROLEMIA GENETIC DETERMINANTS BEYOND LDLR LOCUS

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Background. As many subjects (about 60%) with Familial Hypercholesterolemia (FH) did not demonstrate functional mutations in FH major candidate genes (LDLR, APOB, PCSK9 and LDLRAP1), we assessed FH patients genetic profile through targeted high-throughput sequencing (HTS).
Methods. We analyzed 38 subjects with possible/probable or definite FH, according to the most common diagnostic algorithm, the Dutch lipid score. Targeted HTS (57 genes panel, including loci involved in lipid metabolism, genes supposed to be involved in dyslipidemia, pharmacogenetics of statins, genes related to higher susceptibility for FH polygenic forms, HDL and triglycerides related diseases) through Illumina MiSeq technology was performed.
Results. Thirteen out of 38 patients investigated showed pathogenetic/likely pathogenetic mutations in LDLR gene. Three out 13 LDLR mutation-positive patients also carried likely pathogenetic/uncertain significance mutations in APOB and LDLRAP1 genes. In patients without LDLR mutations (n=25), at least 2 rare variants were identified in 16 patients (64%), and at least 3 rare variants were identified in 9 patients (36%). In these patients, a total of 58 rare variants in other genes beyond LDLR, known to be involved in FH, hypertriglyceridemia, other forms of familial dyslipidemia, polygenic FH or pharmacogenetics (APOB, CELSR2, GHR, ABCG5, LMF1, SLC22A1, GCKR, CREB3L3, ABCB1, LRP1, APOA4, ITIH4, PON1, LIPC, SCARB1, PCSK9, DAB2, PPP1R17, SREBF1/2, HFE, LCAT, LPL, ABCG2, NYNRIN, SLCO1B1, LIPI) were observed. Patients with or without pathogenetic mutations in LDLR gene were comparable for age, sex, and LDL cholesterol levels, whereas Dutch score was significantly higher in LDLR mutation positive patients.
Conclusions. Results from the present study suggest the possible effect of multiple rare variants on the clinical phenotype, thus supporting the contribution of a polygenic predisposition to lipid profile alteration. Conversely, even if significant, our preliminary data support a less severe weight of cardiovascular manifestations and family history in patients with polygenic forms of FH.

RECOMBINANT LCAT (LECITHIN:CHOLESTEROL ACYLTRANSFERASE) PARTIALLY RESTORES THE ALTERED HDL-MEDIATED CARDIOPROTECTION IN ACUTE CORONARY SYNDROME

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Background. Low plasma HDL-C is associated with unfavorable prognosis in patients with acute coronary syndrome (ACS). After an acute myocardial infarction, an early reperfusion is the most effective strategy for improving the clinical outcome; however, the restoration of blood flow can induce injury. HDL are able to preserve cardiac function when given before ischemia or at reperfusion by acting on cardiomyocites. During ACS, HDL lose their protective proprieties, like the ability to stimulate endothelial nitric oxide (NO) release, because of an altered composition due to a reduction in LCAT concentration/activity.
Aim. To evaluate if the incubation with recombinant LCAT restores the HDL-mediated cardioprotection during ACS.
Methods. 20 STEMI patients were enrolled by Intensive Cardiac Care Unit of Niguarda Hospital and blood samples were collected at hospital admission and 72 hours after the event. LCAT concentration and activity, and cholesterol esterification rate (CER) were analyzed. HDL were purified by ultracentrifugation after incubation of patients' plasma with or without recombinant LCAT and used to incubate rat cardiomyoblast cells (H9c2) in a hypoxia/re-oxygenation model. ROS production, apoptosis and cell viability were evaluated by standardized methods.
Results. LCAT concentration and activity and CER were reduced in ACS patients at 72 hours from the event (-22%, p=0.011; -15%, p=0.015; -33%, p<0.01 vs admission, respectively). HDL isolated after incubation of plasma with rhLCAT showed a significantly reduced ROS production stimulation (p=0.023) at 72 hours and a decreased apoptosis induction at admission (p<0.01) compared to untreated HDL. No effect of rhLCAT was observed on HDL capacity to influence cell viability.
Conclusions. The impairment of the cholesterol esterification system during ACS at least partially explains the loss of cardiac anti-oxidant and cytoprotective activities of HDL. rhLCAT can partially restore HDL functionality, making LCAT a new target in ACS.

GLYCOPROTEIN ER-CHAPERONS SURVEILLANCE CHANGES DURING ATHEROGENESIS

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Background. Protein glycosylation is a post-translation modification consisting on the enzymatic attachment of glycans that are covalently attached to a nascent polypeptide chain. It starts in endoplasmic reticulum (ER) where 14 glucans are transferred as "en block" to a lipid linked anchor in a polypeptide chain. This is followed by enzymatic trimming of glucosidase I and II that can hydrolyze two molecules of glucose. This process allows a proper glycan configuration that is recognized by ER chaperons calnexin and calreticulin which are known to assist in quality control and protein folding. Only corrected glycoproteins with eight- or nine-glycans are exported in Golgi while incorrected glycoproteins are retained in ER to complete the folding or they are targeted for degradation. Any disruption in glycosylation affects protein folding and can lead to unfolded protein response (UPR) and ER stress. Aim of this work was to investigate changes in N-glycosylation maturation of proteins during atherosclerotic plaque development in animal models.
Methods. Shotgun proteomics was performed in aorta from LDL-R KO mice, fed in chow or WTD diet, using orbitrap Fusion™ Tribrid ™ Mass Spectrometer followed by protein inference, label free quantification. Total RNA was reverse transcribed into cDNA and gene expression analysis by qRT-PCR was performed.
Results. Cholesterol (Chol) and triglycerides (TG) plasma levels were 211.64±24.85mg/dl and TG 126.76±0.92 mg/dl on chow diet and increased to 518.9± 53.41mg/dl and TG 266,16±56,86 mg/dl on WTD respectively. Following shotgun proteomics, from 1134 proteins identified, 1102 were quantified and matched for biological enrichment pathways where fatty acid metabolism (z-score=3.18). The second activated pathway was metabolism of proteins (z-score=1.48) where the unfolded protein repose was significantly activated followed by activation of "conformational modification of proteins" pathway. Within the unfolded proteins response pathway observed on WTD mice, several enzymes were associated with the modulation in glycosylation of mice. Firstly, ribophorin unit that transfer the glycans in lipid anchored oligosaccharide was significantly upregulated, (an index of glycan assembly in ER); secondly glucosidase enzymes that tries to make a proper conformation of glycoproteins were significantly up-regulated (Ganab, p-value=0.03). In line with this we observed the up-regulation of chaperons such as calnexin, calreticulin and GRP78 (p-value=0.01) both at the mRNA and at the protein level, In addition, we have observed a significant increase in VCP which is known to participate to protein degradation through ERAD pathways. Moreover, the abundance and side-specific N-glycosylation of integrin β-1, laminin subunit γ -1, integrin α-8 were reduced in the plaque of WTD fed mice.
Conclusion. Our data suggest that in aorta of LDL-R KO mice, fed in WTD there is an increased glycan assembly in ER and the up-regulation of glycoprotein chaperon expression, probably to compensate an uncorrected folding of glycoproteins. This is paralleled by N-glycosylation proteins reduction in the plaque of WTD fed mice.

USE OF VOLANESORSEN IN A PATIENT WITH FAMILIAL CHYLOMICRONEMIA SYNDROME (FCS)

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Introduction. Familial chylomicronemia syndrome (FCS) is a rare recessive monogenic disorder (1-2:1.000.000) characterized by reduced or absent lipoprotein lipase (LPL) activity due to homozygous or compound heterozygous mutations in LPL gene and/or in its modulators. LPL catalyzes the hydrolysis of TG-rich lipoprotein and its functional impairment leads to persistence of circulating chylomicrons resulting in severe hypertriglyceridemia. Patients with FCS have a high risk of severe recurrent acute pancreatitis, a potentially life-threatening condition. Volanesorsen is a second-generation antisense oligonucleotide inhibiting transcription/translation of the LPL-inhibitor apoC-III. We evaluated the efficacy and safety of Volanesorsen in a patient with FCS.
Materials and Methods. A 56-year-old female with an homozygous mutation of the LPL gene and severe defect in the enzyme activity was followed up in our lipid clinic. From the age of 20, she has had plasma triglyceride levels consistently higher than 2000 mg/dl despite adequate dietary therapy and drug therapy with fibrate and omega 3 fatty acids. She performed therapeutic apheresis for a year, suspended for symptomatic anemia. In her clinical history one episode of acute edematous pancreatitis was described. Since January 2019 therapy with Volanesorsen was started (285 mg/week and after 3 months 285 mg every two weeks).
Results. The treatment resulted in a 95% reduction in triglyceride levels (from 2400 mg/dl to 129 mg/dl after two months of weekly Volanesorsen administration, with stabilization at levels of 500-700 mg/dl after switching to biweekly injections. The platelet count was monitored every two weeks and was never below 130x109 per liter. Treatment tolerability was good, only mild fever responsive to paracetamol occurred shortly after the first two administrations.
Conclusions. Volenasorsen therapy in a patient with FCS has been shown to be effective in significantly reducing triglyceride levels below the risk threshold for acute pancreatitis without causing significant side effects.

DIETARY RECOMMENDATIONS: THE FIRST STEP FOR MANAGEMENT OF CHILDREN WITH HYPERCHOLESTEROLEMIA

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Background and Aims. Cardiovascular diseases represent the most important cause of mortality. Primary prevention of atherosclerosis must be implemented as early as possible through the promotion of a healthy lifestyle. We aimed to assess if a qualitative nutritional intervention can improve the eating behaviours and the lipid profile of children with primary hypercholesterolemia, in follow-up in our Lipid Clinic.
Methods. We examined 86 children (43M, 43F, average age of 8.4 years) with severe hypercholesterolemia, mainly (74%) normal weight, 73% with genetically-confirmed Familiar Hypercholesterolemia. At baseline we evaluated complete lipid profile (after 12 hour fasting) and eating habits using food frequency questionnaire. No child had previously received dietary counselling, vitamin supplementation or drug therapy. We gave the following dietary recommendations, based on Mediterranean diet: low fat content (<30%, saturated <7%), carbohydrates (45-60%), proteins (14%); 4 main meals plus 1 snack, dividing the calories into: 20% for breakfast and snack, 40% for lunch, 10% for snack, 30% for dinner.
Results. At baseline, mg/dl CT 293, LDL-C 227, triglycerides 82, non-HDL 242, HDL-C 52, vitB12 660 pg/ml, folic acid 7 ng/ml, vitD 23 ng/dl (deficient). Weekly frequencies were: fruits and vegetables 13.9, blue fish 1.7, whole grains 1.6, legumes 1.5, low-fat cheeses 3.3, junk food 9.7, cold cuts 2.9, meat 4.7, egg 0.5. After 6 months of dietary individualized recommendations, weekly frequencies were: fruits and vegetables 21.5 (+54.8%), blue fish 2.4 (+38.7%), whole grains 3 (+85%), legumes 1.9, (+20.1%), low-fat cheeses 1.6 (-53%), junk food 4.9 (-49.7%), cold cuts 1.8 (-39.2%), meat 3.8 (-18.8%), egg 0.8 (+52.1%). The lipid profile was mg/dl CT 287 (-2.1%), LDL-C 219 (-3.3%), triglycerides 78 (-4.1%), non-HDL 234 (-3.3%), HDL-C 53 (+3.7%).
Conclusion. The dietary counselling is safe and well applicable and it has improved the diet and the lipid profile of our patients. This is the first step for management of children with hypercholesterolemia. The long-term goal is to establish the nutritional quality and pattern of food consumption by children and their families.

THE ADAPTATION OF LIPID PROFILE OF HUMAN FIBROBLASTS TO ALGINATE 2D FILMS AND 3D PRINTED SCAFFOLDS

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Background. The development of innovative active materials is currently widely studied in several research and application fields. Here, a targeted lipidomic approach has been used to explore the early impact of alginate (ALG) hydrogel architecture (2D films or 3D printed scaffolds) and the type of gelling agent (CaCl2 or FeCl3) on the lipid profile of human fibroblasts.
Methods. 2D and 3D ALG scaffolds were characterized in terms of mechanical resistance and morphology through scanning electron microscope analysis before seeding human skin fibroblasts for 8 days. Using a liquid chromatography-triple quadrupole-tandem mass spectrometry approach, selected ceramides (CER), lysophosphatidylcholines (LPC), lysophosphatidic acids (LPA) and free fatty acids (FFA) were analyzed.
Results. The highly abundant CER d18:1-16:0 is more expressed on 2D ALG gelled with CaCl2 vs 2D ALG gelled with FeCl3 and vs 3D ALG gelled with CaCl2. Conversely, CER d18:1-22:0 present an opposite trend. The short chain ceramides (CER d18:1-16:1, CER d18:1-20:0) present higher expression on the 2D films gelled with CaCl2 compared to 3D ALG gelled with CaCl2, while the opposite occurs for long chain ceramides (CER d18:1-22:2, CER d18:1-22:5). LPC, FFA and LPA relative trends are more maintained during cell culture on biomaterial and only minor differences are observed as a function of scaffold geometry or gelling agent.
Conclusions. These data suggest that CER are significantly affected by both the structure of the scaffolds and the gelling agents employed, whereas LPC, FFA and LPA are not susceptible of significant influence neither from the geometry nor from the gelling agent we selected. These data allow to extend the knowledge of the behavior of selected lipids in cell-biomaterial interactions.

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