35° CONGRESSO NAZIONALE SISA

VIRTUAL EDITION
LIVE STREAMING
28-30 NOVEMBRE 2021
ON DEMAND
28 NOVEMBRE 2021
28 FEBBRAIO 2022

SOCIETA' ITALIANA
PER LO STUDIO
DELL'ATEROSCLEROSI

RIASSUNTO DELLE COMUNICAZIONI PRESENTATE AL 35° CONGRESSO NAZIONALE S.I.S.A.

IN VITRO STUDIES ON THE MECHANISMS UNDERLYING THE EFFECTS ON CHOLESTEROL HOMEOSTASIS EXERTED BY A MIXTURE OF LACTOBACILLUS PLANTARUM ALONE OR IN COMBINATION WITH BERBERINE AND FERMENTED RED RICE

L. Arnaboldi[1], S. Mazza[1], E. Frisa[1], A. Granata[1], F. Bordonaro[1], F. Di Vincenzo[2], P. Sperandeo[2], A. Polissi[2], A. Corsini[1]
[1]Lab. di Farmacologia Cellulare dell'Aterosclerosi, DISFeB, Università degli Studi di Milano; [2]Lab. di Microbiologia Molecolare, DISFeB, Università degli Studi di Milano
E-mail: lorenzo.arnaboldi@unimi.it

In order to reduce mild-moderate cholesterolemia, even in statin-intolerant patients, beyond already utilized nutraceuticals (e.g fermented red rice, phytosterols, artichoke and bergamot extracts, etc.) recently a new therapeutical possibility emerged. Lactobacillus plantarum (LP), a strain of Lactobacilli commonly present in human intestine, possesses cholesterol-lowering properties both in animal and human studies. The aim of the present work is to demonstrate the mechanism(s) underlying this effect on cholesterol homeostasis exerted by a LP fixed formula. In some experiments, this mix has also been associated with berberine and fermented red rice. LP mix demonstrated to be effective on at least three investigated parameters:

a) Removal of cholesterol from colture medium Growing or heat-inactivated LP are able to remove up to 50-60% of free cholesterol (diluted in ethanol; final medium concentrations 20-200 ug/ml), after 20 hours of incubation (experiments conducted with gas-chromatographic techniques). In our experimental conditions, this effect is neither saturable nor specific for cholesterol. Addition of betasitosterol or stigmasterol exerts the same effect.
b) Bile Salt Hydrolase (BSH) activityLP's BSH, by hydrolyzing conjugated intestinal bile salts, prevents free cholesterol reabsoption, thus contributing to lowering its plasma concentrations. As shown both by semiquantitative TLC and quantitative colorimetric analysis, BSH activity is only present in living LP, after incubation with taurocholic- and glycocholic acids for 20 hours (0.3% in colture medium).
c) Cholesterol biosynthesis inhibition 15% conditioned medium from living LP added for 20 hours to the colture medium of human hepatocarcinoma HuH-7 cells reduces cholesterol biosynthesis by 15-20%. The association with berberine (15 µg/ml) and fermented red rice (titrated by HPLC at 0,1-1 µM in monacoline K) additionally reduces cholesterol biosynthesis (up to 70%). In summary, these in vitro effects corroborate the data on the clinical efficacy of LP strains, and also suggest their use in a possible therapeutical association in the case of more sustained cholesterolemia.

THE ACHILLES TENDON ULTRASONOGRAPHY IN FAMILIAL HYPERCHOLESTEROLEMIA: RESULTS FROM A SUB-STUDY OF THE LIPID TRANSPORT DISORDERS ITALIAN GENETIC NETWORK (LIPIGEN)

A. Baragetti[1], M. Casula[2], P. Scarinzi[3], F. Ristè[4], R. Scicali[5], M. Biolo[6], S. Lugari[7], M. Dall'Agata[8], M. Gazzotti[2], E. Olmastroni[2], Asiiat Alieva[9], F. Nascimbeni[7]
[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; [2]Epidemiology and Preventive Pharmacology Service (SEFAP), Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; [3]U.O.C. Clinica Medica 1, Dipartimento di Medicina DIMED, Univeristà di Padova; [4]U.O. Medicina Interna Universitaria, Dipartimento di Medicina Interna, Università di Ferrara; [5]Dipartimento di Medicina Clinica e Sperimentale, Medicina Interna, Univeristà di Catania; [6]U.O.C. Clinica Medica 1, Dipartimento di Medicina DIMED, Università di Padova; [7]Unità di Medicina Interna e Medicina Metabolica, Ospedale Civile di Baggiovara, Università di Modena, Reggio Emilia, Modena; [8]U.O. Medicina Interna Univeristaria, Dipartimento di Medicina Interna, Università di Ferrara; [9]Almazov National Medical Research Centre, Saint Petersburg, Russia
E-mail: andrea.baragetti@unimi.it

Introduction. The detection of Achilles tendon xanthomas during physical examination contributes the score for the detection of Familial Hypercholesterolemia (FH), included in the Dutch Lipid Clinic Network score (DLCN). We aimed to evaluate whether the ultrasound-based identification of Achilles tendon xanthomata improved the identification of subjects with FH.
Methods. The ACTUS-FH (AChilles Tendon UltraSonography in Familial Hypercholesterolemia) is a multicentre observational study developed within the LIpid transPort disorders Italian GEnetic Network (LIPIGEN). Clinical, biochemical and genetic parameters were collected. The presence of ultrasound-based Achilles tendon xanthomata was recorded.
Results. In 769 subjects with clinically diagnosis of FH, ultrasonography improved xanthomata detection to 33.2% vs 9.8% at physical examination. By adding the ultrasound evidence in the DLCN score, the proportion of subjects classified as definite FH according to DLCN increased from 32.5% to 43.2%. Subjects with xanthomata detected only by ultrasound were more likely to have a positive genetic diagnosis of FH (FH/M+) (94.4%) as compared to subjects without Achilles tendon xanthomas (65.4%, p<.0001).
Conclusions. Ultrasonography identifies patients with tendon xanthomata that cannot be detected by physical examination, improving the number of patients correctly identified as definite FH according to DLCN score and those with proven genetic mutations.

PROC3 DERIVED SCORES FOR FIBROSIS PREDICT CARDIOVASCULAR EVENTS IN NAFLD PATIENTS

F. Baratta[1], D. Pastori[1], G. Tozzi[2], A. Colantoni[3], N. Cocomello[1], S. Cecchi[4], M. Coronati[1], F. Angelico[5], M. Del Ben[1]
[1]Department of Clinical Internal, Anesthesiological and Cardiovascular Sciences, Sapienza - University of Rome; [2]Division of Metabolism and Research Unit of Metabolic Biochemistry, Department of Pediatrics, IRCCS Bambino Gesù Children's Hospital; [3]Department of Anatomical, Histological, Forensic Medicine and Orthopedic Sciences, Sapienza - University of Rome; [4]Hepatology, Gastroenterology and Nutrition Unit, IRCCS "Bambino Gesù" Children's Hospital; [5]Department of Public Health and Infectious Diseases, Sapienza - University of Rome
E-mail: francesco.baratta@uniroma1.it

Introduction. Cardiovascular diseases are the most common cause of death in NAFLD patients and accounts for twice the deaths in comparison to liver-related ones. Recently, a growing body of evidence showed that liver fibrosis is associated with poor survival in NAFLD patients. Despite this, liver fibrosis is underdiagnosed due to the high invasiveness, cost and interobserver variability of liver biopsy. Several clinical/biochemical scores were proposed in the last years to non-invasively diagnose the presence and the severity of liver fibrosis. Serum levels of PRO-C3 have been shown to have a good correlation with biopsy-proven liver fibrosis. PRO-C3 has been also included in some risk scores, namely ADAPT, FIB-C3 and ABC3D to predict liver fibrosis. Aim of the study was to investigate the association between serum PRO-C3 levels, scores including PRO-C3, and cardiovascular events (CVEs) incidence in the large cohort of NAFLD patients enrolled in the prospective Plinio Study.
Methods. The present study is a post hoc analysis of Plinio Study (Progression of LIver Damage and Cardiometabolic Disorders in Non-alcoholic Fatty Liver dIsease: an Observational Cohort study. ClinicalTrials.gov Identifier: NCT04036357). In this analysis were included only patients with US evidence of liver steatosis who consent the blood sampling, with no data missing for the analysis and who complete at least 6 months of follow-up. PRO-C3 assay and PROC-C3 derived score calculation: PRO-C3 was dosed using a commercial elisa kit. Age, Diabetes, PRO-C3 and platelets panel (ADAPT), FIBC3 panel and Age, body mass index (BMI), PRO-C3 and Diabetes panel (ABC3D) were calculated as previously described.Follow up: Data on CVEs were prospectively collected during follow-up. After enrolment, patients underwent periodical phone calls (every six months) and visits (every 12 months) in the outpatient clinic. Only the first CVE registered during follow-up was used in the analysis. The type of CVE was confirmed by medical records (imaging or discharge letter). In case of a fatal event, information was obtained from relatives or general practitioners.
Results. Plinio patients included in the analysis were 663. Mean age was 55.4±12.0 years and 38.6% were female. Median BMI of 30.2±4.9 kg/m^2, 59.7% and 29.9% of the patients had metabolic and type 2 diabetes mellitus, respectively. Median follow up length was 47.8 [25.3-72.8] months yielding 2786.5 person-years of observation. During the follow-up, 41 patients (1.5% year) experienced CVEs. Patients who experienced CVEs were more frequently male (p=0.010) and had higher prevalence of type 2 diabetes (p=0.006), metabolic syndrome (p=0.002) and of prior CVEs (p<0.001). There was no difference in median PROC-C3 according to CVEs. Instead, we found a higher prevalence of impaired ADAPT (p=0.038), FIBC3 (p=0.015) and ABC3D (p<0.001) in patients with CVEs. At multivariate cox regression analysis, CVEs were associated with ABC3D>3 (HR: 2.29, p<0.05) in the whole population and with FIBC3 (HR: 1.40, p<0.05) and ABCD3 (HR: 1.40, p<0.05) in patients in primary prevention. The AUROC for the prediction of CVEs was 0.65 [0.56-0,73] for both FIBC3 and ADAPT scores and 0.63 [0.54-0,73] for ABC3D.
Conclusions. PROC3 levels didn't predict CVEs incidence, differently from PROC3 derived scores. ADAPT, FIB-C3 and ABC3D include cardiovascular risk estimators as variables to calculate the scoring. As previously supposed, these scores both detect advanced liver fibrosis and predict CVEs including shared risk factors in the algorithm. In conclusion, these scores detect patients at high risk for both liver and cardiovascular complications, identifying a sub-group of NAFLD patients who needs a multidisciplinary management.

LYSOSOMAL ACID LIPASE (LAL) INHIBITION AFFECTS DENDRITIC CELLS MATURATION

R. Bellini[1], F. Bonacina[1], M. Gomaraschi[2], G.D. Norata[3]
[1]Department of Pharmacological and Biomolecular Sciences, University of Milan; [2]Centro Grossi Paoletti, Department of Pharmacological and Biomolecular Sciences, University of Milan; [3]Department of Pharmacological and Biomolecular Sciences, University of Milan; Center for the Study of Atherosclerosis, E. Bassini Hospital, Cinisello Balsamo (MI)
E-mail: rossella.bellini@unimi.it

Background. Lysosomal acid lipase (LAL) hydrolyzes apoB-carried cholesterol esters and triglycerides in the lysosome providing free cholesterol and fatty acids, which can be directed to several cellular pathways and used for cellular metabolism. In humans, severe LAL deficiency results in Wolman Disease with extensive accumulation of lipids within the lysosome, while mutations resulting in a partly dysfunctional LAL (Cholesteryl Ester Storage Disease-CESD). As LAL plays a key role not only in hepatocytes but also in immune cells, including macrophages and T lymphocytes, the aim of this project was to investigate the role of LAL during the development and maturation of dendritic cells (DCs).
Methods. Dendritic cells (DCs) were differentiated from PBMC-derived monocytes of healthy donors or bone marrow cells of WT mice, following the treatment with GM-CSF and IL-4 for 6 days. Cells were then incubated with LPS to induce DCs maturation, in the presence or absence of Lalistat, a selective LAL inhibitor. Flow cytometry immunophenotyping paralleled by the evaluation of LAL activity during DCs maturation were performed to profile DCs characteristics.
Results. LAL activity increases in both human and mouse DCs during differentiation (+70%, p<0,01) and is reduced during LPS-induced DCs maturation (-26%). LAL inhibition promotes the reduction of the expression of the costimulatory receptor CD80 (an index of DCs maturation) in maturating DCs (-58%, p<0,05).
Conclusions. Lysosomal acid lipase, a checkpoint protein in lysosomal lipid homeostasis, bridges lipid metabolism and immunometabolic reprogramming during DCs differentiation and maturation. Ongoing studies are now aimed at elucidating the immunometabolic effects of targeting LAL during DCs maturation.

LOW BRACHIAL ARTERY FLOW-MEDIATED DILATION PREDICTS WORSE PROGNOSIS IN HOSPITALIZED PATIENTS WITH COVID-19

V. Bianconi[1], M.R. Mannarino[1], F. Figorilli[2], E. Schiaroli[3], E. Cosentini[1], G. Batori[1], E. Marini[1], A. Sahebkar[4], F. Grignani[1], A. Gidari[3], D. Francisci[3], M. Pirro[2]
[1]Unit of Internal Medicine, Department of Medicine, University of Perugia; [2]Unit of Internal Medicine, Department of Medicine, University of Perugia; [3]Unit of Infectious Diseases, Department of Medicine, University of Perugia; [4]Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran, Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
E-mail: v.bianconi.vb@gmail.com

Introduction. Endothelial injury can be induced by coronavirus disease 2019 (COVID-19) and seems to exert a crucial pathogenic role in its most severe clinical manifestations. We aimed to investigate the association between brachial artery flow-mediated dilation (bFMD), as a clinical and non-invasive measure of endothelial function, and in-hospital prognosis of COVID-19 patients.
Methods. Brachial artery flow-mediated dilation was assessed in hospitalized COVID-19 patients within 48 hours since hospital admission. The association between bFMD and either intensive care unit (ICU) admission or in-hospital death was explored using univariable and multivariable analyses.
Results. Four hundred and eight patients were enrolled. Significantly lower bFMD values emerged in COVID-19 patients with either radiographic signs of pneumonia, respiratory distress, or the need for non-invasive ventilation, as compared to those without ($p<0.001$, $p=0.001$, and $p<0.001$, respectively). Forty-two (10%) patients were admitted to ICU, 76 (19%) patients died, and 118 (29%) patients met the composite endpoint of ICU admission/in-hospital death. At unadjusted Cox regression analysis patients with bFMD <4.4% (the median value) had a significantly higher risk for the composite endpoint of ICU admission/in-hospital death as compared to those with bFMD ≥4.4% (HR 1.675, 95% CI 1.155-2.428, $p=0.007$). At multi-adjusted Cox regression analyses low bFMD was independently associated with a 1.519-to-1.654-fold increased risk for the composite endpoint of ICU admission/in-hospital death.
Conclusions. Low brachial artery flow-mediated dilation can predict an unfavorable in-hospital prognosis in COVID-19 patients. Measurement of bFMD may be clinically useful in the prognostic stratification of COVID-19 patients upon hospital admission.

ANGPTL3 FIBRINOGEN-LIKE DOMAIN FACILITATES LIPOLYSIS IN 3T3-L1 CELLS COMMITTED TO ADIPOCYTES ACTIVATING THE INTRACELLULAR ERK PATHWAY

S. Bini, V. Pecce, A. Di Costanzo, I. Minicocci, A. Ghadiri, L. Polito, M. Arca, L. D'Erasmo
Department of Translational and Precision Medicine, Sapienza University of Rome, Policlinico Umberto I
E-mail: simone.bini@uniroma1.it

Background. Angiopoietin-like protein 3 (ANGPTL3) is proved to stimulate lipolysis in adipocytes, moreover its C-terminal fibrinogen-like domain (ANGPTL3-fld) was found to interact with the cellular integrin receptor $\alpha3\beta v$, possibly transmitting intracellular signaling. Here we investigate the possible intracellular pathway by which ANGPTL3-fld stimulates lipolysis in 3T3-L1 cells.
Material and Methods. Murine 3T3-L1 cells were differentiated to adipocytes. Lipolysis was analyzed in basal conditions and after treatment with ANGPTL3, Isoproterenol or ANGPTL3 pretreatment + isoproterenol. Intracellular kinases were investigated via western blotting or a proteome profiler. In order to investigate the ERK signaling, 3T3-L1 cells were also treated with Vemurafenib or DMSO for 48h in the 4 experimental conditions
Results. ANGPTL3-fld alone is not able to activate lipolysis in 3T3-L1 cells committed to adipocytes; however, ANGPTL3-fld pretreatment determines FFA medium enrichment by two-fold when compared to isoproterenol treatment alone. Western blotting highlighted a similar activation of ERK in the three examined conditions if compared to untreated control. Hormone-sensitive lipase (HSL) phosphorylation was investigated: pSer565-HSL was activated in ANGPTL3-fld, whereas pSer660-HSL was predominant in the other conditions. Vemurafenib-mediated ERK pathway inhibition resulted in the inactivation of several MAPKs, untreated cells and isoproterenol-treated cells. Differently, in the ANGPTL3-fld treatment, upregulation of p38, PLC-gamma and PDGFR phosphorylation is evident and in the case of double treatment, the intracellular signaling completely shifts on Akt.
Discussion. ANGPTL3-fld activates intracellular AMPK and the ERK-pathway, whereas isoproterenol stimulation activates PKA signaling and ERK as well. The phosphokinase profile under Vemurafenib treatment suggests that while under isoproterenol treatment the metabolic change depends greatly on ERK1/2 activation, the ANGPTL3-fld treatment results in several intracellular signals upregulated under ERK-pathway inhibition that might be involved in different cellular activities. In conclusion, ANGPTL3 stimulation enhances β-adrenergic lipolysis through multiple intracellular pathways involved in the ERK signal.

NEW ERA IN HYPERCHOLESTEROLEMIA TREATMENT, INCLISIRAN: EARLY AND SUSTAINED LOW DENSITY LIPOPROTEIN-CHOLESTEROL REDUCTION WITH A TWICE PER YEAR ADMINISTRATION

A. Brambilla, S. Venezia, M. Latella, A. Garavaglia, E. Grillo
Medical department, cardio-renal-metabolic area,
Novartis Farma S.p.A
E-mail: andrea.brambilla@novartis.com

Inclisiran, small-interfering RNA (siRNA), works with the RNA interference (RNAi) mechanism which regulates proteins expression. Inclisiran binds PCSK9 mRNA, leads to its degradation thus decreasing PCSK9 protein expression and lowering LDL-C level. High cholesterol levels and prolonged time of exposure enhance risk of CV events, therefore also timing became crucial for atherosclerotic cardiovascular disease (ASCVD) patients. The early and effective LDL-C lowering action of inclisiran, together with the life-long LDL-C reduction, are positively correlated with CV risk reduction. The siRNA is conjugated with a triantennary GalNAC leading to specific hepatic delivery, therefore the compound is undetectable in plasma after 24-48 h from the injection. The LDL-C level drop is already significant at 14 days, and by day 30 the mean reduction is about 50% (ORION-1 trial). Chemical modifications at the siRNA back-bone level, protect inclisiran from degradation by liver nucleases. In the cytoplasm, RNAi mechanism occurs by the siRNA - RISC protein complex coupling. Physiologically, this bond last for long and the inclisiran modifications further enhance the complex stability. Therefore, one siRNA-RISC complex has an effect on multiple PCSK9 mRNAs, allowing inclisiran administration twice per year (after initial dose at baseline and 3 months), with an early, sustained and effective LDL-C level reduction. A pooled analysis of the 3 phase III trials (ORION-9/10/11) shows a time averaged (18 months) LDL-C reduction of 50.5% on top of therapy with statins±ezetimibe.Inclisiran provides effective evidence-based results on lowering LDL-C levels in different high CV risk populations (HeFH/established ASCVD/ASCVD-risk equivalent), which is demonstrated to be crucial for CV risk reduction. Furthermore, twice per year administration may positively improve adherence, thereby simplifying patient management and follow-up control. Based on these findings, we are stepping into a new era of biologic therapeutics, where inclisiran represents the new, effective and safe therapeutic candidate for lowering LDL-C levels.

INCLISIRAN REDUCES LOW DENSITY LIPOPROTEIN-CHOLESTEROL INDEPENDENT OF GENOTYPE IN SUBJECTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLAEMIA

F.J. Raal[1], D. Kallend[2], K.K. Ray[3], T. Turner[4], W. Koenig[5], R.S. Wright[6], P.L.J. Wijngaard[7], D. Curcio[7], M.J. Jaros[8], L.A. Leiter[9], J.J.P. Kastelein[10]
[1]Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; [2]DalCor Pharmaceuticals, Montreal, Quebec, Canada; [3]the Imperial Centre for Cardiovascular Disease Prevention, Department of Primary Care and Public Health, Imperial College, London, UK; [4]Medpace Reference Laboratories, Cincinnati, USA; [5]Deutsches Herzzentrum München, Technische Universität München, and German Center for Cardiovascular Research, Munich Heart Alliance, Munich, and the Institute of Epidemiology and Medical Biometry, University of Ulm, Ulm, Germany; [6]The Division of Preventive Cardiology and the Department of Cardiology, Mayo Clinic, Rochester, MN, USA; [7]The Medicines Company, Parsippany, NJ, USA; [8]Summit Analytical, Denver, USA; [9]Li Ka Shing Knowledge Institute, St. Michael's Hospital, University of Toronto, Toronto, Canada; [10]The Department of Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands
E-mail: andrea.brambilla@novartis.com

Introduction. HeFH disease severity is linked to the causative mutation type and inclisiran was previously shown to reduce LDL-C in these patients. Important mutations in HeFH include those with two variants; double heterozygous (DH) and compound heterozygous (CH), which are phenotypically similar to HoFH; and functional mutations in LDLR (negative, defective and unknown). This analysis explored the efficacy of inclisiran on these genotypes relative to the genotypes examined previously.
Methods. ORION-9 randomized 482 patients with HeFH 1:1, to receive inclisiran 284 mg or placebo. Randomization was based on mutations in LDLR and APOB, PCSK9, LDLRAP1 genes. Endpoints included percent and absolute change in LDL-C and PCSK9 from baseline at Day 510 across the genetic variants.
Results. Compared with other variants, a greater proportion of patients with DH (33.95%) and LDLR negative (35.6%) had ASCVD. Higher baseline LDL-C was seen in CH, LDLR negative and pathogenic patients. The absolute and percent LDL-C reduction from baseline with inclisiran ranged from 1.4-2.1 mmol/L and 37-56% respectively across genotypes, with lower values corresponding to patients with CH. Reductions in LDL-C across severe genotypes, including DH, LDLR negative and pathogenic, were comparable to less severe genotypes. The degree of LDL-C reduction across the genotypes was unrelated to absolute and percent reduction in PCSK9.
Conclusion. While potential bias from sample size variability between subgroups cannot be excluded, the robust LDL-C reductions provided by inclisiran across a range of HeFH genotypes suggests that preventing PCSK9 synthesis provides therapeutic benefit independent of causative mutation.

NEXT GENERATION SEQUENCING IN SEVERE HYPERTRIGLICERIDEMIA: IDENTIFICATION OF A NOVEL NONSENSE MUTATION OF CREB3L3 GENE

F. Brucato[1], R. Scicali[2], C. Scrimali[1], M. Gueli Alletti[1], R. Spina[1], V. Ingrassia[1], G.I. Altieri[1], D. Noto[1], G. Misiano[1], A. Giammanco[1], C.M. Barbagallo[1], A. Ganci[1], A.B. Cefalù[1], F. Purrello[2], M.R. Averna[1]
[1]Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza "G. D'Alessandro" (PROMISE), Università degli Studi di Palermo; [2]Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Catania
E-mail: federicabrucato21@gmail.com

Introduction. Hypertriglyceridemia (HTG) is a common form of dyslipidemia associated with an increased risk of cardiovascular disease and pancreatitis. The severe forms are characterized by very high plasma levels of triglycerides (TG) (>1000 mg/dL -11.2 mmol/l). Monogenic autosomal recessive forms are characterized by homozygous or compound heterozygous loss-of-function mutations of genes involved in the intravascular lipolysis of the triglyceride-rich lipoproteins, namely lipoprotein lipase (LPL), apolipoprotein C2 (APOC2), apolipoprotein A5 (APOA5), glycophosphatidylinositol (GPI)-anchored high-density lipoprotein-binding protein 1 (GPIHBP1), lipase maturation factor 1 (LMF1), and glycerol-3-phosphate dehydrogenase 1 (GPD1). Mutations in CRE-binding protein 3-like 3 (CREB3L3) and glucokinase regulator (GCKR) have been associated to dominant familial hypertriglyceridemia.
Materials and Methods. We performed a Next Generation Sequencing (NGS) analysis to study the coding exons and intron/exon boundaries of genes affecting the main pathways of triglyceride synthesis and metabolism in outpatients with severe hypertriglyceridemia.
Results and Conclusion. In the majority of subjects no functionally relevant mutations in the LPL, APOC2, APOA5, GPIHBP1, and LMF1 genes were detected. Two patients were found to be carriers of mutations in CREB3L3 gene. A 54 years old woman with very high TG levels (up to 1900 mg/dL) was found to be carrier of a novel nonsense heterozygous mutation (c.610C>T p.Arg204Ter) while a 51 years old woman with TG levels up to 1000 mg/dL was heterozygous for an already known pathogenic mutation (c.718G>A p.Glu240Lys). The p.Arg204Ter variant is predicted to result in the formation of a premature stop codon and synthesis of a truncated protein devoid of function. NGS is a powerful tool for the genetic diagnosis of HTG and mutations of CREB3L3 gene may be associated with severe forms of hypertriglyceridemia.

METABOLIC CHANGES IN SARS-COV-2 INFECTION: CLINICAL DATA ON ALTERATIONS OF LIPID PROFILE IN COVID-19 PATIENTS

D. D'Ardes[#*1,5], I. Rossi[#*1], B. Bucciarelli[1], M. Allegra[1], M. Gentile[1], F. Troiano[1], P. Vizzarri[1], F. Bianco[2], B. Sinjari[3], M. Marchioni[4], M. Di Nicola[4], F. Santilli[1,5], M.T. Guagnano[*1,5], F. Cipollone[*1,5], M. Bucci[*1,5]
[1]"Clinica Medica" Institute, Department of "Medicine and Science of Aging", "G. d'Annunzio" University, Chieti; [2]Pediatric Cardiology and Adult Congenital Heart Disease, Azienda Ospedaliero-Universitaria "Ospedali Riuniti" of Ancona; [3]Department of Innovative Technologies in Medicine and Dentistry, "G. d'Annunzio" University, Chieti-Pescara; [4]Laboratory of Biostatistics, Department of "Medical, Oral and Biotechnological Sciences", "G. d'Annunzio" University, Chieti-Pescara, Chieti; [5]Azienda Sanitaria Locale n. 2 Abruzzo Lanciano-Vasto-Chieti
[#]These authors share first authorship
**These authors share senior authorship*
**Corresponding Authors: Damiano D'Ardes and Ilaria Rossi*
E-mail: mbucci@unich.it

It seems that during SARS-CoV-2 infection total cholesterol, LDL-C, HDL-C values decrease and lipids could play a fundamental role in viral replication. We performed a retrospective analysis of 118 hospitalized patients with COVID-19, comparing pre-infection li-pid profile (53 patients) to those measured on admission, with the aim of evaluating whether SARS-CoV-2 in-fection could be involved in lipid profile alterations and to study possible correlations with disease severity and clinical outcome. Median baseline values at the admission time were: total cholesterol 136.89±42.73 mg/dl, LDL-C 81.53±30.35 mg/dl, HDL-C 32.36±15.13 mg/dl, triglycerides 115.00±40.45 mg/dl, non-HDL-C 104.53±32.63 md/dl. Median values of pre-infection total cholesterol and HDL-C were significantly higher than those measured at the admission time (p value <0.05). C-reactive protein (CRP) negatively correlated with LDL-C (p=0,013) and HDL-C (p=0,05). Our data suggest a possible relation between COVID-19 and lipid profile with a negative correlation between CRP and LDL-C and HDL-C values, proposing the hypothesis that lipid lowering could follow the rising of COVID-19 inflammatory state.

THE CHALLENGE OF LIPID-MODIFYING THERAPIES IN THE ACHIEVEMENT OF OPTIMAL LDL-C LEVELS IN HIGH AND VERY HIGH CV RISK PATIENTS: STILL AN OPEN QUESTION, WAITING FOR BEMPEDOIC ACID

B. Bucciarelli#*[1], F. Bianco#*[2], D. D'Ardes[1,3], I. Rossi[1,3], F. Troiano[1], P. Vizzarri[1], M. Caporale[1], F. Cipollone[1,3], M. Bucci[1,3]
[1]"Clinica Medica" Institute, Department of "Medicine and Science of Aging", "G. d'Annunzio" University, Chieti; [2]Pediatric Cardiology and Adult Congenital Heart Disease, Azienda Ospedaliero-Universitaria "Ospedali Riuniti" of Ancona; [3]Azienda Sanitaria Locale n. 2 Abruzzo Lanciano-Vasto-Chieti
E-mail: mbucci@unich.it

Background. Hypercholesterolemia is one of the main modifiable atherosclerotic cardiovascular disease (ASCVD) risk factors. Thus, optimal treatment is essential, namely in patients with high or very high CV risk, in order to achieve the LDL-C levels recommended by European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) guidelines. Our purpose was to evaluate the efficacy of lipid-modifying therapies (LMT) and the achievement of treatment goals in a cohort of patients at high and very high CV risk, followed for 8±2 months. All patients were enrolled at our Lipid Clinic to evaluate the actual efficacy of the currently available LMT for these patients.
Methods. We enrolled 27 consecutive patients, referred to our Lipid Clinic Center in Chieti between November 2020 and January 2021. Inclusion criteria were: age over 18 and high or very high CV risk, estimated with ESC-SCORE (Systematic Coronary Risk Estimation) charts. The follow-up ended in September 2021. Five patients were excluded due to missing laboratory data during follow-up; 23 patients (mean age 60±13 years old, 74% of males) constituted the final sample of the present analysis.
Results. The majority of patients (n=17/23, 74%) were treated with Ezetimibe, while Rosuvastatin was the most utilized statin (n=10/23, 43%); 9 subjects were treated with both. At the follow-up, the entire population experienced a significant reduction of total cholesterol and LDL-C (p=0.014 and p=0.011, respectively), with a change from the baseline of -28 [-103, 13 (Q1, Q3)] and -34 [-92, 11 (Q1, Q3)] mg/dL. According to the ESC/EAS recommendations, n=11/23 (50%) participants achieved the treatment goal. At the multivariable analysis, the adding of PCSK9i (more frequently Evolocumab) was the main LMT that lead to the achievement of treatment goal [(OR 2.42, 95% CI: 0.26, 5.12 (p=0.043)].
Conclusions. In our cohort of high and very high CV risk patients, the adding of PCSK9i shows to be the most effective LMT to achieve the therapeutic target. Future studies will able to evaluate the role of bempedoic acid, a promising, less expensive LMT, in this clinical scenario.

THE COMPLICATED RELATIONSHIP BETWEEN FAMILIAL HYPER-CHOLESTEROLEMIA AND STATINS IN THE ERA OF BIOLOGICAL DRUGS

M. Caporale*[1], F. Troiano*[1], P. Vizzarri[1], I. Rossi[1,2], D. D'Ardes#[1,2], F. Cipollone[1,2], M. Bucci[1,2]
[1]"Clinica Medica" Institute, Department of "Medicine and Science of Aging", "G. d'Annunzio" University, Chieti;
[2]Azienda Sanitaria Locale n. 2 Abruzzo Lanciano-Vasto-Chieti;
#These authors share first authorship;
**These authors share senior authorship*
E-mail: mbucci@unich.it

Familial Hypercholesterolemia (FH) is the most common lipidic disorder inducing early cardiovascular disease (CV). It is a hereditary disease with predominantly autosomal dominant transmission, but new mutations have been recently identified resulting in a lower liver sensitivity to LDL, as well as a lower response to treatment with statins. After the arrival of the PCSK9is, the need to identify which of the mutations associated with FH are more or less correlated with a reduction in cardiovascular risk and which of them is more or less responsive to traditional drugs or to innovative ones is growing.In this context, the aim of our study was to evaluate the therapeutic efficacy of statins/ezetimibe alone and in association with PCSK9i, by measuring and comparing LDL values in different genetic polymorphisms. Starting from our database of LIPIGEN study, we included 10 patients, 4 women, 6 men, (mean age 48,6 yo). All participants belong to a restricted territorial cluster (Sulmona, AQ) in which 3 patients are carriers of the FH mutation and poor responders to statins, 7 have FH mutation alone but with evident resistance to statin therapy. The study was conducted at our Lipid Clinic of Chieti University by carrying out a blood sample for the genetic study of any mutations and serial checks of LDL levels. The results have shown a poorer control of LDL values during treatment with statin/ezetimibe used alone with an average of 224,1 mg/dL; meanwhile, a marked improvement in lipid profile control (average =50,3 mg/dl) has been noted in all patients after the adding of PCSK9i. Moreover, the treatment with PCSK9i was associated with relevant reduction of LDL values (median 72%); this trend was not affected by the presence of the gene mutation associated to poor response to statins. Particularly, in 2 patients of the same family, with the same mutation, the response to PCSK9i in terms of reduction of LDL was comparable, even if one of the patients carried the mutation of statin intolerance and the other one didn't have it.
In conclusion, the simultaneous use of both drugs could be associated with better control of LDL levels and consequently with a lower risk of cardiovascular events independently from mutations, especially in those patients with FH mutation and poor response to statin treatment. This preliminary report could represent a start point that anyway needs further investigation to confirm our emerging data.

HIGHER ORAL PORPHYROMONAS GINGIVALIS (PG) ABUNDANCE IS ASSOCIATED WITH PREVIOUS ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN HIGH-RISK PATIENTS AND IN PATIENTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

P. Magni*[1,2], P. Pignatelli*[3], D. D'Ardes[#4], L. Scorpiglione[4], F. Cipollone[4], A.L. Catapano[1,2], A. Piattelli[3], M.C. Curia*[1], M. Bucci*[4]
[*]Equally contributed; [1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; [2]IRCCS MultiMedica, Sesto S. Giovanni, Milan; [3]Department of Medical, Oral and Biotechnological Sciences, Università degli Studi "Gabriele d'Annunzio" di Chieti-Pescara, Chieti; [4]Regional Center for the Study of Atherosclerosis, Hypertension and Dyslipidemia, "S.S. Annunziata" Hospital - ASL Chieti; Ce.S.I.-Met, Università degli Studi "Gabriele d'Annunzio" di Chieti-Pescara, Chieti; [#]These authors share first authorship; [*]These authors share senior authorship
E-mail: mbucci@unich.it

Introduction. Low-grade chronic inflammation, promoted by dysbiosis of gut and oral microbiota, is a risk factor for atherosclerotic cardiovascular disease (ASCVD), It may play a role in the individual susceptibility to ASCVD observed in the general population and in heterozygous familial hypercholesterolaemia (FH). High Porphyromonas gingivalis (PG) concentrations have been associated with clinical and experimental atherosclerosis. PG induces HDL-C oxidation and stimulates atherosclerosis-related gene expression in macrophages and foam cells in the presence of oxLDL.
Aim. We assessed oral PG abundance in very high-risk patients with FH (with/without previous ASCVD) and in patients in secondary ASCVD prevention.Methods: In this cross-sectional study, 21 patients with genetically proven FH (7/21 with previous ASCVD, 20 non-FH patients in secondary ASCVD prevention and 20 healthy controls, were selected from the patients cohort enrolled by Lipid Clinic of Chieti in the LIPIGEN study to quantify oral PG abundance through qPCR and assess the oral health status.
Results. The 3 groups did not differ for gender distribution, BMI, smoking, alcohol intake, gingival index. Non-FH patients were older than FH patients (mean difference: -12.4 years) and controls (11.3). TG, TC, HDL-C were similar between FH and non-FH groups. LDL-C was higher in FH patients (p=0.002). There was a trend to a higher plaque index (p=0.075), a reduced number of teeth (p=0.116) and a greater use of mobile prostheses (p=0.101) in non-FH patients vs FH patients. Oral PG abundance was higher in non-FH vs FH (p=0.084) and in non-FH vs controls (p= 0.021) (Kruskal-Wallis Test with DSCF pairwise comparisons). PG was higher among FH patients with previous ASCVD events (p=0.040, Mann-Whitney test). Oral PG quantity and TG levels were positively correlated (p=0.079; rho=0.391), while PG and HDL-C were inversely correlated (p=0.139; rho=0.326) in the FH group.
Conclusions. Higher oral PG abundance is present in high-risk patients, with or without FH, and may be related ASCVD events. Whether this finding relates to an increased risk of ASCVD events in FH patients remains to be addressed.

EFFECTIVENESS OF THE DUTCH LIPID CLINIC NETWORK SCORE (MODIFIED FOR ITALY) IN A CASE OF SUBJECTS OF AGE> 18 YEARS AND COMPARISON WITH THE SUBPOPULATION BETWEEN 18 AND 45 YEARS OF AGE

P. Vizzarri[#*1], D. D'Ardes[#*12], I. Rossi[1,2], F. Troiano[1], M. Caporale[1], F. Cipollone[1,2], M. Bucci[1,2]
[1]"Clinica Medica" Institute, Department of "Medicine and Science of Aging", "G. d'Annunzio" University, Chieti; [2]Azienda Sanitaria Locale n. 2 Abruzzo Lanciano-Vasto-Chieti; [#]These authors share first authorship; [*]These authors share senior authorship
E-mail: mbucci@unich.it

Background. Familial hypercholesterolemia (FH) is the most frequent genetic disorder among family dyslipidemias. Therefore, an early diagnosis and the development of clinical tools such as the Dutch Lipid Clinic Network Score (DLCNS) (1) are essential for identifying FH subjects as soon as possible and to avoid the onset of acute cardiovascular (CV) events. Our aim was to evaluate the performance of the DLCNS modified for Italy (DLCNS-I) (2) in identifying patients with genetically defined FH, particularly in the subgroup of patients <45 years.
Methods. The DLCNS-I and the result of genetic analysis were assessed retrospectively on a group of patients followed at the Chieti Lipid Clinic and already enrolled in the LIPIGEN project.
Results. The DLCNS-I was calculated on a population of 96 subjects >18 years old (average =49.04 years). Within the subcategory with clinically defined FH (score >8), 80.95% of patients presented a positive genetic analysis for FH or other variants of uncertain clinical significance (VUS). In the subgroup 18-45 years (n=34, mean age= 34), among patients with clinically defined FH, 93.3% presented a positive genetic analysis for FH or VUS. Compared to the total population, among 18-45 years old patients, those with missing data for the calculation of DLCNS-I were less numerous (22.9% vs 35.3% had no missing criteria; 30.2% vs 38.2% had 1 missing criterion); whereas patients with 2, 3 or 4 missing criteria (respectively 24.0% vs 23.5%; 13.5% vs 0%; 9.4% vs 2.9%) were more numerous in the sub-population >45 years. If we have no more than one missing diagnostic criterion (as observed in subjects 18-45 yo), despite lower average of LDL-C levels and less frequent CV events, DLCNS-I proved to be more effective thanks to a more complete anamnestic collection and a better physical examination.
Conclusions. The DLCNS-I has confirmed its usefulness in the diagnosis of FH, but it is certainly complex as regards a complete and effective compilation. Positive predictive value of DLCN-I is higher if performed by expert doctors.

References

1. Nordestgaard BG et al. Eur Heart J. 2013; 34: 3478-90a.
2. Casula M et al. Atherosclerosis. 2018; 277: 413-418.

STATIN USE IN ITALIAN CHILDREN: A RETROSPECTIVE AUDIT OF A SINGLE PEDIATRIC HOSPITAL

P.S. Buonuomo, I. Rana, M.V. Gonfiantini, M. Macchiaiolo, D. Vecchio, A. Coretti, A. Bartuli
Bambino Gesù Children's Hospital, Rome;
E-mail: psabrina.buonuomo@opbg.net

Background. The leading cause of mortality worldwide is atherosclerotic cardiovascular disease (ASCVD). Atherosclerotic lesions begin during childhood and can place individuals at greater risk for ASCVD. There is increasing evidence that the administration of statins early in life is beneficial in preventing or reducing atherosclerosis. Anyway, guidelines for the use of statins in children are currently only in place for FH and are fairly universal. According to local policy, atorvastatin is approved for use in children with heterozygous FH from the age of 10, with only pravastatin approved in children from the age of 8. Simvastatin is suggested in BNF for children from the age of 5 and rosuvastatin for children aged 6 years or older but these indications are not always receipted from local authorities. This study aimed to perform an audit of the use of statins in Italian children.
Methods. A retrospective audit of patients prescribed statins in a single children's hospital (Bambino Gesù Children's Hospital, Rome, Italy) was performed. Patients were identified through hospital patient records. Statin use (dose, type), as well as medical history and side effects, were recorded.
Results. A total of 256 patients (126 females, 130 males) under the age of 18 were included in the audit. The most common reasons for being prescribed a statin included a diagnosis of familial hypercholesterolemia (FH) (94%) or history of renal disease (Chronic kidney disease o Nephrotic syndrome, 4%) and diabetes (2%). Four statins were prescribed: atorvastatin (n=158), pravastatin (n=80), rosuvastatin (n=15), simvastatin (n=3). Interestingly, 29/80 pts (35%) initially treated with pravastatin were shifted to atorvastatin at the age of 10 due to low response to treatment. In a little percentage of patients (4%), statins were used in very young children (1-7 years old).
Conclusions. This retrospective hospital audit shows that statins, without a uniform choice of type, are prescribed to hypercholesterolemic children other than FH, as well as very young children, only in a small number of patients. Since pediatricians may play an active and determinant role in preventing the progression of risk factors and future ASCVD events, actions are needed to increase the number of treated children and at an earlier age.

DIETARY CHOLINE CONTENT AND HDL LEVELS CONTRIBUTE TO A PRO-ATHEROGENIC PLASMA METABOLOMIC PROFILE IN MICE

M. Busnelli[1], E. Franchi[1], S. Manzini[1], A. Colombo[1], M.A. García-Rivera[2], J. Kirwan[2], X. Zhang[3], P. Gérard[3], G. Chiesa[1]
[1]Department of Pharmacological and Biomolecular Sciences - Università degli Studi di Milano;
[2]Max Delbrück Center for Molecular Medicine, Berlin, Germany;
[3]Micalis Institute - INRAE, Jouy-en-Josas, France
E-mail: marco.busnelli@gmail.com

Gut microbiota can influence atherosclerosis development by metabolizing dietary choline: experimental and observational studies have highlighted a positive correlation between increased plasma choline-derived TMAO concentrations and adverse cardiovascular events. This study was aimed at investigating how the plasma metabolome of mice prone to atherosclerosis development was modulated by HDL levels and the dietary intake of choline.Low-fat, no cholesterol diets with different choline content (0.09% or 1.2%) were administered for 16 weeks to two groups of atherosclerosis-prone female mice:

1) extremely low-HDL mice, deficient for both murine apoA-I and apoE (DKO);
2) high-HDL mice, deficient for both apoA-I/apoE, but overexpressing human apoA-I (DKO/hA-I).

At sacrifice, atherosclerosis was evaluated, and a targeted metabolomics of plasma was performed. As expected, atherosclerosis, evaluated at the aortic sinus, was strongly increased in DKO vs DKO/hA-I mice. Surprisingly, although the high-choline diet resulted into elevated plasma TMAO levels in both genotypes, choline supplementation significantly worsened plaque development only in DKO/hA-I mice. Noteworthy, high-choline diet led to an increased concentration of plasma lipids only in DKO/hA-I mice: mainly triglycerides and hexosylceramides, but also ceramides and sphingomyelins. Several markers of increased cardiovascular disease risk and compromised renal function such as asymmetric dimethylarginine, symmetric dimethylarginine, indoxyl sulfate, creatinine and the microbiota-derived metabolite phenylacetylglutamine were increased only in high-choline-fed DKO/hA-I mice. Interestingly, the antioxidant histidine-containing dipeptide carnosine and its methylated form anserine were also increased by high-choline diet in DKO/hA-I.
In conclusion, our results indicate that dietary choline supplementation worsens atherosclerosis development only in the presence of HDL. Plasma metabolomics clearly indicated that choline supplementation increases the concentration of different lipid classes as well as of different metabolites indicative of augmented cardiovascular risk and impaired kidney function. Further studies are under way to assess the impact of the gut microbiota composition in regulating the biosynthesis of these molecules.

PROPROTEIN CONVERTASE SUBTILISIN KEXIN TYPE 9 INHIBITORS REDUCE PLATELET ACTIVATION MODULATING OX-LDL PATHWAYS

V. Cammisotto[1], F. Baratta[2], V. Castellani[2], S. Bartimoccia[3], C. Nocella[2], L. D'Erasmo[4], N. Cocomello[2], C. Barale[5], R. Scicali[6], A. Di Pino[6], S. Piro[6], M. Del Ben[7], I. Russo[8], F. Purrello[9], M. Arca[10], R. Carnevale[11], F. Violi[12], D. Pastori[13], P. Pignatelli[14]
[1]Department of General Surgery and Surgical Speciality "Paride Stefanini"Sapienza University of Rome; [2]Department of Clinical, Internal Medicine, Anesthesiological and Cardiovascular Sciences, Sapienza University of Rome; [3]Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome; [4]Department of Translational and Precision Medicine, Sapienza University of Rome; [5]Department of Clinical and Biological Sciences, University of Turin; [6]Department of Clinical and Experimental Medicine, Internal Medicine, University of Catania; [7]Department of Clinical, Internal Medicine, Anesthesiological and Cardiovascular Sciences, Sapienza University of Rome; [8]Department of Clinical and Biological Sciences, University of Turin; [9]Department of Clinical and Experimental Medicine, Internal Medicine, University of Catania; [10]Department of Translational and Precision Medicine, Sapienza University of Rome; [11]Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Rome, Italy; Mediterranea Cardiocentro, Napoli; [12]Mediterranea Cardiocentro, Napoli; [13]Department of Clinical Internal, Anesthesiological and Cardiovascular Sciences, Sapienza University of Rome; [14]Department of Clinical Internal, Anesthesiological and Cardiovascular Sciences, Sapienza University of Rome; Mediterranea Cardiocentro, Napoli
E-mail: vittoria.cammisotto@uniroma1.it

Background. Proprotein convertase subtilisin kexin type 9 inhibitors (PCSK9i) lower LDL-cholesterol, slow atherosclerosis preventing cardiovascular events. While it is known that circulating PCSK9 enhances platelet activation (PA) and that PCSK9i reduce it, the underlying mechanism is not still clarified.
Methods. In a multicenter before-after study in 80 heterozygous familial hypercholesterolemia (HeFH) patients on treatment with maximum tolerated statin dose ± ezetimibe, PA, soluble-NOX2-derived peptide (sNOX2-dp) and oxidized-LDL (ox-LDL) were measured before and after 6 months of PCSK9i treatment. In vitro study investigates the effects of plasma from HeFH patients before and after PCK9i on PA in washed platelets (wPLTs) from healthy subjects.
Results. Compared to baseline, PCSK9i reduced the serum levels of LDL-c, ox-LDL, Thromboxane (Tx) B2, sNOX2-dp and PCSK9 ($p<0.001$). The decrease of TxB2 correlates with that of ox-LDL while ox-LDL reduction correlated with PCSK9 and sNOX2-dp delta. In vitro study demonstrated that wPLTs resuspended in plasma from HeFH after PCSK9i treatment induced lower PA and sNOX2-dp release than those obtained using plasma before PCSK9i treatment. This reduction was vanished by adding ox-LDL. ox-LDL-induced PA was blunted by CD36, LOX1 and NOX2 inhibition.
Conclusions. PCSK9i treatment reduces PA modulating NOX2 activity and in turn ox-LDL formation in HeFH patients.

EFFECTS OF ANTI-PCSK9 MONOCLONAL ANTIBODIES ON PROPROTEIN CONVERTASE SUBTILISIN-KEXIN TYPE 9 ASSOCIATION TO LOW-DENSITY LIPOPROTEINS

L. Canclini[1], A. Baragetti[1], N. Jabnati[2], A.M. Malvandi[3], L. Grigore[4], A.L. Catapano[1]
[1]IRCCS MultiMedica, Milan, Department of Pharmacological and Biomolecular Sciences, University of Milan; [2]Department of Pharmacological and Biomolecular Sciences, University of Milan; [3]IRCCS MultiMedica, Milan; [4]IRCCS MultiMedica, Milan, Center for the Study of Atherosclerosis, Bassini Hospital, Cinisello Balsamo (MI)
E-mail: laura.canclini@unimi.it

Background and Aim. Proprotein convertase subtilisin-kexin type 9 (PCSK9) promotes the intracellular degradation of the hepatic low-density lipoprotein receptor (LDLR), thus increasing LDL-C plasmatic levels. PCSK9 inhibition using monoclonal antibodies (mAbs) produces a large drop in LDL-C levels, with a consequent reduction in cardiovascular events. Our previous studies showed the existence of PCSK9-LDL complexes in plasma. Prompted by this observation, we aimed at establishing whether if anti-PCSK9 mAbs administration could modify the PCSK9-LDL association.
Material and Methods. We enrolled patients treated with Alirocumab 75 mg (n=3)/150 mg (n=2) or Evolocumab 140 mg (n=12). We isolated their lipoproteins (LPs) with Iodixanol-gradient ultracentrifugation before the first mAbs administration (T0, n=17) and 1 (T1, n=14), 3 (T3, n=15) and 6 (T6, n=12) months after therapy. The PCSK9 content of the LP fractions obtained was quantified by ELISA (R&D Systems); cholesterol and triglycerides were measured using colorimetric assays.
Results and Conclusions. LDL-C levels, 143.8±71.3 mg/dL at T0, decreased to 59.7±36.8 mg/dL at T1, 62.3±52.6 mg/dL at T3, 49.4±24.7 mg/dL at T6. PCSK9 levels in plasma increased from 416.9±133.9 ng/mL at T0 to 3758.4±1175.3 ng/mL at T1, 3970.5±975.8 ng/mL at T3 and 4039±1118.7 ng/mL at T6. At baseline (T0), 10±4.6% of total recovered PCSK9 was in the LDL fraction. 6.5±3.4% (T1), 5.7±3.7% (T3) and 5±3.7% (T6) of PCSK9 was found in the LDL fraction after treatment, indicating that the PCSK9-LDL association remains despite the dramatic reduction of LDL-C due to the mAbs therapy. PCSK9 was recovered in a specific LDL sub-fraction both before and after mAbs administration. The absolute amount of PCSK9 LDL-bound increases more than 10 fold after therapy. Further studies are required to define the reason of this increased affinity and the possible biological consequences of the PCSK9-LDL association.

NEXT-GENERATION SEQUENCING HIGHLIGHTS NEW APOB VARIANTS AS A POTENTIAL CAUSE OF FAMILIAL HYPERCHOLESTEROLEMIA

G. Cardiero[1], D. Palma[1], U. Galicia-Garcia[2], A. Larrea-Sebal[2], M.D. Di Taranto[1], C. Martin[2], G. Fortunato[1]
[1]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli;
[2]CEINGE S.C.a r.l. Biotecnologie Avanzate, Napoli;
[2]Fundación Biofisika Bizkaia, Leioa, Spagna
E-mail: cardiero@ceinge.unina.it

Aim. APOB mutations are a rare cause of familial hypercholesterolaemia (FH). The introduction of next-generation sequencing (NGS) has revealed the presence of new variants in regions of the gene that were not analyzed by direct sequencing. Most of APOB variants remain of uncertain significance (VUS) for the absence of functional studies. The aim of study is to highlight the frequency of APOB rare variants and the crucial role of functional characterization in pathogenicity assessment.
Materials and Methods. Eighty patients with clinical suspicion of FH, previously analyzed with direct sequencing for the FH causative genes, were screened by NGS. In vitro characterization of five VUS found in APOB gene was performed by different approaches: proliferation assay of U937 cells, evaluation of LDL uptake in hepatoma cells labelling the patient's LDL with FITC and affinity assay of the patient's LDL for LDLR measuring the EC50 by solid-phase immunoassay.
Results. NGS analysis revealed the presence at heterozygous state of 36 VUS in the APOB gene. The U937 proliferation assay performed for only five APOB variants (p.Thr3785Ile, p.Ala2790Thr, p.Ser3801Thr, p.Asn4107Ser and p.Thr4179Ser) revealed a decreased cell growth (less than 75% of wild-type) for cells incubated with patient LDL carrying.This results were confirmed by decreased uptake of patient's LDL (less than 50% for p.Thr3785Ile, p.Ala2790Thr and p.Ser3801Thr; less than 70% for p.Asn4107Ser and p.Thr4179Ser). Finally, the affinity of the patient's LDL for LDLR was lower (with an EC50 approximately double than wild-type for most variants).
Conclusions. All the five rare variants in the APOB gene were shown to affect protein function, even though to a different degree. The functional data allows to attribute an important pathogenicity criteria that change the VUS into a likely pathogenic variant. These characterizations improve the genetic diagnosis of FH.

SERUM PCSK9 LEVELS AND VASCULAR DISEASE IN TYPE 2 DIABETIC PATIENTS

G. Armentaro[1], V. Cassano[1], E. Clausi[1], G. Mannino[1], T.V. Fiorentino[1], M. Perticone[1], E. Succurro[1], F. Andreozzi[1], F. Carbone[2], F. Montecucco[2], G. Sesti[3], A. Sciacqua[1]
[1]Department of Medical and Surgical Sciences, University Magna Græcia of Catanzaro; [2]Department of Internal Medicine and medical specialties, University of Genova; [3]Department of Clinical and Molecular Medicine, University of Rome-Sapienza, Rome
E-mail: velia.cassano@libero.it

Background. Recent studies proposed Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) as a new biomarker for atherosclerosis, due to its role in the degradation of low-density lipoprotein receptor (LDL-R). An over expression of the PCSK9 gene is related with increased of LDL cholesterol (LDL-C) plasma concentration and cardiovascular (CV) risk. Moreover, PCSK9 is related with arterial stiffness. The aim of the present study was to investigate the correlation between circulating levels PCSK9 serum and arterial stiffness in a large cohort of Caucasian patients affected by type 2 diabetes mellitus (T2DM), with no previous CV events.
Methods. We enrolled 401 T2DM, hypertensive, Caucasian patients. All patients presented normal renal function (GF>60 ml/min/1.73 m^2). PCSK9 was measured by colorimetric Elisa test. Arterial stiffness as the measurement of the carotid–femoral pulse wave velocity (PWV).
Results. Patients were divided in three tertiles according to increasing level of circulating PCSK9. From the I to the III tertiles, there was a statistically significant increase of high sensitivity C-reactive Protein (hs-CRP), fibrinogen and White Blood Cells (WBC) and a reduction of Estimated Glomerular Filtration Rate (e-GFR). Patients with higher levels of PCSK9 presented increased systolic blood pressure (SBP) ($p<0.0001$), diastolic blood pressure (DBP) ($p<0.0001$) and PWV ($p<0.0001$). PWV was significantly and directly correlated with fibrinogen ($p<0.0001$), WBC ($p<0.0001$), age ($p=0.024$), PCSK9's levels ($p=0.003$), and indirectly correlated with body mass index (BMI) ($p=0.001$), diuretic therapy ($p=0.001$). PCSK9 was the major predictor of PWV, justifying 16.9% of its variation.
Conclusion. For the first time, our study demonstrated a close association between circulating levels of PCSK9 and PWV, in T2DM, hypertensive, Caucasian subjects without previous CV events. In conclusion, PCSK9 could be a biomarker for CV risk stratification in diabetic subjects.

OXIDATIVE STRESS AND LEFT VENTRICULAR PERFORMANCE IN PATIENTS ACCORDING TO DIFFERENT GLYCOMETABOLIC PHENOTYPES

V. Cassano[1], S. Miceli[1], G. Armentaro[1], K. Barbara[1], D. Crescibene[1], V. Condoleo[1], G. Severini[1], V. Monaco[1], M. Perticone[1], F. Andreozzi[1], G. Sesti[2], A. Sciacqua[1]
[1]Department of Medical and Surgical Sciences, University Magna Græcia of Catanzaro; [2]Department of Clinical and Molecular Medicine, University of Rome-Sapienza, Rome
E-mail: velia.cassano@libero.it

Background. Recent studies demonstrated that in normoglucose-tolerant subjects (NGT), 1-h post load plasma glucose value ≥155 mg/dl, during oral glucose tolerance test (OGTT), identifies a worse cardio-metabolic risk profile and with increased risk for Type 2 Diabetes Mellitus (T2DM). T2DM patients present increased oxidative stress, due to high blood glucose levels, which plays a central role in the development of CV complication. The global longitudinal strain (GLS) is able to identify early alterations in the subendocardial longitudinal fibres, highlighting left ventricular systolic dysfunction long before the alteration of ejection fraction (EF). The aim of the present study was to evaluate the correlation between oxidative stress and subclinical myocardial damage, assessed with speckle tracking echocardiography, in normal glucose tolerant patients with 1-hour plasma glucose values ≥155 mg/dl (NGT≥155), comparing them to NGT<155, impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) newly diagnosed patients.
Methods. We enrolled 100 Caucasian patients. All subjects underwent OGTT. The serum values of the markers of oxidative stress (8-isoprostane and Nox-2) were assessed with ELISA test. Echocardiographic recordings were performed using an E-95 Pro ultrasound system.
Results. We observed significant differences, among the four groups, for fasting plasma glucose (p<0.0001), 1-h post load (p<0.0001), and 2-h post load plasma glucose levels (p<0.0001), fasting insulin (p<0.0001), 1-h insulin (p=0.029) and 2-h insulin values (p<0.0001) during OGTT. As compared with NGT<155, NGT≥155 exhibited significantly higher 1-h (p<0.0001), 2-h post load plasma glucose levels (p=0.045). No significant differences were observed between NGT≥155 and IGT patients. A significant increase of 8-isoprostane (p<0.0001) and Nox-2 (p<0.0001) was observed from the first to fourth group, indicating an increase in oxidative stress with the worsening of the metabolic status. Serum levels of 8-isoprostane and Nox-2 were significantly increased in NGT≥155 compared to NGT<155 group, but similar to IGT. The Global Longitudinal Strain (GLS), appeared progressively lower proceeding from NGT<155 group to T2DM group (p<0.0001). For similar values of ejection fraction (EF), NGT≥155 presented reduced GLS compared to NGT<155 (p=0.001), but similar to IGT patients.
Conclusions. Our study demonstrated that NGT≥155 subjects present functional alterations of myocardial contractile fibers, and these alterations are correlated with increased oxidative stress.

A SUBGROUP ANALYSIS OF THE ODYSSEY APPRISE STUDY: SAFETY AND EFFICACY OF ALIROCUMAB IN THE ITALIAN COHORT

A.B. Cefalù[1], R. Garbellotto[2], G. Mombelli[3], M. Pirro[4], P. Rubba[5], M. Arca[6], C. Borghi[7], A. Guerrasio[8], R. Nuti[9], K. Massaroni[10], G. Tirone[10], M. Averna[1]
[1]Department of Health Promotion Sciences, Maternal and Infant Care, Internal Medicine and Medical Specialties, University of Palermo; [2]Unit of General Medicine, Presidio Ospedaliero di Vittorio Veneto; [3]Centro Dislipidemie, ASST Grande Ospedale Metropolitano Niguarda, Milan; [4]Unit of Internal Medicine, Angiology and Arteriosclerosis Diseases Department of Medicine and Surgery University of Perugia; [5]Dipartimento di Medicina Clinica e Chirurgia, Università "Federico II" di Napoli; [6]Department of Translational and Precision Medicine "Sapienza" University of Rome; [7]Medical and Surgical Sciences Dept., Alma Mater Studiorum University of Bologna; [8]Department of Clinical and Biological Sciences, University of Turin, Regione Gonzole 10, Orbassano; [9]Department of Medicine, Surgery and Neuroscience, Policlinico Le Scotte, University of Siena; [10]Sanofi, Milano
E-mail: abaldassare.cefalu@unipa.it

Background and Aims. ODYSSEY APPRISE trial evaluated efficacy and safety of alirocumab in 994 patients with hypercholesterolemia and high CV risk in a real-life setting. The aim of the present report is to detail on the Italian cohort enrolled and treated in the trial.
Methods and Results. The methodology of the of the multinational, single-arm, Phase 3b open-label ODYSSEY APPRISE (Clinicaltrials. gov: NCT00730236) has been previously reported. 255 Italian patients were enrolled and treated according to the trial protocol. Overall mean exposure to alirocumab was 83.3±27.7 weeks. At week 12, LDL-C decreased by 51.3±23.1% and this reduction was overall maintained for the duration of the study. A similar reduction was observed in patients with and without heterozygous familial hypercholesterolemia (HeFH 50.7%±23.9 vs. non-FH, 53.6%±19.6). LDL-C was reduced below 1.8 mmol/L and/or by ≥50% reduction from baseline in 62% of patients overall (61 % in HeFH and 67 % in non-FH). Alirocumab was similarly well tolerated in the Italian cohort as in the entire study population and the more common treatment emergent adverse events (TEAEs) were influenza, myalgia and nasopharyngitis. The incidence LDL-C levels <25 mg/dl and <15 mg/dl, was 8.2% and 2.9% respectively.
Conclusion. The efficacy and safety of alirocumab in a real-life setting, in the Italian subgroup of patients are consistent with findings in the entire study population and confirm that alirocumabis a beneficial approach to further reduce LDL-C levels in patients at high CV risk on maximally tolerated conventional lipid lowering treatment.

LOMITAPIDE REDUCES TRIGLYCERIDE (TG) LEVELS IN FAMILIAL CHYLOMICRONEMIA SYNDROME (FCS)

A.B. Cefalù[1], L. D'Erasmo[2], A. Giammanco[1], A. Montali[2], G. Iannuzzo[3], A. Zambon[4], F. Forte[3], D. Noto[1], P. Suppressa[5], C.M. Barbagallo[1], S. Giannini[6], M. Arca[2], M. Averna[1]
[1]University of Palermo; [2]"Sapienza" University of Rome; [3]"Federico II" University of Naples; [4]University of Padua; [5]University of Bari; [6]Careggi Hospital, Florence
E-mail: abaldassare.cefalu@unipa.it

Background. FCS is a rare autosomal recessive disorder caused by impaired lipoprotein lipase (LPL) function, resulting in elevated TG levels, intense abdominal pain, hepatosplenomegaly and recurrent episodes of acute pancreatitis. Treatment requires a strict, extremely low-fat diet (<10% fat/day) to control TG levels <750-1000 mg/dL, which does not fully control the disease. Lomitapide is a microsomal triglyceride transfer protein (MTP) inhibitor that prevents assembly of triglycerides (TGs) into chylomicrons, in addition to very low-density lipoproteins and thereby reduces circulating levels of TGs.
Methods. This open-label, single arm 'LOCHNES' study of lomitapide in FCS, enrolled adult patients ≥18 years with genetically confirmed FCS, elevated fasting TG≥750 mg/dL and a history of pancreatitis, across 3 Italian centres. Patients were administered escalating-doses of lomitapide to maximum tolerated dose (MTD) for 26 weeks. The primary endpoint was the percent change in TGs from baseline to Week 26, with lomitapide in combination with other lipid lowering therapy
Results. Eighteen patients were enrolled in the study (mean ±SD: age 46.6±16.7y; body mass index 23.7±4.1 kg/m^2). Median baseline TG levels were 1804 mg/dL (range 810-4151 mg/dL). Lomitapide dose increased from standard starting dose 5 mg/day at baseline to mean 32.8±17.8 mg/day at Week 26. Median TGs reduced to 305mg/dL (range 70-1818 mg/dL) at Week 26. This equates to a 70.5% reduction in median fasting triglyceride levels. At Week 26, 14 patients achieved TG levels <1000 mg/dL and 13 of these achieved TGs ≤750 mg/dL. Treatment with lomitapide was generally well tolerated with no patient discontinuations. Adverse events were mild to moderate and were mainly related to gastrointestinal tolerability (n=9) and ALT/AST enzyme elevations ≥3x upper limit of normal (n=4). Where available (n=13), liver MRI imaging revealed increases in hepatic fat in some patients (n=5/13), and three patients with a baseline hepatic fat >20% (range 22-30%), experienced increases to 30-50% hepatic fat at 26 weeks. No patient experienced an episode of acute pancreatitis or severe abdominal pain during lomitapide treatment. One patient who temporarily interrupted lomitapide treatment due to an episode of diarrhoea, experienced acute pancreatitis during the treatment interruption period.
Conclusions. Lomitapide is effective in reducing triglycerides in FCS and preventing the recurrence of acute pancreatitis in this pilot study. The extent of the benefit of lomitapide to patients with FCS should be further evaluated in a larger prospective clinical trial.

EFFECT OF HDL/APOA-I DEFICIENCY ON CORONARY ATHEROSCLEROSIS, EXTRAVASCULAR LIPID DEPOSITION AND IMMUNE-INFLAMMATORY PROFILE

A. Colombo[1], M. Busnelli[1], S. Manzini[1], E. Franchi[1], F. Arnaboldi[2], E. Donetti[2], E. Scanziani[3], G.D. Norata[1], G. Chiesa[1]
[1]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano; [2]Department of Biomedical Sciences for Health, Università degli Studi di Milano; [3]Department of Veterinary Medicine, Università degli Studi di Milano
E-mail: alice.colombo@unimi.it

Aim. HDL and its main protein component, apolipoprotein A-I, exert a pivotal role in regulating cell cholesterol homeostasis and in modulating inflammatory response and immune cell activation. This study was aimed at investigating the impact of genetic manipulation of HDL/apoA-I levels on lipid deposition in heart vessels and extravascular tissues in relation to local and systemic immune-inflammatory activation.
Methods. ApoE deficient (EKO) mice, apoE/apoA-I double deficient (DKO) mice, DKO mice overexpressing human apoA-I (DKO/hA-I) and C57Bl/6 control mice were fed chow diet until 30 weeks of age. Plasma lipids were quantified, atherosclerosis development at the aortic sinus and in coronary arteries was measured, skin ultrastructure was evaluated by electron microscopy. Blood and lymphoid organs were characterized through histological, immunocytofluorimetric and whole transcriptome analyses.
Results. DKO mice were characterized by an almost complete lack of plasma HDL-cholesterol and by total cholesterol levels comparable to those of control mice. Only DKO mice showed severe alterations of skin morphology and skin-draining lymph nodes, whose transcriptome analysis revealed increased activation of the immune system and an unbalanced expression of genes involved in energy metabolism. An increased presence of CD4+ T effector memory cells was detected in blood, spleen and in the skin-draining lymph nodes of DKO mice. A worsening of atherosclerosis at the aortic sinus and coronary arteries was also observed in DKO mice vs EKO mice. Human apoA-I overexpression in the DKO background was able to rescue the skin phenotype and to halt atherosclerosis development.
Conclusions. HDL deficiency, in the absence of hyperlipidemia, is associated with severe alterations of skin morphology, coronary atherosclerosis, local and systemic inflammation.

GUT MICROBIOTA AND ATHEROSCLEROSIS: WHAT PERSPECTIVES FOR THE GENERAL PRACTITIONER?

M. Zagami[1], E. Sartori[1], M.M. Coronelli[2]
[1]Studio Medico di Medicina Generale di Bereguardo (PV); [2]Università degli Studi di Pavia
E-mail: mauriziomaria.coronelli@unipv.it

Intestinal dysbiosis is known to cause the systemic release of inflammatory mediators. Inflammation is one of the main causes of the atherosclerotic process. From the literature and new clinical evidence, there appears to be a correlation between dysbiosis and atherosclerosis. Our study aims to investigate this correlation in order to understand if, indeed, it is possible for the general practitioner to have an additional tool for the primary prevention of cardiovascular diseases. For our study, we took a fecal sample from 45 patients already undergoing treatment for atherosclerosis and who had at least one comorbidity (diabetes or hypertension). The collected samples were then subjected to metagenomic analysis. Each patient underwent an echo TSA in order to investigate the presence of intimal plaques or thickenings at the carotid level; a search was conducted for the possible presence of hepatic steatosis and the blood values of TMAO and Zonulin were analyzed, as well as numerous other clinical and biochemical parameters that would allow an adequate assessment of the dyslipidemic state.
Finally, each patient was subjected to a questionnaire relating to their eating habits, in order to be able to correlate this information with the composition of their intestinal microbiota. We also analyzed similar data relating to patients with, until that moment, subclinical atherosclerosis and no comorbidities that had already been collected in the context of the PLIC study. Our study leads the way for considering the presence of dysbiosis as an additional risk factor for atherosclerosis. For the treatment of patients suffering from atherosclerosis, it follows having to aim at a correction of the diet not only with a view to containing the dyslipidemic state, but also to correct the dysbiosis.

PCSK9, CARDIAC METABOLISM AND HFpEF

L. Da Dalt[1], L. Castiglioni[2], A. Baragetti[3], M. Audano[1], M. Svecla[1], F. Bonacina[1], S. Pedretti[1], P. Uboldi[1], P. Benzoni[4], F. Giannetti[4], A. Barbuti[4], F. Pellegatta[5], S. Indino[6], E. Donetti[6], L. Sironi[2], N. Mitro[1], A.L. Catapano[3], G.D. Norata[7]
[1]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano; [2]Department of Pharmaceutical Sciences, Università degli Studi di Milano; [3]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, IRCCS Multimedica Hospital, Sesto San Giovanni, Milan; [4]Department of Biosciences, Università degli Studi di Milano; [5]Centro SISA per lo studio dell'Aterosclerosi, Ospedale Bassini, Cinisello Balsamo (MI); [6]Department of Biomedical Science for Health, Università degli Studi di Milano; [7]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Centro SISA per lo studio dell'Aterosclerosi, Ospedale Bassini, Cinisello Balsamo (MI)
E-mail: lorenzo.dadalt@unimi.it

Introduction. PCSK9 is a glycoprotein released into the circulation mainly by the liver which, interacting with homologous and non-homologous LDLR receptors, including CD36, can regulate their functionality, thus favoring lysosomal degradation. PCSK9 deficiency induces an increase in the uptake of lipoproteins, promoting the removal of circulating lipids and their accumulation in different tissues.
Aim. Given the impact of PCSK9 inhibitors on lipoprotein metabolism, the present work is aimed to evaluate its role in cardiac functionality and metabolism.
Methods and Results. Mouse models WT, Pcsk9 KO, liver selective KO and Pcsk9/Ldlr double KO (DKO) were fed an SFD diet for 20 weeks. Exercise intolerance, muscle strength and cardiac structure were evaluated. Pcsk9 KO displays a reduced running endurance associated with echocardiographic alteration suggestive of heart failure with preserved ejection fraction (HFpEF). The lack of PCSK9 has therefore shown an impact on cardiac metabolism reflected by reduced mitochondrial oxygen consumption and ATP production. A similar phenotype was observed in the Pcsk9/Ldlr DKO models, thus excluding a contribution for LDLR on heart damage observed in Pcsk9 KO mice. The cardiac function profile in the liver selective PCSK9 KO model further ruled out the involvement of circulating PCSK9 in the development of HFpEF, indicating a possible role for locally expressed PCSK9. In vitro study on freshly isolated cardiomyocytes from mice model confirm that CD36, in PCSK9 deficiency states and, in the presence of VLDL, is upregulated and associated with neutral lipid accumulation and mitochondrial mass depletion. In line with what has been demonstrated, carriers of the R46L variant with loss of function for PCSK9 have an increase in left ventricular mass, but a similar ejection fraction compared to control subjects.
Conclusions. PCSK9 deficiency, modulating other receptors than the LDLR, can influence cardiac lipid metabolism, can favor the mitochondrial damage and the development of HFpEF.

TREATMENT OF HIGH- AND VERY HIGH-RISK PATIENTS FOR THE PREVENTION OF CARDIOVASCULAR EVENTS IN EUROPE: BASELINE ITALIAN DATA FROM THE MULTINATIONAL, OBSERVATIONAL SANTORINI STUDY

M. Arca[1], F. Romeo[2], A. Fischetti[2], I. Haq[3], A. Bilitou[3], K.K. Ray[4], A.L. Catapano[5]
[1]Department of Translational and Precision Medicine, Sapienza University of Rome; [2]Daiichi Sankyo Italia, Rome; [3]Daiichi Dankyo Europe, Munich, Germany; [4]Imperial Centre for Cardiovascular Disease Prevention, Department of Primary Care and Public Health, Imperial College London, London, UK; [5]Department of Pharmacological and Biomolecular Sciences, University of Milan and Multimedica IRCCS, Milan
E-mail: letizia.dasacco@daiichi-sankyo.it

Background. The ESC/EAS 2019 guidelines recommend lower goals for LDL-C, especially in patients with high (<70 mg/dl) and very high CV risk (<55 mg/dl). As a consequence more intensive lipid-lowering therapies (LLTs) will be requested. Real-world studies in Europe have demonstrated suboptimal achievement of ESC/EAS 2016 guideline LDL-C goals, but to what extent practice has changed since the last ESC/EAS guideline is uncertain. The SANTORINI study (NCT04271280) was designed to assess in Europe the implementation of the new ESC/EAS 2019 guidelines in the management of dyslipidaemia in high- and very high-risk patients followed in a real-world setting.
Methods. The SANTORINI is a multinational, prospective, observational, non-interventional study enrolling patients aged ≥18 years at high and very high CV risk (as assigned by the investigators) requiring LLTs from 14 European countries across primary and secondary care settings. Patients' characteristics, medical history, major risk factors, current LLT as well as major lipid parameters (total, LDL-C, HDL-C and total triglycerides) were collected at baseline and again at follow-up, approximately 12 months after baseline data collection. Here, we reported baseline data of the Italian cohort enrolled into the SANTORINI study.
Results. From March 2020 to February 2021 in Italy were enrolled 1977 patients. The vast majority of them (77.4%) were classified at very high risk and the ESC/EAS guidelines were the most commonly used reference for risk classification. At baseline, 30.1% of patients were receiving LLT monotherapy, while the combination therapy was limited to 15.9% of patients; 32.6% of patients were not receiving any LLTs. The recorded mean baseline LDL values were 95.5 mg/dl in very high risk and 112.3 mg/dl in high risk patients.
Conclusions. This large registry in patients at high and very high CV risk suggests that, in Italy, more potent LLTs (e.g. combination therapies) are largely underused in high and very-high risk patients and that in these patients LDL-C levels remain substantially higher than those recommended by the ESC/EAS guidelines.

INSIGHTS INTO INDIVIDUAL SUSCEPTIBILITY TO SARS-CoV-2 INFECTION AND DISEASE SEVERITY: THE POTENTIAL ROLE OF DIFFERENT HOST GENETIC PROFILES IDENTIFIED BY NEXT GENERATION SEQUENCING

R. De Cario, E. Sticchi, A. Kura, S. Suraci, T. Capezzuoli, M. Giannini, A. Bertelli, A.A. Rogolino, A.M. Gori, R. Marcucci, B. Giusti
Department of Experimental and Clinical Medicine, University of Florence; Atherothrombotic Diseases Center, A.O.U. Careggi, Florence
E-mail: decariorosy@hotmail.it

Background. First reported in the Wuhan Chinese region in December 2019, COronaVIrus Disease 2019 (COVID-19), a new form of severe acute respiratory syndrome (SARS) caused by anovel strain of coronavirus (SARS coronavirus 2 [SARS-CoV-2]), was declared as a pandemic in 2020 leading to ~4 million deaths all over the world to date. Clinical manifestations range from asymptomatic forms to respiratory failure and death. The infection is also associated with a high incidence of thrombotic complications. Alongside age, comorbidities and gender, host genetic factors have been suggested to play a role in the severity of the disease outcomes, their identification potentially representing a useful prognostic tool. In this study, genetic characterization of 33 Covid-19 patients referred to the Advanced Molecular Genetics Laboratory, Atherothrombotic Diseases Center, Careggi Hospital-University of Florence, was made by Next Generation Sequencing (NGS) in order to identify genetic profiles potentially representing prognostic factors modulating the susceptibility to virus and thrombotic risk.
Methods. NGS was performed by Illumina MiSeq and Haloplex protocol targeting 7 virus entry-related genes (ACE2, TMPRSS2, CTSL, CTSB, HSPA5, IL6, FURIN) and 11 genes influencing the thrombotic phenotypic profile (PROC, PROS1, FGA, FGB, FGG, SERPINC1, F2, F5, F10, PLAT, PLG).
Results. Thirty-seven heterozygous rare variants [Minor Allele Frequency (MAF) <0.01 in the gnomAD European database] were identified among 25 patients involving 7/7 virus entry-related genes (14/40 variants) and 9/11 genes influencing the thrombotic phenotypic profile (23/40 variants). Loss-of-function mutations leading to a diminished activity of those molecular components involved in the virus attachment/entry to the host cells (i.e. ACE2), may be interpreted as protective factors while gain-of-function variants, positively modulating the expression of those genes, may increase susceptibility to infection (i.e. TMPRSS2). Moreover, previously described or new variants involving those genes implicated in the coagulation cascade, may determine a different clinical course (i.e. F10, F5) and response to therapy (i.e. SERPINC1) especially in those patients developing thrombotic complications which require long-term hospitalization and treatment in intensive care.
Conclusions. Our data suggest how individual genetic profiles may participate in modulating the susceptibility to SARS-CoV-2 infection and the wide range of clinical manifestations associated with the disease. Additional studies on the role of single genetic variant together with the evaluation of the potential contribution of common variants may enable the definition of an allelic risk score allowing the identification of subjects at a greater risk of developing severe complications.

TRANSENDOTHELIAL TRANSPORT OF HIGH-DENSITY LIPOPROTEIN: ROLE OF SPHINGOSINE 1-PHOSPHATE AND ITS RECEPTORS

M. Dessena[1], F. Poti[1], R. Feuerborn[2], J.-R. Nofer[2], B. Hesse[3], A. Lukasz[3], M. Simoni[4], A. von Eckardstein[5]
[1]Department of Medicine and Surgery-Unit of Neurosciences, University of Parma, Parma; [2]Central Laboratory Facility, University Hospital of Münster, Germany; [3]Department of Medicine D, Division of General Internal Medicine, Nephrology, and Rheumatology, University Hospital Münster, Münster, Germany; [4]Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena; [5]Institute of Clinical Chemistry, University of Zurich and University Hospital of Zurich, Switzerland
E-mail: mattia.dessena@unipr.it

Background and Goals. Sphingosine 1-phosphate (S1P) is a bioactive lysosphingolipid carried by HDL. In these lipoproteins, S1P is mainly bound to apolipoprotein M (apoM) and this complex effectively activates the five S1P receptors (S1P1-5). The scavenger receptor class B type 1 (SR-BI) facilitates the interaction between HDL-S1P and its receptors on the cell membrane. It has been shown that stimulation of S1P1 is fundamental for vascular homeostasis, promoting the development and maintenance of the endothelial barrier. However, it is not known whether S1P can regulate transendothelial transport of HDL and the possible mechanisms involved in this process remain unknown.We developed a peculiar animal model overexpressing S1P1 specifically in endothelial cells (S1P1-iECKI mice), contributing to clarify the transport of HDL between the different compartments when S1P/S1P1 axis signaling is amplified.
Materials and Methods. Fluorescently labelled HDL or Evans Blue (albumin tracer) were administered to S1P1-iECKI and control mice by intravenous injection (tail vein). Subsequently, the mice were sacrificed and peritoneal lavage was performed. The presence of fluorescent particles or Evans Blue was assessed by fluorimetric-spectrophotometric techniques. In parallel, S1P1 overexpressing animals and related controls were sacrificed and their aortas isolated and processed for immunofluorescence imaging through confocal laser scanning microscopy.
Results. Compared to control mice, S1P1-iECKI mice showed an increased transport of HDL and a decreased transport of Evans Blue from the blood to the peritoneal cavity. The analysis of the aortic endothelium through confocal microscopy confirmed the overexpression of the S1P1 receptor, and most importantly demonstrated an increased expression of SR-BI in the endothelial cells of S1P1-iECKI mice, compared to controls.
Conclusions. The stimulation of S1P/S1P1 axis promotes transendothelial transport of HDL. The opposite effect on transendothelial transport of albumin suggests that HDL can cross the endothelial barriers mainly through specific mechanisms rather than through passive filtration.

THE SELECTIVE ACTIVATION OF FORMYL PEPTIDE RECEPTOR 2 PREVENTS THE INFLAMMATORY AND PRO-CALCIFIC DIFFERENTIATION OF INTERSTITIAL AORTIC VALVE CELLS

M. Donato, C. Bolego, E. Faggin, A. Bressan, F. Cinetto, C. Felice, C. Agostini, M. Rattazzi
Università degli Studi di Padova
E-mail: maristella.donato@studenti.unipd.it

Background. Calcific aortic valve disease (CAVD) is the most common valvulopathy in the general population; however, no effective pharmacological therapy has proven to halt or delay its progression.Aim: We aimed to investigate the effects of the activation of Formyl Peptide Receptor 2 (FPR2), a known receptor for lipoxins, resolvins and anti-inflammatory molecules, on the pro-calcific and inflammatory differentiation of interstitial aortic valve cells (VICs).
Methods. A clone of primary VICs seeded on culture plates or type I collagen scaffolds was treated with lipopolysaccharide (LPS, 500 ng/mL) for 12 days to acquire a pro-calcific profile, with or without the concomitant supplementation of two synthetic FPR2 agonists (MMK1, 50 μM and Ac2-26, 3.2 μM). At the end of the treatment, proteins and RNA were extracted for western blotting and gene expression analysis (RT-PCR). Alkaline phosphatase (ALP) activity and calcium deposition in collagen scaffolds were determined through colorimetric assays. The expression of FPR2 on human pathological and healthy aortic valves was investigated through immunohistochemistry and gene expression analysis.
Results. The gene expression analysis on human valves showed an increase in ALP, IL-6 and FPR2 levels in pathological valves compared to healthy tissue. Concurrently, the in vitro exposure of VICs to LPS increased the expression of inflammatory cytokines (such as IL-1beta, IL-6, TNF-alpha), ALP, BMP2 and FPR2. Moreover, it induced the deposition of calcium on collagen scaffolds. The treatment of cultured VICs with two selective FPR2 agonists (MMK1 and Ac2-26) reduced the overexpression of ALP ($p<0.05$), BMP2 ($p<0.05$) and inflammatory cytokines (such as IL6, $p<0.05$) induced by LPS. The same treatments were effective in reducing the deposition of calcium in collagen scaffolds ($p<0.05$).
Conclusions. The receptor FPR2 is overexpressed under pathological conditions and its selective activation is associated with reduced pro-calcific and inflammatory differentiation of VICs. These promising data offer new insights for a novel therapeutic strategy for CAVD.

NOVEL PATHOGENIC VARIANTS OF THE LDLR GENE IDENTIFIED IN PUTATIVE FH SUBJECTS

E. Bonelli[1], C. Trenti[1], E.A. Negri[1], R. Gnoni[1], S. Bertolini[2], S. Calandra[3], T. Fasano[1]
[1]AUSL-IRCCS di Reggio Emilia; [2]University of Genova; [3]University of Modena and Reggio Emilia
E-mail: tommaso.fasano@ausl.re.it

Introduction. Familial hypercholesterolemia (FH) is a common inherited disorder of low-density lipoprotein (LDL) catabolism causing elevated LDL-cholesterol (LDL-C) and premature atherosclerotic cardiovascular disease. FH is typically caused by deleterious variants of LDLR, APOB or PCSK9 genes. Recent data showed a heterozygous FH prevalence of 1:300 in the general population. Despite effective treatments, FH remains underdiagnosed and undertreated.Aim of this study was the genetic characterization of suspected FH patients referred to the Lipid Clinic and the cascade screening of their relatives.
Methods. From 2014 to 2019 we collected 186 subjects with suspected FH (122 index cases and 64 relatives, aged ^{3}**18** years) who were clinically examined at the Lipid Clinic and tested by Next Generation Sequencing for genes associated with FH (LDLR, APOB, PCSK9, APOE, LDLRAP1, ABCG5, ABCG8, LIPA, CYP27A1, MYLIP).
Results. Overall, 107 subjects (54 index patients/53 relatives) resulted to be heterozygous carriers of pathogenic variants of LDLR (103, 96.3%), APOB (3, 2.8%), or PCSK9 (1, 0.9%) genes. Five (likely)pathogenic variants of LDLR were not reported previously. Three of these, respectively due to nucleotides deletion, deletion/insertion and duplication, caused frameshift with the occurrence of a premature termination codon (Gln33Profs*17, Cys243Trpfs*12, Val365Argfs*20). The other two were missense variants (Pro608His, Ala684Asp), involving highly conserved amino acids, which were found to be deleterious by "in silico" analysis (REVEL score 0.962 and 0.817, respectively).
Conclusion. Clinical and genetic identification of FH patients represents a challenging task in clinical practice. From a genetic point of view, a major challenge is the demonstration of pathogenicity of a newly identified variant. In the present study we report 5 novel LDLR variants. Three of them can be regarded as deleterious due to the formation of a truncated protein. Clinical phenotypes and "in silico" analysis suggested that the two novel missense mutations can also be considered pathogenic.

NON-INVASIVE INSTRUMENTAL EVALUATION OF COENZYME Q10 PHYTOSOME ON ENDOTHELIAL REACTIVITY IN HEALTHY NON-SMOKING YOUNG VOLUNTEERS: A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED CROSS-OVER CLINICAL TRIAL

F. Fogacci, A. Di Micoli, M. Veronesi, C. Borghi, A.F.G. Cicero
University of Bologna
E-mail: federicafogacci@gmail.com

Coenzyme Q10 (CoQ10) is a natural antioxidant compound that prevents the vascular damage induced by free radicals and the activation of inflammatory signaling pathways. Supplementation with CoQ10 is safe though its bioavailability is generally low, as far as variable depending on the pharmaceutical form of preparation. Recently, the development of phytosome technology has improved the bioavailability of CoQ10 and definitely facilitated its effective use in clinical. The present double-blind, randomized, placebo-controlled, cross-over clinical study aimed to investigate the effect on endothelial reactivity and total antioxidant capacity (TAC) of either acute and chronic supplementation with CoQ10 phytosome in a sample of 20 healthy young not smoking subjects. The immediate acute effect of dietary supplementation with CoQ10 phytosome on pulse volume (PV) was sustained in the actively treated group in comparison with placebo and the baseline ($p<0.05$). Chronic supplementation of the tested pharmaceutical formulation of CoQ10 significantly improved mean arterial pressure and TAC compared to placebo and baseline values ($p<0.05$ for both comparisons). In the actively treated group, the effect on dietary supplementation with CoQ10 phytosome on PV was also sustained when compared to the baseline ($P<0.05$).

RUPATADINE TREATMENT IS ASSOCIATED TO ATHEROSCLEROSIS WORSENING AND ALTERED T LYMPHOCYTES RECRUITMENT IN APO-E DEFICIENT MICE

E. Franchi[1], M. Busnelli[1], S. Manzini[1], A. Colombo[1], F. Bonacina[1], G.D. Norata[1], S. Castiglioni[1], C. Andronis[2], E. Lekka[2], E. Scanziani[3], G. Chiesa[1]
[1]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano; [2]Biovista, Athens, Greece; [3]Mouse and Animal Pathology Laboratory (MAPLab), Fondazione UniMi, Milano
E-mail: elsa.franchi@unimi.it

Background and Aims. Rupatadine is a second-generation antihistamine and a PAF antagonist, currently employed for the treatment of allergies. It displays anti-inflammatory properties through the inhibition of macrophages and granulocytes recruitment. The anti-inflammatory and antiplatelet effects showed by rupatadine could be exploited against atherosclerosis development.
Methods. Apolipoprotein E-deficient female mice (n=15 per group) were fed Western-type diet, with (Rupatadine) or without (Control) 0,017% w/w rupatadine for 12 weeks.
Results. Weight gain, food and water intake and organ weights were similar in both groups. Also, plasma cholesterol and triglyceride levels were comparable. Atherosclerotic plaque extent in the aorta was comparable between groups. Unexpectedly, rupatadine treatment worsened plaque development in the aortic sinus, without altering necrotic core area, extracellular matrix and neutral lipids deposits and the presence of macrophages. The treatment increased the levels of T lymphocytes intraplaque (+70%) and around the aortic sinus (+80%). Rupatadine effects on T cells were also evaluated with in vitro tests, which showed that rupatadine did not affect cell proliferation, but promoted the polarization of CD4+ towards Th1 and Th2 subsets. No difference in inflammatory infiltrates was detected in liver, lung, kidney, lymph node and spleen.
Conclusion. In conclusion, rupatadine treatment in EKO mice fed Western diet resulted in a moderate worsening of atherosclerosis development and an altered T lymphocyte activation.

IMPACT OF THE COVID-19 PANDEMIC ON THE MANAGEMENT OF PATIENTS WITH CHRONIC CARDIOVASCULAR THERAPIES IN LOMBARDY

F. Galimberti[1], E. Olmastroni[2], A.L. Catapano[3], E. Tragni[2], M. Casula[3]
[1]IRCCS MultiMedica, Via Milanese 300, 20099, Sesto S. Giovanni (MI); [2]Servizio di Epidemiologia e Farmacologia Preventiva (SEFAP), Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Università degli Studi di Milano; [3]IRCCS MultiMedica, Sesto S. Giovanni (MI), Servizio di Epidemiologia e Farmacologia Preventiva (SEFAP), Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Università degli Studi di Milano
E-mail: federica.galimberti@multimedica.it

The COVID-19 pandemic has posed major challenges to healthcare systems and public policies. We aimed to investigate its impact on the management of chronic cardiovascular therapies (lipid-lowering, antihypertensive, antidiabetic, and anticoagulant drugs) using administrative databases of Lombardy Region. The study period between January and June 2020 was compared with the control period January-June 2019. For all adult patients (≥40 years) with at least one prescription of the selected drugs, the percentage change in drug consumption, adherence to therapy (calculated as proportion of subjects with PDC=100%), and access to healthcare services (blood tests, diagnostic investigations, or specialist visits for disease monitoring) was evaluated. A total of 911,566 patients on lipid-lowering therapy (mean age: 70.9 years; males: 52.2%), 2,147,386 on antihypertensives (mean age: 70.1 years; males: 47.7%), 392,678 on antidiabetics (mean age: 70.7 years; males: 56.4%), and 621,976 on anticoagulants (mean age: 78.8 years; males: 51.1%) were enrolled and compared with 879,881, 2,128,334, 381,752, and 601,204 controls, respectively. Overall, there was a small change in the number of dispensed packages (lipid-lowering drugs: +3.8%; antihypertensives: -1.8%; antidiabetics: -5.9%; anticoagulants: -5.2%); however, in all the cohorts, a slight increase was observed in the first two bimesters, with a sharp decrease in May-June (lipid-lowering drugs: -6.7%; antihypertensives: -11.4%; antidiabetics: -21.3%; anticoagulants: -22.6%). Likewise, adherence to treatments showed an increase in March-April, and a reduction during the following two months. Conversely, there was a dramatic drop in healthcare services utilization in each patient cohort (lipid-lowering drugs: -23.2%; antihypertensives: -29.6%; antidiabetics: -25.6%; anticoagulants: -20.4%), with a negative spike in March/April (lipid-lowering drugs: -65.2%; antihypertensives: -66.0%; antidiabetics: -63.5%; anticoagulants: -53.9%). The COVID-19 pandemic has negatively affected the access to healthcare services by patients with chronic cardiovascular diseases. We observed a tendency to accumulate medicines at the beginning of the lock-down, and a decreased use of health services for disease monitoring compared to the control period.

MANAGEMENT OF A PATIENT WITH DELAYED DIAGNOSIS OF HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

A. Giammanco[1], R. Scicali[2], C. Scrimali[1], A. Di Pino[2], F. Brucato[1], R. Spina[1], S. Piro[2], D. Noto[1], A.B. Cefalù[1], F. Purrello[2], M. Averna[1]
[1]Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza "G. D'Alessandro" (PROMISE), Università degli Studi di Palermo; [2]Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Catania
E-mail: agiamman@gmail.com

Background. Homozygous familial hypercholesterolaemia (HoFH) is a rare, life-threatening genetic disorder characterized by extremely high low-density lipoprotein-cholesterol (LDL-C) levels and severe and accelerated atherosclerotic cardiovascular disease (ASCVD). We describe the clinical management and molecular characterization of a subject with severe hypercholesterolemia.
Methods The proband is a 50-year-old man with family history of early cardiovascular disease and hypercholesterolemia (both parents and his two children). Severe hypercholesterolemia (LDL-C: 450 mg/dL) was first documented when he was 32 and treatment with statin was begun. When he was 47, over a routine cardiologic workup, a coronary angiography revealed diffuse stenotic coronary disease and he underwent to BACG procedure. The LDL-C levels were 230 mg/dl while treated with high-intensity statin at the maximum tolerated dose in combination with ezetimibe. Genetic analysis of FH candidate genes was carried out by NGS. Additional hypolipidemic therapeutic options were considered for the management of this patients.
Results. The genetic analysis revealed that the patient was compound heterozygous of two already known pathogenic mutations of the LDLR gene classified as receptor-defective (c.1118G>A - p.Gly373Asp - and c.1195G>A - p.Ala399Thr). Cascade screening was performed and four family members were found to be heterozygous carriers. Before the genetic data were available, the patient was treated with Alirocumab 150 mg every other week on top of standard care. Once the genetic diagnosis of HoFH was made, alirocumab was switched to Evolocumab 420 mg once a month. Although an effective reduction of LDL-C levels, the lipid goal was not reached and therefore we decided to potentiate hypolipidemic treatment by adding a low dose of Lomitapide (5 mg/daily) which was well tolerated and very effective (LDL-C: 42 mg/dL).
Conclusions. In this HoFH case, the combination of Evolocumab with low-dose Lomitapide an effective and well-tolerated add-on therapeutic option in HoFH carriers of defective mutations of the LDL-R gene.

EXTRACELLULAR VESICLES ENRICHED IN PCSK9 ARE INDICATIVE OF PRO-ATHEROGENIC PHENOTYPE

M.F. Greco[1], C. Macchi[1], L. Cantone[1], M. Hoxha[1], C. Banfi[2], F. Zimetti[3], M.P. Adorni[3], I. Giusti[4], V. Dolo[4], A. Corsini[1], C.R. Sirtori[1], N. Ferri[5], V. Bollati[1], M. Ruscica[1]
[1]Università degli Studi di Milano; [2]Centro Cardiologico Monzino; [3]Università degli Studi di Parma; [4]Università degli studi dell'Aquila; [5]Università degli Studi di Padova
E-mail: mariafrancesca.greco@unimi.it

Background. Extracellular vesicles (EVs) are a heterogeneous population of particles wrapped by a phospholipid bilayer, secreted into the extracellular space by several cell types, carrying proteins, lipids and nucleic acids. EVs are classified into exosomes, microvesicles and apoptotic bodies. EVs deliver their biologically active molecular cargo to the recipient cells. EVs play a significant role in the process of atherosclerotic cardiovascular diseases (ASCVD) through different mechanisms: by promoting endothelial dysfunction and vascular wall inflammation; by increasing expression of surface adhesion molecules; by favouring smooth muscle cell differentiation and by determining the release of pro-inflammatory cytokines. In the context of the pathophysiology of ASCVD, proprotein convertase subtilisin/kexin type 9 (PCSK9) appears to play a crucial role. PCSK9 is not only a key-player in the regulation of low-density lipoprotein-cholesterol (LDL-C) but it also plays pleiotropic effects on atherosclerosis. PCSK9 is expressed in cultured human endothelial cells, vascular smooth muscles cells (VSMC) and in human atherosclerotic plaques. Furthermore, PCSK9 positively influences VSMC differentiation, migration and proliferation.
Aim. To unveil the impact of EVs derived from human smooth muscle cells overexpressing PCSK9 (hSMC-PCSK9) on the inflammatory milieu, migratory capacity, oxidized LDL (oxLDL) uptake and mitochondrial respiration of human monocytes THP-1 and human derived-THP-1 macrophages.
Methods. EVs are isolated from cell culture media of hSMC overexpressing or not PCSK9 (EVs-PCSK9 and EVs-CTR) by ultracentrifugation and are characterized by high-resolution flow cytometry and Western blot (WB) analyses. Number and size of EVs have been measured by Nanoparticle Tracking Analysis (NTA), morphology and size by transmission electron microscopy (TEM). Proteomic analysis has been carried out by liquid chromatography mass spectrometry. Mitochondrial respiration has been assessed by Seahorse analysis.
Results. EVs-CTR and EVs-PCSK9 express CD9 and CD63 tetraspanins as well as Alix and Beta1-Integrin, all markers of exosomes and microvesicles. No differences in concentration were found between EVs-CTR and EVs-PCSK9, 1.2*1010 particles/ml and 1.3*1010 particles/ml, respectively. Similar conclusions were reached in the case of size, namely, 152.3 nm (EVs-CTR) and 160.7 nm (EVs-PCSK9). This evidence was further confirmed by TEM, namely, EVs-CTR and EVs-PCSK9 have the same morphology and size (approximately 100 nm in diameter). Untargeted proteomic analysis has shown that EVs-PCSK9 carry a higher amount of PCSK9. 24-h exposure to EVs-PCSK9 raised gene expression of MCP-1/chemokine (C-C motif) ligand 2 (+27 fold), interleukin (IL)-1α (+28 fold), IL-1beta (+25 fold), IL-6 (+94 fold), and IL-8 (+4 fold) in both monocytes and macrophages. EVs-PCSK9 increased the phosphorylation of STAT3 and decreased that of SOCS3 in both cell lines, whereas no differences were found for NLRP3. EVs-PCSK9 increased oxLDL uptake, compared to their counterpart (EVs-CTR) in derived-THP-1 macrophages and decreased basal and maximal respiration achieved by THP-1. The migratory

capacity of THP-1 monocytes and macrophages was raised upon incubation with EVs-PCSK9.
Conclusions. EVs enriched in PCSK9 appear to be characterized by a pro-atherogenic phenotype, with a raised inflammatory milieu, cell migratory capacity, oxLDL uptake and impairment in mitochondrial respiration.

POST PRANDIAL METABOLISM OF LIPOPROTEINS IN FAMILIAL CHYLOMICRONEMIA PATIENTS TREATED WITH LOMITAPIDE AND TIPARVOVEC

M. Gueli Alletti[1], P. Bigolin[2], F. Brucato[1], C. Scrimali[1], R. Spina[1], V. Ingrassia[1], G.I. Altieri[1], F. Fayer[1], D. Noto[1], G. Misiano[1], A. Giammanco[1], C.M. Barbagallo[1], A. Ganci[1], A.B. Cefalù[1], A. Zambon[2], M.R. Averna[1]
[1]Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza "G. D'Alessandro" (ProMISE), Università degli Studi di Palermo; [2]Dipartimento di Medicina - DIMED Università degli Studi di Padova
E-mail: mariagrazia.guelialletti@gmail.com

Background. Familial chylomicronemia syndrome (FCS) is a rare recessive monogenic disease characterized by triglycerides (TG) levels >10 mmol/L. Although FCS is causally associated with mutations in candidate genes (LPL, LMF1, GPIHBP1, ApoAV and ApoCII), most patients with FCS have mutations in lipoprotein lipase (LPL). Defects in LPL enzyme result in reduced clearance of chylomicrons (CM) from plasma and development of acute pancreatitis. Treatment of FSC patients is based on combined action of a lipid- and carbohydrate-reduced diet in addition to available hypolipidemic therapies (fibrates, statins, omega-3 fatty acids) that often fails to achieve a desired TG levels. Recently several innovative drugs have been developed: tiparvovec (gene therapy), lomitapide (MTP inhibitor), volanesorsen (antisense oligonucleotide that inhibits the formation of apoC-III) and monoclonal antibodies (anti ApoCIII and ANGPTL3).
Material and Methods. Five patients carrying familial chylomicronemia causative mutations of the major candidate genes were collected. Each patient was given a modified oral fat load to avoid a risk of pancreatitis induced by postprandial hyperchylomicronemia but sufficient to assess the change in postprandial chylomicron levels. The meal was supplemented with retinol palmitate (RP) as CM biomarker. We compared TG and RP levels after administration of an oral fat load before and after lomitapide or tiparvovec. The trend in postprandial TG levels was evaluated by taking hourly samples for nine hours and a single sample at 24 hours later.
Risultati e Conclusioni. Here we present preliminary data of four patients treated with lomitapide for twenty-six weeks and the only patient that received tiparvovec in Italy.Area Under Curve of patients on lomitapide therapy were reduced roughly by 87% for TG, 27% for non-HDL-C, while no improvement was observerd for tiparvovec. Lomitapide was effective in improving post prandial metabolism of lipoproteins in subjects with FCS. No benefits were observed for tiparvovec.

RISK STUDY: ROLE OF BIOLOGICAL MARKERS AND TRANSCRIPTOMICS PROFILE FROM CEREBRAL THROMBI AND PERIPHERAL VENOUS BLOOD IN PATIENTS WITH ACUTE ISCHEMIC STROKE

A. Kura[1], G. Cassioli[1], A. Sodero[2], E. Sticchi[1], A. Magi[1], S. Suraci[1], R. De Cario[1], A. Consoli[3], A. Rosi[3], S. Nappini[3], L. Renieri[3], N. Limbucci[3], B. Piccardi[2], F. Arba[2], C. Sarti[2], D. Inzitari[2], S. Mangiafico[3], R. Marcucci[1], A.M. Gori[1], B. Giusti[1]

[1]Univ., Dept. Exp. Clin. Med, Florence; [2]AOUC Careggi, Stroke Unit, Florence; [3]AOUC Careggi, Interventional Neuroradiol, Florence

E-mail: ada-kura@hotmail.it

Background. Acute ischemic stroke (AIS) remains a major cause of death and disability worldwide. AIS therapies consist of cerebral blood restoration by i.v. rt-PA thrombolysis and/or mechanical thrombectomy (MT). Few previous studies showed that thrombus composition can correlate with both AIS pathophysiology and outcomes. Moreover, it has been observed the important role of MMP and other inflammatory biomarkers for prediction of unfavorable outcomes in patients undergoing thrombolysis.

Aims of the study were:

1) to investigate the global gene expression profile of cerebral thrombi (CT) and venous peripheral blood (PB) in order to identify markers of different pathophysiological mechanisms of AIS and/or determinants of clinical outcomes (haemorrhagic transformation, 24 h edema, modified 3 months Rankin scale-mRS, death);
2) 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. Blood samples were collected before and 24 hours after MT in tubes containing liquid for RNA stabilization. A blood withdrawal for assessment of MMPs, cyto/chemokines and myeloperoxidase (MPO) was performed. We evaluated a gene expression profile of 52 AIS patients. The thrombus obtained during MT was collected in RNA later. 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. Circulating biomarkers were determined in serum by using a multiplex assay (Bioplex Instruments). For statistical analysis we used SPSS v.25 software

Results. As concerns transcriptomics data, the average of analyzable probe sets numbered 440,085 in CT and 602,874 in PB samples. In the two different type of specimens 20,341 symbols were common, whereas 3 symbols were unique in CT and 562 symbols were unique in PB. The Gene Ontology (GO) enrichment analysis allowed to identify common and peculiar features and biological processes; really 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. Concerning PB, significant enriched terms associated with regulation and activation of transcriptomes of cells are particularly significant. Biomarkers evaluation showed 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. In conclusion, our results obtained from both transcriptomics profile and biomarkers evaluation in ischaemic stroke patients treated with thrombolysis provided interesting insights into the mechanisms underlying the ischaemic stroke and the response to thrombolysis.

AN INNOVATIVE BERBERINE FORMULATION IS ABLE TO REDUCE PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 PLASMA LEVELS IN MICE

M.G. Lupo[1], G. Panighel[1], I. Ferrarese[1], E. Brilli[2], G. Tarantino[2], S. Dall'Acqua[1], N. Ferri[3]
[1]Department of Pharmaceutical and Pharmacological Sciences, University of Padua; [2]PharmaNutra S.p.A., Siena; [3]Department of Medicine, University of Padua
E-mail: mariagiovanna.lupo@unipd.it

Background: Increased proprotein subtilisin/kexin type 9 (PCSK9) plasma levels may hide a subclinical cardiovascular risk (CVR) due to increase low-density lipoprotein cholesterol (LDLc) level. Berberine (BBR) is a natural hypocholesterolemic alkaloid able to reduce circulating PCSK9 by hampering its transcription *via* the inhibition of hepatic nuclear factor 1α (HNF1α) transcription factor. Moreover, BBR prolongs LDL receptor (LDLR) mRNA half-life. Despite its proven efficacy as hypocholesterolemic agent, BBR has a poor bioavailability. The aim of this study was to evaluate the efficacy of a new BBR formulation (BBR-U) both *in vitro* and *in vivo*.
Methods: HuH7 human hepatocarcinoma cells were incubated for 24h with increased concentration (10 – 20 – 40μg/mL) of two different BBR formulations: not formulated BBR (BBR) and BBR-U. Simvastatin 40μM and BBR chloride 40μM were use as positive controls. RT-qPCR, western blotting, and LDL-uptake assays were performed to evaluate the efficacy of the new BBR formulation. Twenty male wild-type C57BL/6 mice (age 6 weeks at starting time) were randomly divided in 4 groups (5 mice each) in order to receive standard diet (SD) or high fat diet (HFD) for 16 weeks and oral BBRs gavage (or vehicle) for the last 8 weeks, according to this scheme: group 1 (G1), SD + vehicle; G2, HFD + vehicle; G3, HFD + BBR; G4, HFD + BBR-U. The daily oral gavage was provided from Monday to Friday. BBR and BBR-U dosage was initially 50mg/kg/die and 6.25mg/kg/die respectively, according to the different Cmax observed. Glucose tolerance test (GTT) was performed the day before sacrifice. At sacrifice, liver, kidney, brain, and blood were collected. Pcsk9 serum levels was measured thanks to commercially available ELISA kit. BBR distribution among tissues were analyzed via mass spectrometry (MS)
Results: *In vitro*, BBR-U produced a significant decrease in PCSK9 mRNA, intracellular and extracellular protein levels at each tested concentration ($p < 0.001$ *vs* control), and a significant dose-dependent increase in LDLR mRNA levels and in LDL uptake by the cells. *In vivo*, BBR-U 6.25mg/kg/die proved very effective in reducing Pcsk9 serum levels in HFD mice (-50% *vs* SD mice), compared to BBR 50mg/kg/die. In addition, BBR-U 6.25mg/kg/die proved as effective as BBR 50mg/kg/die in ameliorating GTT profile compared to vehicle-gavaged-HFD mice. MS analysis showed a different distribution of BBR and its metabolites among tissues in BBR-U derived murine tissues compared to BBR ones, with the brain being the most affected.
Conclusion: The innovative formulation of BBR (BBR-U) proved highly effective in reducing PCSK9 expression and in boosting LDL uptake in HuH7 cell line. Moreover, a very low-dosage of BBR-U oral gavage on mice under HFD regimen resulted as effective as BBR at standard dosage in reducing PCSK9 serum level and in ameliorating GTT profile, compared to HFD mice, thus proving the innovative formulation being a very succesful tool to improve the low BBR bioavailability. In addition, the different distribution of BBR and its metabolites in tissues from BBR-U group (es. brain), may pave the way to effectively repurpose BBR to treat other diseases, such as Alzheimer's, in whose onset and progression cholesterol seems to play a role, and in which BBR has been already proved to be effective.

DEPRESSION AND CARDIOVASCULAR RISK IN OBESITY: IMPACT OF PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 AND BRAIN-DERIVED NEUROTROPHIC FACTOR ON ADIPOCYTES

C. Macchi[1], P. Amadio[2], M.F. Greco[1], M. Buoli[3], L. Vigna[4], A. Ieraci[5], A. Corsini[1], S. Barbieri[2], C.R. Sirtori[1], V. Bollati[6], M. Ruscica[1]
[1]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano; [2]Unit of Brain-Heart Axis: Cellular and Molecular Mechanisms, Centro Cardiologico Monzino IRCCS, Milan; [3]Department of Pathophysiology and Transplantation, Università degli Studi di Milano; [4]Occupational Medicine Unit, Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, Milan; [5]Laboratory of Neuropsychopharmacology and Functional Neurogenomics, Department of Pharmaceutical Sciences, Università degli Studi di Milano; [6]EPIGET Lab, Department of Clinical Sciences and Community Health, Università degli Studi di Milano
E-mail: chiara.macchi@unimi.it

Introduction. Obesity, raising Worldwide, increases the susceptibility to cardiovascular diseases (CVDs) and mood disorders. Among these, depression enhances the CVD risk and it is approximately twice as prevalent in women. Obesity, depression and CVDs often come hand in hand, although a mechanistic link among these three conditions remains not well defined. Aim. To unravel molecular pathways beneath these liaisons.
Methods. In 642 obese individuals, of the cross-sectional SPHERE (Susceptibility to Particle Health Effects, miRNAs and Exosomes) study, we evaluated possible mediators of the link between depression and obesity (proprotein convertase subtilisin/kexin type 9 (PCSK9) and Brain-Derived Neurotrophic Factor (BDNF)). We have deepened the molecular mechanisms contributing to this association by taking advantage of an in vitro model of human adipocytes (SW872 cells).
Results. In the SPHERE cohort, PCSK9, a key-regulator of cholesterol, mediated 11% of the relationship between depression and insulin resistance, a CVD risk factor. This association was lost in carriers of the loss-of-function PCSK9 R46L variant, confirming a possible causal role for PCSK9 in the link between depression and insulin resistance. Since SPHERE cohort comprises obese individuals, the effects of PCSK9 on SW872 cells were investigated. The silencing of PCSK9 raised the adipocyte differentiation process. Since BDNF Val66Met human polymorphism is involved in the onset of depression, in CVD risk and in adipose tissue pathophysiology, we measured circulating BDNF levels in the SPHERE cohort. Circulating BDNF was negatively associated with depression (BDI-II score), while positively associated with insulin and HOMA-IR, an index of insulin resistance, in females. Treatment of SW872 cells with ProBDNFMet synthetic peptide impaired adipogenesis and the insulin signaling pathway.
Conclusions. PCSK9 and BDNF may share biological mechanisms underlying the association between depression and insulin resistance. This suggests how they may be intertwined in modulating CV risk factors in the presence of an obesity-driven depressive-like phenotype.

RESTRING: MANAGING FUNCTIONAL ENRICHMENT OF COMPLEX EXPERIMENTAL DESIGNS MADE EASY

S. Manzini[1], M. Busnelli[1], A. Colombo[1], E. Franchi[1], P. Grossano[2]
[1]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano; [2]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano
E-mail: stefano.manzini@unimi.it

Functional enrichment analysis is an analytical method to extract biological insights from gene expression data, popularized by the ever-growing application of high-throughput techniques. Typically, expression profiles are generated for hundreds to thousands of genes/proteins from samples belonging to two experimental groups, and after ad-hoc statistical tests, researchers are left with lists of statistically significant entities, possibly lacking any unifying biological theme. Functional enrichment tackles the problem of putting overall gene expression changes into a broader biological context, based on pre-existing knowledge bases of reference: database collections of known expression regulation, relationships and molecular interactions. STRING is among the most popular tools, providing both protein-protein interaction networks and functional enrichment analysis for any given set of identifiers. For complex experimental designs, manually retrieving, interpreting, analyzing and abridging functional enrichment results is a daunting task, usually performed by hand by the average wet-biology researcher. We have developed restring (https://github.com/Stemanz/restring), a cross-platform, open-source software that seamlessly retrieves from STRING functional enrichments from multiple user-supplied gene sets, without any need for specific bioinformatics skills. As a core capability, it aggregates all such findings into human-readable table summaries, with built-in features to easily produce user-customizable publication-grade clustermaps and bubble plots. Everything is managed through reString's straightforward graphical user interface in just a few clicks and seconds of processing times. The software is backed with a comprehensive online documentation, YouTube installation tutorials, sample input files, online support, an upcoming publication and more.

CEREBROSPINAL FLUID AND SERUM HDL CHOLESTEROL EFFLUX CAPACITY ARE IMPAIRED IN NEURODEGENERATIVE DISORDERS

C. Marchi[1], M.P. Adorni[2], E. Conti[3], L. Tremolizzo[4], F. Bernini[1], L. Calabresi[5], F. Zimetti[6]
[1]Department of Food and Drug, University of Parma;
[2]Department of Medicine and Surgery, University of Parma;
[3]Lab of Neurobiology, School of Medicine and Surgery and Milan Center of Neuroscience, University of Milano-Bicocca, Monza;
[4]Lab of Neurobiology, School of Medicine and Surgery and Milan Center of Neuroscience, University of Milano-Bicocca, Monza, Neurology Unit, San Gerardo Hospital, ASST Monza;
[5]Centro E. Grossi Paoletti, Department of Pharmacological and Biomolecular Sciences, University of Milano;
[6]Department of Food and Drug, University of Parma
E-mail: cinzia.marchi2@studenti.unipr.it

Background. Alterations of cholesterol homeostasis in the central nervous system (CNS) have been associated to various neurodegenerative disorders, including Alzheimer's disease (AD). In the brain, cholesterol transport is mediated by lipoproteins similar to plasma HDL that have been identified in the cerebrospinal fluid (CSF). These particles, as occurs in plasma, interact with membrane transporters such as ABCA1 and ABCG1 both expressed on astrocytes, and promote the efflux of cholesterol towards the neurons. The overall objective of the present study is to characterize CSF and serum of AD patients, by evaluating their HDL cholesterol efflux capacity (CEC), in comparison with non-AD dementia patients and controls.
Material and Methods. CSF and serum from AD (n=36), non-AD dementia (n=13) and from control subjects (n=14) have been collected by lumbar puncture and blood withdrawal, respectively. CEC was evaluated by a radioisotopic technique, using specific cellular models. In particular, human astrocytoma cells U373 were used as models of astrocytes to evaluate CSF-CEC. For serum evaluation we used ABCG1 overexpressing CHO and ABCA1 overexpressing J774 macrophages.
Results. CSF-CEC measured in astrocytes was reduced both in AD and non-AD dementia patients compared to controls (-40%, p=0.0193; -38%, p=0.0308, respectively). By analyzing serum from the same patients, we found that the total efflux from macrophages did not differ among the three groups. However, by separately evaluating the single cholesterol efflux pathways, we observed that ABCA1-CEC in AD and non-AD dementia patients was reduced compared to controls (-19.5%, p=0.0151 and -28.4%, p=0.0015, respectively). In addition, serum ABCG1-CEC was lower in AD patients compared to controls (-19.5%, p=0.0153). By considering AD and non-AD patients as a whole group, we found a direct correlation between serum ABCG1-CEC and the MMSE score evaluated at sixth month of follow-up (r=0,47; p=0.03).
Conclusion. These preliminary results suggest that neurodegenerative disorders may be associated to alterations of brain cholesterol transport. These alterations are also detectable in serum correlating with the degree of cognitive decline. Further evaluations will be necessary to establish whether CSF or serum CEC may represent valid biomarkers of disease or a novel pharmacological target.

VALUTAZIONE DELLA DISPERSIONE DEGLI INDICI DI RIPOLARIZZAZIONE NEI PAZIENTI AFFETTI DA COVID-19

M. Marino, F. Spadaro, D. Sinicropi, M. Sparacino, M. Cinquegrani, E. Rullo Venenzi, C. Morace, G. Nunnari, G. Squadrito, V. Viapiana, G. Mandraffino
Internal Medicine Unit Department of Clinical and Experimental Medicine University of Messina
E-mail: marinom04@gmail.com

L'OMS ha dichiarato l'infezione da SARS-CoV-2 come emergenza sanitaria di interesse internazionale nel gennaio 2020. L'infezione da SARS-CoV-2 si presenta con diverse caratteristiche che potrebbero essere associate alla genesi dell'instabilità elettrica e all'allungamento dell'intervallo QT (1). La malattia può agire in chiave pro-aritmica non solo per l'effetto patogeno diretto o indiretto associato alla risposta infiammatoria (2), ma anche per la terapia farmacologica di fondo utilizzata o per il suo trattamento (3). Abbiamo valutato retrospettivamente i dati clinici ed elettrocardiografici di 75 pazienti affetti da COVID-19 ricoverati presso l'Unità di Malattie Infettive dell'Azienda Ospedaliera Universitaria G. Martino di Messina nel periodo compreso tra marzo e maggio 2020.Sono stati considerati l'età e l'anamnesi farmacologica (OLT, corticosteroidi, azitromicina, idrossiclorochina, EBPM, fondaparinux, anticoagulanti orali, altri farmaci potenzialmente in grado di allungare l'intervallo QT) (4). Il rischio di aritmie ventricolari e morte cardiaca improvvisa è stato valutato calcolando gli intervalli QT e QTc, (5) ed indici da questi derivati quali la dispersione e la DS di QT e QTc (6), sia al momento del ricovero che durante la degenza ospedaliera, secondo il metodo raccomandato da Priori et al. (7). Il 44% dei pazienti (33 su 75) inclusi nello studio assumeva - come terapia consolidata - farmaci riconosciuti come potenzialmente in grado di allungare l'intervallo QT (antipsicotici, antiepilettici, antidepressivi), spesso in combinazione; inoltre, al 62,67% delle persone incluse (47 su 75) è stato prescritto il protocollo azitromicina/idrossiclorochina come raccomandato nel periodo di riferimento. Non abbiamo trovato alcuna associazione significativa tra i parametri elettrocardiografici e i farmaci prescritti. Abbiamo testato ogni parametro elettrocardiografico come potenziale predittore di mortalità intraospedaliera in pazienti con COVID-19. Non sono stati stimati modelli significativi per i parametri QT e QTc e le misure derivate, ad eccezione della dispersione relativa di QTc (beta 0,651, p=0,003), identificata pertanto come potenziale predittore di mortalità.I dati ottenuti supportano il suggerimento di valutare gli indici di dispersione della ripolarizzazione ventricolare per stimare il rischio proaritmico nei pazienti COVID-19. Potrebbe essere utile un follow-up elettrocardiografico più stretto durante la degenza ospedaliera per poter riconoscere precocemente i pazienti a maggior rischio di morte cardiaca.

Bibliografia

1. Yenerçağ M, Arslan U, Doğduş M, Günal Ö, Öztürk ÇE, Aksan G, Erdoğan G, Gül S, Yontar OC, Şen A. Evaluation of electrocardiographic ventricular repolarization variables in patients with newly diagnosed COVID-19. J Electrocardiol. 2020; 62: 5-9.
2. Lazzerini PE, Laghi-Pasini F, Acampa M, Boutjdir M, Leopoldo Capecchi P. IL-6 (Interleukin 6) Blockade and Heart Rate Corrected QT Interval Prolongation in COVID-19. Circ Arrhythm Electrophysiol. 2020; 13(9).
3. Bazoukis G, Yeung C, Wui Hang Ho R, Varrias D, Papadatos S, Lee S, Ho Christien Li K, Sakellaropoulou A, Saplaouras A, Kitsoulis P, Vlachos K, Lampropoulos K, Thomopoulos C, Letsas

KP, Liu T, Tse G. Association of QT dispersion with mortality and arrhythmic events-A meta-analysis of observational studies. J Arrhythm. 2019; 36(1): 105-115.
4. Zhu S, Wang J, Wang Y, Chu J, Liu Y, Chen X, Chen X. QTc prolongation during antiviral therapy in two COVID-19 patients. J Clin Pharm Ther. 2020; 45(5): 1190-1193.
5. Kosmopoulos M, Roukoz H, Sebastian P, Kalra R, Goslar T, Bartos JA, Yannopoulos D, Benditt DG. Increased QT Dispersion Is Linked to Worse Outcomes in Patients Hospitalized for Out-of-Hospital Cardiac Arrest. J Am Heart Assoc. 2020; 9(16): e016485.
6. Kautzner J, Yi G, Camm AJ, Malik M. Short- and long-term reproducibility of QT, QTc, and QT dispersion measurement in healthy subjects. Pacing Clin Electrophysiol. 1994; 17(5 Pt 1) 928-37.
7. Priori SG, Napolitano C, Diehl L, Schwartz PJ. Dispersion of the QT interval. A marker of therapeutic efficacy in the idiopathic long QT syndrome. Circulation. 1994; 89(4): 1681-9.

COMPARING DIETARY CALORIC INTAKE AND DIETARY INFLAMMATORY POTENTIAL

E. Mattavelli[1], R. Domenighini[2], L. Redaelli[2], C. Tidone[2], A. Pirillo[3], L. Grigore[2], F. Pellegatta[2], P. Magni[2], A.L. Catapano[4], A. Baragetti[1]
[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università di Milano; [2]SISA Centro per lo Studio dell'Aterosclerosi, Ospedale Bassini, Cinisello Balsamo, Milano; [3]SISA Centro per lo Studio dell'Aterosclerosi, Ospedale Bassini, Cinisello Balsamo, Milano, IRCCS Ospedale Multimedica Milano; [4]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università di Milano, IRCCS Ospedale Multimedica Milano
E-mail: elisa.mattavelli@unimi.it

Introduction. The increasing access to a variety of nutrient-dense food is believed to impact on the risk CVDs worldwide, despite it is still unresolved whether this association is merely reflecting the caloric intake or it implies an inflammatory effect of the macro-nutrients and food component.To preliminary get into these aspects, we analyzed how the whole caloric dietary intake or the quantitative intake of specific macronutrients associate with a set of immune-inflammatory circulating markes that we previously associated with CVD occurrence.
Materials and Methods. Clinical, anthropometrical, biochemical information of 336 subjects (PLIC cohort) were collected. The analysis of seven day-dietary records provided two parameters that were independently used in the analysis: a) the whole energy intake (kcal) was derived from and b) the estimated pro-/anti-inflammatory score of diet Dietary Inflammatory Index (DII) (that sums the quantitative intake of each dietary component, multiplied for a correction factor indicative of the in vitro inflammatory effect of the component). In addition, to evaluate the estimated DII indepently from caloric intake, Energy Adjusted-Dietary Inflammatory Index (EA-DII) was also calculated. A panel of 368 inflammatory systemic markers were evaluated; Parameters were quantitated by mass spectrometry and their changes were normalized (Normalized Protein eXpression; NPX). Also complete hematocrit formula were crossed with dietary data.
Results and Conslusion. We observed that higher caloric intakes (> median 1.700 Kcal/day), that were more likely explained by the quantitative daily intake of fats, were associated with a significant amount of immune-inflammatory markers (up to 50). Similarly to the whole caloric intake, also higher DII values (indicating a more pro-inflammatory potential of diet) were explained by higher intakes of dietary fats, although they were associated with different immune-inflammatory markers and more significantly with leukocytes from hematocrit. These findings hold true also when considering (EA-DII). This preliminary study, one of the fews harnessing quantitative analysis of dietary intakes, calls for the formulation of future algorhitms, to be tested on larger populations, to asses the inflammatory relevance of diet.

HOMEMADE FOOD, ALCOHOL, AND BODY WEIGHT: CHANGE IN EATING HABITS IN YOUNG INDIVIDUALS AT THE TIME OF COVID-19 LOCKDOWN

S. Maurotti[1], Y. Ferro[1], E. Mazza[2], G. Boragina[3], A. Mirarchi[2], J. Khoory[4], A. Pujia[5], T. Montalcini[3]
[1]Department of Health Science, University Magna Græcia; [2]Department of Medical and Surgical Science, University Magna Græcia; [3]Department of Clinical and Experimental Medicine, University Magna Græcia; [4]Nutrition Unit, University Magna Grecia; [5]Department of Medical and Surgical Science, University Magna Graecia
E-mail: smaurotti@unicz.it

Background. The 2019 coronavirus disease (COVID-19) lockdown has caused significant changes in everyday life. This study evaluated the effect of the COVID-19 quarantine on dietary and alcohol consumption habits and body weight of Italian university students.
Materials and Methods. An online cross sectional survey was carried out among university students than 18 years in July 2020. The online self administered questionnaire included demographic and anthropometric data (reported weight and height), weight, and dietary habits changes during of the COVID-19 lockdown.
Results. A total of 520 respondents have been included in the study. A total of 393 (~76%) were female, 3.8% was obese, and the mean age was 23±4 years. Numerous students reported a change in their eating habits during the lockdown with an increase in consumption of chocolate (40%), ice cream, and desserts (34%), but most of all an increase of homemade bread and pasta (60%), pizza (47%), and homemade sweets (55%). The students also reported an increase of vegetables, fresh fruit, legumes, eggs, and coffee, but also of processed meat, fried foods, cheeses, butter, and sweet beverage, and a reduction in alcohol intake. The weight gain was observed in 43.8%, and males have greater weight gain than females (57% vs. 46%, respectively; P=0.04). A greater increase in body weight was observed in obese as compared to those with normal weight (77% vs. 44%, respectively; P<0.001).
Conclusions. Our data highlighted the need for dietary guidelines to prevent weight gain during the self-isolation period, particularly by targeting people who are overweight and obese, with increased atherosclerotic and cardiometabolic risk. Therefore, targeted interventions are needed to maintain optimal body weight for maximum benefits already in young people, to reduce these dietary errors that lead to early atherosclerosis.

ATHEROSCLEROSIS, NON-ALCOHOLIC FATTY LIVER DISEASE AND SARCOPENIA: A DANGEOURS TRIAD

S. Maurotti[1], Y. Ferro[1], E. Mazza[2], M. Prastaro[2], G. Boragina[3], A. Mirarchi[2], R. Mare[2], A. Pujia[2], T. Montalcini[3]
[1]Department of Health Science, University Magna Græcia; [2]Department of Medical and Surgical Science, University Magna Græcia; [3]Department of Clinical and Experimental Medicine, University Magna Græcia
E-mail: smaurotti@unicz.it

Background. While it is well recognized that cirrhotic patients are affected by sarcopenia, it is currently unexplored the association between non-alcoholic fatty liver disease and sarcopenia. Myostatin levels significantly increased in both conditions. It modulates fibrogenic phenotype of human liver cells and promotes atherosclerotic damage. Grip strength is a parameter of muscle function and is the primary measure for the diagnosis of sarcopenia, which also influences immune response and inflammation mechanisms. The relationship between muscle deterioration and hepatic steatosis are extensively investigated in the Asian population, but not in Caucasians. We thus assessed the association between muscle function, liver steatosis and atherosclerosis in an adult population.
Methods. We retrospectively evaluated 388 ambulatory patients of both genders. Dynamometer, transient elastography and atherogenic index of plasma were performed. Relative handgrip strength was assessed as the handgrip strength divided by the body mass index. The population was divided into tertiles.
Results. A total of 207 participants had non-alcoholic fatty liver disease. The prevalence of hepatic steatosis was higher among the participants with the lowest relative handgrip strength (tertile I) than those with a higher (tertile III) (64% vs 46%, p=0.02). Compared to the tertile III (reference), the tertile I and tertile II had significantly higher ORs of having non-alcoholic fatty liver disease (OR=5.30, 95% CI=2.24-12.57, p<0.001; and OR=2.56, 95% CI=1.17-5.59, p=0.01; respectively). Furthermore, atherogenic index of plasma was significantly higher only in the adult men of tertile I than those in the tertile III (4.54±0.6 vs 0.33±0.06, p=0.05; respectively).
Conclusion. We observed a greater risk of non-alcoholic fatty liver disease in Caucasian individuals with lower handgrip strength. Several metabolic alteration are common in liver disease, atherosclerosis and sarcopenia. The muscle function assessment might be useful in identify individuals at risk for liver steatosis and atherosclerosis especially in men.

CIRCULATING LIPOOLYSACCHARIDES AND IMPAIRED ANTIOXIDANT STATUS IN PATIENTS WITH ATRIAL FIBRILLATION. DATA FROM THE ATHERO-AF STUDY

D. Menichelli, R. Carnevale, C. Nocella, V. Cammisotto, V. Castellani, S. Bartimoccia, G. Frati, F. Violi, P. Pignatelli, D. Pastori
Sapienza University of Rome
E-mail: danilo.menichelli@uniroma1.it

Background. Atrial fibrillation (AF) is characterized by an oxidative imbalance, which is associated with an increased risk of cardiovascular events (CVEs). It is unclear whether low grade endotoxemia may contribute to the impaired antioxidant status in AF patients. We investigated the relationship between circulating lipopolysaccharides (LPS) and antioxidant status in AF patients.
Patients and Methods. Post-hoc analysis from the ongoing prospective observational cohort ATHERO-AF study including 907 patients. Antioxidant status was evaluated by the activity of glutathione peroxidase 3 (GPx3) and superoxide dismutase (SOD). Patients were divided into two groups to evaluate the risk of CVEs:
1) LPS below median and GPx3 above median (n=254);
2) LPS above median and GPx3 below median (n=263).
Results. The mean age was 73.5±8.3 years, and 43.1% were women. Median LPS and GPx3 were 50.0 pg/mL (interquartile range [IQR] 15-108) and 20.0 U/mL (IQR 10.0-34.0), respectively. Patients of Groups 2 were older, with a higher prevalence of heart failure. LPS above the median was associated with reduced GPx3 (Odds Ratio for LPS 1.752, 95% Confidence Interval [CI] 1.344-2.285, p<0.001) and SOD (OR 0.525, 95%CI 0.403-0.683) activity after adjustment for CHA2DS2VASc score. In a mean follow-up of 54.0±36.8 months, 118 CVEs occurred, 42 in Group 1 and 76 in Group 2 (Log-Rank test p=0.001). At multivariable Cox regression analysis, Group 2 was associated with a higher risk of CVEs (Hazard Ratio [HR] 1.644, 95%CI 1.117-2,421, p=0.012), along with age ≥75 years (HR 2.035, 95%CI 1.394-2.972, p<0.001), diabetes (HR 1.927, 95%CI 1.280-2.900, p=0.002), and previous cerebrovascular disease (HR 1.895, 95%CI 1.251.
Conclusions. Our study indicates that circulating LPS may contribute to impaired antioxidant status in patients with AF. Patients with coincidentally high LPS and reduced GPx3 activity showed the highest risk of CVEs.

CONSEQUENCES OF ANGPTL3 DEFICIENCY ON HEPATIC AND EXTRAHEPATIC FAT DISTRIBUTION: A COMPARISON OF ANGPTL3 GENE LOSS-OF-FUNCTION CARRIERS

I. Minicocci[1], L. D'Erasmo[2], T. Neufeld[3], M. Di Martino[4], T. Fraum[5], A. Di Costanzo[6], S. Bini[6], M. Maranghi[6], G. Pigna[6], G. Labbadia[7], J. Zheng[8], P. Commean[5], A. Montali[6], F. Ceci[9], C. Catalano[10], A. Arca[6], N. Stitziel[11]
[1]Department of Translational and Precision Medicine, Sapienza, University of Rome; [2]Department of Translational and Precision Medicine, Sapienza, University of Rome; [3]Cardiovascular Division, Department of Medicine, Washington University School of Medicine, Saint Louis, Missouri, USA; [4]Department of Radiological Sciences, Oncology, Anatomical Pathology, Sapienza, University of Rome; [5]Mallinckrodt Institute of Radiology, Washington University School of Medicine, Saint Louis, Missouri, USA; [6]Department of Translational and Precision Medicine, Sapienza, University of Rome; [7]Department of Internal Medicine and Medical Specialties, Sapienza University of Rome; [8]Mallinckrodt Institute of Radiology, Washington University School of Medicine, Saint Louis, Missouri, USA; [9]Department of Experimental Medicine, Sapienza, University of Rome; [10]Department of Radiological Sciences, Oncology, Anatomical Pathology, Sapienza, University of Rome; [11]Cardiovascular Division, Department of Medicine; Department of Genetics and McDonnell Genome Institute, Washington University School of Medicine, Saint Louis, Missouri, USA;
E-mail: ilenia.minicocci@hotmail.it

Introduction. Loss-of-function (LOF) mutations in ANGPTL3 cause familial combined hypolipidemia (FHBL2) characterized by very low levels of all major lipoprotein fractions (LDL-C, TGs,HDL-C and ApoB). Genetic or pharmacological inactivation of ANGPTL3 has emerged as a new therapeutic opportunity to lower two causal risk factors (LDL-C and TG) for coronary heart disease (CHD) with potentially favorable metabolic effects. It is unknown, however, if inhibiting ANGPTL3 will result in adverse consequences. We aimed to ascertain changes in fat liver content as well as ectopic fat distribution in subjects showing partial or total ANGPTL3 deficiency due to the presence of inactivating mutations in ANGPTL3 gene.
Methods. We studied individuals carrying LOF mutations in ANGPTL3 resulting in complete (N=6) or partial (N=28) ANGPTL3 deficiency along with 76 wild-type controls. Magnetic resonance spectroscopy (MRS) and chemical shift magnetic resonance imaging (csMRI) were used to quantify hepatic triglyceride content in ANGPTL3 LOF carriers and non-carriers.
Results. The mean hepatic fat fraction (HFF) measured by MRS was not significantly different in ANGPTL3 LOF mutation carriers as compared with non-carrier controls [8.1%±13.3% (IQR 0.1%-9.2%) vs. 11.9%±16.3% (IQR 0.1%-21.6%), respectively, P=NS]. Similar results were found by csMRI [5.6%±3.8% (IQR 2.6%-7.1%) vs. 7.3%±6.2% (IQR 2.7%-9.7%), respectively, P=NS]. In a multivariate model including ANGPTL3 genotype, age, gender, triglycerides, BMI and HOMA-IR, we found that only BMI and HOMA-IR were independently associated with increased HFF.
Conclusion. In the present study, we have shown how loss of ANGPTL3 function in vivo is not associated with hepatic fat accumulation thus suggesting that the pharmacological inhibition of ANGPTL3 may be not associated with increased risk of fatty liver.
Disclosures. This work was funded by the United States National Institutes of Health (R01HL131961). NS has received an investigator-initiated research grant from Regeneron Pharmaceuticals.

AGING-RELATED DECLINE OF AUTOPHAGY IN PATIENTS WITH ATRIAL FIBRILLATION. A POST-HOC ANALYSIS FROM THE ATHERO-AF STUDY

C. Nocella[1], F. Versaci[2], V. Valenti[2], M. Forte[3], V. Cammisotto[4], S. Bartimoccia[5], L. Schirone[5], S. Schiavon[5], D. Vecchio[5], L. D'Ambrosio[5], G. Spinosa[5], A. D'Amico[6], I. Chimenti[5], F. Violi[7], G. Frati[8], P. Pignatelli[9], S. Sciarretta[8], D. Pastori[9], R. Carnevale[10]
[1]Department of Clinical, Internal Medicine, Anesthesiological and Cardiovascular Sciences, "Sapienza" University of Rome; [2]Department of Cardiology, Santa Maria Goretti Hospital, Latina; [3]IRCCS Neuromed, Pozzilli (IS); [4]Department of General Surgery and Surgical Speciality Paride Stefanini, Sapienza University of Rome; [5]Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina; [6]Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Rome; [7]Mediterranea Cardiocentro, Naples; [8]IRCCS Neuromed, Pozzilli (IS), Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina; [9]Department of Clinical, Internal, Anesthesiological and Cardiovascular Sciences, "Sapienza" University of Rome; [10]Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Mediterranea Cardiocentro, Naples
E-mail: cristina.nocella@uniroma1.it

Background. Aging is an independent risk factor for chronic disorder. Recent evidence suggests that autophagy plays a role in delaying aging and improving cardiovascular function in aging.
Methods. Post-hoc analysis of the prospective ATHERO-AF cohort study including 150 atrial fibrillation (AF) patients and 150 healthy subjects (HS). The population was divided into three age groups: <50-60, 61-70 and >70 years. Oxidative stress (Nox2 activity and hydrogen peroxide, H2O2), platelet activation (PA) by sP-selectin and CD40L, endothelial dysfunction (nitric oxide, NO) and autophagy parameters (P62 and ATG5) were assessed.
Results. Nox2 activity and H2O2 production were higher in AF patients then HS; conversely, antioxidant capacity was decrease in AF patients compared HS. Compared to HS, NO bioavailability in AF patients was decreased. Moreover, sP-selectin and CD40L were higher in AF patients then HS. Autophagy process was also significantly impaired in AF patients. Moreover, a significant difference in oxidative stress, PA, endothelial function and autophagy across age groups was found. A simple linear regression analysis showed that autophagy markers were associated with oxidative stress, PA and endothelial dysfunction both AF patients and HS.
Conclusions. This study provides evidence that autophagy process may represent a mechanism for the increased cardiovascular risk in the AF population.

STUDY OF THE ROLE OF MATURE NATURAL KILLER CELLS IN EXPERIMENTAL ATHEROSCLEROSIS

J. Nour[1], F. Bonacina[1], A. Moregola[1], R. Bellini[1], C. Garlanda[2], G.D. Norata[3]
[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; [2]Dipartimento di Scienze Biomediche, Istituto Clinico Humanitas, IRCCS, Rozzano (MI); [3]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; Centro SISA, Ospedale Bassini, Cinisello Balsamo (MI)
E-mail: jasmine.nour@unimi.it

Aim. Natural killer cells are innate lymphoid cells, playing a key role in the antiviral and antitumoral response. Mature Natural Killer 2 (mNK2, CD27-CD11b+) is a subset of NK known for its cytotoxic activity towards unhealthy cells and is increased following Il-1r8 (TIR-8) deficiency. Our aim is to study mNK2 in experimental atherosclerosis.
Methods. 8 weeks old-LDLR KO and Il-1r8/LDLR double KO (DKO) male mice were fed with standard diet (STD) or western-type diet (WTD) for 12 weeks. Plasma lipid profiling, extensive immunophenotyping by flow cytometry and histological analysis of the atherosclerotic plaques were then performed to evaluate the progression of atherosclerosis.
Results. Circulating mature Natural Killer 2 cells significantly increase in LDLR KO mice when fed for 12 weeks with cholesterol-enriched diet when compared to animals on STD ($p<0,01$). In mice fed standard diet, Il-1r8 deficiency did not impact neither circulating and tissue-resident immune cell distribution, nor plasma lipids. When mice were fed with WTD, DKO mice presented a significant increase in circulating mNK2 ($p<0,0001$) and monocytes ($p<0,05$), as compared to LDLR KO animal, whereas plasma cholesterol and triglyceride levels were similar. The increase in mNK2 cells however did not impact the development of atherosclerotic plaques which presented similar atheroma area, collagen deposition and macrophage infiltration.
Conclusion. These data suggest that mature Natural Killer 2 cells do not impact the development of atherosclerosis.

SEPARATE AND COMBINED EFFECTS OF BODY MASS INDEX AND POLYGENIC PREDISPOSITION TO HIGH BMI ON THE RISK OF DEVELOPING TYPE 2 DIABETES

E. Olmastroni[1], B.A. Ference[2], Q. Guo[2], D.L. Bhatt[3], N.J. Samani[4], K.K. Ray[5], S.J. Nicholls[6], T.B. Ference[2], F. Galimberti[7], G.D. Smith[8], M.V. Holmes[9], C. Baigent[9], S.E. Nissen[10], C.T. Ruff[11], M.S. Sabatine[11], A.L. Catapano[7], N. Sattar[12], J.E. Deanfield[13]

[1]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan; [2]Centre for Naturally Randomized Trials, University of Cambridge, Cambridge, UK; [3]Brigham and Women's Hospital Heart and Vascular Center, Harvard Medical School, Boston, MA; [4]Department of Cardiovascular Sciences, University of Leicester, Leicester, UK & National Institute for Health Research Leicester Cardiovascular Biomedical Research Centre, Leicester, UK; [5]Imperial Centre for Cardiovascular Disease Prevention, Department of Primary Care and Public Health, School of Public Health, Imperial College London, London UK; [6]Victorian Heart Institute, Monash University, Melbourne, Australia; [7]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy & Multimedica IRCCS, Milano; [8]MRC Integrative Epidemiology Unit, University of Bristol, Bristol, UK; [9]MRC Population Health Research Unit and the Clinical Trial Service Unit and Epidemiological Studies Unit, Nuffield Department of Population Health, University of Oxford, Oxford, UK; [10]Cleveland Clinic Coordinating Center for Clinical Research, Department of Cardiovascular Medicine, Cleveland Clinic, Cleveland, Ohio; [11]Thrombolysis in Myocardial Infarction (TIMI) Study Group, Division of Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, USA; [12]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK; [13]Institute of Cardiovascular Science, University College London, London, UK

E-mail: elena.olmastroni@unimi.it

Background. Type 2 diabetes (T2D) is a chronic metabolic disorder characterized by dysglycemia leading to microvascular and macrovascular complications. Whether measurements of body mass index (BMI) and polygenic predisposition to high BMI can be combined to better estimate risk of type 2 diabetes or identify the optimal timing for interventions to prevent diabetes is unknown. To address this issue, we sought to evaluate the separate and combined effects of BMI and a PGS on the risk of developing T2D. In addition, we sought to compare the effect of lifelong exposure to increased BMI as compared to BMI changes later in life on the risk of T2D to assess whether BMI has a cumulative effect on the risk of diabetes over time.

Methods. The primary outcome was type 2 diabetes, defined as the diagnosis of diabetes after the age of 35 years. We compared the separate and combined effects of BMI and a polygenic score (PGS) composed of 2,037,596 variants on the risk of T2D among 445,765 participants enrolled in UK Biobank. Cumulative lifetime risk of T2D was estimated using Kaplan-Meier curves, with age as the time scale, for participants within each quintile of PGS, and within each quintile of measured BMI. To evaluate the combined effect of measured BMI and polygenic predisposition to high BMI on the risk of T2D, the proportion of participants diagnosed with T2D was compared after ordering participants by quintiles of BMI and PGS, respectively. To make inferences about the optimal timing of interventions to prevent diabetes, we compared the effect of genetically determined lifelong exposure to increased BMI using a 96-variant instrumental variable BMI genetic score with the effect of increased BMI measured in middle-life on the risk of T2D.

Results. The mean age of participants at enrolment was 57.3 years (54.3% women), the median follow-up time was 8.1 years, and a total of 28,563 participants (6.4%) were diagnosed with T2D after the age of 35 years. A 1 standard deviation (SD) increase in BMI (4.77 units) as measured at the time of enrolment into UK Biobank was associated with an OR for diabetes of 2.26 (95%CI: 2.23-2.28). One SD increase in this score was associated with a OR of 1.50 (95%CI: 1.49-1.52). Both increasing quintiles of PGS and increasing quintiles of BMI were associated with increasingly steeper trajectories of lifetime risk for diabetes. Participants in the highest compared to lowest BMI quintile had an OR of 12.57 (95%CI: 11.43-13.34) for the risk of T2D, while participants in the highest compared to lowest PGS quintile had an OR of 3.09 (95%CI: 2.96-3.22). In stratified analyses, the risk of T2D varied by at least 10-fold within each quintile of PGS depending on differences in BMI. By contrast, ordering by BMI quintile stratified by PGS quintile appeared to separate participants into distinct categories of risk. In observational analyses, a 1-unit increase in BMI measured in middle life was associated with an OR for T2D of 1.21 (95%CI: 1.21-1.22). In Mendelian randomization analyses, a one-unit increase in genetically determined lifetime exposure to BMI was associated with an OR of 1.23 (95%CI: 1.21-1.25), suggesting that lifetime exposure to one-unit increase in BMI had a very similar effect on the risk of T2D as the same change in BMI measured in middle-life.

Conclusion. We found that BMI is a much stronger risk factor for T2D than polygenic predisposition to high BMI and that leads to reversible metabolic changes that do not accumulate over time. Therefore, most cases of diabetes can potentially be prevented or reversed if clinical interventions are put in place in the early stages of the disease.

NADPH OXIDASE-2 ACTIVATION AND GUT-DERIVED LIPOPOLYSACCHARIDES IN PATIENTS WITH CORONARY MICROVASCULAR ANGINA

F. Orlando[1], V. Ivanov[2], N. Ciobanu[2], M. Ivanov[2], C. Nocella[1], V. Cammisotto[1], A. Paraninfi[1], P. Ciacci[1], E. Maggio[1], A. D'Amico[3], S. Bartimoccia[4], F. Barillà[5], F. Violi[6], R. Carnevale[4], L. Loffredo[1]
[1]Department of Clinical, Internistic, Anaesthetic and Cardiovascular Sciences, Sapienza University of Rome; [2]Moldavian Research Institute of Cardiology, Chisinau, Republic of Moldova; [3]Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Rome; [4]Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina; [5]Dipartimento di Medicina dei Sistemi, Università degli Studi "Tor Vergata", Roma; [6]Cardiocentro Mediterranea, Napoli
E-mail: fedeorlando91@gmail.com

Background. Coronary microvascular angina (MVA) is characterized by typical chest pain, ischemic EKG alterations and the absence of obstructive coronary artery disease. Endothelial dysfunction and oxidative stress were hypothesized to be involved in the pathogenesis of this disease. In particular NADPH oxidase-2 (NOX2) activation could provoke increased oxidative stress and endothelial dysfunction but data in MVA have not been provided yet. The aim of this study was to evaluate NOX2 activation, serum lipopolysaccharide (LPS) levels as well as oxidative stress production and endothelial dysfunction in MVA patients and control group (CT).
Methods. In this study we wanted to compare serum levels of soluble NOX2-dp (sNOX-2-dp), H2O2 production, hydrogen peroxide breakdown activity (HBA), nitric oxide (NO) bioavailability, endothelin-1 (ET-1), zonulin and LPS in 80 consecutive subjects, including 40 MVA patients and 40 CT matched for age and gender.
Results. Compared with CT, MVA patients had significant higher values of sNOX-2-dp, H2O2, ET-1, LPS and zonulin; conversely HBA and NO bioavailability was significantly lower in MVA patients. Simple linear regression analysis showed that sNOX2 was associated with serum LPS (Rs=0.629; p<0.001), serum zonulin (Rs=0.641; p<0.001), H2O2 (Rs=0.590; p<0.001) and ET-1 (Rs=0.410; p<0.001); furthermore, an inverse correlation between sNOX2 and HBA (Rs=-0.646; p<0.001) and nitric oxide bioavailability (Rs=-0.312; p=0.005) was observed. Multiple linear regression analysis showed that LPS, HBA, zonulin emerged as the only independent predictive variables associated with sNOX2 (R2=61%).
Conclusions. This study provides the first report attesting that patients with MVA have an impairment of gut permeability, high LPS levels, NOX-2 activation and an imbalance between pro-oxidant and antioxidant systems, in favor of the oxidizing molecules that could be potentially implicated in the endothelial dysfunction and vasoconstriction of this disease.

B-TYPE AND NT-PROBNP NATRIURETIC PEPTIDES AS A PROGNOSTIC MARKER OF COVID-19 DISEASE SEVERITY AND OUTCOME: WHICH ONE IS THE BEST PERFORMER?

D. Palma[1], R. de Falco[2], M. Vano[2], G. Cardiero[1], C. Anastasio[2], P. Autiero[2], M. Savoia[2], G. Fortunato[1]
[1]Dip. di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli; [2]CEINGE S.C.a r.l. Biotecnologie Avanzate, Napoli
E-mail: palmad@ceinge.unina.it

Introduction. In March 2020, the World Health Organization (WHO) declared a pandemic for coronavirus disease (COVID 19). In September 2021, WHO data recorded about 220 million positive cases with a mortality rate of about 2% (over 4 million deaths). Several studies investigated the possible correlation between markers of cardiac disease and the worsening of COVID-19 severity (1, 2). Diffuse inflammation is the hallmark of the disease and could lead to atherosclerosis-based cardiovascular disease, as previously demonstrated for other infectious diseases (3). Increased concentrations of BNP and NT-proBNP in COVID-19 patients have already been reported. The aim of this study was to evaluate the usefulness of these markers and any potential difference between them in predicting COVID-19 prognosis.
Materials and Methods. We retrospectively collected and analyzed data about 174 consecutive adult patients affected with COVID-19. The clinical course of COVID-19 before hospitalization and its related complications were also acquired. In particular, the presence of pre-existing diseases related to cardiac and pulmonary functions was recorded, alongside with diabetes and hypertension. BNP and NT-proBNP of each patients were collected at admission in hospital. BNP plasma concentrations were measured by chemiluminescent microparticle immunoassay on the ARCHITECT i2000SR system (Abbott Laboratories, Wiesbaden, Germany). NT-proBNP was also measured on the ARCHITECT i2000SR system by using the Alere assay (Roche Diagnostics GmbH, Mannheim, Germany). Results: BNP and NT-proBNP values were higher in in-hospital non-surviving patients (p<0.001). Despite a high correlation obtained by Spearman's rank correlation coefficient between these two variables (rho =0.716, p<0.001), receiver operating characteristics (ROC) curve analysis showed that NT-proBNP (AUC =0.951) performed better (p=0.01) than BNP (AUC =0.777). Kaplan-Meier analysis was performed by dividing the population into groups, based on whether NT-proBNP and BNP concentrations at admission were higher than the cut-offs resulting from ROC curves. Both log rank tests resulted significant (p<0.001), in the group of patients with NT-proBNP admission values lower than the cut-off showing an absence of fatal outcome, whereas the subgroup of patients with BNP admission values lower than cut-off included 53.84% of all non-survivors of this study.
Conclusion. NT-proBNP proved to be a better prognostic tool than BNP for fatal outcome in COVID-19 patients. In particular, our study highlighted that a value of NT-proBNP below the cut-off of 511 ng/L at admission led to no in-hospital mortality in our population.

References

1. Mueller C, Giannitsis E, Jaffe AS, Huber K, Mair J, Cullen L, Hammarsten O, Mills NL, Mockel, M, Krychtiuk K, et al. Cardiovascular biomarkers in patients with COVID-19. European heart journal. Acute cardiovascular care 2021.
2. Dawson D, Dominic P, Sheth A, Modi M. Prognostic value of

Cardiac Biomarkers in COVID-19 Infection: A Meta-analysis. Research square 2020; 10.21203/rs.3.rs-34729/v1.
3. Musher DM, Abers MS, Corrales-Medina VF. Acute Infection and Myocardial Infarction. The New England journal of medicine. 2019; 380: 171-176.

PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 (PCSK9) ALTERS CHOLESTEROL HOMEOSTASIS IN CELL MODELS OF ASTROCYTES AND NEURONS

B. Papotti[1], C. Marchi[1], N. Ferri[2], M.P. Adorni[3], N. Ronda[1], F. Zimetti[1], F. Bernini[1]
[1]Department of Food and Drug, University of Parma; [2]Department of Pharmaceutical and Pharmacological Sciences, University of Padova; [3]Department of Medicine and Surgery, University of Parma
E-mail: bianca.papotti@unipr.it

Introduction. Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) seems to be involved in Alzheimer's disease (AD), although the underlying mechanisms are not fully clarified. This study aims to investigate the influence of PCSK9 in cholesterol transport from astrocytes to neurons, essential to maintain neuronal functions.
Materials and Methods. The following cellular models have been utilized:
1) human astrocytoma U373 cells exposed to exogenous PCSK9;
2) human neuroblastoma SH-SY5Y cells differentiated to neurons overexpressing or not PCSK9.

Amyloid β (Aβ) fibrils were used to reproduce in vitro AD-like conditions. Cholesterol synthesis, efflux and uptake were evaluated by radioisotopic assays; cholesterol content by a fluorimetric assay. Gene and protein expression were evaluated through qRT-PCR and WB, while the interaction between fluorescent apolipoprotein E (apoE) and cells by confocal microscopy. Cell viability was evaluated through MTT assay.
Results. In U373 PCSK9 significantly increased endogenous cholesterol synthesis ($p<0.05$), either in the absence or presence of Aβ, and reduced LDLR and ApoER2 expression ($p<0.05$). PCSK9 and Aβ both reduced intracellular cholesterol content ($p<0.05$ and $p<0.01$, respectively), with a more pronounced effect when incubated together ($p<0.001$). PCSK9 did not influence ABCA1- and ABCG1-mediated cholesterol efflux, while Aβ reduced ABCA1-mediated efflux ($p<0.001$), and ABCA1 protein expression. In PCSK9-overexpressing SH-SY5Y cells, the uptake of apoE-HDL was reduced ($p<0.001$) compared to control cells, either in the absence or presence of Aβ. PCSK9 overexpression also reduced LDLR and ApoER2 expression ($p<0.05$) and the interaction between fluorescent apoE and cells. Finally, PCSK9 overexpression in Aβ-treated cells furtherly reduced neuronal viability compared to control cells ($p<0.05$).
Conclusion. In conclusion, PCSK9, possibly in cooperation with Aβ, impairs brain cholesterol transport, with negative consequences on neuronal survival. Hence, PCSK9 may be considered a pathogenic factor in AD.

THE ROLE OF ADHERENCE TO LIPID-LOWERING THERAPIES IN ACHIEVING LIPID TARGET: FINDINGS FROM REAL-WORLD ANALYSIS IN ITALY

L. Degli Esposti[1], C. Borghi[2], M. Galvani[3], E. Giacomini[1], P. Manotti[4], A. Marra[5], A. Passaro[6], V. Perrone[1], F. Pieraccini[7], D. Sangiorgi[1], A. Navazio[8]
[1]CliCon Srl, Bologna; [2]Medicine Department of Medical and Surgical Sciences University of Bologna; [3]U.O.C. Cardiologia Ospedale Morgagni-Pierantoni Forlì, AUSL Romagna, Forlì; [4]Dipartimento Cure Primarie, AUSL di Reggio Emilia; [5]U.O. Farmacia Ospedaliera Dipartimento Biotecnologie, Trasfusionale e di Laboratorio Azienda Ospedaliero Universitaria S. Anna, Ferrara; [6]Azienda Ospedaliera-Universitaria S. Anna. Ferrara; [7]Assistenza farmaceutica AUSL della Romagna, Forlì; [8]U.O. Cardiologia Ospedaliera Presidio Ospedaliero ASMN Azienda USL di Reggio Emilia - IRCCS, Reggio Emilia
E-mail: valentina.perrone@clicon.it

The objective of the present real-world analysis was to evaluate the impact of adherence to lipid-lowering drugs in reaching the lipid target in settings of clinical practice in Italy.The analysis was based on administrative and laboratory databases of selected Healthcare Units in Italy covering approximately 10% of Italian population. Adult patients prescribed with statins and with at least a low-density lipoprotein (LDL) determination were included between 2012 and 2019. The index date was defined as the first prescription for statins within the inclusion period. Patients fell into 4 clusters collectively exhaustive and mutually exclusive based on their characteristics assessed during the year prior index date. Patients were considered as adherent if they had a proportion of days covered (PDC) ≥80%. Among overall patients prescribed statins and with a LDL determination, 1% was with familial hypercholesterolemia, 28% with previous cardiovascular events, 21% with diabetes and 50% in primary prevention. Regardless their risk profile, the increasing of adherence was related to a higher achievement of LDL-target, with an increment of +53.2% among familial hypercholesterolemia, +43.1% in diabetes and +30% in previous cardiovascular events and primary prevention clusters while progressing from low (PDC<40%) to high (PDC≥80%) levels of adherence. However, while in diabetes and primary prevention clusters 80% and 86% of adherent patients, respectively, had their cholesterol level under control, in the familial hypercholesterolemia and previous cardiovascular events clusters only 46% of adherent patients achieved the lipid target. The analysis showed adherence to be a key factor for cholesterol control. However, our findings underline a therapeutic need for patients that, although adherent, fail to achieve the lipid target, especially among patients with previous cardiovascular events (that have low level of LDL to achieve) and with familial hypercholesterolemia (that have high LDL basal level), suggesting therapeutic intensification should be applied.

REAL-WORLD ANALYSIS ON THE ECONOMIC VALUE OF REACHING LIPID TARGET IN ITALY

L. Degli Esposti[1], C. Borghi[2], M. Galvani[3], E. Giacomini[1], P. Manotti[4], A. Marra[5], A. Passaro[6], V. Perrone[1], F. Pieraccini[7], D. Sangiorgi[1], A. Navazio[8]
[1]CliCon Srl, Bologna; [2]Medicine Department of Medical and Surgical Sciences University of Bologna; [3]UOC Cardiologia Ospedale Morgagni-Pierantoni Forlì, AUSL Romagna, Forlì; [4]Dipartimento Cure Primarie, AUSL di Reggio Emilia; [5]U.O. Farmacia Ospedaliera Dipartimento Biotecnologie, Trasfusionale e di Laboratorio Azienda Ospedaliero Universitaria S. Anna, Ferrara; [6]Azienda Ospedaliera-Universitaria S. Anna. Ferrara; [7]Assistenza farmaceutica AUSL della Romagna, Forlì; [8]U.O. Cardiologia Ospedaliera Presidio Ospedaliero ASMN Azienda USL di Reggio Emilia - IRCCS, Reggio Emilia
E-mail: valentina.perrone@clicon.it

The study aimed to evaluate the healthcare direct costs for Italian National Health System of patients treated with lipid-lowering drugs that do not achieve the low-density lipoprotein (LDL)-cholesterol target compared to those reaching their targets, and to analyze costs according to the distance from LDL target by using real-world data. An observational analysis was performed on administrative and laboratory data from selected Italian Healthcare Departments. Patients were included if they presented at least one laboratory LDL test between 2012 and 2019 and if they were prescribed lipid-lowering drugs during 6 months prior the last LDL detection (index date). Mean annual direct costs were evaluated in the 12 months before index date in terms of all drugs prescribed, all-cause hospitalizations and all outpatient services. Distance to LDL target was calculated as difference between the index LDL level and LDL target. Total mean annual healthcare direct cost for patients that did not reach LDL target was higher compared to total cost of patients achieving LDL target (€ 3,678 vs € 2,906). Costs were mainly driven by hospitalization (€ 1,330) followed by drugs (€ 1,012) and outpatient services (€ 563) expenditure. Mean total annual healthcare costs increased with the distance from LDL target, specifically from € 3,004 for patients with 10% distance from LDL target up to € 4,823 for those 50% or more distance from LDL target. This trend was particularly evident for the cost item related to hospitalization, that went from € 1,486 to € 2,819 moving from 10% to ≥50 distance from LDL target.
Results from this real-world study highlighted the higher economic burden for patients that do not reach the therapeutic LDL target, that tend to rise along with increasing distance from the LDL target. Overall, our findings could suggest that reducing the distance from LDL target could have a positive impact also on the economic outcomes for these patients.

THE MANAGEMENT OF CHOLESTEROL LEVEL CONTROL WITH LIPID-LOWERING DRUGS IN ITALIAN CLINICAL PRACTICE: FINDINGS FROM THE STREAM (SUPPORTING WITH THE REAL-WORLD EVIDENCE THE ASSESSMENT OF MEDICINES AND HEALTH TECHNOLOGIES) STUDY

L. Degli Esposti[1], C. Borghi[2], M. Galvani[3], E. Giacomini[1], P. Manotti[4], A. Marra[5], A. Passaro[6], V. Perrone[1], F. Pieraccini[7], D. Sangiorgi[1], A. Navazio[8]
[1]CliCon Srl, Bologna; [2]Medicine Department of Medical and Surgical Sciences University of Bologna, Bologna; [3]UOC Cardiologia Ospedale Morgagni-Pierantoni Forlì, AUSL Romagna, Forlì; [4]Dipartimento Cure Primarie, AUSL di Reggio Emilia; [5]U.O. Farmacia Ospedaliera Dipartimento Biotecnologie, Trasfusionale e di Laboratorio Azienda Ospedaliero Universitaria S. Anna, Ferrara; [6]Azienda Ospedaliera-Universitaria S. Anna, Ferrara; [7]Assistenza farmaceutica AUSL della Romagna, Forlì; [8]U.O. Cardiologia Ospedaliera Presidio Ospedaliero ASMN Azienda USL di Reggio Emilia, IRCCS, Reggio Emilia
E-mail: valentina.perrone@clicon.it

The study aimed to evaluate the proportion of patients not reaching their low-density lipoprotein (LDL)-cholesterol target according to their risk profile in real-world settings of Italian clinical practice. This observational analysis was based on administrative and laboratory databases from a pool of Italian Entities covering around 10% of Italian population. All patients included had at least one laboratory LDL test between 2012 and 2019. Presence of lipid-lowering drugs was analyzed in the 6 months before index date (last LDL test detected), during which time adherence to these therapies was measured as proportion of days covered (PDC). Risk profile was assessed based on ESC guidelines (1). Among patients with LDL tests that received lipid-lowering drugs, 49.7% were deemed as very high-risk (VHR), 38.3% at high risk (HR), 12% with other risks (OR). Overall, 80% of patients did not reach their LDL-cholesterol target: 87.2% in the HR-cohort, 82.9% in the VHR-cohort (LDL level target 70 mg/dl and 55 mg/dl, respectively) and 49.6% in OR-cohort (LDL level target 116 mg/dl). Statin and ezetimibe combination was observed only in 6.5% of HR and OR and 10.3% of VHR patients, while patients were mainly in monotherapy with statins (87.5 VHR, 91.2% HR, 90.6% OR). Furthermore, patients adherent to treatment (PDC≥80%) accounted for the 52% of VHR-cohort, 47.2% of HR-cohort and 39.1% of OR-cohort. Our findings highlight the need to optimize the management of cholesterol control, especially among patients at risk. Despite the high proportion of patients not reaching LDL target, sub-optimal levels of adherence and a low use of combination regimens were observed, thus suggesting LDL-control could be supported by increasing adherence and/or the use of combination therapies and, if the target is not yet achieved, by the utilization of more recent therapies.

Reference

1. European Heart Journal. 2020; 41, 111188.

DISCORDANT RESPONSE OF LDL-CHOLESTEROL AND LIPOPROTEIN(A) PLASMA LEVELS TO PCSK9I THERAPY IN A COHORT OF HYPERLP(A) PATIENTS: A SINGLE CENTRE EXPERIENCE

G. Petracca, E. Sani, L. Santi, D. Travia, M.G. Zenti
Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, University and University Hospital of Verona
E-mail: grazianapetracca1@gmail.com

Background. Lipoprotein(a) (Lp(a)) is an independent risk factor for atherosclerotic cardiovascular disease. No effective therapies that target Lp(a) exist currently but some clinical-trials have shown that proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) reduce Lp(a) levels by 25% to 30%, irrespective of baseline-levels.
Method. Monocentric observational cohort study aiming at evaluating the efficacy of PCSK9i-therapy in lowering Lp(a) and LDL-C plasma-levels in a real-world-setting.
Results. 145 patients (88 males, 57 females; median age: 65.6 years [interquartile range: 40-74]). All subjects were at very-high cardiovascular risk (CVR): 45% had Heterozygous familial hypercholesterolemia (HeFH), 65% coronary heart disease (CHD), 50% peripheral vascular disease (PVD), 19% diabetes mellitus (DM), 6% impaired fasting glucose (IFG) and 61% were statin-intolerant. Baseline mean LDL-C and median Lp(a) were 149±53 mg/dL and 36 mg/dL [17-88], respectively. LDL target (<55 mg/dL) was reached in 56% patients at 6 months, 56% at 12months, 54% at 24 months follow-up. Furthermore, LDL-C target was reached in 66.7% males vs 26.7% females (p<0.001), 60% patients with CHD vs 34% without CHD (p<0.01), 62% patients with PVD vs 41% without PVD (p<0.036). HyperLp(a), defined as Lp(a) >50 mg/dl, was observed in 57 patients: baseline median Lp(a) 89 mg/dL [62-129], with an increasing trend in the follow up: 88 mg/dl [70-129] at 6months, 114 mg/dl [77-267] at 12 months (+28%) and 133 mg/dl [80-333] at 24 months (+49%).
Conclusion. In our experience PCSK9i-therapy failed to reduce lp(a) plasma level, indeed we observed a further increase in the follow-up. Awaiting the new drugs on the way, such as the antisense oligonucleotides against apolipoprotein(a), the first step is the personalization of the therapy for intensive management of all CVR factors (i.e. glucose-metabolism and blood pressure). In the meantime lipoprotein Apheresis, which is the golden standard treatment, should be considered.

LIPOPROTEIN(A) MASS LEVELS PREDICT HEPATIC FIBROSIS BUT NOT CARDIOVASCULAR OUTCOMES IN NONALCOHOLIC FATTY LIVER DISEASE PATIENTS

M. Ruscica[1], M. Meroni[2], M. Longo[2], R. Lombardi[2], E. Paolini[1], C. Macchi[1], A. Corsini[1], C. Sirtori[1], A.L. Fracanzani[2], P. Dongiovanni[3]
[1]Università degli Studi di Milano; [2]Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico; [3]Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano
E-mail: massimiliano.ruscica@unimi.it

Background. Dyslipidemia and cardiovascular complications are comorbidities of nonalcoholic fatty liver disease (NAFLD), which ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis and cirrhosis. Although, genome wide association, epidemiological, and clinical studies have established Lp(a) levels as a causal risk factor for cardiovascular diseases, whether Lp(a) is causal in NAFLD patients remains to be clarified.
Methods. Circulating Lp(a) mass levels were assessed in 600 patients with biopsy-proven NAFLD. The association of Lp(a) with liver damage was explored by categorizing serum Lp(a) into quartiles. ROC curves were used to analyse the prediction of Lp(a) on CVD outcomes and hepatic fibrosis. Hepatic expression of LPA was evaluated by RNA-seq in a 183 patients of whom Lp(a) dosage was available.
Results. In NAFLD patients, elevated Lp(a) levels were modestly associated with circulating lipids, carotid plaques and hypertension. Conversely, patients with low serum Lp(a) displayed insulin resistance ($p<0.05$), transaminases elevation ($p<0.05$) and increased risk to develop severe fibrosis ($p=0.007$) and cirrhosis ($p=0.002$). The diagnostic accuracy of Lp(a) to predict fibrosis increased by combining it with transaminases (AUC F4=0.87, $p<0.0001$). Hepatic LPA expression reflects serum Lp(a) levels ($p=0.018$), and both were reduced with the progression of NAFLD ($p<0.05$). Hepatic LPA mRNA levels correlate with those of genes involved in lipoprotein release, lipid synthesis and fibrogenesis ($p<0.05$). The TM6SF2 rs58542926, ApoE rs445925 and PCSK9 rs7552841 variants, known to affect circulating lipids, influence significantly serum Lp(a) mass levels.
Conclusions. Lp(a) mass levels predict hepatic fibrosis but not cardiovascular outcomes in patients with NAFLD.

LIRAGLUTIDE FOR WEIGHT MANAGEMENT IN A REAL-WORLD SETTING: EXPERIENCE FROM A SINGLE OBESITY CENTER

E. Sani, G. Petracca, L. Santi, G. Da Prato, M.G. Zenti
Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, University and University Hospital of Verona
E-mail: elenasani@live.it

Purpose. Several randomized controlled clinical trials have shown that the use of Liraglutide, in combination with diet and exercise, leads to a significant weight loss and improvement of blood pressure and metabolic profile. This retrospective study aimed at evaluating the effectiveness of Liraglutide therapy in a real-world setting.
Methods. A cohort of 58 patients (46 females, 12 males), mean ± DS age 47.1±11.7 years, referring to our Obesity Center, all obese or overweight with weight-related comorbidities, started Liraglutide therapy from May 2016 to January 2021. 39/58 (67.2%) patients reached the maximum dose of 3 mg. At 3 months 43/58 (74.1%) patients continued therapy, at 6 months 26/58 (44.8%), at 12 months 11/58 (19%).
Results. The mean weight loss at 3 months was -5.8 kg (-5.6%), at 6 months -7.5 kg (-7.4%), at 12 months -10.0 kg (-8.9%). The proportion of patients achieving 5-10% weight loss was 20.7% at 3 months, 10.3% at 6 months, while the proportion achieving ≥10% weight loss was 13.8% at 3 months, 15.5% at 6 months, 10.3% at 12 months. We observed a significant weight loss at 3, 6 and 12 months. There was also a significant reduction of systolic blood pressure and HbA1c at 3 and 6 months, while total cholesterol and LDL-cholesterol significantly declined only at 3 months. The majority of patients (37.9%) discontinued treatment for economic concerns. The overall incidence of adverse events was 46.6% (27/58) and the most reported was nausea or vomiting.
Conclusion. In a real-world setting, Liraglutide therapy was associated with a clinical meaningful weight loss at 3, 6 and 12 months, with also a significant improvement in systolic blood pressure and metabolic profile in the short term, which may contribute to the reduction of cardiovascular risk. Economic concerns still represent an important barrier for access to therapy.

NON-HDL CHOLESTEROL AND APOLIPOPROTEIN B IN A HYPERTENSIVE POPULATION: ROLE OF ADIPOSITY AND INSULIN RESISTANCE

R. Sarzani[1,2], F. Giulietti[1,2], S. Biondini[2], E. Fausti[2], M. Allevi[2], F. Spannella[1,2]
[1]*Internal Medicine and Geriatrics, IRCCS INRCA, Ancona;*
[2]*Department of Clinical and Molecular Sciences, University "Politecnica delle Marche", Ancona*
E-mail: r.sarzani@univpm.it

Introduction. Non-HDL cholesterol (non-HDLc) and apolipoprotein B (ApoB) indirectly measure all atherogenic or potentially atherogenic circulating lipoproteins. In patients with essential hypertension, atherogenic dyslipidemia and insulin resistance (IR) due to overweight/obesity are highly prevalent.
Aim. Evaluate how adiposity and IR affect non-HDLc and ApoB levels in essential hypertensive patients.
Methods. We performed a cross-sectional study on 272 consecutive patients referred to our Hypertension Centre and not taking lipid-lowering drugs. Body mass index (BMI) and waist circumference (WC), measured to the nearest 0.1 cm at the midpoint between the lowest rib and the iliac crest, were used to assess adiposity. IR was evaluated with HOMA-IR index, calculated according to the formula: HOMA-IR = [glucose] (mmol/l) × [insulin] (μU/ml)/22.5.
Results. Mean age: 50.2±14.5 years; male prevalence: 65.1%; mean BMI: 27.9±4.8 kg/mq; mean WC: 99.1±13.1 cm; mean Non-HDLc: 156.2±48.6 mg/dl; mean ApoB100: 113.8±36.4 mg/dl; median HOMA-IR index: 2.4 (1.7-4.0). The prevalence of overweight/obesity and IR was 76.0% and 48.6%, respectively. We found a fair correlation between non-HDLc and ApoB (r=0.588; p<0.001). Overweight/obese patients showed higher prevalence of IR (57.4% of overweight/obese). We found no linear association of both BMI and WC neither with ApoB nor with non-HDLc (all p>0.05), while a negative correlation was found with HDLc (r=-0.295; p<0.001 for BMI and r=-0.224; p=0.009 for WC) and a positive correlation with triglycerides (r=0.222; p=0.004 for BMI). A significant correlation emerged between HOMA-IR index and ApoB100 (r=0.280; p=0.016), independently of BMI.
Conclusion. In our study, the excess adiposity is directly linked to HDLc and triglycerides, while IR is directly linked to ApoB levels. Although there is a close association between IR and obesity, not all overweight/obese patients had IR, IR that probably plays a major role in atherogenic lipoprotein levels than just obesity.

RECLASSIFICATION OF CARDIOVASCULAR RISK ACCORDING TO SCORE2 AND PREVALENCE OF TARGET LDL-CHOLESTEROL RECALCULATED WITH MORE RECENT EQUATIONS IN A LARGE HYPERTENSIVE POPULATION

F. Spannella[1,2], F. Giulietti[1], M. Allevi[2], P. Turri[2], S. Sarnari[2], S. Re[2], R. Sarzani[1,2]
[1]*Internal Medicine and Geriatrics, IRCCS INRCA, Ancona;*
[2]*Department of Clinical and Molecular Sciences, University "Politecnica delle Marche", Ancona*
E-mail: r.sarzani@univpm.it

Introduction. Until recently, SCORE was the most commonly used model for cardiovascular risk (CVR) assessment and Friedewald was the most widely used formula to calculate low-density lipoprotein cholesterol (LDLc) in clinical practice. Recently, an updated CVR model (SCORE2) and several apparently more accurate equations have been proposed and validated.
Aim. Evaluate the impact of both SCORE2 on CVR stratification and of new formulas on prevalence of LDLc control in a wide hypertensive population.
Methods. Cross-sectional study on 1192 consecutive hypertensive outpatients. LDLc was calculated using the Friedewald formula (F), the modified Friedewald formula proposed by Martin SS. (M) and the equation proposed by Sampson M. (S). SCORE and SCORE2 have been used for individual CVR stratification. LDLc control was defined according to the 2019 ESC/EAS Dyslipidemia Guidelines.
Results. Mean age: 56.5±13.7 years. Mean LDLc calculated using the three formulas: 122.8±35.1 mg/dl (F), 124.4±34.1 mg/dl (M) and 125.4±35.1 mg/dl (S), respectively. The three LDLc equations showed high correlations (r=0.99, p<0.001 for all the three comparisons). According to SCORE2, there is a significant re-classification of the individual CVR (p<0.001). The 57.6% and the 4.5% of patients with low-moderate risk at SCORE moved to high risk and very high risk according to SCORE2, respectively, while the 4.0% and the 47.6% of patients with high risk according to SCORE moved to low-moderate risk and very high risk according to SCORE2, respectively. All patients with very high risk at SCORE (27.3% of all the studied population) was confirmed at SCORE2. Within patients at target for LDLc according to SCORE and F, the 39.3% and the 41.1% was not at target according to SCORE2 and S or M, respectively (all p<0.001).
Conclusion. We found non-negligible differences in CVR reclassification and LDLc control in our hypertensive population, after the application of SCORE2 and new LDLc equations. Our findings show how the application of these new tools may significantly affect the management of dyslipidemia and therefore the CV prevention in clinical practice.

COVID-19 SWAB COLLECTION AND SEROLOGICAL SCREENING IN LIPOPROTEIN APHERESIS UNIT

F. Sbrana[1], B. Dal Pino[2], F. Bigazzi[2], M. Pianelli[2], R. Luciani[2], A. Ripoli[2], T. Sampietro[2]
[1]*Fondazione Toscana "Gabriele Monastreio";*
[2]*Fondazione Toscana "Gabrielel Monasterio"*
E-mail: francesco.sbrana@ftgm.it

Background. Assessing evolution of SARS-CoV-2 immune response among patients receiving therapeutic apheresis can define its durability in a highly clinically relevant context because these patients share the characteristics of persons most susceptible to SARS-CoV-2 infection. In our Lipoprotein Apheresis (LA) Center 36 subjects (mean age 64±10 years; male 25/36) are currently being treated for inherited dyslipidemias; more than 500 treatment/year are performed.
Methods. To protect patients and healthcare team against the COVID-19 outbreak, a dedicated protocol was applied:
1) telephone call performed two days before LA treatment, in order to identify clinical symptoms relate to COVID-19 disease;
2) real-time PCR assays for SARS-CoV-2 on upper respiratory specimens collected the day before LA;
3) body temperature check when the patient arrives at the entrance of the facility;
4) all patients undergoing LA are wearing surgical mask and maintaining a social distanced of 1.8 meters (including bed space);
5) serological testing (Coronavirus Disease 2019 Antibody Combined Test Kit - Medical Systems Biotechnology®) is periodically done.

Results. In 14th months 5 patients out of 36 on treatment (14%) had SARS-CoV-2 infection: one patient required intensive care unit admission, 3 had mild symptoms and the last one had asymptomatic infection. The serological tests performed monthly before LA treatment and at least 21 days after positive results on RT-PCR, revealed that, after a peak (2 months after infection), there was a continuous decline in the median value with a response that persisted for at least 6 months (IgG Receptor Binding Domain: 1st month 4.16±1.02-7th month 0.85±0.33; R2 0.9246; IgG index value ≥1.4).
Conclusion. Our data show that the protocol in use was effective in containing COVID-19 outbreak in patients receiving LA and that serological trend in patients with COVID-19 infection is in line with other clinical settings. We are further implementing our protocol dosing Ig response to the spike protein antigen after vaccination.

OPTIC NERVE HEAD BLOOD FLOW ACUTELY INCREASES UPON CHOLESTEROL REMOVAL. THE EYES MIRROR OF THE HEART?

T. Sampietro[1], B. Dal Pino[1], F. Bigazzi[1], F. Sbrana[1], A. Ripoli[1], E. Fontanelli[2], M. Pianelli[1], R. Luciani[1], A. Lepri[2], G. Calzetti[3]
[1]*Fondazione Toscana "Gabriele Monasterio";*
[2]*Università di Pisa;* [3]*Università di Parma*
E-mail: francesco.sbrana@ftgm.it

Background. Lipoprotein apheresis (LA) acutely increases coronary microcirculation blood flow (BF) measured with different techniques, most of which are time-consuming, costly and invasive. The ocular vasculature may be assumed as an easily accessible window to systemic microcirculation. Recent advances in imaging techniques enable to quantify ocular microcirculation BF in a quick and non-invasive manner, which represents a wonderful opportunity to study the short-term changes in optic nerve head (ONH) BF after LA in inherited hypercholesterolemia (IH).
Methods and Results. The study was performed at Italian Reference Centre for inherited dyslipidemias of Tuscany and included 22 patients with IH, the same cohort that was previously studied for coronary microcirculation. Laser Speckle Flowgraphy (LSFG) was used to measure rest ONH BF before and after lipoprotein removal by LA. Main outcomes were:
1) tissue average BF (referred to as 'Mean Tissue', MT);
2) arteriolar/venular average BF (referred to as 'Mean Vessel', MV).

Eyes were statistically clustered in two groups based on pre-LA ONH blood flow values.After each LA treatment, parallel to the reduction of plasma lipids, all ONH microcirculatory parameters significantly increased, more significantly in eyes with lower pre-apheresis ONH BF values (MT +7.0%, $p<0.005$; MV +7.2%, $p<0.05$).
Conclusions. A single LA session resulted in a statistically significant increase in short-term ONH BF rest. These findings, evaluated together with previous coronary microcirculation data, suggest a similar ocular and coronary BF response to LA. Ocular microcirculation BF might represent a versatile biomarker to evaluate microcirculatory system health, including coronary microcirculation. It is therefore plausible that plasma lipoproteins levels may influence ONH BF.

IS TREATING SEVERE HE FH SO EASY? A COMBINED TREATMENT BETWEEN LIPOPROTEIN APHERESIS AND PCSK9 INHIBITORS

B. Dal Pino, F. Sbrana, F. Bigazzi, T. Sampietro
Fondazione Toscana "Gabriele Monasterio"
E-mail: francesco.sbrana@ftgm.it

Background. Despite advance in pharmacotherapy of lipid disorders, many heterozygous Familial Hypercholesterolemia patients do not achieve a desirable lipid target to significantly reduce the risk of atherosclerotic cardiovascular disease. The aim of the present work is to evaluate the interaction between Lipoprotein apheresis (LA) and PCSK9i in a small FH cohort in which the guidelines therapeutic target is not achieved.
Methods. We evaluate 14 patients (mean age 66±9 years, male 71%) with HeFH and ischemic heart disease (IHD) on maximally tolerated lipid lowering therapy and chronic LA. Pre-LA lipid values plus PCSK9i administered immediately after LA (PCSK9i-0) or 7 days later LA (PCSK9i-7) were compared.
Results. The PCSK9i administration 7 days after the LA, counteracting the LA lipid rebound effect, allowed to achieve the pre-LA lipid values at a significantly longer time interval (LDL cholesterol: PCSK9i-0 102±35 mg/dl vs. PCSK9i-7 103±50 mg/dl, p=0.474; mean LDL cholesterol: PCSK9i-0 71±28 mg/dl vs. PCSK9i-7 71±40 mg/dl, p=0.985; median inter-LA interval: PCSK9i-0 14 [14-15] days vs. PCSK9i-721 [14–28] days, p= 0.025). By PCSK9i and LA therapy combination, as here reported, we obtain LDL-cholesterol reduction higher than 50% when comparing with lipid levels without the injection therapy. During one year, together with a complete adherence to PCSK9i therapy, we recorded a mean reduction by 3 LA-treatment per patient.
Conclusion. This therapeutic approach suggests:
1) the possibility of increasing the number of patients treated with LA;
2) the improvement of patient quality of life;
3) the costs reduction for the single patient-treatment.

ACUTE CORONARY SYNDROME AND LIPID-LOWERING THERAPY IN A REALISTIC DIAGNOSTIC-THERAPEUTIC CARE PATHWAY

T. Sampietro, B. Dal Pino, F. Sbrana, F. Bigazzi, C. Palmieri, S. Berti
Fondazione Toscana "Gabriele Monasterio"
E-mail: francesco.sbrana@ftgm.it

Background. Optimal use of lipid-lowering therapy (LLT) after acute coronary syndromes need to be intensified.Fondazione Toscana Gabriele Monasterio (FTGM) is a cardiopulmonary tertiary-level Institute, with cath-lab hub for acute coronary syndrome, adult and pediatric cardiac surgery center, referral facility for heart failure, primitive pulmonary hypertension patients and reference center for inherited dyslipidemias, rare lipid disorders with lipoprotein apheresis unit.
Study design. To optimize the LLT, in FTGM, we developed a diagnostic-therapeutic care pathway (DTCP) for subjects admitted with acute coronary syndrome because:
1) inherited atherogenic dyslipidemias have an expected prevalence up to 75% among coronary patients under 65 years of age and are usually unrecognized;
2) in the POSTER study on italian patients with a recent coronary artery event, heterozygous familial hypercholesterolemia has about 5.1% prevalence, which indicates the need of early identification to improve their cardiovascular risk management;
3) plasma levels of Lp(a) are definite risk factor for atherosclerotic cardiovascular disease and their measurement, in the cascade screening of familial hypercholesterolemia, are strongly recommended;
4) statin-associated muscle symptoms are a major determinant of poor treatment adherence and/or statin discontinuation.

The DTCP in inherited dyslipidemias involves dialogue between the cardiologist who manages acute coronary syndrome and the lipidologist, in order to assess the correct diagnosis, to initiate the cascade screening, to proceeded with the best LLT and to perform patient's personalized follow-up.
Conclusion. DTCP have a pivotal role to improve the LLT effectiveness; the first cardiovascular event must signal the opportunity to start a personalized LLT and an unmissable opportunity to activate the cascade testing for studied case and relatives.

DIRECT AND INDIRECT EFFECT OF SARS-COV-2 PANDEMIC IN SUBJECTS WITH FAMILIAL HYPERCHOLESTEROLEMIA: A SINGLE LIPID CENTER REAL WORLD EVALUATION

R. Scicali[1], V. Ferrara[2], M.G. Farina[3], S. Conti[3], S. Piro[3], F. Purrello[3], A. Di Pino[3]
[1]U.O.C. Geriatria - ARNAS Garibaldi Catania; [2]U.O.C. Medicina Interna, Azienda Ospedaliera Policlinico Catania; [3]UOC Medicina Interna, ARNAS Garibaldi, Catania
E-mail: robertoscicali@gmail.com

Background and Aims. The clinical manifestations of SARS-CoV-2 infection broadly differ among the affected subjects; about half of the infected subjects remain asymptomatic, the majority of the symptomatic subjects experience influenza-like symptoms and 10-15% of these develop a severe disease (COVID-19) characterized by a widely clinical scenario from pneumonia to acute respiratory distress syndrome (ARDS) and disseminated intravascular coagulation. Over the respiratory tract, COVID-19 could also affect the cardiovascular system. Beyond the reported direct damage of SARS-CoV-2 infection, an increasing attention has been focused on the indirect effects of SARS-CoV-2 pandemic due to the healthcare public system restructuring. In this context, the reduced cardiovascular screening may be deleterious in subjects at high cardiovascular risk such as familial hypercholesterolemia (FH). In this study we evaluated the direct and indirect effects of SARS-CoV-2 pandemic in a cohort of FH subjects.
Methods. This was a retrospective observational study involving patients aged over 18 years with a genetically confirmed FH diagnosis and enrolled from the Lipid Centre University Hospital of Catania, Italy from 4th June 2021 to 9th August 2021. All partecipants obtained a telephone survey concerning their lipid profile values [total cholesterol, high-density lipoprotein cholesterol (HDL), triglycerides (TG), LDL-C], lipidologist and cadiologist visits, carotid and femoral ultrasound evaluation and lipid lowering therapy adherence in the 12 months before and after the italian lockdown (9th March - 3rd June 2020); moreover, all partecipants confirmed or not the SARS-CoV-2 infection from 9th March 2020 to the 12 months after the end of italian lockdown (3rd June 2020).
Results. The percentage of FH subjects who analyzed the lipid profile was lower after COVID lockdown (56.5% vs 100.0%, p<0.01); moreover, FH subjects had a significant reduction of HDL cholesterol (47.78±10.12 vs 53.2±10.38 mg/dL, p<0.05) ed and increasing of Non HDL cholesterol (117.24±18.83 vs 133.09±19.01 mg/dL, p<0.05); finally, a reduced percentage of FH subjects reached the recommended LDL cholesterol target after COVID-19 lockdown (31.2% vs 40.4%, p=0.09). Of note, a reduction of lipid lowering therapy adherence was observed after COVID-19 lockdown (85.4% vs 95.4%, p=0.08). Moreover, it was observed a reduced percentage of FH subjects who performed lipidologist, cardiologist and vascular imaging follow-up after lockdown (for lipidologist follow-up 33.5% vs 100.0%, p<0.001; for cardiologist follow-up 22.3% vs 60.8%, p<0.01; for vascular imaging follow-up 19.6% vs 100.0%, p<0.001). The most frequent reason of the reduced health care follow-up was the COVID contagion fear. Finally, the percentage of FH subjects who was affected by SARS-CoV-2 was 7.3%; of these, the majority were in secondary prevention and didn't reach the recommended LDL cholesterol target and none of FH subjects was hospitalized.
Conclusion. In our retrospective study, the indirect effects of SARS-CoV-2 pandemic was deleterious in a cohort of subjects at high cardiovascular risk as FH.

UNCOMMON PRESENTATION OF CHOLESTERYL ESTER STORAGE DISEASE (CESD): DESCRIPTION OF A CASE AND GENETIC CHARACTERIZATION BY NEXT GENERATION SEQUENCING

C. Scrimali[1], F. Brucato[1], M. Caserta[2], M. Gueli Alletti[1], C. Castana[2], R. Spina[1], V. Ingrassia[1], G.I. Altieri[1], F. Fayer[1], D. Noto[1], G. Misiano[1], A. Giammanco[1], C.M. Barbagallo[1], A. Ganci[1], M. Piccione[3], A.B. Cefalù[1], M.R. Averna[1]
[1]Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza "G. D'Alessandro" (PROMISE), Università degli Studi di Palermo; [2]Pediatric Clinic, ARNAS Civico Di Cristina Benfratelli, Palermo; [3]Regional Reference Center for the Control and Treatment of Down Syndrome and Other Chromosomal and Genetic Diseases, Medical Genetics University of Palermo, Villa Sofia/Cervello-United Hospitals, Palermo
E-mail: chiara.scrimali@libero.it

Introduction. The LIPA gene located on chromosome 10 encodes the lysosomal acidic lipase (LAL), an enzyme which hydrolyzes cholesterol esters (CE) and triglycerides (TG). Cholesteryl Ester Storage Disease (CESD) is a rare recessive disease caused by mutations in LIPA gene which result in residual LAL activity. Complete LAL deficiency is associated with a more severe form of disease known as Wolman's disease. Hyperlipidemia and liver steatosis are common clinical features of CESD.
Materials and Methods. The proband, a 2 years old child, was evaluated for microcephaly. Routine laboratory data showed total high cholesterol levels (243 mg/dL) and triglycerides (272 mg/dL). Next generation sequencing was carried out on an Ion GeneStudio S5 Plus System using the Ion 540 Chip. We designed a custom panel to analyze 50 candidate genes related to LDL, HDL e triglycerides metabolism.
Results and Conclusions. No pathogenic mutations were identified in the major candidate genes for familial hypercholesterolemia and hypertrygliceridemia. However, the proband was found to be carrier of two mutations in LIPA gene (c.883C>T -p.His295Tyr- and c.929G>A - p.Trp310Ter). This result prompted to the assay of LAL activity by Dried Blood Spot Analysis. LAL activity was <5% of the normal range. The His295Tyr variant is an already known pathogenic missense mutation associated with CESD, while the Trp310Ter variant has been previously identified in homozygosity in two newborns of Sicilian origin with Wolman's disease. The family cascade screening revealed the presence of His295Tyr mutation in the proband's father and the Trp310Ter in the proband's mother. We report a case of CESD with uncommon clinical presentation features compound heterozygous for two mutations in LIPA gene.

SERUM ENDOCAN LEVELS IN PATIENTS WITH COVID-19

D. Sinicropi[1], F. Spadaro[1], M.S. Franzè[1], M. Marino[1], G. Caliò[2], G. Nunnari[3], A. David[2], G. Squadrito[1], G. Mandraffino[1]
[1]Department of Clinical and Experimental Medicine Internal Medicine Unit, University of Messina; [2]Department of Clinical and Experimental Medicine Intensive Care Unit, University of Messina; [3]Department of Clinical and Experimental Medicine Infectious Disease Unit, University of Messina
E-mail: sinicropidavide@gmail.com

COVID-19 pandemic due to the novel SARS-CoV-2 coronavirus caused -and is still causing- a high number of deaths worldwide. The clinical course of COVID is very heterogeneous and unpredictable, ranging from mild and self-limiting disease, up to more severe forms characterized by acute respiratory distress syndrome (ARDS), pulmonary embolism, multi-organ failure and death. The more severe course of the disease seems to be supported by an inappropriate inflammatory response, resulting of a direct and indirect pathogenic effect that SARS-CoV-2 exerts on endothelial cells, with consequent dysfunctional activation in a pro-thrombotic way. Under these lights, to identify a biochemical profile associated to the higher risk of evolution towards the most severe forms could be of help to recognize early the patients needing higher intensity of care.Endocan is a molecule recently acknowledged as a potential immune-inflammatory marker associated with cardiovascular events. Higher levels of endocan have been reported in some inflammatory diseases with endothelial involvement, suggesting its possible role as a marker of endothelial dysfunction.In this work we evaluated endocan serum levels in two small groups of patients with COVID-19, admitted to a standard care ward and ICU, looking for any difference and the potential correlations between endocan levels with the other variables (including medical therapy, laboratory tests and the need for oxygen therapy). The results seem to confirm a significant correlation between endocan serum levels and the need for intensive care in patients hospitalized for COVID-19.

ACHIEVING THERAPEUTIC GOAL ON LDL IN HIGH AND VERY HIGH RISK PATIENTS IN A DEDICATED OUTPATIENT CLINIC

B. Napolitano[1], A. Maretta[2], V. Spagnuolo[3]
[1]Internship Student Internal Medicine, A.O. Cosenza;
[2]Institute of Pharmacy, University of Catanzaro, Catanzaro;
[3]Lipid Center Cosenza, A.O. Cosenza
E-mail: vitalianos@yahoo.com

Background. High LDL cholesterol levels represent an important risk factor for development of cardiovascular disease. Reduction of LDL reduces cardiovascular risk. Current guidelines (ESC 2019) recommend ambitious LDL levels for high and very high risk patients. But, in clinical practice, just a part of these patients achieve the ESC 2019 goals (DA VINCI Study).
Patients and Methods. We wanted to evaluate. in the first 105 patients, at high and very high cardiovascular risk, consecutively afferent to our dedicated clinic for hyperlipidaemia (Lipid Center CS), the achievement of the therapeutic goal of LDL according to ESC 2019 guidelines. All patients were on lipid-lowering drug treatment from at least twelve months and the last blood tests were no less recent than three months.
Results. Of all patients, 61 were female. The mean age was 64.49±10.22 years. Twenty patients were in secondary prevention. Of all patients, 77% reached the therapeutic goal (65% with very high risk and 84% with high risk). 71% were taking high-intensity lipid-lowering therapy. 43% were taking combination therapy. 35% were treated with PCSK1 inhibitors.
Discussion. Taking note of the limited number of the sample, our therapeutic results achieved are higher than that achieved in the DA VINCI study in which only 60% of patients from west Europe reached the therapeutic goal. A dedicated clinic for hyerlipidaemia and the high use of combination therapy with intensive drugs and extensive use of PCSK1 inhibitors may justify the results of our study. Our center did not participated in the DA VINCI study.

FIXED COMBINATION OF MONACOLIN K AND OCTACOSANOL REDUCE LDL LEVELS IN MODERATE CARDIOVASCULAR RISK PATIENTS AND UNWILLING TO STATINS

B. Napolitano[1], A. Maretta[2], V. Spagnuolo[3]
[1]Internship Student Internal Medicine, A.O. Cosenza;
[2]Institute of Pharmacy, University of Catanzaro;
[3]Lipid Center Cosenza, A.O. Cosenza
E mail: vitalianos@yahoo.com

Background. Monacolin k is a bioactive element of fermented red rice which acts with a mechanism of inhibition of HMG-CoA reductase reducing LDL values (~ 20%). Octacosanol is an aliphatic alcohol that has a synergistic action with monacolin K inhibiting synthesis of HMG-CoA reductase. The combined use may be effective in reducing LDL values in subjects unwilling to statin therapy.
Patients and methods:
We wanted to evaluate efficacy in reducing LDL values in 25 hypercholesterolemic patients, with moderate cardiovascular risk (SCORE> 1% <5% at 10 years), unwilling to statin therapy, who received monacolin K at 10 mg with octacosanol 12 mg once daily for six weeks.

In Table I are reported initial mean values of the 25 patients.

Table I	Age (years)	Body mass index (kg/m^2)	LDL (mg/dl)
All (25)	61.5±9.5	26.5±2.12	125.8±29.9
Females (16)	63.7±9.7	23.3±3.1	116.9±29.8
Males (9)	56.2±7.4	26.5±2.1	147.2±17.9

Results
Table II shows the mean values after six weeks of therapy with monacolin 10 mg and octacosanol 12 mg in fixed combination.

Table II	Body mass index (kg/m^2)	LDL (mg/dl)
All (25)	26.5±2.12	95.2±29.8
Females (16)	23±3.3	92.25±32.4
Males (9)	26.5±2.1	102.4±23.9

Discussion. After six weeks of therapy, the mean reduction in LDL was 24% (females 21.13%; males 30.44%). This reduction is higher respect this reduction is greater than that reported in the literature with monacolin K alone.
Despite the limited number of patients evaluated, the good result on the reduction of LDL values supports the combined use of monacolin 10 mg and octacosanol 12 mg in patients at moderate cardiovascular risk and not compliant with statins therapy.

FAMILIAL HYPERCHOLESTEROLEMIA GENETIC CHARACTERIZATION: WHAT ELSE BEYOND LDLR?

E. Sticchi[1], A. Kura[2], R. De Cario[2], G. Barbieri[2], A. Magi[3], S. Suraci[2], A.M. Gori[1], F. Cesari[4], G.M. Scaturro[5], E. Lotti[4], R. Marcucci[1], B. Giusti[1]
[1]University of Florence, Department of Experimental and Clinical Medicine, Atherothrombotic Diseases Center, Careggi Hospital, Florence; [2]University of Florence, Department of Experimental and Clinical Medicine, Florence; [3]University of Florence, Department of Information Engineering, Florence;
[4]Atherothrombotic Diseases Center, Careggi Hospital, Florence;
[5]Metabolic Disease Unit, AOU Meyer
E-mail: elenasticchi@hotmail.com

Background. As many subjects (about 60%) with Familial Hypercholesterolemia(FH) did not demonstrate functional mutations in FH major candidate genes (LDLR, APOB, PCSK9, LDLRAP1), we genetically characterized FH patients through targeted high-throughput sequencing (HTS).
Method. We analysed 79 patients with clinically possible/probable/definite FH according to Dutch Lipid Score. Targeted HTS (57 genes including those involved in lipid metabolism, supposed to be involved in dyslipidemia, pharmacogenetics of statins, related to FH polygenic forms, HDL and triglycerides related diseases) was assessed by Illumina technology.
Results. Among 79 patients, 35 carried a rare variant in LDLR gene, whereas 44 were LDLR-negative. Talmud score evaluation (Talmud 2013) in both groups showed a higher mean value in patients without LDLR mutations, with respect to LDLR-positive (0.977±0.203 vs 0.937±0.184). HTS analysis revealed that 8 LDLR mutation-positive patients also carried likely pathogenetic/uncertain significance mutations in APOB or LDLRAP1 genes. In patients without LDLR mutations, at least 2 rare variants were identified in 20 patients (46%), and at least 3 rare variants were identified in 14 patients (32%). In these patients, 82 rare variants with uncertain significance/conflicting interpretation of pathogenicity were identified in 34 different genes (APOB, PCSK9, LDLRAP1, ABCB1, ABCG2, ABCG5, ABCG8, ANGPTL3, APOA4, CELSR2, CETP, CREB3L3, DAB2, GCKR, GHR, HFE, ITIH4, LCAT, LIPC, LIPI, LMF1, LPA, LPL, LRP1, MTTP, NPC1, NYNRIN, PON1, PPP1R17, SCARB1, SLCO1B1, SLC12A4, SREBF1 SREBF2). 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. The present study suggest the possible contribution of multiple loci, beyond LDLR gene, in influencing lipid profile alterations and cardiovascular risk. Further expansion of present data might allow a better comprehension of major/modifier genes role, as well as of accumulation of common small-effect LDL-C raising alleles in determining LDL-C levels and cardiovascular events.

ROLE OF THE ASIALOGLYCOPROTEIN RE CEPTOR 1 ON LIPOPROTEIN METABOLISM AND ATHEROSCLEROSIS

M. Svecla[1], A. Moregola[1], P. Uboldi[1], L. Da Dalt[1], F. Bonacina[1], A.L. Catapano[2], G.D. Norata[3]
[1]Department of Pharmacological and Biomolecular Sciences, University of Milan; [2]Department of Pharmacological and Biomolecular Sciences, University of Milan; [2]IRCCS Multimedica Hospital, Sesto San Giovanni (MI); [3]Department of Pharmacological and Biomolecular Sciences, University of Milan; [3]Centro SISA, Ospedale Bassini, Cinisello Balsamo (MI)
E-mail: monika.svecla@unimi.it

Background. The hepatic asialoglycoprotein receptor 1 (ASGR1) is involved in the removal from circulation of several desialylated glycoproteins including vitamin B12, alkaline phosphatase, low-density lipoproteins (LDL), chylomicron remnants, fibronectin, IgA but also platelets, apoptotic cells. Loss of function in ASGR1 results in lower levels of plasma non-HDL-cholesterol compared to non-carriers and a 34% reduction in the risk of coronary artery disease.
Aim. To investigate the molecular mechanism of ASGR1 in controlling lipoprotein catabolism and liver metabolism under hypercholesterolemic conditions.
Methods. Fast protein liquid chromatography (FPLC) was performed in the plasma of LDL-R-/- and ASGR1-/-/LDL-R-/- mice, fed in western type diet (WTD) for 16 weeks, and shotgun proteomics using orbitrap Fusion™ Tribrid™ Mass Spectrometer was performed in the liver of LDL-R-/- and ASGR1-/-/LDL-R-/- mice followed by protein inference, label-free quantification, and pathway enrichment analysis.
Results. Plasma cholesterol was reduced in ASGR1-/-/LDL-R-/- compared with LDL-R-/- (p-value=0.03). When the analysis was focused on enzymes for N-glycosylation cascade in the liver, Ddost, which controls glycans transfer from Dolicol-P-P to asparagine in ER, was down-regulated (p<0.001) while glucosidases (Ganab, Prkcsh) which favor proper protein folding in concert with the lectin chaperon Calnexin/Calreticulin and ERp57 were downregulated. In parallel ERGIC-53 that operates the transport of glycoproteins from ER to Golgi was significantly down-regulated while Derlin-1 (p-value<0.01) which increases during unfolding proteins response was up-regulated. These data suggest that reduced production of glycosylated proteins occurs during atherosclerosis coupled with a reduction in transporting proteins from ER to Golgi.
Conclusion. Our data suggest that an alteration in protein N-glycosylation takes place within the liver of WTD fed LDL-R vs ASGR1/LDL-R KO mice. This can further affect the synthesis of apolipoproteins secreted in plasma.

PLASMA AND CEREBROSPINAL FLUID LIPID PROFILE AND LIPOPROTEINS CHARACTERIZATION IN ALZHEIMER'S DISEASE

M. Turri[1], C. Pavanello[1], E. Conti[2], F. Gastoldi[1], L. Tremolizzo[3], L. Calabresi[1]
[1]Centro E. Grossi Paoletti, Department of Pharmacological and Biomolecular Sciences, University of Milano; [2]Lab of Neurobiology, School of Medicine and Surgery and Milan Center of Neuroscience, University of Milano-Bicocca, Monza; [3]Neurology Unit, San Gerardo Hospital, ASST Monza
E-mail: marta.turri@unimi.it

Aim. The inverse association between the risk of developing Alzheimer's disease (AD) and plasma HDL-C levels has been demonstrated in different epidemiological studies. The mechanism by which plasma HDL could influence the pathogenesis and progression of AD is still unsolved. It has been suggested a direct involvement of plasma HDL on brain cholesterol homeostasis, related to the ability of specific HDL subclasses to cross the blood-brain barrier. Despite the consistent knowledge of the relation with plasma HDL-C levels, a qualitative analysis of plasma and brain HDL is still lacking. In this study HDL subclasses have been characterized in plasma and cerebrospinal fluid (CSF) of AD patients.
Materials and Methods. 42 AD patients (19M/23F, 75±7 y.o.) and 17 non-AD demented (8M/9F, 69±8 y.o.) were recruited at the memory clinics of the San Gerardo hospital and 5 cognitively-intact control subjects (3M/2F, 70±8 y.o.) were recruited from patients undergoing knee replacement surgery. Blood and CSF samples have been collected and plasma separated by low-speed centrifugation at 4°C. A complete plasma lipid-lipoprotein profile was determined using a Roche Integra c311 analyzer. CSF total cholesterol (TC) and unesterified cholesterol (UC) content have been measured by HPLC. ApoE and apoA-I in CSF were determined by SDS-page electrophoresis, PL content by commercial fluorometric assay kit. HDL subclasses have been characterized in plasma and CSF by non-denaturing two-dimensional (2D)-electrophoresis, followed by immunodetection with polyclonal antibodies against human apoE, apoA-I and apoA-II.
Results. Plasma UC/TC ratio of AD patients (0.19±0.08) is similar to controls (0.20±0.03), but UC/TC in AD CSF (0.45±0.13) is higher than controls (0.40±0.12), suggesting a defect in esterification process in CSF of AD patients. Plasma HDL subclass distribution in AD patients is similar to that of healthy controls. Three different classes of apoA-I-containing HDL are detected; they comprise small pre-β HDL, and α-migrating HDL2 and HDL3. Curiously, CSF apoE-containing lipoproteins showed only α-migrating particles in all samples. CSF apoA-I-containing lipoproteins of AD patients are characterized by a high amount of α-migrating HDL; in addition, pre-α-migrating apoA-I-containing lipoproteins have been detected in CSF samples from AD patients. The migration of CSF lipoproteins containing apoA-II is similar to apoA-I lipoproteins and their profile looks analogue to plasmatic HDL.
Conclusions. Since it is known that apoA-I and apoA-II mRNA are not present in central nervous system, this qualitative characterization of HDL together with their quantitative determination supports the hypothesis of a direct role of plasma HDL in brain cholesterol homeostasis. Further studies are necessary to evaluate if parameters related to HDL-C may represent biomarkers of disease's risk and progression.

EFFECT OF LIPID-LOWERING THERAPIES ON C-REACTIVE PROTEIN LEVELS: A META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

S. Xie[1], F. Galimberti[2], E. Olmastroni[1], M. Casula[2], A.L. Catapano[2]
[1]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan; [2]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan, IRCCS MultiMedica, Sesto San Giovanni (MI)
E-mail: sining.xie@unimi.it

Cardiovascular diseases are widely recognized as having an inflammatory component. Yet, it is not clear whether inflammation simply accompanies the atherosclerotic process or represents the causal driver. Our study aimed to assess the anti-inflammatory effect of lipid-lowering drugs on C-reactive protein (CRP) levels, in addition to lipid reduction. We conducted a meta-analysis according to the PRISMA reporting guidelines. PubMed, Web of Science, EMBASE, Cochrane Library and ClinicalTrial.gov were searched since inception to June 2021.
Inclusion criteria were:
1) randomized controlled trials (RCTs) in human, phase II, III or IV;
2) English language;
3) reporting the effects on CRP levels;
4) with intervention duration more than 3 weeks;
5) and sample size (for each arm) over than 100 subjects.

Pooled estimates were assessed by random-effects model. Between-study heterogeneity was tested by Cochrane's Q test and measured with the I2 statistics. For statins, 11 RCTs were included in the meta-analysis, accounting for 46,499 participants. An additional 1.10 mg/L (95%CI, -1.11 to -1.10) absolute reduction of CRP concentration was observed for statin-treated group compared to placebo. For lipid profile, there was a significant decrease of 51.28 mg/dL for LDL-C levels and 19.85 mg/dL for TG levels. For PCSK9 inhibitors, 5 trials were included with 47,709 participants. CRP level was slightly increased by 0.06 mg/L (95%CI, 0.06 to 0.06). LDL-C and TG levels were significantly reduced by 62.28 mg/dL and 26.70 mg/dL, respectively. For Ezetimibe, also 5 trials were included with 15,505 participants. A 0.66 mg/L (95%CI, -0.68 to -0.64) absolute decrease of CRP level was found in the ezetimibe-combined treatment group compared to single-drug treatment group. Both LDL-C and TG were decreased (-17.28 mg/dL and -13.32 mg/dL, respectively).In conclusion, that statin and ezetimibe administration reduce serum CRP concentration while, patients treated with PCSK9 inhibitors experience no significant changes in CRP levels.

INDICE DEGLI AUTORI