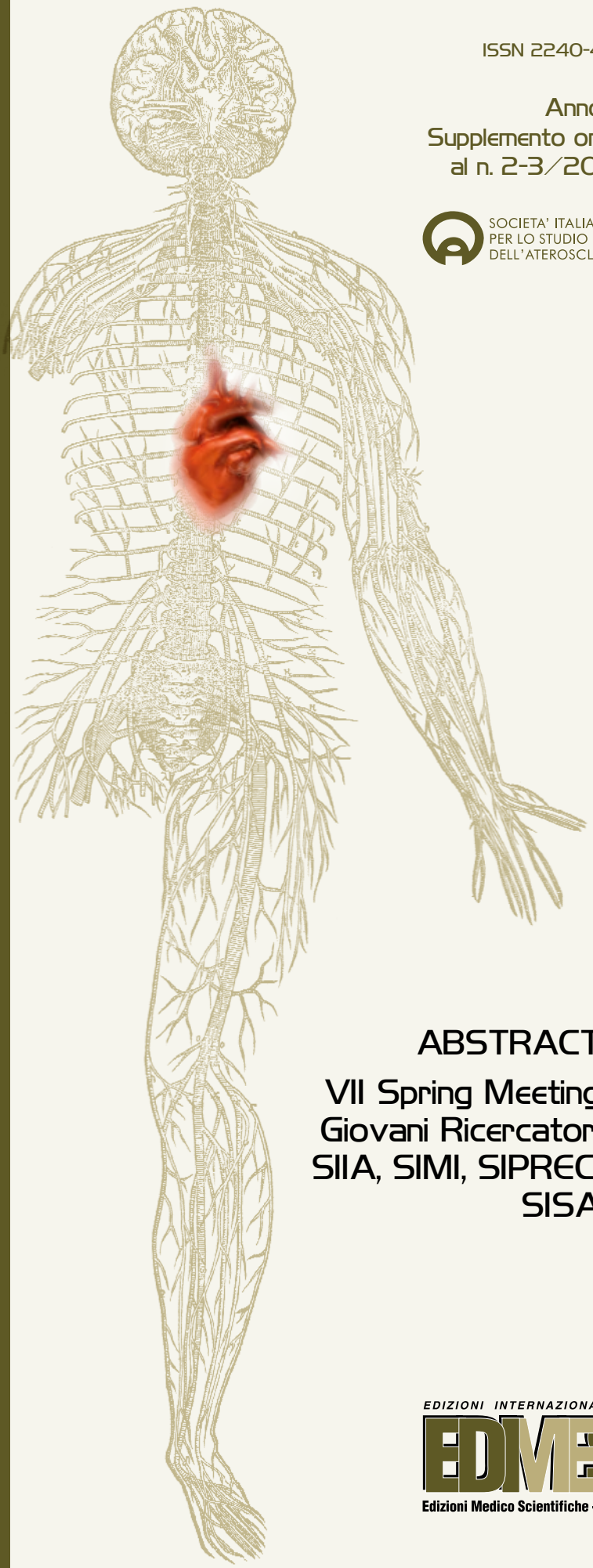
GIORNALE ITALIANO dell'ARTERIOSCLEROSI

ISSN 2240-4821

Anno 13
Supplemento online
al n. 2-3/2022

SOCIETA' ITALIANA
PER LO STUDIO
DELL'ATEROSCLEROSI



ABSTRACT

VII Spring Meeting
Giovani Ricercatori
SIIA, SIMI, SIPREC,
SISA

EDIZIONI INTERNAZIONALI srl
EDIMES
Edizioni Medico Scientifiche - Pavia

SOCIETÀ ITALIANA PER LO STUDIO DELLA ATEROSCLEROSI (SISA)

Spring Meeting Giovani Ricercatori SIIA, SIMI, SIPREC, SISA 2022

La ricerca in ambito cardiometabolico: ritorno al futuro

5-7 Giugno 2022

Dopo due anni, è tornato finalmente in presenza lo Spring Meeting dei Giovani Ricercatori che si è svolto per la sua settima edizione a Rimini dal 5 al 7 giugno 2022. Come nelle scorse edizioni, l'evento ha coinvolto i gruppi giovani della SISA (Società Italiana per lo Studio dell'Aterosclerosi), SIMI (Società Italiana Medicina Interna), SIIA (Società Italiana dell'Ipertensione Arteriosa) e SIPREC (Società Italiana per la Prevenzione Cardiovascolare).

Essenziale e prezioso è risultato il supporto delle società coinvolte che hanno creduto ancora una volta nei propri gruppi giovani, sostenendoli nell'organizzazione dell'evento.

Moltissimi sono stati gli abstract inviati, a conferma dell'entusiasmo dei giovani per il congresso. Tra questi 25 sono stati selezionati per le comunicazioni orali e 44 per i poster, tutti di ottimo livello e risorsa di interessanti spunti di discussione.

Per il secondo anno consecutivo, a completamento dell'attività congressuale in presenza, è stata utilizzata una piattaforma digitale (accessibile al link https://padlet.com/FondazioneSisa/SPRING2022) dove gli autori hanno potuto condividere un breve video di presentazione per farsi conoscere e per mostrare i dati del proprio lavoro oltre che promuovere la discussione, anche "off-line" prima dell'apertura ufficiale dei lavori.

Non sono mancate le classiche relazioni su invito, in cui cinque giovani relatori delle diverse società hanno mostrato i risultati della loro ricerca di base e clinica, affrontando argomenti di grande interesse generale.

Questi gli argomenti trattati: "Tossicità cardiovascolare in pazienti oncologici trattati con chemioterapia" (Allegra Battistoni, Università La Sapienza - Roma), "NAFLD from pathogenesis to clinic (Rosa Lombardi, Università degli Studi di Milano), "Il deficit di LCAT: un modello di ricerca traslazionale" (Chiara Pavanello, Università degli Studi di Milano), "La complessità della risposta sistemica alle infezioni gravi: nuovi scenari e prospettive future" (Alessandro Russo, Università della Magna Graecia - Catanzaro) "Hypertension mediated organ damage: from bench to bedside" (Giacomo Pucci, Università di Perugia).

Parte del congresso, come da format consolidato da anni, è stata dedicata ai workshop, che si sono occupati di temi di interesse trasversale alle diverse società.

Il primo workshop ha trattato il tema dell'informazione scientifica "Information Vs Disinformation: How to survive the infodemic" (Viola Guardigni), un argomento che ha riguardato tutti i ricercatori durante la pandemia da COVID-19 e che è stato oggetto di ampia discussione. Il secondo workshop "Come selezionare e sviluppare un buon quesito scientifico" (Simone Birocchi) ha fornito ai discenti alcuni strumenti di base per individuare un valido quesito scientifico nei diversi ambiti di ricerca e impostare un metodo per utile per trovare una risposta. L'ultimo workshop, infine, ha fornito interessanti spunti, sia per la ricerca di base che per la ricerca clinica, su "Il valore del patient engagement nella ricerca e nella pratica clinica" (Caterina Bosio), portando a discutere circa le possibili modalità per ottenere un coinvolgimento attivo del paziente nel processo di cura.

Nella tavola rotonda sui "Forthcoming project: condivisione di progetti di ricerca tra le tre società", sono stati discussi 9 diversi progetti con possibile sviluppo inter-societario (SIMI-COVID, REPOSI, COVID-Burnout, Survey ECO Torace per SIMI; Appropriatezza prescrittiva per SIIA; Controllo da remoto del rischio CV durante la pandemia da COVID-19 per SIPREC; Lipigen Pediatrico, PCSK9-CKD e FCS LIPIGEN per SISA).

Infine, un momento cruciale del programma sono risultate le varie attività di networking, pianificate e non, che hanno permesso la nascita di nuove collaborazioni societarie ed inter-societarie e l'ideazione di ulteriori proposte progettuali.

Complessivamente hanno partecipato al meeting 91 persone di cui 22 tra relatori, moderatori, comitato organizzatore e comitato scientifico e 69 iscritti.

39 partecipanti risultano iscritti alla SISA, 10 alla SIIA, 15 alla SIMI, 1 alla SIPREC, 1 a SIMI e SISA e 1 a SIIA e SIPREC, 1 a SIIA e SIMI e 1 a SIIA, SIMI e SISA

Il successo dell'evento è stato confermato da molteplici punti di vista: tutte le sessioni sono state partecipate con grande entusiasmo e caratterizzate da una vivace discussione; i feedback raccolti in presenza hanno mostrato la soddisfazione dei partecipanti e degli organizzatori.

Lo Spring Meeting si è riconfermato così un momento importante per dare visibilità e opportunità di confronto, crescita, e collaborazione ai giovani ricercatori.

L'appuntamento è quindi allo Spring Meeting 2023!

A nome di tutti i membri del comitato gruppo giovani ricercatori della SISA
Chiara Pavanello, Vanessa Bianconi, Mario Luca Morieri, Manuela Casula, Laura D'Erasmo, Antonella Giammanco, Fabio Nascimbeni

COMUNICAZIONI ORALI

INCREASED RISK OF NEW ONSET OF ARTERIAL HYPERTENSION IN CARLFIZOMIB USE FOR MULTIPLE MYELOMA TREATMENT

G. De Fazio[1], A. Melaccio[1], M. Cavallo[1], A. Vacca[2], R. Ria[1], S. Cicco[1]
[1]Unit of Internal Medicine "Guido Baccelli", Department of Biomedical Sciences and Human Oncology, University of Bari "Aldo Moro";
[2]Division of Internal Medicine, Department of Medicine, University of Udine

Aim. Several drugs are used today to counter Multiple Myeloma (MM). Some of these are loaded with an increased risk of developing cardiovascular disease. It is described cardiovascular disease due to Carfilzomib, a known proteasome inhibitor. However, many factors may lead to LV dysfunction such as arterial Hypertension. Purpose is to evaluate if patients who underwent to Carfilzomib treatment may present an increased incidence of arterial Hypertension.
Methods. We evaluate 34 patients (Group K, 20M, aged 65.87±5.98) treated with Carfilzomib. Among them, 25 take 56 mg/m^2 (group 14M, aged 67.30±5.98) while 9 were treated with 27 mg/m^2 (group 2, 6M, aged 63.00±5.39). As controls, 34 patients (16M, aged 69.80±9.13) who underwent in the same period to MM treatment without Carfilzomib.
Hypertension was identified as new onset or disease worsening in patients who were already affected (need to double the dose used, add a new drug, or no pressure control in at least 3 drugs treatment).
Results. At baseline, Controls presented an increased arterial hypertension rate (76.47% vs GroupK 47.06%, p=0.07). There was no difference in any other CVD risk factor. There was no difference in LV mass, volumes and ejection fraction. Group K experienced an increased incidence of arterial hypertension compared to controls (Controls 0% vs GroupK 41.17%, p=0.001). This result is not related to the drug dose. Finally, there was no difference in mortality between the two groups also analysing CV death.
Conclusion. Carfilzomib is a potent drug useful in MM. Its cardiovascular toxicity relates to a vascular and endothelial damage leading to a new onset of arterial hypertension. This observation may be useful in preventing evolution of CV dysfunction in survivals and long survivals, as well as a good question to answer in studying vascular damages in arterial hypertension pathophysiology.

THE FIBRINOGEN-LIKE DOMAIN OF ANGPTL3 FACILITATES LIPOLYSIS IN 3T3-L1 CELLS BY ACTIVATING THE INTRACELLULAR ERK PATHWAY

S. Bini, V. Pecce, A. Di Costanzo, L. Polito, A. Ghadiri, I. Minicocci, F. Tambaro, S. Covino, M. Arca, L. D'Erasmo
Department of Translational and Precision Medicine "Sapienza" University Rome

Background. Angiopoietin-like protein 3 (ANGPTL3) is a key player in lipoprotein metabolism. Also, it has been reported that ANGPTL3 stimulates lipolysis in adipocytes and its C-terminal fibrinogen-like domain (ANGPTL3-Fld) is able to bind the cellular integrin receptor $\alpha 3\beta v$. We evaluated the effect of ANGPTL3-Fld in stimulating lipolysis and characterized the intracellular kinases involved in this action.
Material and Methods. 3T3-L1 differentiated adipocytes were treated with ISO, ANGPTL3-Fld, or a combination of both. Lipolysis was evaluated through the release of FFA in the culture medium using ELISA. We also evaluated the intracellular activation of the hormone-sensitive lipase. The phosphorylation status of intracellular kinases was evaluated using a commercial proteome profiler with and without inhibition of the ERK arm of MAPK pathway.
Results. Although the treatment with ANGPTL3-Fld alone was not able to activate lipolysis in 3T3-L1 adipocytes, the combination of ANGPTL3-Fld and ISO determines a 2-fold enrichment of free fatty acids in the culture medium with an incremental effect compared to that obtained with ISO alone. Adipocytes treated with ANGPTL3-Fld alone showed a higher inhibitory phosphorylation of HSL that was activatory in the case of ISO alone. The co-treatment determined a marked increase in the activatory phosphorylation of HSL. Then, we identified that ANGPTL3-Fld generates an intracellular signal that activates the MAPK-ERK pathway possibly acting through the PDGFRβ - PLCγ axis and thus activating AMPK.
Conclusion. ANGPTL3-Fld appears to act as lipolysis facilitator in adipocytes and this effect was driven by the activation of a transduction signal different from the canonical β-adrenergic dependent pathway.

EFFECT OF CONTINUOUS POSITIVE AIRWAY PRESSURE TREATMENT ON GLYCEMIC AND LIPID PROFILES IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

F. Cattazzo[1], M.F. Pengo[2], A. Giontella[1], D. Soranna[2], J. Karalliedde[3], L. Gnudi[3], P. Minuz[1], C. Lombardi[2,4], G. Parati[2,4], C. Fava[1]
[1]Department of Medicine, University of Verona, Section of General Medicine and Hypertension, Verona;
[2]IRCCS, Istituto Auxologico Italiano; Department of Cardiovascular, Neural and Metabolic Sciences, S. Luca Hospital, Milan;
[3]School of Cardiovascular Medicine & Sciences, Section Vascular Biology and Inflammation, Unit for Metabolic Medicine, King's College London, UK;
[4]Department of Medicine and Surgery, University of Milano-Bicocca, Milan

Background and Aim. Continuous Positive Airway Pressure (CPAP) is the main therapy for obstructive sleep apnea (OSA). Nevertheless, uncertainty remains about the effectiveness of CPAP in improving OSA-related metabolic dysregulation. This meta-analysis of randomized controlled trials (RCTs) aimed to investigate whether CPAP, as compared to other control treatments, could improve glucose metabolism and/or lipid metabolism in OSA patients.
Methods. Relevant articles were searched in three different databases (MEDLINE, EMBASE and Web of Science) from inception to 6stFeb 2022 through specific search terms and selection criteria.
Results. From a total of 5,962 articles, 32 RCTs were included. CPAP treatment significantly decreased fasting plasma glucose (FPG) (standardized difference in means [SDM]=-0.11 [95%CI, -0.17- -0.33], p=0.004) and improved insulin sensitivity reducing both fasting plasma insulin (SDM=-0.17 [95%CI, -0.28- -0,05], p=0,005) and HOMA-IR (SDM=-0.19 [95%CI, -0.29- -0,08], p=0,001). In subgroup analysis, prediabetic patients and those that demonstrated either a greater CPAP usage (≥4/h per night) or an apnoea-hypopnea index≥30 events/h at baseline showed the greatest response to CPAP. Regarding lipid metabolism, CPAP was associated with a significant reduction of both total cholesterol (TC; SDM=-0.19 [95%CI, -0.28- -0,1], p=<0.0001) and LDL-cholesterol (SDM=-0.11 [95%CI, -0.2- -0,01], p=<0.03). In sensitivity analysis, favorable metabolic effects were present in patients that exhibited a greater adherence to CPAP, in those that presented more severe nocturnal oxygen desaturations (Minimum SatO2 <77%) and an apnoea-hypopnea index ≥30 events/h at baseline. CPAP treatment did not affect glycated haemoglobin, triglycerides, and HDL-cholesterol levels.
Conclusion. CPAP treatment, even if with low effect size, improves insulin sensitivity and reduces FPG, TC and LDL-cholesterol levels in OSA patients. Prediabetic patients and those that exhibited a greater CPAP usage as well as apnoeic events or oxygen desaturations at baseline may benefit the most from CPAP.

IS GENOTYPE DETERMINED LP(a) SUPERIOR TO MEASURED LP(a) CONCENTRATION AS PREDICTOR OF CARDIOVASCULAR RISK?

F. Galimberti[1], E. Olmastroni[1,2], A.L. Catapano[1,2], B.A. Ference[3]
[1]IRCCS MultiMedica, Sesto San Giovanni (Milano);
[2]University of Milan, Epidemiology and Preventive Pharmacology Service (SEFAP) - Department of Pharmacological and Biomolecular Sciences, Milan;
[3]Centre for Naturally Randomized Trials at the University of Cambridge, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom

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

EARLY IMPAIRMENT IN MITOCHONDRIAL QUALITY CHECK AND FUNCTION PRECEDES THE DEVELOPMENT OF CARDIAC PHENOTYPES IN A MOUSE MODEL OF FABRY DISEASE

J. Gambardella[1,2], A. Fiordelisi[1], F. Cerasuolo[1], R. Avvisato[1], C. Perrino1, S. D'Apice[1], R. Paolillo[1], D. Sorriento[1], G. Iaccarino[1,2]
[1]Università degli Studi di Napoli Federico II, Naples;
[2]Centro Interdipartimentale di Ricerca in Ipertensione Arteriosa e Patologie Correlate

Background. Fabry disease (FD) is caused by genetic deficiency of α-GalA activity with accumulation of globotriaosylceramide (GB3). FD-patients develop left ventricular hypertrophy and dysfunction, whose pathogenesis is unclear. Beside GB3 accumulation, impairment of energetic metabolism and mitochondria is a putative mechanism, suggested by reduced respiration in fibroblasts from FD-patients.
Aim. To explore the pathogenic mechanism of cardiac dysfunction in FD evaluating the role of mitochondria.
Methods. R301Q-GLA [Tg)/mα-GalA knockout mice were used as humanized FD-model. On WT and FD MEFs we assessed mitochondrial phenotype. In-vivo, we performed cardiac ultrasounds (CUS), then, ex-vivo histological and biochemical studies, and contractility-analysis ex-vivo.
Results. FD-MEFs showed an impairment of mitochondrial respiration rate and reduced ATP content. Increased mitochondrial levels of DRP1, LC3II, and MFN-ubiquitination indicated the presence, in FD-cells, of damaged mitochondria targeted for mitophagy that did not get removed, confirmed by accumulation of fragmented mitochondria and autophagosomes. Mitophagy-induced mitochondrial biogenesis was also affected as demonstrated by reduced PGC-1α. Altogether, these data suggest impaired mitophagy and quality check of FD-mitochondria. CUS showed that FD-mice developed diastolic dysfunction at 9 months with increased **E/E'**. At the same age, we observed cardiomyocytes hypertrophy, confirmed by increasing BNP and ANP-levels. Accordingly, FD-CMs showed higher basal contractability compared to WT-CMs and reduced inotropic response to adrenergic stimulation. Cardiac alterations of mitochondrial function were observed already at 3 months, including increased mitochondrial MFN2-ubiquitination and decreased PGC-1α-levels. Starting from 6 months, mitochondrial damage was obvious as we observed increased mitochondrial levels of DRP1 and LC3II with accumulation of disarranged- mitochondria and autophagosomes, confirming the progressive deposition of damaged mitochondria. Impairment of mitochondrial respiration in FD-CMs and reduced myocardial ATP-content was also detected, together with increased cytosolic levels of Cit-C, marker of mitochondrial damage and permeabilization.
Conclusions. Mitochondrial dysfunction in FD, due to impaired autophagy/mitophagy, is early and progressive, and precedes late cardiac hypertrophy and dysfunction.

CORONARY CT AND NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD). IS HEPATIC STEATOSIS AN INDEPENDENT FACTOR IN THE ASSESSMENT OF CARDIOVASCULAR DISEASE?

E. Lustri[1], G. Sinatti[1], A. Cirella[1], A. Bracci[2], L. Evangelista[2], S.J. Santini[1], E. Di Cesare[2], C. Balsano[1]
[1]Department of Clinical Medicine, Life, Health & Environmental Sciences-MESVA, University of L'Aquila;
[2]Department of Applied Clinical Sciences and Biotechnology, University of L'Aquila

Aim. The natural history of NAFLD is variable and patients may progress from simple hepatic steatosis (HS) to steatohepatitis up to end-stage liver disease. Growing evidence indicates that the presence of NAFLD increases cardiovascular (CV) morbidity and mortality.
Among non-invasive procedures, computed tomography (CT) is routinely used to detect and characterize liver steatosis. NAFLD is associated with increased coronary calcifications.
The aim of this study is to evaluate the correlation between the degree of NAFLD and coronary calcification using coronary CT, in terms of prognostic stratification of asymptomatic NAFLD patients at risk of developing coronary heart disease (CHD).
Methods. A retrospective cohort of 270 patients (181 males and 89 females), who underwent coronary CT in the period between 2017 and 2021, was enrolled. Images were acquired by prospective cardio-synchronous technique; pre-contrastographic scan performed for Calcium Score (CS), was extended to the upper abdomen for assessment of liver density. CS (using Agatston score), hepatic steatosis (in terms of density in HU) and degree of fibrosis (using FIB-4 score) were evaluated.
Results. Data on 270 patients were analyzed. On coronary CT images, the association between the Agatston score and the grading of hepatic steatosis was significant (p 0.03). The CS also showed a close correlation with the degree of fibrosis as assessed by the FIB-4 score (p 0.0091), higher in males (p 0.003) than in females. Age was also strongly associated with CS ($p<0.001$).
Conclusions. Our study suggest that should be considered the opportunity to perform coronary CT to patients with high NAFLD fibrosis score in order to prevent the onset of major cardiovascular events in NAFLD asymptomatic patients.

CORRELATION BETWEEN VASCULAR INFLAMMATION MARKERS, DIASTOLIC DYSFUNCTION AND CARDIOVASCULAR RISK IN PATIENTS WITH TAKAYASU ARTERITIS

S. Cicco[1], V. Desantis[1,2], A. Vacca[3], G. Cazzato[4], A.G. Solimando[1], A. Cirulli[1], S. Noviello[1], G. Brosolo[3], C. Catena[3], R. Ria[1,†,*], M. Montagnani[2,†]

[1]Unit of Internal Medicine and Clinical Oncology, Department of Biomedical Sciences and Human Oncology (DIMO), University of Bari Aldo Moro Medical School, Bari;
[2]Department of Biomedical Sciences and Human Oncology, Pharmacology Section, Medical School, University of Bari Aldo Moro, Bari;
[3]Division of Internal Medicine, Department of Medicine, Building 8, University of Udine;
[4]Section of Pathology, Department of Emergency and Organ Transplantation (DETO), University of Bari "Aldo Moro", Bari

Aim. Takayasu arteritis (TAK) increases vascular stiffness and arterial resistance. Abnormal immune response is a crucial factor in the pathogenesis of TAK. We investigated:

1) vascular and cardiac ultrasonography parameters as increased cardiovascular risk in TAK patients, compared to atherosclerotic patients;
2) Treg and Th17 cells frequency in TAK-refractory patients treated with infliximab.

Methods. Clinical, instrumental and biochemical data in patients with active TAK were compared in a case control study to age- and sex-matched atherosclerotic patients. In a subpopulation of TAK patients, Treg/Th17 cells percentage was measured before (T0) and after 18 months (T18) of infliximab treatment. Echocardiogram, supra-aortic Doppler ultrasound, and lymphocytogram were carried out in all patients. Histological and immunohistochemical analysis were performed to correlate vessel wall patho-morphology and clinical/laboratory results

Results. TAK patients displayed increased aortic valve dysfunction and diastolic dysfunction compared to atherosclerosis. Moderate-to-severe aortic regurgitation correlates with the highest serum levels of uric acid in TAK patients. A significant increase in aortic stiffness was associated with peripheral T lymphocyte levels. Increase in CD3+CD4+ and CD8+ infiltration and HMGB1 was significantly higher in TAK group. CD15+ neutrophils were significantly higher in TAK, suggesting an association with inflammation-related vascular damage. Flow cytometric Tregs percentage was significantly reduced in TAK patients. Patients treated with infliximab, Treg significantly increased at T18 compared to T0 while CD3+CD4+IL-17+ cells behaved in the opposite way. Supporting the specific pathogenetic mechanisms of vessel damage in TAK, we found an increased risk of cardiovascular disease that correlates directly with the degree of inflammatory cell infiltration in the vessel wall.

Conclusions. These observations strengthen the clinical efficacy of infliximab in TAK patients, supporting the idea that biologic therapy may achieve a better control of TAK progression and help to stabilize the Treg/Th17 score toward values similar to those found in atherosclerotic patients.

INCREASED LIPOPROTEIN PRODUCTION PREVENTS LIVER MITOCHONDRIAL CHOLESTEROL ACCUMULATION AND METABOLIC IMPAIRMENT

L. Da Dalt[1], M. Pedrelli[2], C. Pramfalk[2], M. Olin[2], G.D. Norata[1,3], P. Parini[2,4]

[1]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano;
[2]CardioMetabol Unit, Department of Laboratory Medicine and Department of Medicine, Karolinska Institute, Huddinge, Sweden;
[3]Centro SISA per lo studio dell'Aterosclerosi, Ospedale Bassini, Cinisello Balsamo (Milano);
[4]Medicine Unit Endocrinology, Theme Inflammation and Ageing, Karolinska University Hospital, Stockholm, Sweden

Background and Aim. Mature normal hepatocyte express only SOAT2 and mainly use mitochondrial oxidative metabolism for energy production. Hence, depletion of SOAT1 in Human Hepatoma HepG2 cells better resembles the normal human hepatocytes intracellular cholesterol and lipoprotein metabolism. The aim of this study was to investigate in SOAT2-only-Hepg2 cells whether the changes in intracellular cholesterol, due to the overactivation of SOAT2 in the presence of human serum, impacts mitochondrial metabolism and promotes the development towards a more mature hepatocyte phenotype.

Methods. SOAT2-only-HepG2 and wild-type HepG2 cells were cultured with human serum (HS, 2%) pooled from 8 healthy individuals or with fetal bovine serum (10%). qPCR, mitochondrial isolation, flow-cytometry, and Seahorse analysis were performed.

Results. In SOAT2-only-HepG2 cells cultured either in FBS or HS the expression of all mitochondrial fusion and fission genes, as well as of genes involved in mitochondrial metabolic pathways, were increased. Further, SOAT2-only-HepG2 cells cultured in HS have an increased mass of active mitochondria. These mitochondria contain less free cholesterol compare to those of wild-type HepG2 cells cultured in FBS. The increased active mitochondrial mass in SOAT2-only-HepG2 cells determines an increased metabolic activity and might reduce the proliferation rate.

Conclusion/Discussion. Given the role of free cholesterol in maintaining the mitochondrial inner and outer membrane shape, the modulation of intracellular cholesterol level by SOAT2 seems to be critical for preserving the low mitochondrial cholesterol levels needed for the metabolic preference toward mitochondrial Oxidative Phosphorylation OXPHOS and for the normal adult hepatocyte phenotype, explaining also why tumoral human hepatocytes express both SOAT1 and SOAT2.

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

M. Gueli Alletti, P. Bigolin, F. Brucato, C. Scrimali, R. Spina, V. Ingrassia, G.I. Altieri, F. Fayer, D. Noto, G. Misiano, A. Giammanco, C.M. Barbagallo, A. Ganci, A.B. Cefalù, A. Zambon, M.R. Averna
Dipartimento PROMISE - Università degli Studi di Palermo

Aim. 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).
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.
Results. 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.
Conclusions. Lomitapide was effective in improving post prandial metabolism of lipoproteins in subjects with FCS. No benefits were observed for tiparvovec.

LIPOPROTEINS AND CENTRAL NERVOUS SYSTEM: HDL METABOLISM IN ALZHEIMER'S DISEASE

M. Turri[1], C. Pavanello[1], F. Gastoldi[1], E. Conti[2], D. Emide[3], A. Barbiroli[3], L. Tremolizzo[2-4], L. Calabresi[1]
[1]Centro Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano;
[2]Scuola di Medicina e Chirurgia e centro di Neuroscienze di Milano (NeuroMI), Università di Milano-Bicocca, Monza;
[3]Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Università degli Studi di Milano;
[4]Unità di Neurologia, Ospedale San Gerardo, Monza

Background and Aim. A growing number of evidence indicates a strong inverse association between the risk of developing Alzheimer's disease (AD) and plasma HDL-C levels, suggesting an involvement of cholesterol metabolism in the etiology and progression of AD, however the mechanism is still unclear. Within this study the HDL metabolism in plasma and CSF of AD patients was investigated.
Materials and Methods. 50 AD (19M/31F, 74±7 y.o.), 20 non-AD dementia (11M/9F, 70±8) and 5 cognitively-intact control subjects (3M/2F, 75±10) were recruited at the San Gerardo hospital with a complete neurological examination. Plasma lipid-lipoprotein profile was determined using a Roche Cobas c311 analyzer. The esterification process in CSF has been evaluated as total cholesterol (TC) and unesterified cholesterol (UC) content measured by HPLC, apolipoproteins content by Western blot. HDL subclasses have been characterized in plasma and CSF by non-denaturing two-dimensional (2D)-electrophoresis.
Results. In AD and non-AD dementia plasma HDL-C levels are normal and interestingly HDL-C levels are inversely associated to cognitive decline (measured with Mini-Mental State Exam). Plasma HDL subclass distribution is analogous to that of healthy controls. CSF apoE-containing lipoproteins showed only α-migrating particles. Curiously CSF apoA-I and apoA-II-containing lipoproteins are very similar to plasma HDL in AD, even if these apolipoproteins are not synthetized in the CNS. UC/TC ratio in AD CSF (0.51±0.13) is higher than controls (0.40±0.12) and directly associated to cognitive decline.
Conclusions. This study supports the hypothesis of a direct role of plasma HDL in brain cholesterol homeostasis and a defect of cholesterol esterification in AD.

INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) AS PREDICTOR OF CARDIOVASCULAR MORTALITY IN HEART FAILURE PATIENTS: DATA FROM THE T.O.S.CA. REGISTRY

G. Landi, G. Crisci, A. De Giorgi[1], M. Iacoviello[3], V. Triggiani[4], G. Rengo[5], F. Cacciatore[6], C. Maiello[7], G. Limongelli[8], D. Masarone[9], F. Perticone[10], P. Perrone Filardi[11], S. Paolillo[12], A. Mancini[13], M. Volterrani[14], O. Vriz[15], R. Castello[16], A. Passantino[17], M. Campo[18], P.A.o Modesti[19], A. Salzano[20], R. D'Assante[21], M. Arcopinto[22], V. Raparelli[23], F. Fabbian[24], A. Sciacqua[25], A. Colao[26], T. Suzuki[27], E. Bossone[28] A.M. Marra[2], A, Cittadini[29], On behalf of T.O.S.CA. Investigators

[1]Clinica Medica Unit, Azienda Ospedaliero-Universitaria S. Anna, Ferrara;
[2]Department of Translational Medical Sciences, Federico II University, Naples;
[3]Clinical Outcome Research and Reporting Program (I-CORRP) - Center for Pulmonary Hypertension, Thorax clinic at Heidelberg University Hospital, Heidelberg, Germany;
[4]Cardiology Unit, Department of Medical and Surgical Sciences, University of Foggia;
[5]Interdisciplinary Department of Medicine-Section of Internal Medicine, Geriatrics, Endocrinology and Rare Diseases. University of Bari 'A Moro', Bari;
[6]Department of Translational Medical Sciences, Federico II University, Naples - Istituti Clinici Scientifici Maugeri SpA Società Benefit - IRCCS - Scientific Institute of Telese Terme (Benevento);
[7]Department of Translational Medical Sciences, Federico II University, Naples;
[8]Heart Transplantation Unit, Monaldi Hospital, Azienda Ospedaliera dei Colli, Naples;
[9]Division of Cardiology, Monaldi Hospital, Azienda Ospedaliera dei Colli, University of Campania L. Vanvitelli, Caserta;
[10]Division of Cardiology, Monaldi Hospital, Azienda Ospedaliera dei Colli, University of Campania L. Vanvitelli, Caserta;
[11]Department of Medical and Surgical Sciences; University Magna Græcia of Catanzaro;
[12]Department of Advanced Biomedical Sciences, Federico II University, Naples, Italy - Mediterranea Cardiocentro, Naples;
[13]Department of Advanced Biomedical Sciences, Federico II University, Naples, Italy - Mediterranea Cardiocentro, Naples;
[14]Operative Unit of Endocrinology, Catholic University of the Sacred Heart, Rome;
[15]Department of Medical Sciences, IRCCS San Raffaele Pisana, Rome;
[16]Heart Center Department, King Faisal Hospital & Research Center, Riyadh, Kingdom of Saudi Arabia;
[17]Division of General Medicine, Azienda Ospedaliera Universitaria Integrata, Verona, Italy, Scientific Clinical Institutes Maugeri, IRCCS, Pavia;
[18]Unit of Endocrinology and Metabolic Diseases, Department of Medical and Surgical Sciences, University of Foggia;
[19]Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi di Firenze;
[20]Italian Clinical Outcome Research and Reporting Program (I-CORRP) - IRCCS SDN, Diagnostic and Nuclear Research Institute, Naples;
[21]Department of Translational Medical Sciences, Federico II University, Naples - Italian Clinical Outcome Research and Reporting Program (I-CORRP);
[22]Department of Translational Medical Sciences, Federico II University, Naples - Italian Clinical Outcome Research and Reporting Program (I-CORRP);
[23]Department of Translational Medicine, University of Ferrara;
[24]Clinica Medica Unit, Azienda Ospedaliero-Universitaria S. Anna & Department of Medical Sciences, University of Ferrara;
[25]Department of Medical and Surgical Sciences; University Magna Græcia of Catanzaro;
[26]Clinical Medicine and Surgery Department, Federico II University, Naples;
[27]Department of Cardiovascular Sciences and NIHR Leicester Biomedical Research Centre, University of Leicester, Glenfield Hospital, Leicester, UK;
[28]Italian Clinical Outcome Research and Reporting Program (I-CORRP) - Cardiology Division, A. Cardarelli Hospital, Naples;
[29]Department of Translational Medical Sciences, Federico II University, Naples - Italian Clinical Outcome Research and Reporting Program (I-CORRP)

Introduction. Data from the "Trattamento Ormonale nello Scompenso CArdiaco" (T.O.S.CA) registry showed that heart failure (HF) represents a complex clinical syndrome with different hormonal alterations. Renal failure represents a frequent com-plication in HF. We evaluated the relationship between renal function and Insuline-like Growth Factor-1 (IGF-1) deficiency and its impact on cardiovascular mortality (CVM) in patients enrolled in the T.O.S.CA. registry.

Methods. At the enrolment, all subjects underwent chemistry examinations, include circulating hormones, and cardiovascular functional tests. COX regression analysis were used to evaluate factors related to CVM during the follow-up period in all populations, in high-risk patients and in the young-adult population. Also, we evaluate the effects of renal function on the CVM.

Results. 337 patients (41 deceased) were analyzed. CVM were related to severe renal dysfunction (HR stages IV-V=4.86), high-risk conditions (HR 2.25), serum IGF-1 (HR 0.42), and HF etiology (HR 5.85 and HR 1.63 for valvular and ischemic etiology, respectively). In high-risk patient s, CVM were related to IGF-1 levels, severe renal dysfunction and valvular etiology, whereas in young patients CMV was related to the high-risk pattern and serum IGF-1 levels.

Conclusions. Our study showed the clinical and prognostic utility of the IGF-1 assay in patients with HF.

SREBP-1C IS A TARGETABLE CHECKPOINT IN REGULATORY T CELLS BY COUPLING FATTY ACID METABOLISM TO IMMUNOREGULATORY FUNCTION

S. Fraire[1]*, F. Bonacina[1]*, M. Audano[1], S. Pedretti[1], G. Stucchi[1], A. Moregola[1], A.L. Catapano[1,2], N. Mitro[1], G.D.N. Norata[1,3]
[1]Department of Excellence of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano;
[2]IRCSS MultiMedica, Milano;
[3]SISA Atherosclerosis Center, Bassini Hospital, Cinisello Balsamo (Milano);
**equally contributed*

Aim. Regulatory T cells (Tregs) have an immunosuppressive role over atherosclerosis-associated inflammation. Numerous evidence have shown that reduced numbers and dysfunction of Tregs are involved in atherosclerosis pathogenesis, thus suggesting that improving Treg immunosuppression may reduce disease progression. Since suppressive Tregs rely mostly on lipid metabolism, the aim of this project was to investigate the impact of fatty acid metabolism on Tregs functional profile taking advantage of a KO mouse model for SREBP-1c.
Methods. Treg cells were isolated from lymphoid tissues (natural, nTreg) of SREBP-1c WT and KO mice or differentiated in vitro starting from conventional T cells (induced, iTreg) in the presence of IL-2 and TGF-β. nTregs and iTreg were characterized through flow cytometry analysis, in vitro functional assays and metabolic assays (Seahorse analysis).
Results. SREBP-1c KO mice showed reduced levels of Tregs in the blood (-66%, p<0,01) and in the secondary lymphoid tissues (-67%, p<0,01) paralleled to a significant decreased expression of typical Treg functional markers (CD25, PD-1 and Helios). Analysis of in vitro suppression revealed a reduced suppressive potential of SREBP-1c KO Tregs compared to WT cells (-21%, p<0,01), however KO Tregs showed an increased migratory capacity toward the inflammatory chemokines CXCL10 (+46%, p<0,05) and CCL19 (+34%, p<0,05). Seahorse data unveiled a metabolic shift of SREBP-1c KO Tregs toward anaerobic glycolysis compared to WT cells (Glycolysis stress test), which was in line with metabolomics data that showed an increased amount of lactate (+16%, p<0,05) in the medium of KO Tregs.
Conclusion. our data demonstrate that the deficiency of SREBP-1c in Tregs leads to a metabolic reprogramming toward anaerobic glycolysis which is also associated with a functional shift from suppressive to migratory behaviour. Therefore SREBP-1c target genes could be potentially exploited as pharmacological targets to boost Tregs immunosuppressive response in the atherosclerotic plaque.

EFFECT OF PCSK9 INHIBITORS TREATMENT ON SERUM LIPOPROTEIN FUNCTIONS IN SUBJECTS WITH FAMILIAL HYPERCHOLESTEROLEMIA: A MULTI-LIPID-CENTER REAL-WORD EVALUATION

M. Palumbo[1], M.P. Adorni[2], F. Zimetti[1], C. Pavanello[3], A. Giammanco[4], A. Baldassarre Cefalù[4], D. Noto[4], S. Piro[5], A. di Pino[5], M. Averna[4], L. Calabresi[3], Franco Bernini[1], F. Purrello[5], R. Scicali[5]
[1]Department of Food and Drug, University of Parma;
[2]Department of Medicine and Surgery, Unit of Neurosciences, University of Parma;
[3]Department of Pharmacological and Biomolecular Science, "E. Grossi Paoletti" Center, University of Milan;
[4]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties "G. D'Alessandro" (PROMISE), University of Palermo;
[5]Department of Clinical and Experimental Medicine, Internal Medicine, "Garibaldi-Nesima" Hospital, University of Catania

Aim. Proprotein convertase subtilisin/kexin type 9 (PCSK9), beyond regulating plasma cholesterol levels, exerts several pleiotropic effects modulating lipid metabolism in extrahepatic cells, including macrophages. Indeed, PCSK9 may directly modulate both macrophage cholesterol efflux and cholesterol uptake. However, macrophage cholesterol homeostasis also strictly depends on serum lipoprotein functions, including the HDL capacity to promote cell cholesterol efflux capacity (CEC) and the serum capacity to promote cell cholesterol loading capacity (CLC). The aim of the present study was to evaluate the effect of PCSK9 monoclonal antibodies treatment on HDL-CEC and serum CLC in a cohort of heterozygous Familial Hypercholesterolemia (HeFH) patients.
Methods. N=31 patients with a diagnosis of heterozygous FH have been recruited. Blood was collected and serum isolated at baseline and after six months of treatment with PCSK9 inhibitors (evolocumab/alirocumab). HDL-CEC through the main pathways was evaluated with a radioisotopic cell-based assay. Serum CLC was assessed fluorimetrically in human monocyte-derived macrophages THP-1.
Results. After treatment with PCSK9 inhibitors, total cholesterol and LDL-c significantly decreased (-40.3%, p<0.0001 and -50.4%, p<0.0001 respectively), while no changes were observed in HDL-c and TG levels. Total HDL-CEC was not different between patients before and after treatment. Conversely, despite no changes in HDL-c levels between the groups, ABCG1 HDL-CEC significantly increased after treatment (+22.2%, p<0.0001) as well as HDL-CEC by aqueous diffusion (+7.8%, p=0.0008). No significant changes of ABCA1 CEC between the groups occurred after treatment. PCSK9 inhibition significantly decreased serum CLC (-6.6%, p=0.0272). This effect was only partly related to the reduction of LDL-c levels.
Conclusions. Treatment with PCSK9 inhibitors had a positive impact on both quantitative and functional lipid profile as it increased aqueous diffusion and ABCG1 HDL-CEC and reduced the serum CLC. All these effects may contribute to the reduction of CV risk obtained after PCSK9 inhibitors treatment in FH patients.

SERUM PARATHORMONE, VITAMIN D AND CARDIOVASCULAR RISK FACTORS AND MARKERS

I. Fucile, M.V. Manzi, M. Lembo, B. Migliaccio, C. Mancusi
Hypertension Research Center & Department of Advanced Biomedical Science, Federico II University Hospital, Naples

Background. Vitamin D deficiency is a common cause of secondary hyperparathyroidism, particularly in elderly people. There are conflicting reports in the literature regarding whether 25 OHD and PTH may play a role in atherosclerosis. The aim of this study is to evaluate the associations of serum 25-OHD and PTH concentrations with blood pressure and carotid intima-media thickness (IMT).

Methods. This observational retrospective study included 81 patients admitted at Hypertension Research Center of Federico II University Hospital in Naples with GFR >60 mL/min. We collected serum 25-OHD, PTH, creatinine, BMI, lipid profile, blood glucose and office blood pressure. All patients underwent carotid doppler ultrasound and echocardiography.

Results. Mean age of the participants was 55±18 years and 74% were men. Mean (SD) 25-OHD and PTH were 21±9 ng/mL and 82±41 pg/mL, respectively. 69% of the patients had hypertension, 43% dyslipidemia and 12 % diabetes.

Pearson's correlation analysis indicated that PTH levels correlated directly with age, diabetes, dyslipidemia, hypertension, fasting glucose, and inversely with the GFR and vit D level (all $p<0.05$). Vit. D levels correlated inversely with PTH and diastolic blood pressure. No significant association of PTH and vit D were found with carotid IMT or left ventricular mass (both $p>0.05$).

Multivariate regression models were built to assess main determinant of PTH and Vit. D. PTH was significantly associated with higher fasting glucose ($p=0.002$), with no effect of age, GFR, diabetes, dyslipidemia, hypertension. Vit D was associated with lower GFR($p=0.007$) and PTH level ($p=0.04$).

Conclusion. In a population at high cardiovascular risk, PTH level was associated with higher fasting glucose while Vit D was associated with lower renal function. Vit. D and PTH are not directly associated with carotid atherosclerosis but might influence association with other cardiovascular risk factors and markers.

INTERPLAY BETWEEN S1P RECEPTORS AND SR-BI IN ATHEROSCLEROSIS RELEVANT CELLS: NEW INSIGHT FROM TRANSGENIC ANIMALS

M. Dessena[1], M. Simoni[2], J.-R. Nofer[3], A. von Eckardstein[4], F. Potì[1]
[1]*Department of Medicine and Surgery-Unit of Neurosciences, University of Parma;*
[2]*Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena;*
[3]*Central Laboratory Facility, University Hospital of Münster, Germany;*
[4]*Institute of Clinical Chemistry, University of Zurich and University Hospital of Zurich, Switzerland*

Aim. Sphingosine 1-phosphate (S1P) is a lysosphingolipid, mainly bound to high-density lipoproteins (HDL), which acts as an extracellular and intracellular signaling molecule by binding to its G protein-coupled receptors, named S1P1-5. The expression pattern of the S1P receptors (S1PRs) is important to regulate the cardiovascular, immune and nervous system functions. SR-BI, an HDL receptor, is widely expressed in different cell types, including endothelial cells and macrophages. SR-BI promotes the selective uptake of cholesterol esters from HDL or other lipoproteins to cells and enhances cell cholesterol efflux to HDL.

It has been demonstrated that the transient interaction between SR-BI and S1PRs, that can activate S1PRs, is stimulated by HDL-bound S1P.

Taking advantage of peculiar animal models overexpressing S1PRs in a tissue-specific manner, we tried to clarify the interplay between S1PRs and SR-BI.

Methods. We generated mice overexpressing S1PRs specifically in endothelial cells and myeloid cells (monocyte/macrophages), through the Cre-LoxP technology.

Animals overexpressing S1P1 in the endothelium (S1P1-iECKI) and related controls were sacrificed, their aortas isolated and processed for immunofluorescence imaging through confocal laser scanning microscopy. Mice overexpressing S1P3 in myeloid cells (S1P3-LyzMCre) were intraperitoneally injected with thioglycolate broth, then sacrificed and their peritoneal macrophages (MPMs) isolated and cultivated under cholesterol normal o loading (acetylated LDL) conditions. Gene and protein expression of target molecules in MPMs were evaluated by real time RT-PCR and Western blot.

Results. Confocal microscopy analysis confirmed the overexpression of the S1PRs, and interestingly showed an increased expression of SR-BI in the endothelial cells of S1P1-iECKI mice, compared to controls. In addition, in S1P3 overexpressing macrophages, quantitative PCR demonstrated the enhanced mRNA expression of SR-BI. Importantly, SR-BI protein expression is also increased in S1P3-LyzMCre macrophages. Treatment with S1PR-modulators also affected SR-BI expression, regardless of AcLDL stimulation.

Conclusions. The modulation of S1PRs may affect the expression of SR-BI in atherosclerosis relevant cells.

COVID-19 AND CARDIOVASCULAR SYSTEM: NOT ONLY HEART BUT ALSO VASCULAR. THE EFFECTS OF THE INFECTION ON ARTERIAL STIFFNESS

E. Gualini, A. Maloberti, S. Scarpellini, M. Algeri, M. Biolcati, E. Grasso, C. Tognola, A. Moreo, C. Giannattasio
[1]*Cardiology 4, Niguarda Hospital, Milan;*
[2]*School of Medicine and Surgery, University of Milano-Bicocca, Milan*

Introduction. SARS-CoV-2 determines a framework of multi-organ dysfunction that can involve the cardiovascular system creating damages of different nature. Among these, endothelial damage could play a key role in increasing arterial stiffness and thus the cardiovascular risk of infected patients. The aim of this study is to evaluate the Pulse Wave Velocity (PWV) of a population of patients after recovery from infection and to compare them with those of a group affected by arterial hypertension.
Methods. This prospective observational monocentric study involved 143 patients with previous diagnosis of Covid-19 who undergone PWV measurement during the follow-up at a median time of 3.8 months after the infection. These patients were compared to a population of 143 patients with hypertension matched by age, sex, Systolic Blood Pressure values and Body Mass Index.
Results. PWV values were higher in COVID-19 group comparing to hypertension group (10.5±3.0 m/s vs 8.9±2.5 m/s). Furthermore, there is a correlation between higher PWV values and lower values of SpO2% at time of admission at the Emergency Department. (R=-0.302; p<0.001).
Conclusions. SARS-CoV-2 infection seems related to increased PWV values. Moreover, higher arterial stiffness seems correlated to a worse oxygen saturation in Emergency Department. More studies with longer follow-up time are necessary to establish whether the vascular damage is reversible and whether it correlates with an increase of long-term cardiovascular risk.

IMPACT OF INTERLEUKIN-1 RECEPTOR 8 DEFICIENCY ON ATHEROSCLEROSIS

J. Nour[1], F. Bonacina[1], A. Moregola[1], P. Uboldi[1], R. Bellini[1], C. Garlanda[2], A.L. Catapano[1,3], G.D. Norata[1,4]
[1]*Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano;*
[2]*Dipartimento di Scienze Biomediche, Istituto Clinico Humanitas, IRCCS, Rozzano (Milano);*
[3]*IRCSS MultiMedica, Milano;*
[4]*Centro SISA, Ospedale Bassini, Cinisello Balsamo (Milano)*

Aim. Interleukin-1 receptor 8 (Il-1r8) dampens the IL-Rs and TLRs response by acting as a negative immune regulator. Intriguingly, its deficiency results in increased frequency of mature natural killer type 2 cells (mNK2) as well as their hyperresponsiveness. Our aim was to study the role of mNK2 in experimental atherosclerosis.
Methods. 8 weeks old-Ldlr-/- and Il-1r8-/- Ldlr-/- (double KO, DKO) male mice were fed with standard diet (STD) or western-type diet (WTD) for 12 weeks. Changes in lipid metabolism and atherosclerosis progression were evaluated through plasma lipid profiling, extensive immunophenotyping by flow cytometry, and histological analysis of the atherosclerotic plaques at the aortic sinus.
Results. Circulating mature natural killer type 2 cells significantly increased in Ldlr-/- mice when fed for 12 weeks with WTD when compared to STD fed mice (p<0,05). Next, to evaluate a pathogenic role of mNK2 in atherosclerosis development, we generated an experimental model presenting higher levels of mNK2 on an atheroprone background, by crossing Il-1r8-/- mice with Ldlr-/- mice (DKO). While plasma lipid profile and circulating immune cell distribution were not affected in DKO compared to Ldlr-/- mice, a decrease in NKs and dendritic cells levels in mediastinal lymph nodes, and an increase in splenic macrophages count were observed. When mice were fed with WTD for 12 weeks, DKO mice presented a significant increase in circulating mNK2 (p<0,05) and monocytes (p<0,05) when compared to Ldlr-/- animals. These differences, however, were not associated with increased atherosclerotic plaque burden, collagen deposition or immune infiltration. In parallel, plasma cholesterol and triglyceride levels were similar.
Conclusions. These data suggest that Il-1r8 deficiency does not impact atherosclerosis development and activated mNK2 cells are redundant during atherogenesis.

IMPAIRED ENDOTHELIAL FUNCTION IN CONVALESCENT PHASE OF COVID-19: A 3 MONTH FOLLOW UP OBSERVATIONAL PROSPECTIVE STUDY

E. Guerrieri[1], L. Giuliani[1], L. Giovenali[1], I. Di Blasi[1], M. Scarponi[1], L. Santoro[2], L. Falsetti[3], V. Zaccone[3], A. Nesci[2], M. Tosato[4], B. Giupponi[5], G. Moroncini[6], A. Gasbarrini[7,8], F. Landi[4,8], A. Santoliquido[2,8]

[1]Emergency Medicine Residency Program, Marche Polytechnic University, Ancona;
[2]Department of Cardiovascular and Thoracic Sciences, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome;
[3]Department of Emergency Medicine, Internal and Sub-Intensive Medicine, Azienda Ospedaliero-Universitaria "Ospedali Riuniti", Ancona;
[4]Geriatrics Department, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome;
[5]Department of Emergency Medicine, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome;
[6]Department of Experimental and Clinical Medicine, Clinica Medica, Azienda Ospedaliero-Universitaria "Ospedali Riuniti", Ancona;
[7]Department of Medical and Surgical Sciences, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome;
[8]Università Cattolica del Sacro Cuore, Rome

Aim. Endothelial dysfunction has a role in acute COVID-19, contributing to systemic inflammatory syndrome, acute respiratory distress syndrome, and vascular events. Evidence regarding COVID-19 middle- and long-term consequences on endothelium are still lacking. Our study aimed to evaluate if COVID-19 severity could significantly affect the endothelial function after three months from the acute phase.
Methods. We assessed endothelial function in outpatients with previous COVID-19 three months after negative SARS-CoV-2 molecular test by measuring flow-mediated dilation (FMD) in patients categorized according to a four-variable COVID-19 severity scale ("home care"; "hospital, no oxygen"; "hospital, oxygen"; "hospital requiring high-flow nasal canula, non-invasive ventilation, invasive mechanical ventilation, or extracorporeal membrane oxygenation"). FMD difference among COVID-19 severity categories was assessed with analysis of variance; we further clarified the relationship between FMD and previous COVID-19 severity with multivariate logistic models.
Results. Among 658 consecutive COVID-19 subjects, we observed a significant linear trend of FMD reduction with the increase of the COVID-19 category ($p<0.0001$). The presence of endothelial dysfunction was more frequent among hospitalized patients (78.3%) with respect to home-care patients (21.7%; $p<0.0001$). COVID-19 severity was associated with increased endothelial dysfunction risk (OR: 1.354; 95% CI: 1.06-1.71; $p=0.011$) at multivariate binary logistic analysis. FMD showed a significant direct correlation with PaO2 ($p=0.004$), P/F ratio ($p=0.004$), FEV1 ($p=0.008$), and 6MWT ($p=0.0001$).
Conclusions. Hospitalized COVID-19 subjects showed an impaired endothelial function three months after the acute phase that correlated with pulmonary function impairment. Further studies are needed to evaluate if these subjects are at higher risk of developing pulmonary disease or future cardiovascular events.

THE ORAL MICROBIOME: A NEW PERSPECTIVE FOR CARDIOVASCULAR RISK PREDICTION

M. Caporale, D. D'Ardes, I. Rossi, P. Vizzarri, D. Di Giacomo, F. Troiano, G. Matarazzo, M. Montagano, F. Cipollone, M. Bucci
Institute of Clinica Medica, Department of Medicine and Science of Aging, "G. d'Annunzio" University of Chieti-Pescara

Background. Dysbiosis of gut and oral microbiota contributes to individual susceptibility to atherosclerotic cardiovascular disease (ASCVD) through low-grade chronic inflammation. Particularly high oral Porphyromonas gingivalis (Pg) and lower Fusobacterium nucleatum (Fn) concentrations have been associated with clinical and experimental atherosclerosis. We assessed oral Pg and Fn abundance in very high-risk patients with previous ASCVD, with or without heterozygous familial hypercholesterolemia (HeFH), and in healthy control subjects.
Methods. In this cross-sectional study, we quantified oral Pg and Fn abundance by qPCR and assess oral health status in three group of patients: 40 patients with previous ASCVD (10 with genetically proven HeFH and 30 without FH), 26 subjects with HeFH in primary prevention and 31 healthy controls
Results. Patients with previous ASCVD showed higher Pg (1101.3 vs 192.4, $p=0.03$), but similar Fn abundance, compared to controls. Even higher concentrations of Pg has been shown in HeFH patients with ASCVD than non-HeFH patients and controls (1770.6 vs 758.4 vs 192.4, respectively; $p=0.048$). No differences were found in Pg and Fn abundance in HeFH subjects in primary prevention, compared to controls. In all patients examinated, BMI was correlated positively with Pg abundance and negatively with Fn abundance.
Conclusions. Very high-risk patients with previous ASCVD, with or without FH, are characterized by higher oral Pg abundance. These data suggest a potential relationship between Pg concentration and CV events. Future studies will assess the predictive value of Pg abundance measurement in ASCVD risk stratification.

PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 IN PRIMARY SJÖGREN'S SYNDROME: FOCUS ON INFLAMMATION, IMMUNE ACTIVATION, AND DISEASE ACTIVITY

E. Cosentini[1], V. Bianconi[1], E. Bartoloni Bocci[2], C. Perricone[2], G. Cafaro[2], F. Figorilli[1], M.R. Mannarino[1], R. Gerli[2], M. Pirro[1]
[1]Unit of Internal Medicine, Department of Medicine and Surgery, University of Perugia;
[2]Unit of Rheumatology, Department of Medicine and Surgery, University of Perugia

Aim. Primary Sjögren's syndrome (pSS) is chronic inflammatory autoimmune disorder with variable multisystemic manifestations. Inflammation induces the expression of PCSK9 which in turn has pro-inflammatory effects. As to whether plasma PCSK9 may be related to inflammation, immune activation and disease activity in pSS has never been explored.
Methods. A cross-sectional study was performed enrolling patients with pSS, either treated or untreated with anti-inflammatory/immune-modulating therapies, and age- and sex-matched healthy controls. Clinical characteristics of the study population were collected from medical records. Plasma PCSK9 levels were determined by ELISA. Inflammation was assessed through plasma C-reactive protein (CRP). Immune activation was evaluated through anti-SSA/Ro and anti-SSB/La antibodies. Disease activity was determined through the EULAR Sjögren's syndrome disease activity index (ESSDAI).
Results. Fifty-two patients with pSS (mean age 56 years ±11, 6% males) and 26 age- and sex-matched healthy controls (mean age 53±14 years, 6% males) were enrolled. Plasma PCSK9 levels were significantly higher in pSS patients as compared to age-and sex-matched healthy controls [162 (79-255) versus 40 (31-91), p<0.001]. In pSS patients, no significant correlation emerged between plasma PCSK9 and CRP (p>0.05). In addition, plasma PCSK9 levels did not differ according to the presence of either anti-SSA/Ro or anti-SSB/La antibodies (p>0.05). Instead, significantly higher plasma PCSK9 levels were observed in patients with medium-high ESSDAI (i.e., <5) as compared to those with low ESSDAI (i.e., <5). Also, plasma PCSK9 levels were higher in pSS patients untreated as compared to those treated with steroid therapy (p=0.006), albeit not differing significantly according to other anti-inflammatory/immune-modulating therapies (i.e., leflunomide, hydroxychloroquine, rituximab).
Conclusions. Plasma PCSK9 is upregulated in pSS. Patients with higher disease activity and untreated with steroid therapy display higher plasma PCSK9 levels.

EVALUATION OF PRESSURE PROFILE AND ORGAN DAMAGE IN YOUNG OUTPATIENTS WITH SYSTOLIC ISOLATED HYPERTENSION

G. Nardoianni[1], L. Carducci[1], B. Citoni[1], M. Salvetti[2], A. Paini[2], M.L. Muiesan[2], M. Volpe[1], G. Tocci[1]
[1]Hypertension Unit, Division of Cardiology, Department of Clinical and Molecular Medicine, University of Rome Sapienza, Sant'Andrea Hospital, Rome;
[2]Department of Clinical and Experimental Sciences, University of Brescia

Introduction. Isolated systolic hypertension (ISHT) is the most common hypertension phenotype among elderly patients, being related to aortic stiffness and endothelial dysfunction. The prognostic significance of ISHT in young outpatients is still unclear.
Aim. To evaluate clinic and 24-hour ambulatory blood pressure (BP) profile and markers of hypertension-mediated organ damage (HMOD) in a cohort of young adults with ISHT compared to a control group of normotensive subjects.
Methods. We prospectively enrolled outpatients of both sexes, aged between 20 and 50 years, who were referred to the Hypertension Unit, Sant'Andrea Hospital, Rome (IT) for hypertension assessment. All patients must have valid clinic and 24-hour ambulatory BP levels and concomitant assessment of vascular and cardiac HMOD. Once included, patients were stratified into 3 hypertension groups, including normotension (NT), ISHT, and systo-diastolic hypertension (HTN).
Results. We considered an overall sample of 185 adult outpatients, among whom 84 (45.4%) had NT, 23 (17.4%) ISHT, and 78 (42.2%) HTN. No significant differences were found between groups in terms of antihypertensive therapy and prevalence of white-coat phenomenon. Clinic (P<0.001) and central (P=0.035) pulse pressure (PP) was significantly higher in ISHT patients compared to other groups. A progressive and significant increase of both systolic 24-hour ambulatory (P<0.001) and central (P<0.001) BP values was observed from NT to patients with ISHT and HTN. No significant differences were found among groups in terms of cardiac HMOD. Patients with HTN showed significantly increased values of peripheral vascular resistance (P<0.001) and PWV (P=0.006) compared to NT individuals, though not significantly different compared to those with ISHT patients.
Conclusions. Young ISHT patients showed significantly higher prevalence of vascular HMOD as defined by increased brachial and central PP. They also showed sustained BP elevation and abnormal peripheral vascular resistances and PWV as observed in HTN patients, thus confirming that this condition should not be viewed as an innocent phenomenon.

COFFEE CONSUMPTION RELATES TO A REDUCTION OF VASCULAR AND HEART DAMAGE IN WELL-CONTROLLED HYPERTENSIVES

M.S. Marozzi[1], M. Di Trani[1], A. Vacca[2], G. Ranieri[1], S. Cicco[1]
[1]Unit of Internal Medicine "Guido Baccelli", Department of Biomedical Sciences and Human Oncology, University of Bari "Aldo Moro";
[2]Division of Internal Medicine, Department of Medicine, Building 8, University of Udine

Aim. Nutrition management of Arterial Hypertension is far to be defined. Coffee is the most used beverage all over the world. Many studies tried to understand the better amount of coffee to use and if there is a role in cardiovascular prevention. Aim is to evaluate heart and vascular damage in well controlled hypertensives according to coffee consumption.
Methods. We evaluate 316 patients (150 F, aged 62.22±10.76) affected arterial essential hypertension. All patients were evaluated for organ damage screening. The number of coffee cups per day was asked during the visit. The median coffee consumption was 2 cups per day.
Results. patients were subdivided into three groups according to tertiles of cups consumed: Group 1 0-1 cups 112 patients (57F; 64.21±10.91), Group 2, 2 cups, 101 patients (53F; 61.95±10.10), Group 3, >2 cups, 103 patients (40F; 60.32±10.95). No difference there were in weight and BMI, nor in blood pressure both systolic and diastolic. Group 1 presents an increased left ventricle mass compared to the other two groups (p=0.0008 and p=0.019 respectively). Similarly, Group 1 presents a significant reduction in E/e' ratio (p=0.042 and p=0.019, respectively). No significance between Group 2 and 3 for any of these two comparisons evaluated. On the contrary, Group 3 results with an increased intima-media thickness (p=0.004 and p=0.005 respectively). We found in a similar proportion of carotid stenosis between the three groups but there was a decreased stenosis percentage in Group 3 (p=0.011 and p=0.039, respectively).
Conclusion. Our data indicate the role of coffee consumption in reduction of heart remodelling, indicating that the consumption of at least 2 cups per day reduce heart mass and diastolic dysfunction. These results may be related to the role on reducing stenosis percentage, despite there is an increase in vessel wall thickness.

INCREASED DIETARY CHOLINE ALTERS THE PLASMA METABOLOME, WORSENS ATHEROSCLEROSIS DEVELOPMENT, BUT DOES NOT MAKE GUT MICROBIOTA PRO-ATHEROGENIC PER SE

E. Franchi[1], M. Busnelli[1], A. Colombo[1], S. Manzini[1], M.A. García-Rivera[2], J. Kirwan[2], X. Zhang[3], P. Gérard[3], G. Chiesa[1]
[1]Università degli studi di Milano, Dipartimento di Scienze Farmacologiche e Biomolecolari, Milano;
[2]Max Delbrück Center for Molecular Medicine, BIH Core Facility Metabolomics, Berlin, Germany;
[3]AgroParisTech, Micalis Institute, Universitè Paris-Saclay, INRAE, 78350 Jouy-en-Josas, France

Aim. Experimental and clinical studies have shown a positive correlation between adverse cardiovascular events and plasma concentrations of TMAO, a metabolite of dietary choline. The study was aimed at investigating whether the levels of other metabolites besides TMAO were affected by dietary choline intake. Additionally, it was studied if dietary choline could modify the intestinal microbiota composition, and, in turn, if the microbiota shaped by the dietary choline content could itself affect atherosclerosis development.
Methods. Female EKO mice were fed two low-fat, no cholesterol diets differing for a low (0.09%) or a high (1.2%) choline content for 16 weeks. Atherosclerosis development was quantified at the aortic sinus, microbiota composition was evaluated, and targeted plasma metabolomics was performed. Furthermore, the cecal microbiota of the two groups was transplanted into germ-free EKO mice which were fed a low choline diet for 16 weeks. Atherosclerosis development was then quantified.
Results. High-choline intake increased plasma TMAO concentration and worsened atherosclerosis development. Plasma choline concentration was not affected by diet. Surprisingly, high-choline feeding was associated with lower plasma levels of the pro-atherogenic metabolite homocysteine and a concomitant increase of its metabolites methionine and sarcosine. Hexoses and carnitine were also increased by high-choline diet. The evaluation of fecal microbiota composition revealed that, overall, dietary choline did not modify the relative abundance of bacterial fila and families, although it modulated clusters belonging to the family Lachnospiraceae. The evaluation of atherosclerosis development in germ-free EKO mice transplanted with the gut microbiota showed no differences both at the aortic sinus and along the entire aorta.
Conclusions. Taken together, our data indicate that a boost of dietary choline led to an altered plasma metabolome associated to atherosclerosis worsening. Dietary choline moderately affected gut microbiota composition and did not increase the pro-atherogenic potential of the intestinal bacterial make-up.

PREDICTORS OF REDUCED OF INDEXED MECHANO-ENERGETIC EFFICIENCY IN HYPERTENSIVE PATIENTS: THE CAMPANIA SALUTE NETWORK

M.V. Manzi, M. Lembo, M.A. Elena Rao, C. Mancusi, R. Izzo
Department of Advanced Biomedical Sciences, Federico II University of Naples

Background. Several studied have shown that a reduced of indexed mechano-energetic efficiency (MMEi), a well-known echo-derived parameter of left ventricular performance, is an independent predict of heart failure (HF) in hypertensive setting. We aimed to analyze the possible predictor of reduced MMEi in a hypertensive population with optimal blood pressure control, during a median follow-up of 5 years.
Methods. The study population included 6.736 hypertensive patients, from the Campania Salute Network, with no prevalent cardiovascular disease, normal baseline left ventricular ejection fraction (≥50%) and MMEi. We compared patients who developed a reduced MMEi (reduction of 10% compared to baseline) during the follow-up to patients with persistently normal MMEi.
Results. Optimal blood pressure control was achieved in more than 80% of patients. A reduction of MMEi was registered in 42.90% of population. They were older and more often smoker, presented lower value of left ventricular mass index (LVMi), systolic and diastolic blood pressure and higher value of glucose and left atrium volume index at baseline than patients with normal MMEi. In addition, they received less often beta-blocker therapy. A logistic regression demonstrated that independent predictor of reduced MMEi were hyperglycemia and smoke, while beta blocker therapy was a protective factor (OR 1.121, p=0.04).
Conclusion. Smoke, hyperglycemia and reduced use of beta-blocker therapy predicted the reduction of MMEi in a population of hypertensive patients with optimal blood pressure control.

PROGNOSTIC IMPACT OF RENIN ANGIOTENSIN ALDOSTERONE SYSTEM INHIBITION IN HYPERTENSIVE PATIENTS UNDERGOING TRANSCATHETER AORTIC VALVE IMPLANTATION

C. Basile, C. Mancusi, A. Franzone, M. Avvedimento, D. Angellotti, D.S. Castiello, Andrea Mariani, R. Manzo, P. Cirillo, G. de Simone, G. Esposito
Department of Advanced Biomedical Science, "University of Naples Federico II"

Introduction. Aortic Stenosis (AS) is the most common heart disease in western countries, with a prevalence that is increasing in tandem with life expectancy. Tissue renin-angiotensin-aldosterone system (RAAS) activation participates to myocardial fibrosis and worse clinical consequences.
Aim. We determine the impact of pre-procedural treatment with RAAS inhibitors (RAASi) on all-cause mortality, in a series of patients with severe AS who underwent transcatheter aortic valve implantation (TAVI).
Methods. We retrospectively examined 373 patients with AS who had undergone TAVI. Analyses were undertaken in the subgroup of hypertensive individuals (n=327) with 2-year follow-up. Univariable and multivariable Cox regression models were built according to baseline RAASi therapy status [RAASi (n=222) vs non-RAASi (n=151)]. A second analysis was undertaken by categorizing patients in angiotensin II receptor blockers (ARBs) (n=110) vs non-ARBs (n=217) or angiotensin-converting enzyme inhibitors (ACEi) (n=112) vs non-ACEi (n=215) recipients.
Results. Among hypertensive TAVI patients, RAASi administration at baseline was significantly related to gender (Women 56.8%), heart failure (25.7%), chronic kidney disease (CKD, 25.7%), atrial fibrillation (19.4), use of calcium-channel blocker (26.6%) or anti-platelet therapy (68.5%) (all p<0,05). All-cause mortality occurred in 23 (10.4%) patients on RAASi (9 on ARBs and 14 on ACEi), and in 21 of 105 patients not taking RAASi (20%).
In univariable COX regression model, overall, treatment with RAASi was associated with 54% reduction in 2-year all-cause mortality (HR=0.46, 95% CI 0.25-0.84 p=0.011). After multivariable control for significant confounders, this association was not statistically significant (95% CI 0.31-1.11 p 0.1). Analyzing pre-procedural ACEi and ARBs separately vs non RAASi recipients, ARB therapy was associated with 58% reduction in all-cause mortality (95% CI 0.18-0.95 p=0.038) that was confirmed in multivariate analysis.
Conclusion. RBs are independently associated with decreased risk of 2-year all-cause mortality in a series of hypertensive patients with severe AS, who underwent TAVI.

ABSTRACT SELEZIONATI

THE LIPIGEN PAEDIATRIC GROUP: FOCUS ON CLINICAL AND GENETIC FEATURES OF FH SUBJECTS UNDER 18 YEARS

M. Gazzotti[1], S. Bertolini[2], C. Pederiva[3], M.E. Capra[4], A.L. Catapano[5,6], M. Casula[5,6] on behalf of the LIPIGEN paediatric group
[1]Fondazione SISA (Società Italiana per lo Studio dell'Aterosclerosi), Milano;
[2]Department of Internal Medicine, University of Genova;
[3]U.O. Clinica Pediatrica, Servizio Clinico Dislipidemie per Lo Studio e La Prevenzione Dell'Aterosclerosi in Età Pediatrica, Ospedale San Paolo, Milano;
[4]Centro Dislipidemie in Età Evolutiva U.O. Pediatria e Neonatologia, Ospedale G. da Saliceto, Piacenza;
[5]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan;
[6]IRCCS MultiMedica, Sesto S. Giovanni (Milano)

Aim. In the last decade, the creation of familial hypercholesterolemia (FH) pathology registries allowed to deepen the knowledge about genetic profile, clinical features, and therapeutic management of FH patients and of specific sub-groups. Here, we report recent advances from the LIPIGEN Paediatric Group in the Italian scenario, with a focus on the genotype-phenotype relation.
Methods. From the LIPIGEN study, we identified 1495 clinically and/or genetically defined FH-subjects under 18 years, followed by 31 Italian LIPIGEN lipid clinics, with at least one pre-treatment LDL cholesterol (LDL-C) measurement and results from genetic testing.
Results. Genetically-positive subjects (70.4%) presented higher LDL-C level (mean±SD) compared to subjects with only variants of uncertain significance (8.4%) or negative diagnosis (21.2%) (246.5±102.1 vs 166.4±56.5 vs 159.9±47.7 mg/dL; p<0.0001). Among subjects with a genetically-positive diagnosis of FH, 1015 individuals presented one causative variant (untreated LDL-C level=231.6±53.4 mg/dL) in the gene encoding for LDL receptor (LDLR, N=1000) or for apolipoprotein B (APOB, N=15), while in 38 subjects two causative variants were detected (untreated LDL-C=645.6±219.9 mg/dL). In heterozygous FH for LDLR, more than 200 different causative variants were detected (the three mainly reported being p.Gly549Asp [LDL-C=245.0±50.6 mg/dL], p.Gly592Glu [LDL-C=198.7±50.1 mg/dL], and p.Asp221Gly [LDL-C=212.2±41.2 mg/dL]), with a great variability in the LDL-C values observed within the same mutation. Stratifying LDLR variants by receptor residual activity, a more severe phenotype was confirmed in children/adolescents with a null-variant compared to defective-variant (245.1±52.0 vs 220.2±51.8 mg/dL; p<0.0001).
Conclusions. Our analysis of the LIPIGEN paediatric cohort highlighted a great genetic variability, resulting in a wide range of LDL-C values based on type and number of variants but also presents among subjects carrying the same variant. This is one of the aspects that makes the diagnosis of the disease challenging, and calls for the design of targeted screening strategies in this sub-population.

DUTCH LIPID SCORE MODIFIED FOR ITALY: EVALUATION OF ITS EFFECTIVENESS ON A COHORT OF PATIENTS FROM CHIETI UNIVERSITY

E. Gigante[1,2], A. Calisi[1,2], D. D'Ardes MD[1,2], I. Rossi MD[1,2], P. Vizzarri MD[1,2], A.F. Damiani Tripolino MD[1,2], F. Troiano MD[1,2], M. Caporale MD[1,2], F. Cipollone MD, PhD[1,2], M. Bucci MD[1,2]
[1]"Clinica Medica" Institute, "SS. Annunziata" Hospital, Chieti;
[2]Department of "Medicine and Science of Aging", "G. d'Annunzio" University, Chieti

Background. Familial hypercholesterolemia (FH) is a common genetic disease caused by mutation of one or more of the genes critical for low-density lipoprotein cholesterol (LDL-C) catabolism. It is also the most frequent genetic disorder among dyslipidemias.
Aim. To evaluate the performance of Dutch Lipid Clinic Network Score (DLCNS) modified for Italy (DLCNS-I) in identifying patients with genetically defined FH, particularly in those ones <45 years.
Methods. The DLCNS-I and the result of genetic analysis were assessed retrospectively on a group of 96 subjects aged >18 (mean 49 years) followed at the Chieti Lipid Clinic and already enrolled in LIPIGEN project.
Results. Within the subcategory with clinically defined FH (DLCNS-I >8), 80.95% of patients presented a positive genetic analysis for FH or variants of uncertain significance (VUS).
In the subgroup aged 18-45 (n=34, mean age=34), 93.3% presented a positive genetic analysis for FH or VUS among patients with clinically defined FH.
Patients aged 18-45 years with missing data to calculate DLCNS-I were less numerous if compared to the total population (respectively 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 were more numerous in 18-45 years subcohort (respectively 24.0% vs 23.5%; 13.5% vs 0%; 9.4% vs 2.9%). DLCNS-I proved to be more effective if we have no more than one missing diagnostic criterion (as between subjects aged 18-45 years), despite lower LDL-C levels and less CV events, probably due to a more comprehensive history collection and more carefully physical examination.
Conclusions. This data confirm that DLCNS-I is usefulness in FH diagnosis, even if its effective compilation still represents a difficulty.

VARIANT CLUSTERS IN FAMILIAL HYPERCHOLESTEROLEMIA: HIGH FREQUENCY AND HIGH IMPACT OF THE VARIANT C.2312-3C>A IN THE LDLR GENE

C. Gianfico[1], G. Cardiero[1,2], M. Ferrandino[1], I. Calcaterra[3], G. Iannuzzo[3], M.N.D. Di Minno[4], M.D. Di Taranto[1], G. Fortunato[1,2]

[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;
[3]Dipartimento di Medicina Clinica e Chirurgia, Università degli Studi di Napoli Federico II, Napoli;
[4]Dipartimento di Scienze Mediche Traslazionali, Università degli Studi di Napoli Federico II, Napoli

Aim. Familial Hypercholesterolemia (FH) is the most common genetic disease associated with high levels of LDL-cholesterol (LDL-c) which lead to early atherosclerosis and increased cardiovascular risk. FH is caused by pathogenic variants in the *LDLR*, *APOB*, *PCSK9* genes. Several variants showed a very high frequencies in specific geographic regions (variants clusters). We aim to report our data about an extremely common splicing variant found in Campania region, the c.2312-3C>A in the *LDLR* gene which causes exon 16 skipping, p.(Ala771_Ile796del) resulting in a protein lacking of transmembrane region.
Methods. A retrospective analysis was performed in 715 unrelated index cases with FH clinical diagnosis (446 adults and 269 children) and in their relatives, i.e. a total of 935 patients (600 adults and 335 children). The variant frequency and LDL-c levels have been evaluated.
Results. The variant c.2312-3C>A in the *LDLR* gene has been identified in 45/715 index cases (6.3%). Cascade screening performed on patient's relatives allowed to identify additional 37 patients reaching a total of 82/935 (8.8% of whole population). Nevertheless, compound heterozygotes carrying the variant were not identified. Among adults, LDL-c levels were higher in patients with the c.2312-3C>A variant than in other heterozygotes, i.e. 292 (254-343) mg/dL vs 256 (214-302) mg/dL, p=0.002. In pediatric patients only a trend of increase was observed in variant carriers.
Conclusions. We confirmed that the variant c.2312-3C>A in the *LDLR* gene constitutes a cluster in our region, probably due to a founder effect. This variant was shown to be causative of a very severe phenotype, compatible with high impact of protein structure alteration. Thanks to the cascade screening it was possible to almost double the number of detected subjects with this heterozygous variant. Therefore, our results suggest that targeted screening protocols should be activated in Campania to increase the variant detection.

IMMUNE-INFLAMMATORY PROTEOMICS ASSOCIATED WITH ELEVATED CARDIOVASCULAR RISK IN GENETICALLY DETERMINED FAMILIAL HYPERCHOLESTEROLEMIA

A. Baragetti[1,2], F. Pellegatta[2,3], A.S. Alieva[1,4], O. Terenghi[1,3], D. Alagia[1,3], M. Gazzotti[5], L. Grigore[2,3], O. Reutova[4], E. Usova[4], E.V. Shlyakhto[4], A.L. Catapano[1,2]

[1]Department of Pharmacological and Biomolecular Sciences, University of Milan;
[2]IRCCS MultiMedica Hospital, Milan;
[3]S.I.S.A. Centre for the Study of Atherosclerosis, Bassini Hospital, Cinisello Balsamo (Milan);
[4]Research Laboratory of Lipid Metabolism Disorders and Atherosclerosis, Almazov National Medical Research Centre, St. Petersburg, Russia;
[5]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan

Aim. Timely identification of Familial Hypercholesterolemia (FH) subjects, at higher Cardiovascular Disease (CVD) when genetically determined (FH/M+) because of their LDL-C "burden" since inception, is still critical in clinics.
Overlap in the clinical phenotype between FH/M+ and clinically defined but not genetically confirmed FH (FH/M-) oftentimes affects the clinical performance.
We sought immune-inflammatory plasma proteins, that linked to CVD trajectories in two general populations, to propose new markers of risk stratification.
Methods. We measured 274 plasma proteins (Proximity Extension Assay, Olink™) in 173 clinically defined FH (according to Dutch Lipid Clinic Network Score, DLCNS) from two independent lipid clinics (Milan, Italy and St. Petersburg, Russia) and in a population-based survey of normolipidemic subjects not on lipid lowering therapies ("controls"). Next generation sequencing of *LDLR* locus identified 133 FH/M+" and 40 "FH/M-".
Results. Both FH/M+ (30 (13-40) y-old) and FH/M- (FH/M+ vs 39 (29-54)) presented significantly elevated levels of up to 250 proteins versus controls (55 (50-61) y-old respectively). Proteomics of FH/M+ vs FH/M- was also different (Bray-Curtis dissimilarity of principal components; PC1=66.7% vs PC2=10%) and was explained by eight hit proteins. These proteins, increased in FH/M+ from both lipid clinics, are involved in hematopoiesis (Stem Cell Factor cell, Interleukin-7), cell proliferation (Epidermal Growth Factor Receptor; Placenta growth factor), damage (Tumor Necrosis Factor Receptor Superfamily member 10; Galectin-9), chemotaxis (Chemokine (C-C) ligand 8 (CCL8); Platelet-derived growth factor-B).
Proteomics better discriminate FH+ from FH- beyond either LDL-C or DLCN alone (AUC of the model with proteomics included =0.972 vs AUC with LDL-C alone 0.862 vs AUC with DLCN alone =0.795 p<0.001).
This performance was not significant in younger FH/M+ (<18 y-old).
Conclusions. A cluster of immune-inflammatory proteins characterizes FH phenotype and genotype. Follow-up studies are required to understand the effect of LDL-C, and its burden since inception, on these markers.

THE ABSENCE OF DCIR2 DOES NOT IMPACT ATHEROGENESIS IN APOE-/- MICE

R. Bellini[1], A. Moregola[1], F. Bonacina[1], G. D. Norata[1,2]
[1]Department of Excellence of Pharmacological and Biomolecular Sciences (DisFeB), Università degli Studi di Milano;
[2]SISA Centre, Bassini Hospital, Cinisello Balsamo (Milano)

Aim. Dendritic cells immunoreceptor 2 (DCIR2) is a c-type lectin receptor, mainly expressed on dendritic cells, in charge for tailoring adaptive immune response to pathogens. Peculiarity of this receptor is the inhibitory intracellular domain which modulates DCs activation and following T cells polarization. Since the emerging differential role of the immune system within atherosclerotic plaque, aim of this work was to investigate the contribution of DCIR2 in atherosclerosis.
Methods. 8-week-old male *Dcir2$^{-/-}$ApoE$^{-/-}$* (double KO, DKO) and *ApoE$^{-/-}$* mice were fed with chow or cholesterol-enriched (WTD) diet for 12 weeks. Plasma lipid characterization and plaque histological analysis were performed, followed by the immunophenotyping of circulating, as well as thymic, immune cells through flow cytometry.
Results. While Dcir2 deletion in *ApoE$^{-/-}$* mice fed a chow diet did not show differences regarding plasma lipid profile, DKO mice exhibited increased circulating T lymphocytes, both CD4$^+$(+36%, $p<0.05$) and CD8$^+$(+42%, $p<0.05$), increase mainly driven by T naïve (+40%, $p<0.05$ and +43%, $p<0.001$ for CD4$^+$ and CD8$^+$ respectively) when compared to *ApoE$^{-/-}$*. Concordantly, as the educational process to T lymphocytes commonly takes place within the thymus, here the amount of both CD4$^+$ and CD8$^+$ naïve T cells augmented (+31%, $p<0.05$ both) in DKO mice compared to control counterpart. To shed light on the contribution of DCIR2 in atherogenesis, matched *Dcir2$^{-/-}$ApoE$^{-/-}$* and *ApoE$^{-/-}$* mice were fed with cholesterol-enriched diet for 12 weeks. Following the administration of WTD, changes in adaptive immunity were no longer observed, while the evaluation of plasma lipid profile and atherosclerotic plaque of DKO mice revealed a similar phenotype to *ApoE$^{-/-}$* mice.
Conclusions. DCIR2 is a critical receptor that maintains immune homeostasis through the modelling of adaptive immunity. However, our data suggest that although its role is evident in low cholesterol condition, its contribution is inconclusive following administration of a cholesterol-enriched diet and this receptor does not affect atherogenesis in *ApoE$^{-/-}$* mice.

COMBINING FAMILY HISTORY OF CORONARY HEART DISEASE AND INDIVIDUAL GENETIC PREDISPOSITION TO MORE ACCURATELY PREDICT THE LIFETIME RISK OF MAJOR CORONARY EVENTS

E. Olmastroni[1,2], F. Galimberti[2], A.L. Catapano[1,2], B.A. Ference[3]
[1]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan;
[2]IRCCS MultiMedica, Sesto S. Giovanni (Milano);
[3]Centre for Naturally Randomized Trials, University of Cambridge, Cambridge, U.K.

Background and Aim. Inherited predisposition to atherosclerosis leads to higher risk for developing coronary heart disease (CHD). We aimed at assessing the impact of family history of CHD and genetic predisposition in predicting the individual lifetime risk.
Methods. Using adjusted Cox proportional hazard models, we estimated the lifetime risk of major coronary events (MCE) associated with parental family history of CHD and genetic predisposition (estimated by a polygenic risk score including 350 variants).
Results. A total of 445,744 UK-Biobank participants were selected (mean age 57 years; 54.3% females). Having one parent (either mother or father) with a history of CHD increased the lifetime risk of MCE by 75% (HR 1.75, 95%CI 1.70-1.82). However, having both parents with a history of heart disease further increased the risk (HR 2.78, 95%CI 2.64-2.92), proving a dose-response relationship. Similarly, a dose-dependent step-wise increase MCE risk was observed moving from the lowest to the highest decile of the polygenic score. Compared to subjects without family history of CHD and with average level of the polygenic score, having a parental history of CHD determines a lifetime risk of MCE comparable to belonging to the highest decile of the polygenic score (HR 1.90, 95%CI 1.82-1.98 and HR 1.89, 95%CI 1.76-2.02, respectively). However, if subjects present both parental family history of heart disease and a very high polygenic predisposition, the risk is even much higher (HR 3.54, 95%CI 3.34-3.75), suggesting an additive contribution.
Conclusions. We described the addictive impact of family history of CHD and individual polygenic predisposition in predicting lifetime risk of MCE. In order to identify subjects at higher risk of having an early event, it is essential to retrieve information about the inherited cardiovascular risk, in addition to the evaluation of all the other cardiovascular risk factors.

HMGB1 IS HIGHLY EXPRESSED IN VESSEL WALL OF COVID19 PULMONARY EMBOLISM: A POST- MORTEM STUDY

F. Albanese[1], A. Vacca[2], G. Cazzato[3], G. Cicco[4], S. Sablone[5], C. Cariddi[6], R. Ria[1], S. Cicco[1]
[1]COVID section, Unit of Internal Medicine "Guido Baccelli", Department of Biomedical Sciences and Human Oncology, University of Bari;
[2]Division of Internal Medicine, Department of Medicine, Building 8, University of Udine, Italy
[3]Section of Pathology, Department of Emergency and Organ Transplantation (DETO), University of Bari "Aldo Moro", Bari;
[4]Department of Medical and Surgical Sciences for Children and Adults, University of Modena and Reggio Emilia, Modena;
[5]Section of Legal Medicine, Department of Interdisciplinary Medicine, University of Bari;
[6]Dipartimento dell'Emergenza e Trapianti d'Organo (DETO), Sezione di Anestesiologia e Rianimazione, Ospedale Policlinico, University of Bari "Aldo Moro"

Aim. Cardiovascular disease is one of the main factors leading a worst prognosis in COVID-19 patients. One of these complications during COVID-19 is pulmonary embolism (PE). It mostly relates to an increase coagulation due to endothelitis. Molecular mechanism are still far to be well understood. A possible mechanism is related to damage- associated molecular patterns (DAMPs) and, in particular, the high-mobility group box 1 (HMGB1). Purpose was the evaluation of the immune cell infiltration and HMGB1 expression in COVID-19 EP tissue samples.
Methods. We evaluated 6 patients who died for pulmonary embolism and were affected by COVID-19 (CPE, 3M, aged 70.86±11.83). As controls, we used data collected from 20 patients affected by PE, died before December 31, 2019 (8F, aged 75.94±18.84). All patients underwent to a complete physical examination, pulmonary computerized tomography, laboratory tests, d-dimers and blood gas analysis at the time of diagnosis. Echocardiogram was evaluated at the ward admission. Died patients underwent to a post- mortem analysis of tissues. Histological analysis was performed to evaluate vessel wall patho-morphology. Immunohistochemical on CD3+, CD4+, CD8+, CD15+ and HMGB1.
Results. No differences between the two groups there is in laboratory tests, in d-dimers, nor in echocardiogram parameters evaluating left or right heart. CPE presents an increased HMGB1 expression ($p<0.05$). No differences is found in CD3+, CD4+, CD8+. On the contrary, CD15 increases in CPE ($p<0.05$). HMGB1inversely relates to CD15 in both groups (Spearman r-0.66 for CPE and -0.79 for controls) but there is a direct correlation to CD4 only in CPE (r 0.74).
Conclusion. Our data indicates that COVID19-PE not appear to have a different clinical setting. The inflammatory infiltration results similar between COVID and non COVID patients. HMGB1 results the only different marker expressed in COVID-19 PE. Therefore, a possible mechanism involved in the disease progression may consider this pathway.

THYROID-STIMULATING HORMONE PREDICTS THE REDUCTION OF TOTAL CHOLESTEROL AND LOW-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS IN PATIENTS WITH COVID-19

F. Figorilli, V. Bianconi, E. Cosentini, C. Colangelo, G. Cellini, M.R. Mannarino, M. Pirro
Unit of Internal Medicine, Department of Medicine and Surgery, University of Perugia

Aim. A complex dysregulation of lipid metabolism has been reported in COVID-19, leading to reduced total cholesterol (TC), LDL cholesterol (LDL-C), and HDL cholesterol (HDL-C) levels. Also, significant alterations of thyroid function have been described in COVID-19. The aim of this study was to explore the association between thyroid-stimulating hormone (TSH) levels during COVID-19 and variation (Δ) of lipid profile parameters between the period before COVID-19 and hospital admission due to COVID-19.
Methods. A cohort of hospitalized COVID-19 patients was retrospectively analysed. Inclusion criteria were:
1) availability of a lipid profile from 1 month to 1 year before COVID-19;
2) availability of a lipid profile and TSH within 24 hours since hospital admission due to COVID-19.

Exclusion criteria were current lipid-lowering therapy and/or thyrosuppressive/thyroid hormone replacement therapy. The association between TSH at hospital admission and either Δ-TC, Δ-LDL-C, or Δ-HDL-C over the selected time frame was assessed through univariable and multivariable analyses accounting for the confounding effect of inflammation and COVID-19 severity.
Results. Three hundred and fifty patients were enrolled (mean age 75, 25 years, 53% males). A significant reduction of plasma TC, LDL-C, and HDL-C was recorded between the two time points ($p<0.001$ for all the comparisons). TSH was directly associated with Δ-TC (rho=0.152, p=0.006), Δ-LDL-C (rho=0.153, p=0.008), Δ-HDL-C (rho=0.135, p=0.019), and inversely associated with CRP (rho=-0.129, p=0.022). CRP was inversely associated with Δ-TC (rho=-0.110, p=0.040), Δ-LDL-C (rho=-0.128, p=0.019), and Δ-HDL-C (rho=-0.183, p=0.001). In addition, TSH decreased while CRP increased significantly with greater COVID-19 severity ($p<0.001$). A significant independent association was found between TSH and either Δ-TC (β=0.116, p=0.042) or Δ-LDL-C (β=0.119, p=0.042) after adjusting for multiple confounders, including CRP and COVID-19 severity.
Conclusions. TSH levels contribute to explain the reduction of TC and LDL-C observed in patients with COVID-19.

ATRIAL FIBRILLATION INCIDENCE IN SARS-CoV-2 INFECTED PATIENTS: PREDICTORS AND RELATIONSHIP WITH IN-HOSPITAL MORTALITY

V. Colombo[1,2], A. Maloberti[1,2], C. Giannattasio[1,2], P. Rebora[3], G. Occhino[3], N. Ughi[4], J. Rizzo[2], S. Fabbri[2], F. Leidi[2], I. Cartella[2], M. Algeri[1], S. Scarpellini[1], C. Rossetti[5], O. M. Epis[4], G. Molon[5], P. Bonfanti[7], M.G. Valsecchi[2], S. Genovesi[2,8]

[1]Cardiology 4, "A. De Gasperis" Cardio Center, ASST GOM Niguarda Ca' Granda, Milan;
[2]School of Medicine and Surgery, University of Milano-Bicocca, Milan;
[3]Bicocca Bioinformatics, Biostatistics and Bioimaging Centre - B4, School of Medicine and Surgery, University of Milano-Bicocca, Milan;
[4]Rheumatology, Multispecialist Medical Department, ASST GOM Niguarda Ca' Granda, Milan;
[5]Nuclear Medicine, ASST GOM Niguarda Ca' Granda, Milan;
[f] Cardiology Department, IRCCS Sacro Cuore Don Calabria Hospital, Negrar (Verona);
[7]Infectious Disease Department, San Gerardo Hospital, Monza;
[8]Cardiology Unit, Istituto Auxologico Italiano, IRCCS, Milan

Background. Among the different CardioVascular (CV) manifestation of the COronaVIrus-related Disease (COVID) particular attention has been paid to arrhythmia and particularly to Atrial fibrillation (AF). The aim of our study was to assess the incidence of AF episodes in patients hospitalized for COVID and to evaluate its predictors and its relationship with in-hospital all-cause mortality.
Methods. We enrolled 3435 cases of SARS-CoV-2 infection admitted in four hospitals in Northern Italy. We collected data on clinical history, vital signs, Intensive Care Unit (ICU) admission, laboratory tests and pharmacological treatment. AF incident and all-cause in-hospital mortality were considered as outcomes.
Results. 145 (4.2%) patients develop AF during hospitalization, with a median time of 3 days (IQR: 0, 11.5) from admission. Incident AF patients were older and had lower eGFR, lower platelet and lymphocytes count and higher C-Reactive Protein (CRP), were admitted more frequently to ICU and more frequently died compared to subjects that didn't present AF. At the Cox regression model significant determinants of incident AF were older age (HR 1.070; 95% CI: 1.048, 1.092), history of AF (HR 2.800; 95% CI: 1.465, 5.351), ischemic heart disease (HR 0.324; 95% CI: 0.130, 0.811) and ICU admission (HR 8.030; 95% CI: 4.511, 14.292). Incident AF was a predictor of all-cause mortality (HR 1.679; 95% CI: 1.170, 2.410), together with age (HR 1.053; 95% CI: 1.042, 1.065), dementia (HR 1.553; 95% CI 1.151, 2.095), platelet count (HR 0.997; 95% CI: 0.996, 0.999) higher CRP (HR 1.004; 95% CI: 1.003, 1.005) and eGFR (HR: 0.991; 95% CI: 0.986, 0.996)
Conclusion. AF present as the main arrhythmia in COVID-19 patients and its development during the hospitalization strongly relates with in-hospital mortality.

RENIN-ANGIOTENSIN-SYSTEM INHIBITORS ARE ASSOCIATED WITH LOWER IN-HOSPITAL MORTALITY IN COVID-19 PATIENTS AGED 80 AND OLDER

F. Spannella[1,2], F. Giulietti[1,2], C. Di Pentima[1,2], M. Allevi[1,2], V. Bordoni[1,2], A. Filipponi[1,2], S. Falzetti[1,2], C. Garbuglia[1,2], S. Scorcella[1,2], A. Massacesi[1,2], A. Gezzi[1,2], P. Giordano[1], R. Sarzani[1,2]

[1]Internal Medicine and Geriatrics, IRCCS INRCA, Ancona;
[2]Department of Clinical and Molecular Sciences, University "Politecnica delle Marche", Ancona

Aim. Older adults are at higher risk of morbidity and mortality for COVID-19. Renin-angiotensin-system-inhibitors (RASi) were found to protect COVID-19 adult patients against mortality. We investigated whether this association was confirmed also in COVID-19 older patients.
Methods. Prospective observational study on 337 hospitalized older adults (aged 80 years and older). We classified the study population according to RASi use before and during hospitalization. A propensity score analysis was also performed to confirm the findings.
Results. Mean age was 87.4±6.1 years. Patients taking RASi at home were 147 (43.6%). During hospitalization, 38 patients (11.3% of the entire study population) discontinued RASi, while 57 patients (16.9% of the entire study population) started RASi. In-hospital mortality was 43.9%. Patients taking RASi during hospitalization (patients who maintained their home RASi therapy + patients who started RASi during hospitalization) had a significant lower in-hospital mortality than untreated patients [HR 0.48 (95% CI 0.34-0.67)], even after adjustment for required respiratory support, functional status, albumin, inflammation and cardiac biomarkers. The analysis on the groups derived from the 'propensity score matching' (58 patients in each group) confirmed these results [HR 0.46 (95% CI 0.23-0.91)].
Conclusions. Despite the high risk of death in older COVID-19 patients, RASi therapy during hospitalization was associated with clinically relevant lower in-hospital mortality, likely due to the benefit of RAS modulation on cardiopulmonary system during the acute phase of the disease. Our findings confirm the protective role of RASi even in COVID-19 patients aged 80 years and older.

ALTERATIONS OF LIPID PROFILE OBSERVED IN COVID-19 PATIENTS

M. Montagano, D. D'Ardes, I. Rossi, P. Vizzarri, F. Troiano, M. Caporale, G. Matarazzo, F. Cipollone, M. Bucci
Institute of Clinica Medica, Department of Medicine and Science of Aging, "G. d'Annunzio" University of Chieti-Pescara

Introduction. It seems that during SARS-CoV-2 infection, total cholesterol, LDL-C, and HDL-C values decrease and lipids could play a fundamental role in viral replication.
Methods and Aim. We performed a retrospective analysis of 118 hospitalized patients with COVID-19, comparing pre- infection lipid profile (53 patients) to those measured on admission. Our aim was to evaluate whether SARS-CoV-2 infection could be involved in lipid profile alterations and study possible correlations with disease severity and clinical outcome.
Results. Median baseline values at the admission time were: total cholesterol 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 and non-HDL-C 104.53±32.63 md/dL. Median values of pre-infection total cholesterol and HDL-C were significantly higher (total cholesterol 158.43±45.18 mg/dL; HDL-C 44.08±17.76 mg/dL) than those measured at the admission time (p value <0.05). The C-reactive protein (CRP) was negatively correlated with LDL-C (p=0.013) and HDL-C (p=0.05).
Conclusion. Our data suggest a possible relation between COVID-19 and lipid profile with a negative correlation between CRP, LDL-C, and HDL-C values, thus proposing the hypothesis that lipid lowering could follow the rising of the COVID-19 inflammatory state.

UNCOVER THE IMMUNOMETABOLIC ROLE OF APOLIPOPROTEIN E FROM KUPFFER CELLS VERSUS HEPATOCYTES ORIGIN

F. Bonacina[1], A. Diroma[1], P. Uboldi[1], M. Ceccon[1], A. Moregola[1], J. Heerin[2], G.D. Norata[1,3]
[1]Department of Excellence of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano;
[2]Department of Biochemistry and Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;
[3]Centro SISA per lo Studio dell'Aterosclerosi, Ospedale Bassini, Cinisello Balsamo (Milano)

Aim. Apolipoprotein E (ApoE), one of the main plasma apolipoproteins, participates to the catabolism of VLDL and LDL lipoproteins. We and others have shown that ApoE is produced by myeloid immune cells where it plays an immunomodulatory role by controlling HDL-mediated efflux of cholesterol. Aim of this project is to depict the contribution of hepatic ApoE versus the protein produced by liver macrophages, Kupffer cells, on systemic metabolism and immune response.
Methods. Hepatocytes and Kupffer cells selective ApoE-KO mice were generated by crossing ApoE flox/flox mice with Albumin-Cre (*ApoE*$^{\Delta Hep}$) or Clec4f-Cre (*ApoE*$^{\Delta KC}$) mice. Lipid (cholesterol and triglycerides) and lipoprotein (FPLC) plasma profile were measured in 8-12 week-old mice fed to standard fat diet. ApoE distribution was evaluated in total plasma and FPLC fractions by Western Blotting (WB). Blood and liver immunophenotyping was performed by flow cytometry.
Results. Total levels of cholesterol (Chol) and triglycerides (TG) were not affected in *ApoE*$^{\Delta KC}$ mice, while *ApoE*$^{\Delta Hep}$ mice showed a significant increase in Chol (+40%) and decreased trend in TG levels (-30%) compared to controls. Lipoprotein distribution by FPLC revealed a slight increase in Chol in HDL of *ApoE*$^{\Delta KC}$, while in *ApoE*$^{\Delta Hep}$ mice Chol was mainly concentrated in LDL and reduced in HDL. Whereas ApoE was not detectable in plasma, LDL and HDL fraction of *ApoE*$^{\Delta Hep}$ mice, *ApoE*$^{\Delta KC}$ mice presented a reduction of ApoE in plasma and HDL fractions. No main differences in systemic and hepatic innate and adaptive subsets were reported in both models.
Conclusion. Our preliminary data suggest a different metabolic role of ApoE produced by Hepatocytes versus Kupffer cells and current studies are ongoing to unveil the impact on atherosclerosis and metabolic diseases.

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[1,2], A. Gezzi[1,2], M. Allevi[1,2], P. Turri[1,2], S. Sarnari[1,2], S. Re[1,2], R. Sarzani[1,2]
[1]Internal Medicine and Geriatrics, IRCCS INRCA, Ancona;
[2]Department of Clinical and Molecular Sciences, University "Politecnica delle Marche", Ancona

Aim. Recently, an updated CVR model (SCORE2, instead of SCORE) and several apparently more accurate equations, instead of Friedewald, 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).
Conclusions. 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.

FREQUENZA CARDIACA A RIPOSO E RIGIDITÀ ARTERIOSA: STUDIO LONGITUDINALE PROSPETTICO IN UNA POPOLAZIONE DI PAZIENTI IPERTESI

M. Monticelli, A. Maloberti, S. Fumagalli, I. Garofani, G. Simone, R. Giacomo, M. Bombelli, C. Giannattasio
Cardiologia 4, Ospedale Niguarda, Milano;
Scuola di Medicina e Chirurgia, Università Milano-Bicocca, Milano;
Medicina Interna, Ospedale di Desio (Monza Brianza)

Aim. The role of resting Heart Rate on the progression of arterial stiffness has not yet been extensively evaluated. The aim of this study is to investigate the relationship between resting HR and baseline arterial stiffness (evaluated by cfPWV) as well as its progression in a population of hypertensive patients over a 3.7 years follow-up period.
Methods. We enrolled 572 hypertensive outpatients 18-80 aged, followed by the Hypertension Unit of St. Gerardo Hospital (Monza, Italy). Anamnestic, clinical and laboratory data, BP and cfPWV (complior) were assessed at baseline and after a median follow-up time of 3.7±0.5 years
Results. At baseline the mean age was 53.9±12.7 years, SBP and DBP were 141.2±17.8 and 86.5±10.5 mmHg, HR was 65.6±10.9 bpm and PWV was 8.6±2.0 m/s. Despite an improvement in BP values (from 141.2/86.5 to 132.6/79.2 mmHg, p<0.001), during follow-up, PWV increased (ΔPWV 0.5±2.2 m/s). In patients with a ΔHR above as compared to those under the median value (9 bpm), ΔPWV was significantly higher (0.82±2.22 vs 0.27±2.25 m/s, p=0.003). At multivariate analysis, HR was among the significant determinants of both baseline PWV and its progression (β=0.031, p<0.001). Furthermore, ΔHR was a significant determinant of ΔPWV (β=0.019; p=0.017).
Conclusions. in hypertensive patients there is a significant relationship between basal resting HR and basal PWV as well as between the increase of HR and the increase of PWV during the follow-up period. Beyond age and BP, resting HR must be considered as an independent determinant of arterial stiffness. This represents a possible mechanism through which HR contributes to the increase in CV risk.

PROGRESSIVE RIGHT VENTRICULAR DYSFUNCTION AND EXERCISE IMPAIRMENT IN PATIENTS WITH HEART FAILURE AND DIABETES MELLITUS: INSIGHTS FROM THE T.O.S.CA. REGISTRY

A. Salzano[1], R. D'Assante[2,3], M. Iacoviello[4], V. Triggiani[5], G. Rengo[2,6], F. Cacciatore[2], C. Maiello[7], G. Limongelli[8], D. Masarone[8], A. Sciacqua[9], P. Perrone Filardi[10,11], A. Mancini[12], M. Volterrani[13], Olga Vriz[14], R. Castello[15], A. Passantino[16], M. Campo[17], P. A Modesti[18], A. De Giorgi[19], Michele Arcopinto[2,3], P. Gargiulo[10], M. Perticone[9], A. Colao[20], S. Milano[21], A. Garavaglia[22], R. Napoli[2], T. Suzuki[23], E. Bossone[3,24], A.M. Marra[2,3,25], A. Cittadini[2,3]

[1]IRCCS SYNLAB SDN, Diagnostic and Nuclear Research Institute, Naples;
[2]Department of Translational Medical Sciences, Federico II University, Naples;
[3]Italian Clinical Outcome Research and Reporting Program (I-CORRP);
[4]Cardiology Unit, Department of Medical and Surgical Sciences, University of Foggia;
[5]Interdisciplinary Department of Medicine-Section of Internal Medicine, Geriatrics, Endocrinology and Rare Diseases. University of Bari 'A Moro', Bari;
[6]Istituti Clinici Scientifici Maugeri SpA Società Benefit (ICS Maugeri SpA SB) - IRCCS - Scientific Institute of Telese Terme, Benevento;
[7]Heart Transplantation Unit, Monaldi Hospital, Azienda Ospedaliera dei Colli, Naples;
[8]Division of Cardiology, Monaldi Hospital, Azienda Ospedaliera dei Colli, University of Campania Luigi Vanvitelli, Caserta;
[9]Department of Medical and Surgical Sciences; University Magna Græcia of Catanzaro;
[10]Department of Advanced Biomedical Sciences, Federico II University, Naples;
[11]Mediterranea Cardiocentro, Naples;
[12]Operative Unit of Endocrinology, Catholic University of the Sacred Heart, Rome;
[13]Department of Medical Sciences, IRCCS San Raffaele Pisana, Rome;
[14]Heart Center Department, King Faisal Hospital & Research Center, Riyadh, Kingdom of Saudi Arabia;
[15]Division of General Medicine, Azienda Ospedaliera Universitaria Integrata, Verona;
[16]Scientific Clinical Institutes Maugeri, IRCCS, Pavia;
[17]Unit of Endocrinology and Metabolic Diseases, Department of Medical and Surgical Sciences, University of Foggia;
[18]Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi di Firenze;
[19]Department of Medical Sciences, School of Medicine, Pharmacy and Prevention, University of Ferrara;
[20]Clinical Medicine and Surgery Department, Federico II University, Naples;
[21]Department of Laboratory Medicine, AOUP P. Giaccone, Palermo;
[22]Novartis Farma S.p.A. - Medical Department;
[23]Department of Cardiovascular Sciences and NIHR Leicester Biomedical Research Centre, University of Leicester, Glenfield Hospital, Leicester, UK;
[24]Cardiology Division, A Cardarelli Hospital, Naples;
[25]Center for Pulmonary Hypertension, Thoraxclinic at Heidelberg University Hospital, Heidelberg, Germany

Aims. The T.O.S.CA. Registry recently reported that impairment of insulin action (insulin resistance - IR or diabetes mellitus - T2D) increases morbidity and mortality in patients with chronic heart failure (CHF). Aims of the present investigation were to explore the separate impact of T2D and IR on left and right chambers morphology and function and cardiopulmonary performance and their longitudinal changes in relation to the cardiovascular outcomes.
Methods. Patients enrolled in the T.O.S.CA. Registry - divided into three groups based on the evidence of insulin impairment: no evidence (EU), evidence of IR (IR), and T2D - performed echocardiography and cardiopulmonary exercise test at baseline and at a patient-average follow-up of 36 months.
Results. Patients Compared with EU and IR, T2D was associated with increased filling pressures (E/e' ratio: 12.0±6.5, 14.5±8.1, and 15.9±8.9 respectively, $p<0.01$) and worse right ventricular (RV)-arterial uncoupling (RVAUC) (TAPSE/PASP ratio 0.6±0.3, 0.6±0.3, and 0.52±0.2, respectively, $p<0.05$). Likewise, impairment in peak oxygen consumption (peak VO_2) in TD2 vs EU and IR patients was recorded (respectively, 15.8±3.8 mL/kg/min, 18.4±4.3 mL/kg/min and 16.5±4.3 mL/kg/min, $p<0.003$). Longitudinal data demonstrated higher deterioration of RVAUC, RV dimension, and peak VO_2 in the T2D group vs baseline (+13% increase in RV dimension, -21% decline in TAPSE/PAPS ratio and -20% decrease in peak VO_2).
Conclusion. The group of HF patients with T2D, who exhibited a higher risk of death and CV hospitalization in the T.O.S.CA. Registry, displayed progressive RV ventricular dysfunction and exercise impairment when compared to non-diabetic CHF patients, supporting the pivotal importance of hyperglycaemia and deterioration of right chambers in HF prognosis.

MULTIMODALITY IMAGING IN CORONARY ARTERY ANOMALIES

M. Lo Monaco, E. Cristiano, F. Cava, A. Berni, M. Volpe
Azienda Ospedaliera S. Andrea. Facoltà di Medicina e Psicologia, Università di Roma Sapienza

Most cases of abnormal coronary artery origin do not manifest clinically and are most often found occasionally during coronary angiography.
The clinical case presented by us concerns an 80-year-old man who came to the emergency room for oppressive chest pain onset at rest. On the EKG: junctional rhythm at HR 48 bpm, ST segment elevation in inferior leads (DII, DIII, avF) and posterior leads (V7-V9), ST segment depression in V2-V3 and in D1 and avL.
During the angiographic examination, an anomalous origin of the circumflex from the right coronary sinus was highlighted. The dominant circumflex artery showed ostial thrombosis and subocclusive thrombosis to the proximal tract with minimal distal flow, TIMI I.
Patient was treated with primary PCI and implantation of two medicated stents on the circumflex artery with good final angiography and downstream flow TIMI III.
An independent origin of the circumflex may be relative frequencies. Its clinical relevance is related to the possibility of compression during valve surgery. There are no studies that demonstrate which coronary anomaly determines a preferential incidence of coronary heart disease, but some authors report that the presence of an anomaly of the circumflex artery exposes it earlier and with greater extension to an atherosclerotic process to the same vessel in the absence of anomalies.
Until now, coronary angiography has represented the technique of choice for the diagnosis of coronary anomalies which, although often benign, could be responsible for anginal-like symptoms due to an ab extrinsic compression along their course, sudden death, arrhythmia-related, among young people or they could create complications during cardio-thoraco-vascular surgery.
In recent years, other techniques in cardiological diagnostic imaging have been developed such as coronary angio-MRI, electron beam tomography (EBT) and TCMS (multislice ct scan) which are and will be increasingly supportive in the non-invasive diagnosis of coronary anomalies and more.

IMPORTANCE OF URIC ACID THRESHOLD IN ITS CORRELATION WITH METABOLIC SYNDROME

S. Fabbri, A. Maloberti, I. Garofani, S. Fumagalli, C.M. Ciampi, O. Paolo, M. Carbonaro, M. Massimiliano, G. Tavecchia, M. Bombelli, C. Giannattasio
[1]*Cardiologia 4, Ospedale Niguarda, Milano;*
[2]*Scuola di medicina e chirurgia, Università Milano-Bicocca, Milano;*
[3]*Medicina Interna, Ospedale di Desio (Monza Brianza)*

Background. The relationship between Hyperuricemia and Cardiovascular risk has been established but whether or not a correlation between Serum Uric Acid (SUA) and Metabolic Syndrome (MS) exists is still a matter of debate. Indeed whether SUA level is part of MS diagnosis or just a pure marker of an unfavourable metabolic profile has not been demonstrated. Besides it's unknown whether SUA's addition to MS definition makes a difference in terms of prognosis. In our study we focused on evaluating in a group of hypertensive patients, the correlation between MS diagnosis and SUA defined with two different cut-off:
1) ≥6 mg/dL for women and ≥7 for men (classic cut-off);
2) >5.6 mg/dL for both sexes (recently proposed by the URRAH Project).

Methods. We enrolled 473 Hypertensive patients followed by the Hypertension Unit of San Gerardo Hospital (Monza, Italy), in which SUA was measured. Patients with Hyperuricemia were identified according to the two different thresholds. NCEP-ATP-III criteria were used for diagnosis of MS.

Results. MS was diagnosed in 33.6% while Hyperuricemia was found in 14.8% of subjects according to the traditional cut-off and 35.9% according to URRAH study's cut-off. Hyperuricemia and MS coexist in 9.7% (traditional cut-off) and 17.3% (URRAH's threshold) of the population. Hyperuricemia was more frequent in MS than in non-MS subjects (29 vs 7.6%, p-value <0.0001 for cut-off 6/7 mg/dL and 51.6 vs 28.0%, p-value <0.0001 for cut-off 5.6 mg/dL). Linear regression models showed that SUA is related to MS diagnosis (β=1.597, p-value <0.0001). At logistic analysis Hyperuricemia was strongly related to MS when defined by the HURRAH's cut-off (OR=0.303, p-value <0.0001). The same relation is weak, although significan, when Hyperuricemia was defined by the classic cut-off (OR=0.182, p-value <0.0001).

Conclusions. Hyperuricemia is related with MS diagnosis especially when defined by the recently defined cut-off of 5.6 mg/dL.

COMPARISON OF THE ADHERENCE TO THE MEDITERRANEAN DIET, TO THE "HEALTHY AND VARIABLE DIET" AND ASSOCIATION WITH MARKERS OF INFLAMMATION

E. Mattavelli[1,2], L. Redaelli[2], R. Domenighini[3], L. Grigore[3], F. Pellegatta[3], A. Pirillo[2], P. Magni[1,3], A.L. Catapano[1,3], A. Baragetti[1,3]

[1]Department of Pharmacological and Biomolecular Sciences, University of Milan;
[2]SISA Center for the Study of Atherosclerosis, Bassini Hospital, Cinisello Balsamo, (Milan);
[3]IRCCS MultiMedica Hospital, Milan

Aim. Mediterranean Diet (Med-Diet) is believed to be protective against cardiovascular diseases (CVD) and other chronic inflammatory diseases. However, two concerns are debated:

1) How much it reflects a "healthy" and variable" diet;
2) whether it associates with changes in circulating inflammatory markers predictive for CVD and chronic inflammatory diseases.

Methods. We preliminary explored the first concern, by analyzing seven-day-dietary records filled from 296 subjects (a representative sample of the PLIC cohort, n=2,606). Subjects reported what they consumed and the relative quantity, during each meal. 581 foods were identified from the analysis of the diaries and were clustered into 28 food groups. We hence discriminated:

1) adherence to higher vs lower Med-Diet (8 as PREDIMED threshold) and
2) the Healthy Food Diversity-Index (HFD-index) (=the variability of "healthy foods" consumed) to discriminate adherence to "healthier and more variable" (HFD-index >0.55) vs that to "less healthy and less variable" (HFD-index <0.55) diet.

To then explore the second concern, 368 plasma inflammatory markers (that we preliminary validated as predictive of CVD risk) were measured (Proximity Extension Assay technology; Olink™).

Results. Subjects with lower Med-Diet adherence consumed less alcoholic beverages, fish and white meat and did not consume more other food groups.

Subjects with lower HFD-index consumed less vegetables, fruit, milk, yogurt and more alcoholic beverages, coffee and tea, alcohol-free beverages, processed-meat and salty snack.

Higher Med-Diet adherence was not related to higher HFD-index (r=–0.048; p=0.415).

Lower Med-diet adherence was associated with 91 increased and 2 decreased inflammatory markers. Lower HFD-index was associated with 13 decreased and 23 increased inflammatory markers. VEGFA and HGF were observed to be increased in both lower Med-diet adherence and lower HFD-index. IL1RL2 was increased in lower Med-Diet adherence and decreased in lower HFD-index.

Conclusions. Adherence to Mediterranean Diet does not reflect a "healthy and variable diet" and their relation with markers of inflammation appears not mutual.

EVALUATION OF THE PREVALENCE OF THE MOST COMMON PSYCHIATRIC DISORDER IN PATIENTS WITH TYPE 2 DIABETES THROUGH THE PATIENT HEALTH QUESTIONNAIRE (PHQ): THE DIA2PSI STUDY

A.E. Claro[1], C. Palanza[2], M. Mazza[1], L. Tartaglione[3], G. Marano[1], M. Rigoni[4], C. de Waure[5], L. Bocchio Chiavetto[6], C. Baldari[6], P.C.M. Muti[4], A. Pontecorvi[7], L. Janiri[1], G. Sani[1], D. Pitocco[3]

[1]Psychiatry Unit, Department of Neurosciences, Università Cattolica del Sacro Cuore,
Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome;
[2]Department of Environmental Biology, Università Sapienza, Rome;
[3]Diabetes Care Unit, Department of Endocrinology and Diabetes, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome;
[4]Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano Statale, Milan;
[5]Department of Experimental Medicine, University degli Studi di Perugia;
[6]Department of Theoretical and Applied Sciences, Università e Campus, Novedrate (Como);
[7]Division of Endocrinology, Department of Endocrine-Metabolic and Dermo-Rheumatology, Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome

Aim. Common Psychiatric Disorder (CPD) are associated with the development of overweight and obesity, the strongest risk factors for the onset and subsistence of Type 2 Diabetes (T2D). T2D determine a widespread early atherosclerosis development and is considered an equivalent of cardiovascular disease (CVD). This study evaluates the prevalence of the CPD in a sample of T2D patients' numerically representative of the Italian population. There are currently no similar studies in Italy.

Methods. This is a monocentric cross-sectional study held at the Fondazione Policlinico Universitario Agostino Gemelli IRCCS (FPUAG). The sample size was determined in 184; patients with T2D, aged 18 to 85 were screened for CPD using the PHQ. The study protocol was approved by the IRB and Ethics Committee of the FPUAG. The expected prevalence was estimated from previously international studies carried out in patients with T2D.

Results. Patients presents T2D from an average of 12 years. They are 64% men, the mean age is 66±11 years, the mean HbA1c 7.13%±1,63%, the mean BMI 28±5 kg/m^2, 40% has a history of CVD, 27% shows CKD, 76% have hypertension.

The 43% tested positive for one or more mental disorders, 25% for depression. These values correspond to the prevalence of CPD described in similar international studies.

Conclusions. The prevalence of CPD and depression in the general Italian population is 7.3% and 3% respectively. The higher prevalence of CPD in patients with T2D results in poor adherence to prescriptions of lifestyle changes and therapy thus having less chance of induce weight loss and remission of T2D. Lack of adherence to therapies determines the development of atherosclerosis and CVD. An integrated psychiatric-diabetological approach to T2D is required in the early years of the pathology, aiming at the remission of the psychiatric and diabetic conditions in order to prevent the development of CVD.

EFFECTS OF TRADITIONAL CIGARETTE AND NEXT GENERATION TOBACCO PRODUCTS ON SMOOTH MUSCLE CELL PHENOTYPIC MODULATION

C. Rossi[1], I. Damiani[1], S. Castiglioni[1], A. Corsini[1,2], S. Bellosta[1]
[1]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano;
[2]IRCCS MultiMedica, Milano

Background and Aims. Vascular homeostasis is maintained by differentiated quiescent smooth muscle cells (SMCs) that, following environmental cues, may undergo transcriptional changes affecting contractile proteins and their proliferative phenotype. One of these risk factor for cardiovascular disease is cigarette smoke. Whole traditional cigarette (TC) smoke contains over 7000 chemical components, some of which are toxic. Thus, alternative next-generation tobacco products (E-cigarettes (E-CIG) and tobacco heating products (THP)) are being developed as less dangerous. We studied the effects of aqueous extracts (AEs) from TC, E-CIG and THP on SMC phenotypic modulation.
Methods. Human aortic SMCs (HSMCs) were incubated for 48 hours with AEs and gene/protein expression analyzed by real-time PCR, western blot analysis, and confocal microscopy. Proliferation activity was measured by coulter counter analysis, migratory activity analyzed by wound healing assay and Boyden's chamber.
Results. AEs stimulated the expression of contractile markers (α-actin, calponin) and of a network of regulatory transcription factors such as myocardin and SRF, which promote a SMC contractile state while reducing the levels of myocardin repressor Krüppel-like factor 4.
E-CIG showed potent and faster induction of SMC proliferation and migratory activity, while TC slowed it down and THP did not show any effect compared to control. The incubation with TC and E-CIG AEs also affected cell morphology, with the extension of filopodia, and increased F-actin levels; in particular, TC increased it by 70% and E-CIG by three-fold, instead THP did not affect the expression of F-actin protein.
Conclusions. AEs have different effects on SMC plasticity. All three smoke extracts stimulate the expression of contractile SMC-related genes. However, E-CIG potently induces SMC proliferation and migration activity, while TC reduces it. Therefore, the real long-term health effects of these next-generation cigarettes will have to be further assessed.

METABOLIC ALTERATIONS OF SKELETAL MUSCLE AND EXERCISE INTOLERANCE IN A MOUSE MODEL OF FABRY DISEASE

A. Fiordelisi[1], J. Gambardella[1,2], F. Cerasuolo[1], R. Avvisato[1], G. Iaccarino[1,3], D. Sorriento[1]
[1]Università degli Studi di Napoli Federico II, Naples;
[2]Albert Einstein College of Medicine, NY, USA;
[3]Centro Interdipartimentale di Ricerca in Ipertensione Arteriosa e Patologie Correlate, Città

Background. Muscle pain and fatigue are commonly reported in Fabry disease (FD). Studies in FD patients suggest direct involvement and primary skeletal muscle abnormalities, with unknown pathogenesis. The disarrangement of energy metabolism could contribute to organ damage in FD, as suggested by the reduced mitochondrial activity found in patient fibroblasts.
Aim. To characterize the muscle phenotype associated with FD and explore its pathogenetic mechanism, evaluating the role of metabolic and mitochondrial alterations.
Methods. GAL-Tg / KO (R301Q-GLA) mice were used as a model of FD. Spontaneous physical activity was assessed by measuring night running on wheels, while in-vivo muscle strength by weight-test. Histological and biochemical analyzes were performed on the ex vivo muscle.
Results. FD-mice show a reduced propensity to spontaneous physical activity compared to WT mice of the same age, indicating a lower tolerance to aerobic activity. However, FD-mice reach a higher score on the weight test, demonstrating greater muscle power during acute-anaerobic exercise. From the histological analysis, it appears that the fast/glycolytic fibers capable of conferring a high contractility, but high fatigue are more represented in the muscle-FD. The counterpart of slow/oxidative fibers is instead reduced in muscle-FD. The biochemical analysis of muscle-FD shows increased expression of Hexokinase-II, lactic dehydrogenase, and pyruvate-kinase, key enzymes of the glycolytic pathway which, together with the accumulation of lactate, suggest an overactivation of anaerobic glycolysis. The expression of the III/IV complexes of the respiratory chain are significantly reduced in the muscle-FD and electron microscopy shows alterations in the mitochondrial ultrastructure. These biochemical and functional alterations of the muscle are precocious with respect to the onset of diastolic dysfunction in FD-mice.
Conclusions. Muscle-FD shows a metabolic disarrangementwith a switch towards glycolysis-anaerobic and impaired exercise tolerance. The alterations in muscle metabolism could depend on alterations in the mitochondria.

GENETICALLY ELEVATED LDL CHOLESTEROL BURDEN RESULTS INTO REDUCED PROGENITOR CELLS WITH IMPAIRED ENDOTHELIAL FORMING POTENTIAL

O. Terenghi[1], D. Alagia[1,2], F. Pellegatta[2,3], L. Grigore[2,3], G.D. Norata[1], A.L. Catapano[1,3], A. Baragetti[1,3]
[1]Department of Pharmacological and Biomolecular Sciences, University of Milan;
[2]S.I.S.A. Center for the Study of Atherosclerosis, Bassini Hospital, Milan;
[3]IRCCS MultiMedica, Milano

Aim. Genetically determined Familial Hypercholesterolemia (FH) leads to lifelong elevated LDL cholesterol (LDL-C) burden and premature coronary vascular endothelial dysfunction. We previously demonstrated that genetically determined FH results into premature hematopoietic cellular aging and now we aim to evaluate whether Endothelial Progenitor Cell (EPCs), in charge of the endothelial formation, display features of premature impairment.
Methods. EPCs were taken by 33 Heterozygous FH (HeFH, Next Generation Sequencing on LDLR) (95,12% on LDL-C lowering treatment), by 10 clinically defined but not genetically confirmed FH ("CD-FH", 11,11% on LDL-C treatment) and by 13 normocholesterolemic subjects ("controls", none on treatment).
EPCs were characterized for:
1) blood count (Flow-Cytometry);
2) ex vivo number of Endothelial Colony Forming Units ("EC-CFUs");
3) proliferation index (Cell-trace Violet).

To also explore whether EPCs reflects the coronary vascular endothelial dysfunction in vivo we correlated, in HeFH, these features with PET-CT Coronary Artery Calcium (CAC) score.
Results. Blood EPCs were reduced in HeFH versus controls (37±53 vs 225±506 cells/mL, p=0,050). Of note, EPCs were comparable between CD-FH and controls (50±43 vs 225±506 cells/mL, p=0,687).
After three days of peripheral blood cells culture, EC-CFUs of HeFH were less versus that of controls (7±8,19 vs 20±27,84 EC-CFU/well, p=0,04). Vice versa, EC-CFUs from CD-FH and controls were comparable (5±4,92 vs 20±27,84 EC-CFU/well, p=0,152).
Proliferation index of EC-CFU from controls increased by cell seeding up to the third day of culture (32,73±18,4%), while this did not hold true for HeFH (25,05±15,13%) and CD-FH (95,32±15,12%).
Of note, blood EPCs did not correlate with CAC scores (R^2=0,001, p=0,932).
Conclusions. A low number of EPCs is present in subjects with genetically elevated LDL-C burden, also with ongoing LDL-C lowering treatment. Lack of relation with CAC score, clinical marker of coronary vascular endothelial dysfunction, implies intrinsic alterations of this cellular compartment in FH, whose origins at the level of the bone marrow entice our future investigations.

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

Aim. 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.
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. 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.
Conclusions. We report a case of CESD with uncommon clinical presentation features compound heterozygous for two mutations in LIPA gene.

DIAGNOSIS AND MANAGEMENT OF FAMILIAL CHYLOMICRONEMIA SYNDROME IN A NEWBORN GIRL

T.M.G. Fasciana, A. Scalzo, C. Scrimali, G. Savarino, F. Brucato, V. Insinga, M. Gueli Alletti, C. Bonacasa, G. Misiano, A. Giammanco, C.M. Barbagallo, M. Carta, D. Noto, A.B. Cefalù, M. Giuffré, M.R. Averna

Dipartimento di Promozione della Salute, Materno-Infantile, di Medicina Interna e Specialistica di Eccellenza "G. D'Alessandro" (PROMISE), Università degli Studi di Palermo

Aim. Familial chylomicronaemia syndrome (FCS) is a rare, severe, monogenic, recessive disorder caused by loss-of-function mutations in both alleles of one or more of the genes that control the intravascular lipolytic cascade of triglyceride (TG) - rich lipoproteins. FCS is characterized by severe hypertriglyceridemia (TGs >10 mmol/L - 886 mg/dL) due to the accumulation of chylomicrons during fasting. FCS patients also develop eruptive xanthomas, lipemia retinalis, recurrent abdominal pain, acute and/or recurrent pancreatitis, hepato-splenomegaly and memory loss. The standard of care of FCS is based on a strict dietary regimen with <10% of energy from fat and supplementation with medium-chain TGs. Long-term adherence to this diet is poor.

Methods. Here we describe a female neonate, born at term of uneventful pregnancy from healthy non-consanguineous parents. At 6th day of life a capillary blood micro-sample showed lactescent serum. TG levels were 3632 mg/dl and the clinical suspicion of FCS was made. A Next Generation Sequencing (NGS) custom panel was used to analyze candidate genes involved in the pathways of triglyceride synthesis and metabolism. Implementation of a nutritional management plan was started.

Results. NGS analysis allowed to identify a previously described homozygous pathogenetic mutation in LPL gene (c.829G>A p.Asp277Asn). Genetic molecular cascade screening allowed to identify the mutation in heterozygosity in both parents.

Milk formula supplemented with medium chain triglycerides (MCT) oil, vitamins and oligoelements ensured an adequate intake of nutrients and TGs were stably <500 mg/dl over the weeks.

At 6 months complementary feeding was introduced with a specific low-fat diet. Feeding has been well tolerated and TG levels have been as low as 339 mg/dl.

Conclusions. In conclusion, early diagnosis and nutritional management of FCS in newborn are crucial to guarantee adequate growth and neuro-psycho-motor development and prevention severe complication.

HIGH FREQUENCY OF APOB VARIANTS DETECTED BY NEXT-GENERATION SEQUENCING: A CASE REPORT

M. Ferrandino[1], G. Cardiero[1,2], C. Gianfico[1], R. Auricchio[3], C. Flagiello[2], M.V. Esposito[2], C. Martin[4], M.D. Di Taranto[1], G. Fortunato[1,2]

[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;
[3]Dipartimento di Scienze Mediche Traslazionali, Università degli Studi di Napoli Federico II, Napoli;
[4]Fundación Biofisika Bizkaia, 48940 Leioa, Spagna

Aim. Familial Hypercholesterolemia (FH) is a very frequent genetic disease (1:250) associated with premature cardiovascular disease. The main causative genes of FH are *LDLR*, *APOB* and *PCSK9* with a codominant inheritance. Most of the causative variants are in *LDLR* and cause the full or partial loss of function of LDLR. Variants in *APOB* causing FH lead to a protein not able to bind LDLR. Next-generation sequencing (NGS) allows to analyze the whole *APOB* gene identifying a large number of variants, mainly classified as variants of unknown significance.

Methods. The studied patient was a male with hypercholesterolemia, reporting hypercholesterolemia also in his child and siblings. The genetic analysis was performed by NGS, using a panel including the main causative gene of FH, the *LDLR*, and the most rarely causative genes *APOB*, *PCSK9*, *APOE* e *STAP1.* A functional assay in HepG2 cells was performed to assess the residual binding of APOB to LDLR.

Results. A rare *APOB* variant, c.11354 C>T-p. (Thr3785Ile) was found in a patient with clinical suspect of FH. No other rare variants were found. The functional characterization of this variant in HepG2 cells showed a residual binding of APOB to LDLR of 40%. Due to this result, to the very low variant frequency and to bioinformatics predictions suggesting an impact on protein function, variant was classified as Likely Pathogenic. The cascade screening allowed to identify the same variant in the patient child and two additional variants in *APOB* in patient siblings, that are still under study.

Conclusions. The NGS allowed to identify a high number of variants in *APOB,* a large gene that wasn't completely analyzed in the past because of its size. Pathogenic assessment based on wide and accurate genetic analyses and on functional assays can help to correctly classify *APOB* variants to define their pathogenic role.

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

Aim. 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.
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. 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.
Conclusions. NGS is a powerful tool for the genetic diagnosis of HTG and mutations of CREB3L3 gene may be associated with severe forms of hypertriglyceridemia.

CORRELATION BETWEEN FAMILIAL HYPERCOLESTEROLEMIA GENOTYPE AND RESPONSE TO LIPID-LOWERING THERAPY

F. Troiano, D. D'Ardes, I. Rossi, P. Vizzarri, A.V. Di Giacomantonio, M. Caporale, G. Matarazzo, M. Michela, F. Cipollone, M. Bucci

[1]Institute of Clinica Medica, Department of Medicine and Science of Aging, "G. d'Annunzio" University of Chieti-Pescara

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

BACTEROIDES THETAIOTAOMICRON PREVENTS ETHANOL-INDUCED GUT-LIVER AXIS PERTURBATIONS AND IMPROVES LIVER METABOLISM IN EXPERIMENTAL ALCOHOL-RELATED LIVER DISEASE

M. Sangineto[1,2], C. Grander[2], F. Grabherr[2], L. Mayr[2], J. Schwärzler[2], M. Dallio[2,3], Vidyasagar Naik Bukke[1], A. Moola[1], Martina Ciarnelli[1], A. Moschetta[4], T.E. Adolph[2], C. Sabbà[4], H.t Tilg[2], G. Serviddio[1]

[1]C.U.R.E. (University Center for Liver Disease Research and Treatment), Liver Unit, Department of Medical and Surgical Sciences, University of Foggia;
[2]Department of Internal Medicine I, Gastroenterology, Hepatology, Endocrinology & Metabolism, Medical University Innsbruck, Innsbruck, Austria;
[3]Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples;
[4]Department of Interdisciplinary Medicine, University of Bari

Aim. Alcohol-related liver disease (ALD) represents a global burden in terms of morbidity and mortality. ALD pathogenesis encompasses a wide spectrum of hepatic metabolic disorders, although the key-role of intestinal homeostasis and microbiota recently emerged. Bacteroides thetaiotaomicron (Bt), one of most common components of human and murine intestinal microbiota, is crucial for intestinal immunity and mucosal barrier development, and was recently proposed as obesity opponent. Considering the prominent functions of Bt in human health and the central role of microbiota in ALD development, we aimed to investigate the involvement of Bt in ALD preclinical model.
Methods. The fecal Bt DNA was quantified in wild-type mice (C57/Bl6) receiving a Lieber-DeCarli diet supplemented with increasing alcohol concentrations (up to 5%) for 15 days. In a second step, ethanol-fed mice were treated by administering living Bt by oral gavage. Gut-liver axis and hepatic histological and biochemical alterations were assessed. Experiments were conducted between the Medical University of Innsbruck (Internal Medicine-I), Austria, and the University of Foggia (Unità di Epatologia), Italy.
Results. Alcohol feeding drastically reduced Bt intestinal amount, hence a potential role of Bt loss in ALD development was supposed. In the second step, Bt intestinal abundance was restored by oral administration in ethanol-fed mice, and interestingly, Bt-treated mice showed lower hepatic steatosis. Ethanol feeding decreased FGF15 intestinal production, while Bt treatment restored FGF15 levels likely by intestinal FXR activation. The cellular energy sensor AMPK, hepatic target of FGF15 receptor signalling, was reduced by alcohol and restored in Bt-treated mice. Consequently, liver bioenergetics was positively affected by the treatment, as Bt improved ATP content and counteracted the lipid metabolism perturbations, reducing fatty acid synthesis (FA), and improving FA oxidation and lipid exportation. Furthermore, mitochondrial biogenesis and fitness were disrupted by alcohol and preserved by Bt supplementation.
Conclusions. Bt recovery in ethanol-fed mice significantly improves steatosis and liver metabolism likely by preventing ethanol-induced gut-liver axis disruption. Bt candidates as a novel probiotic to treat ALD in the future.

HEPATOCELLULAR CARCINOMA AND DIFFERENT IMMUNOLOGICAL MICROENVIRONMENT, A POSSIBLE EXPLANATION FOR NO RESPONDER IMMUNOTHERAPY IN METABOLIC PATIENTS

A. Dalbeni[1,2], L.A. Natola[1,2], S. Cicco[6], F. Cattazzo[1,2], A. Vella[3], M. Bevilacqua[1,2], J. Kassem[2], A. Mantovani[1,2], S. Conci[4], T. Campagnaro[4], A. Guglielmi[4], M. Milella[5], D. Sacerdoti[2]

[1]General Medicine, C Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona;
[2]Liver Unit, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona;
[3]Immunology Unit, University and Azienda Ospedaliera Universitaria Integrata of Verona;
[4]Biliar and Liver Surgery Unit, Department of Surgery, University and Azienda Ospedaliera Universitaria Integrata of Verona;
[5]Department of Oncology, University and Azienda Ospedaliera Universitaria Integrata of Verona;
[6]Department of Biomedical Sciences and Human Oncology (DIMO), Unit of Internal Medicine and Clinical Oncology, University of Bari Aldo Moro Medical School, Policlinico di Bari University Hospital, Bari

Introduction. Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death. Most cases (90%) of HCC arise in the setting of a chronic liver disease. The advanced stages of HCC are amendable only to systemic treatment. Systemic therapy has been revolutionized by immune-based therapies. However, a recent meta-analysis of three randomized phase III clinical trials that tested inhibitors of PD-L1 or PD1 in more than 1,600 patients with advanced HCC, revealed that immune therapy did not improve survival in patients with metabolic HCC.
Aim. The aim of the study was to identify differences in immune cell population of patients with HCC, according to etiopathogenesis of cirrhosis, in order to identify different patterns that may be targeted for immunotherapy.
Patients and Methods. We analyzed 50 leukocyte sub-populations, using flow cytometric technique, in a cohort of 111 consecutive HCC cirrhotic patients with different etiopathogenesis. Differences between groups were analyzed with the Mann-Whitney U test.
Results. We divided HCC patients with cirrhosis into five groups according to etiology of liver disease: metabolic (NAFLD/NASH), alcoholic, viral (HCV/HBV), overlap between etiological factors and other etiology. Leukocyte sub-populations were significantly different in two groups. Specifically in the group of alcoholic etiology there were increased levels of PMN ($p<0.01$), monocytes ($p<0.01$) and NKT cells CD57+RA-(4-8-) ($p=0.011$), while in viral etiology group there were increased levels of T cells CD57-RA+(4-8-) ($p=0.007$) and T cell CD3+ (4-8-) ($p=0.009$). No specific leukocyte subpopulation was significantly increased in the metabolic one.
Conclusions. These data suggest that in liver disease with different etiopathogenesis there are different immunological microenvironments. Innate and innate-like cells are the principal regulators of immune response in alcoholic HCC, while adaptive immune system is predominant in viral etiology. No differences in metabolic HCC. This suggests that differences in cancer-mediated immune escape could explain the different response to immunotherapy and may be helpful to identify new HCC immunotherapies in metabolic setting.

SUBCLINICAL AND CLINICAL ATHEROSCLEROSIS IN NON-ALCOHOLIC FATTY LIVER DISEASE ARE ASSOCIATED WITH THE PRESENCE OF HYPERTENSION

A. Mantovani[1,2,3], F. Cattazzo[1,2], R. Lombardi[4], M. Bevilacqua[1,2], M. Zoncapè[1,2], L. Iogna Prat[3], D. Roccarina[3], L. Fortuna[1], A. Cespiati[4], D. Sacerdoti[1,2], A.L. Fracanzani[4], E. Tsochatzis[3], C. Fava[1], A. Dalbeni[1,2]

[1]General Medicine C, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona;
[2]Liver Unit, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona;
[3]UCL Institute for Liver and Digestive Health, Royal Free Hospital and UCL, London, UK;
[4]Unit of Internal Medicine and Metabolic Disease, Ca' Granda IRCCS Foundation, Policlinico Hospital, Department of Pathophysiology and Transplantation, University of Milan

Aim. Non-alcoholic fatty liver disease (NAFLD) is associated with increased cardiovascular risk. However, whether NAFLD contributes independently to the development of cardiovascular disease is not fully understood. Our study aimed to assess the differences in several indices of atherosclerosis, arterial stiffness, cardiac function and morphology between NAFLD patients with or without hypertension (HT) and patients with HT but no NAFLD through several markers of atherosclerosis.
Methods. One hundred and sixty-nine participants (mean age =50.4±10.2 yrs; males =73.6%) were divided according to the presence of NAFLD and HT in three groups: only-NAFLD (55 patients), only-HT (49 patients) and NAFLD+HT (65 patients). Exclusion criteria were BMI≥35 kg/m^2 and the presence of diabetes mellitus. Carotid ultrasonography was performed to measure markers of arterial stiffness, subclinical and overt atherosclerosis. Cardiac function and morphology were analyzed using transthoracic echocardiography.
Results. The prevalence of subclinical and overt atherosclerosis was significantly higher in the NAFLD+HT patients as compared to the other two groups (atherosclerotic plaques: 43.1%, 10.9%, 22.4% (p<0.001), in NAFLD+HT, NAFLD and HT group). No differences were found among indices of arterial stiffening and cardiac remodeling or dysfunction. In multivariate regression analysis the coexistence of NAFLD and HT was independently associated with overt atherosclerosis (OR=4.88; p=0.03), while no association was found when either NAFLD or HT was considered alone.
Conclusion. Overt atherosclerosis was more pronounced in NAFLD+HT patients. This implies that the impact of NAFLD on vascular structure and function could depend on the coexistence of other major cardiovascular risk factors, such as HT.

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

S. Argeri, S. Cicco, A. Vacca, G. Lauletta
Unit of Internal Medicine "Guido Baccelli", Department of Biomedical Sciences and Human Oncology, University of Bari "Aldo Moro"

Aim. non-alcoholic fatty liver disease (NAFLD) is one of the end-target organ damages of lipid metabolism alterations. There are not clear data about the connection between steatohepatitis and heart involvement. However, many risk factors are similar between the two diseases. Therefore, a possible connection between the two organs may be possible
Purpose is to evaluate risk of cardiovascular disease in NAFLD.
Methods. We evaluate 41 patients (15 F, aged 58.71±13.56) who presented NAFLD according to the recent international guidelines. Using the liver ultrasound we evaluate the liver stiffness as fibrosis marker as stiffness measured by share wave technology. As controls, we compared 88 patients who were NAFLD negative (33F, aged 57.39±9.91). To evaluate the cardiovascular risk we calculate for each patient Atherosclerosis Cardiovascular disease score (ASCVD-10-yr).
Results. in NAFLD group, arterial essential hypertension was in 58.53% (24) and diabetes in 34.15% (14). Similar percentages of comorbidities were found in Controls. Patients with NAFLD has an increased liver fibrosis (median metavir class 1 [IQR 1-3]), compared to controls (metavir 0) (p<0.05) and an increased cardiovascular risk compared to controls (ASCVD-10-yr 17.21±17.99% vs 7.93±7.68, p<0.05). Metavir class was directly correlated to ASCVD-10-yr (Spearman r 0.37, p<0.01). The same direct correlation was found between ASCVD-10-yr and absolute liver stiffness values evaluated in kPa (Pearson r 0.39, p<0.05).
Conclusion. Our data suggest that NAFLD may relate to a reduced life-expectancy mostly due to cardiovascular disease. These data may be useful in evaluation of both cardiovascular disease and liver steatosis due the connection we found between the two parameters. Thus, a comprehensive evaluation may be useful in order to optimize the tailored therapy for these patients. Further prospective studies are needed to confirm our data.

ROLE OF OPA1-MEDIATED MITOCHONDRIAL DYNAMICS IN KUPFFER CELLS ON SYSTEMIC METABOLISM

M. Ceccon[1], A. Diroma[1], A. Moregola[1], L. Da Dalt[1], F. Bonacina[1], G.D. Norata[1,2]
[1]Department of Excellence of Pharmacological and Biomolecular Sciences, University of Milan;
[2]SISA Atherosclerosis Center, Bassini Hospital, Cinisello Balsamo (Milan)

Aim. Kupffer Cells are tissue-resident macrophages of the liver, that play crucial roles in liver physiopathology. In addition to a pure immunology action, they synthesize proteins with a metabolic activity that affects systemic metabolism (i.e., CETP). The OPA1 protein is a member of the dynamine family located in the mitochondrial inner membrane, where it is involved in mitochondrial dynamism, regulating cristae formation, and thus controlling OXPHOS. This project aims to study the role of mitochondrial dynamics in KC and its effect on systemic metabolism taking advantage of mice lacking OPA1 selectively in KC.
Methods. OPA1 flox/flox Clec4F-Cre+ and controls were fed a standard diet for 22 weeks. Metabolic phenotype was assessed by indirect calorimetry through metabolic cages. Blood and liver were collected for immunophenotyping by flow cytometry analysis. Total cholesterol and triglycerides were measured in fresh plasma. Histological analyses of liver were performed
Results. OPA1 flox/flox Clec4F-Cre+ showed less energy expenditure (-9.44%; $p<0.05$), less oxygen consumption (-9.44%; $p<0.05$) and less carbon dioxide production (-9,48%; $p<0.01$), despite an increase in movement (+26.6%) flox/flox Clec4F-Cre- control mice. Systemic immune profile was similar, while the percentage of Kupffer Cells in the liver was reduced in OPA1 flox/flox Clec4F-Cre + compared to OPA1 flox/flox Clec4F- Cre - (-25% $p<0.05$). No significance difference in cholesterol and triglyceride levels were observed between the two groups as well in liver histology.
Conclusions. Our data suggest that opa1-mediated alteration of mitochondrial dynamics affects KC and, in turn, impacts systemic energy phenotype. To unveil whether this pathway plays a role in liver diseases, ongoing studies are addressing the role of OPA1 deficiency in KC in models of diet-induced obesity.

PREDICTORS OF SACUBITRIL/VALSARTAN HIGH DOSE TOLERABILITY IN HFrEF

V. Visco[1], I. Radano[1], A. Campanile[2], A. Ravera[2], A. Silverio[1], D. Masarone[3], G. Pacileo[3], M. Correale[4], P. Mazzeo[4], G. Dattilo[5], F. Giallauria[6], A. Cuomo[6], V. Mercurio[6], C. Gabriele Tocchetti[6,7,8,9], P. Di Pietro[1], A. Carrizzo[1,10], R. Citro[2], G. Galasso[1], C. Vecchione[1,10], M. Ciccarelli[1]
[1]Chair of Cardiology, Department of Medicine, Surgery and Dentistry, Schola Medica Salernitana, University of Salerno;
[2]Department of Cardiology, "San Giovanni di Dio e Ruggi D'Aragona" Hopital-University, Salerno;
[3]Heart Failure Unit, AORN Colli, Naples;
[4]Cardiology Department, Ospedali Riuniti University Hospital, Foggia;
[5]Department of Clinical and Experimental Medicine, Operative Unit of Cardiology, University of Messina;
[6]Department of Translational Medical Sciences, Federico II University, Naples;
[7]Center for Basic and Clinical Immunology Research (CISI), Federico II University, Naples;
[8]Interdepartmental Center for Clinical and Translational Research (CIRCET), Federico II University, Naples;
[9]Interdepartmental Hypertension Research Center (CIRIAPA), Federico II University, Naples;
[10]Vascular Pathophysiology Unit, IRCCS Neuromed, Pozzilli, Isernia

Aim. Sacubitril/valsartan (Sac/Val) demonstrated to be superior to enalapril in reducing hospitalizations, cardiovascular and all-cause mortality in patients with ambulatory heart failure and reduced ejection fraction (HFrEF), in particular when it is maximally up-titrated. Unfortunately, the target dose is achieved in less than 50% of HFrEF patients, thus undermining the beneficial effects on the outcomes. Specifically, in this study, we aimed to evaluate the role of Sac/Val and its titration dose on reverse cardiac remodeling and determine which echocardiographic index best predicts the up-titration success.
Methods. From January 2020 to June 2021, we retrospectively identified 95 patients (65.6 [59.1-72.8] years; 15.8% females) with chronic HFrEF who were prescribed Sac/Val from the HF Clinics of 5 Italian University Hospitals and evaluated the tolerability of Sac/Val high dose as the primary endpoint in the cohort.
Results. A significant improvement in NYHA functional class was reported (NYHA II: from 58% to 74%; NYHA III: from 41% to 14%); After 6 months, LVEF significantly increased from 28.8% [22.2-33] to 35% [29-40]; LVESV significantly decreased from 135 [108-180] to 114 [83-166]; ($p<0.001$), and sPAP decreased from 35 [28.7-48.5] to 31.5 mmHg [23-42.2]. In relation to the primary endpoint, three continuous variables (age, systolic blood pressure, and TAPSE) resulted significantly associated with the study outcome variable with a strong discriminatory capacity (area under the curve 0.876, 95% confidence interval (CI) 0.803-0.949).
Conclusions. Our study is the first to analyze the potential role of echocardiography and, in particular, of RV dysfunction, measured by TAPSE, in predicting Sac/Val maximum dose tolerability. From a clinical point of view, patients with RV dysfunction (baseline TAPSE <16 mm, in our cohort) might benefit of a different strategy during the follow-up (e.g. a more gradual up-titration), in order to maximize the benefit of the treatment with Sac/Val.

IMPACT 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[1,2], A.L. Catapano[1,2]
[1]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan;
[2]IRCCS MultiMedica, Sesto San Giovanni (Milano)

Lipid-lowering therapies (LLTs) may have an effect also on inflammatory mediators, resulting in better clinical outcomes. However, evidence from clinical studies is conflicting. Our study aimed to assess the effect of LLTs on C-reactive protein (CRP), in addition to lipid reduction.
We conducted a meta-analysis according to the PRISMA guidelines, evaluating 6 different classes of LLTs (statins, ezetimibe, PCSK9-inhibitors, CETP-inhibitors, fibrates, and omega-3 fatty acids [omega-3FA]). PubMed, Web of Science, EMBASE, Cochrane Library, and ClinicalTrial.gov were searched from 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 level;
4) with intervention duration more than 3 weeks;
5) and sample size (for each arm) greater than 100 subjects.
Pooled estimates were assessed by a random-effects model. Between-study heterogeneity was tested by Cochrane's Q test and measured with the I2 statistics.
A total of 157,885 participants from 38 RCTs were included in our meta-analysis. CRP concentration was significantly decreased by 0.54 mg/L (95%CI, -1.08 to -0.01) and 1.17 mg/L (95%CI, -2.31 to -0.04) for statins (12 RCTs, 48,008 participants) and ezetimibe (6 RCTs, 16,909 participants) compared with placebo, respectively. A 0.62 mg/L (95%CI, -1.50 to 0.26) absolute reduction was also observed for fibrates (4 RCTs, 3,245 participants), although not statistically significant. For omega-3FA (7 RCTs, 16,064 participants), CRP levels were slightly reduced by 0.15 mg/L (95%CI, -0.26 to -0.04). Instead, a slight increase of CRP concentration was found for PCSK9-inhibitors (0.06 [95%CI, -0.01 to 0.14], not statistically significant; 5 RCTs, 47,709 participants) and CETP-inhibitors (0.07 [95%CI, 0.03 to 0.12]; 4 RCTs, 25,950 participants).
In conclusion, among LLTs, statins, ezetimibe, fibrates, and omega-3FA seemed to reduce serum CRP concentration. The impact of this anti-inflammatory effect in terms of cardiovascular prevention needs further investigation.

IMPACT OF SLCO1B1 AND ABCB1 POLYMORPHISMS ON THE RISK OF ADVERSE STATIN REACTIONS

G. Matarazzo, D. D'Ardes, I. Rossi, P. Vizzarri, A. Michetti, F. Troiano, M. Caporale, M. Montagano, F. Cipollone, M. Bucci
Institute of Clinica Medica, Department of Medicine and Science of Aging, "G. d'Annunzio" University of Chieti-Pescara

Aim. Statins are the gold standard in reducing cardiovascular risk through lowering LDL cholesterols. However, 7 to 29% of statin-treated patients experience myopathy. The SLCO1B1 and ABCB1 gene are responsible for hepatic reabsorption and biliary and renal elimination of statins, respectively. The aim of the study was to evaluate the presence of a correlation between heterozygous rs 4149056 SLCO1B1 or homozygous rs 2032582 ABCB1 mutations and adverse events in patients treated with (rosuva, prava, atorva/simva) statins.
Methods. We monitored through clinical and laboratory parameters two statin metabolism's regulatory genes (SLCO1B1, ABCB1) in patients under statin therapy.
Results. SLCO1B1 gene: in patients treated with atorvastatin/simvastatin, 7 out of 8 patients experienced adverse events such as to discontinue therapy; in those treated with rosuvastatin, 50% in a group of10 patients required a change of therapy while for pravastatin, only 1 out of 5 patients experienced side effects.
ABCB1 gene: there was a marked intolerance towards atorvastatin/simvastatin (6 out of 6 patients discontinued therapy), while for pravastatin 2 out of 3 patients discontinued therapy and, about rosuvastatin, only 1 out of 6 had to discontinue therapy
Conclusions. Despite the small sample size, the SLCO1B1 mutation appears to be strongly associated with an increased probability of adverse reactions from statins in drugs metabolized by cytochrome CYP450 3A4 (simvastatin and atorvastatin). Instead, rosuvastatin minimizes the risk of adverse effects in patients with the ABCB1 polymorphism.
Although metabolism of pravastatin is not influenced by cytochromes, a greater number of adverse events have been observed in patients carrying the ABCB1 mutation.
The genotyping of the polymorphisms rs 4149056 SLCO1B1 and rs 2032582 ABCB1, could help to improve the therapeutic management of the statins in future increasing the safety of the administration, and allowing to implement more and more a "tailored therapy".

LIPID-MODIFYING THERAPIES: EVALUATION OF THEIR EFFICACY IN HIGH AND VERY HIGH CV RISK PATIENTS

A. Calisi[1,2], E. Gigante[1,2], I. Rossi[1,2], D. D'Ardes[1,2], B. Bucciarelli[3], F. Bianco[3], F. Troiano[1,2], P. Vizzarri[1,2], M. Caporale[1,2], F. Cipollone[1,2], M. Bucci[1,2]
[1]"Clinica Medica" Institute, "SS. Annunziata Hospital", ASL02 Abruzzo, Chieti;
[2]Department of "Medicine and Science of Aging", "G. d'Annunzio" University, Chieti;
[3]Azienda Ospedaliero-Universitaria "Ospedali Riuniti", Ancona

Background. Hypercholesterolemia is one of the main modifiable atherosclerotic cardiovascular (CV) disease's risk factors.
Aim. to evaluate the effectiveness of the currently available lipid lowering therapy (LLT) and the achievement of treatment targets (TT) in a cohort of high and very-high CV risk patients treated with maximum tolerated LLT.
Methods. We enrolled 27 consecutive high or very-high CV risk (estimated by ESC-SCORE charts) patients between November-2020 to January-2021 and followed-up them for 8±2 months. Four patients were excluded due to missing laboratory data; 23 patients (mean age 60±13 yo, M=74%) represented our final sample of this analysis.
Results. Ezetimibe was the most utilized therapy (n=17/23, 74%); Rosuvastatin was the most prescribed statin utilized (n=10/23, 43%); 9 subjects were treated with both. At follow-up visit, a significant reduction in Total- and LDL-Cholesterol (p=0.014 and p=0.011, respectively), was experienced by the entire population with a change from baseline of -28 [-103, 13 (Q1, Q3)] and -34 [-92, 11 (Q1, Q3)] mg/dL. Half of participants (n=11/23, 50%) achieved the TT according to the 2019 ESC/EAS recommendations. At the multivariable analysis, PCSK9-i was the most effective LLT to achieve the TT [(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 addition of PCSK9-i still showed to be the most effective LLT to achieve TT but this is often too expensive. The challenge is that future less expensive therapeutic strategies, currently not available in Italy, could allow the TT to be reached equally.

MEASUREMENT OF CHOLESTEROL IN THE CEREBROSPINAL FLUID

F. Gastoldi[1], M. Turri[1], C. Pavanello[1], E. Conti[2], D. Emide[3], A. Barbiroli[3], L. Tremolizzo[2-4], L. Calabresi[1]
[1]Centro Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano;
[2]Scuola di Medicina e Chirurgia e centro di Neuroscienze di Milano (NeuroMI), Università di Milano-Bicocca, Monza;
[3]Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Università degli Studi di Milano;
[4]Unità di Neurologia, Ospedale San Gerardo, Monza

Background and Aim. Cholesterol in the CNS is essential for brain development and functioning. Different studies suggested an involvement of cholesterol in neurodegenerative disorders such as Alzheimer's disease (AD). While the measurement of cholesterol in plasma is a simple routinary analysis, the cerebrospinal fluid (CSF) cholesterol is difficult to quantify because this matrix contains about 300 times less cholesterol than plasma, meaning that a specific analytical method is needed.
The aim of this study has been to quantify unesterified cholesterol (UC) and total cholesterol (TC) in CSF of 30 AD patients.
Materials and Methods. CSF samples of 30 AD patients have been collected by lumbar puncture. To quantify TC an aliquot of CSF has been incubated with cholesterol esterase for 1 hour at 37 °C to convert the esterified cholesterol in UC. To quantify both TC and UC an esterase-treated and an untreated aliquot were extracted in duplicate with 2-propanol (1:5, v:v). The extracted aliquots were then analysed by RP-HPLC in isocratic conditions with a Jupiter 4u Proteo 90A 150 x 4.6 mm column and acetonitrile: 2-propanol 2:1 as mobile phase operating with a flux of 0.8ml/min. The chromatogram was obtained at λ=210 nm.
Results. 30 CSF samples of AD patients were analysed. Unesterified cholesterol is eluted 8 minutes after sample injection into the column. For quantification, a calibration curve was constructed using standard cholesterol solubilized in 2-propanol. TC and FC were measurable in all the tested samples. The average TC value was 0,336±0,077mg/dl and the average UC value was 0,174±0,033 mg/dl.
Conclusions. The described method allows the measurement of TC and UC under isocratic conditions in CSF samples; obtained results are in line with those previously reported in the literature, confirming the use of HPLC for the measurement of cholesterol in CSF.

IN SILICO PREDICTION OF ANGPTL3-ENDOTHELIAL LIPASE INTERACTION

L. Montavoci, O. Ben Mariem, S. Saporiti, T. Laurenzi, U. Guerrini, A. Ossoli, L. Palazzolo, L. Calabresi, I. Eberini
Laboratorio di Biofisica e Biochimica Computazionale, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano

Aim. ANGPTL3 (Angiopoietin-like protein 3) is a plasma protein that plays a main role in the metabolism of lipoproteins. It inhibits the lipoprotein lipase, responsible for the hydrolysis of triglycerides on VLDL, and the endothelial lipase (EL), responsible for the hydrolysis of phospholipids on HDL. The lack of ANGPTL3, in subjects with genetic deficiency, has been demonstrated to induce a hypolipidemic and antiatherogenic profile, related to low LDL-C levels. Accordingly, there is a growing interest in developing new pharmacological therapies aimed at inhibiting ANGPTL3. However, whereas its role in ApoB-containing lipoprotein metabolism has been already characterized, its role in HDL metabolism has not been fully clarified. More specifically, there is a lack of structural information on the N-terminal domain of ANGPTL3 and on the interaction with EL. The aim of this study is to in silico investigate and characterize, at an atomistic level, the interaction between these two proteins.
Methods. The experiments were carried out using homology modeling, ab initio techniques, molecular dynamics (MD) simulations and molecular docking.
Results. In absence of experimentally solved structures, three-dimensional structure of ANGPTL3 and EL was predicted, using homology modeling for EL and using ab initio techniques for the N-terminal domain of ANGPTL3. Both these structures were then equilibrated by MD simulations. Subsequently, their interaction was investigated via protein:protein docking, analyzing the most conserved interactions of the top-scoring poses. Finally, the stability of the interactions of the the selected pose was tested with MD simulations.
We identified three amino acids of ANGPTL3 that could play a key role in the interaction with EL.
Conclusions. These results lay the basis for future studies aimed at identifying specific surfaces that could be targets for future drugs.

ELEVATED LIPOPROTEIN(A) LEVELS AS PREDICTOR OF THE SEVERITY AND COMPLEXITY OF CORONARY ARTERY DISEASE IN PATIENTS WITH PREMATURE CORONARY EVENTS

A. Cesaro, F. Fimiani, A. Vergara, G. Signore, G. Raucci, V. Acerbo, A. de Pasquale, E. Blasi, F. Gragnano, E. Moscarella, P. Calabrò
University of Campania "L. Vanvitelli", Caserta

Aim. Elevated lipoprotein(a) (Lp[a]) is a risk factor for premature coronary artery disease (CAD). Lp(a) mediates cardiovascular risk through prothrombotic, proinflammatory, and proatherogenic properties. This study aims to investigate the relationship of Lp(a) with the severity and complexity of coronary artery disease using the SYNergy between percutaneous coronary intervention with TAXUS and Cardiac Surgery (SYNTAX) and Gensini scores in patients with premature CAD.
Methods. Lp(a) concentration was reported in mg/dL, and a serum concentration ≥30 mg/dL was considered elevated. Based on Lp(a) concentration, the study population was divided into three groups defined as 'normal' (<30 mg/dL), 'high' (≥30 and <60 mg/dL), or 'very high' (≥60 mg/dL) Lp(a). SYNTAX and Gensini scores were independently assessed by 2 investigators.
Results. A total of 223 consecutive patients with premature coronary events (aged <60 years) who underwent coronary angiography, according to guidelines indications, were enrolled. The mean SYNTAX score was 8 in the "normal" group, 13 in the "high" group, and 21 in the "very high" group ($P<0.001$). The proportion of patients with elevated Lp(a) was significantly higher in patients with higher SYNTAX and Gensini scores ($P<0.05$). Multivariate analysis (adjusting for age, diabetes, hypertension, and previous coronary event) showed that elevated Lp(a) remained significant, independent predictors of higher SYNTAX and Gensini scores ($P<0.05$).
Conclusion. In patients with premature CAD, elevated Lp(a) is an independent predictor of the severity and complexity of CAD. Lp(a) should be routinely screened for younger patients presenting to the coronary care unit, especially those who do not have classic cardiovascular risk factors.

TESTOSTERONE DEFICIENCY INDEPENDENTLY PREDICTS ALL-CAUSE MORTALITY IN WOMEN WITH HEART FAILURE AND REDUCED EJECTION FRACTION: INSIGHTS FROM THE T.O.S.CA. REGISTRY

G. Crisci[1], A.M. Marra[1], A. Salzano[2], R. D'Assante[1], M. Iacoviello[3], V. Triggiani[4], G. Rengo[1], F. Cacciatore[1], C. Maiello[5], G. Limongelli[6], D. Masarone[6], F. Perticone[7], A. Cimellaro[7], P. Perrone Filardi[8], S. Paolillo[9], A. Mancini[10], M. Volterrani[11], O. Vriz[12], R. Castello[13], A. Passantino[14], M. Campo[15], P.A. Modesti[16], A. De Giorgi[17], I.P. Monte[18], A. Puzzo[19], A. Ballotta[20], M. Arcopinto[1], P. Gargiulo [8], A. Sciacqua[7], R. Napoli[1], A. D'Agostino[2], V. Valente[1], G. Giardino[1], G. Landi[1], A. Colao[21], T. Suzuki[22], V. Raparelli[23], A.M. Isidori[23], E. Bossone[1], A. Cittadini[1]

[1]Department of Translational Medical Sciences, Federico II University, Naples;
[2]IRCCS SDN, Diagnostic and Nuclear Research Institute, Naples;
[3]Cardiology Unit, Department of Medical and Surgical Sciences, University of Foggia;
[4]Interdisciplinary Department of Medicine-Section of Internal Medicine, Geriatrics, Endocrinology and Rare Diseases, University of Bari 'A Moro', Bari;
[5]Heart Transplantation Unit, Monaldi Hospital, Azienda Ospedaliera dei Colli, Naples;
[6]Division of Cardiology, Monaldi Hospital, Azienda Ospedaliera dei Colli, University of Campania Luigi Vanvitelli, Caserta;
[7]Department of Medical and Surgical Sciences; University Magna Grecia of Catanzaro, Italy;
[8]Department of Advanced Biomedical Sciences, Federico II University, Naples, Italy;
[9]Mediterranea Cardiocentro, Naples;
[10]Operative Unit of Endocrinology, Catholic University of the Sacred Heart, Rome, Italy; [11]Department of Medical Sciences, IRCCS San Raffaele Pisana, Rome;
[12]Heart Center Department, King Faisal Hospital & Research Center, Riyadh, Kingdom of Saudi Arabia;
[13]Division of General Medicine, Azienda Ospedaliera Universitaria Integrata, Verona, Italy;
[14]Scientific Clinical Institutes Maugeri, IRCCS, Pavia;
[15]Unit of Endocrinology and Metabolic Diseases, Department of Medical and Surgical Sciences, University of Foggia;
[16]Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi di Firenze; [17]Department of Medical Sciences, School of Medicine, Pharmacy and Prevention, University of Ferrara;
[18]Department of General Surgery and Medical-Surgery Specialties, University of Catania;
[19]IRCSS Oasi Maria SS, Troina, Enna;
[20]IRCCS Policlinico San Donato, San Donato Milanese, Milano;
[21]Department of Public Health, Federico II University, Naples;
[22]Department of Cardiovascular Sciences and NIHR Leicester Biomedical Research Centre, University of Leicester, Glenfield Hospital, Leicester, UK;
[23]Department of Translational Medicine, University of Ferrara; University of Alberta, Faculty of Nursing, Edmonton, Alberta, Canada;
[24]Department of Experimental Medicine, "Sapienza" University of Rome

Aim. Testosterone Deficiency (TD) is associated with increased morbidity and mortality in Heart Failure with reduced ejection fraction (HFrEF). However, data in women are scanty. Aim of this study was to investigate the clinical impact of Testosterone Deficiency (TD) on women affected by HFrEF.

Patients and Methods. Women prospectively enrolled in the T.O.S.CA. (Terapia Ormonale Scompenso CArdiaco) Registry, a prospective, multicentre, observational study involving 19 Italian centres were included in this analysis. Patients were divided according to the presence of TD (TD+), which was defined as serum testosterone levels lower than 25 ng/dL. Data regarding clinical status, echocardiography, exercise performance, cardiovascular hospitalization and survival were analysed.

Results. Thirty patients (31,9%) displayed TD. TD was associated with lower level of tricuspid annular plane excursion (TAPSE) to pulmonary arterial systolic pressure PASP ratio (TAPSE/PASP) (p: 0,008), peak oxygen consumption (VO2 peak) (p: 0,03) and lower values of estimated glomerular filtration rate (p: 0.0001). TD resulted an independent predictor of the combined endpoint of all-cause mortality/cardiovascular hospitalization (HR: 10,27; 5-95% CI: 3,18-16,81; p: 0,001) and also of separate components of the endpoint (HR: 8,17; 5-95%: 5,14-15,81; p: 0,04 for all-cause mortality and HR: 2,23; 5-95% CI: 1,11-4,48; p: 0,02 for cardiovascular hospitalization).

Conclusions. Testosterone Deficiency impacts remarkably on morbidity and mortality of women with HFrEF. Moreover, TD is associated with worse exercise capacity, right ventricular-pulmonary arterial coupling and renal function. These results prompt attention on the possibility of a replacement therapy in this subset of patients.

THE ABSOLUTE AMOUNT OF PCSK9 ASSOCIATED TO LDL IS INCREASED AFTER anti-PCSK9 MABS ADMINISTRATION

L. Canclini[2,1], A. Baragetti[2,1], M. S. Donghi[2], S. Franchin[2], E. Yzeiraj[2], L. Grigore[1,3], A. L. Catapano[1,2]
[1]IRCCS MultiMedica, Milano;
[2]Department of Pharmacological and Biomolecular Sciences, University of Milan;
[3]Center for the Study of Atherosclerosis, Bassini Hospital, Cinisello Balsamo (Milan)

Aim. Proprotein convertase subtilisin-kexin type 9 (PCSK9) plays a key role in determining plasma LDL-C levels. PCSK9 is known to associate with LDL in plasma. Anti-PCSK9 monoclonal antibodies (mAbs) inhibit PCSK9 activity, thus reducing LDL-C levels and cardiovascular events. The aim of our study was to address whether the administration of mAbs targeting PCSK9 modify the PCSK9-LDL association.
Methods. We collected plasma of mAbs treated subjects before treatment (T0, n=21) and 1 (T1, n=18), 3 (T3, n=18) and 6 (T6, n=16) months after the first mAb administration. Lipoprotein (LP) fractions were isolated from plasma with Iodixanol gradient ultracentrifugation. The PCSK9 and cholesterol content of each fraction was quantified with ELISA and a clinical-grade colorimetric assay, respectively. We studied the LP fractions also for their composition and electrophoretic migration.
Results. Plasma PCSK9 levels increased after therapy from 479.8±177.9 ng/mL to 3945.6±1054.4 ng/mL; LDL cholesterol levels decreased from 141.9±65.3 mg/dL to 54.1±37.3 mg/dL. We found PCSK9 associated with a specific LDL subfraction both before and after anti-PCSK9 mAbs therapy. The % of association was 10.5±4.4%, 6.6±3.1%, 5.9±3.6% and 5.3±3.8% of total recovered PCSK9 at T0, T1, T3 and T6, respectively. Despite the large LDL-C reduction, post therapy the absolute amount of bound PCSK9 increased more than 10 fold.
Conclusions. The PCSK9-LDL association remains after anti-PCSK9 mAbs administration. The absolute amount of bound PCSK9 increases after treatment: it remains to be determined whether this is due to the large increase in PCSK9 plasmatic levels or to other factors that increase the PCSK9-LDL affinity. Further studies are required to characterize the LDL subclass that shows enhanced affinity for PCSK9.

PCSK9 RAISED IN ANOREXIA NERVOSA

F. Bigazzi[1], C. Macchi[2], C.F. De Pasquale[3], S. Maestro[3], C. Corciulo[1], B. Dal Pino[1], F. Sbrana[1], C.R. Sirtori[4], M. Ruscica[2], T. Sampietro[1]
[1]U.O. Lipoapheresis and Center for Inherited Dyslipidemias, Fondazione Toscana Gabriele Monasterio, Pisa;
[2]Dipartimento di Science Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milano;
[3]Fondazione Stella Maris, IRCCS Istituto Scientifico per la Neuropsichiatria dell'Infanzia e dell'Adolescenza, Pisa;
[4]Dyslipidemia Center, A.S.S.T. Grande Ospedale Metropolitano Niguarda, Milano

Aim. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is one of the major regulators of low-density-lipoprotein cholesterol (LDL-C). Among the physiological regulators of PCSK9 gene expression, nutritional status represents one of the open issues to be addressed and evaluation of PCSK9 levels in human eating disorder appears of high interest. Some studies have suggested that also the hormone leptin could be involved in the pathophysiology of atherosclerosis, probably modulating LDL receptor expression through PCSK9 pathway. We have studied subjects affected by Anorexia Nervosa (AN) to increase understanding of the metabolic alterations in this disease.
Methods. We managed a case-control observational study, enrolling 20 anorexic adolescent's females and 20 normal adolescent's females as control group, age and sex matched. Lipids profile, plasma PCSK9 levels and plasma leptin concentrations were analyzed.
Results. As expected, AN subjects showed lower BMI. The lipoproteins analysis shows lower concentrations of total cholesterol and LDL-C in AN vs control groups, and, in spite of the marked dietary restriction in AN, lipid levels are within normal range. Furthermore, in adolescent girls with AN, we measured higher PCSK9 (+24%, $p<0.005$) and lower leptin levels (-43%, $p<0.01$) in comparison to the control group.
Conclusions. In our clinical settings of adolescent anorexia, data on lipid profile, on the increased levels of PCSK9 and reduced of leptin, stimulate to address further research to unravel the role of the liver and adipose tissue in the handling of PCSK9/ LDL metabolism.

ROLE OF LYSOSOMAL ACID LIPASE IN PPAR-MEDIATED REDUCTION OF LIPID ACCUMULATION IN HEPATOCYTES

F. Cetti, A. Ossoli, L. Da Dalt, G. Danilo Norata, L. Calabresi, M. Gomaraschi
Centro E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano

Aim. Lysosomal acid lipase (LAL) catalyzes the hydrolysis of cholesteryl esters and triglycerides in the lysosomal compartment. Recently, it was shown that NAFLD patients can develop an acquired LAL deficiency which is rescued by PPAR-alpha activation. Aim of the study was to investigate the role of other isoform-specific and dual PPAR agonists on lipid accumulation and LAL activity using an in vitro model of steatosis.
Methods. To induce lipid accumulation, HepG2 cells were loaded with free fatty acids (FAs) and incubated with selective agonists of PPAR alpha (fenofibric acid), gamma (pioglitazone) and delta (seladelpar) receptors or with dual alpha/gamma (saroglitazar). LAL activity, lipid accumulation and the activation of autophagy were assessed.
Results. All the tested agonists significantly reduced lipid accumulation, but the effect was completely lost when LAL was blocked by a specific inhibitor. PPAR agonists promoted TFEB expression, with consequent lysosomal biogenesis and activation of autophagy, as shown by the expression of p62 and LC3. Pioglitazone, seladelpar and saroglitazar were more effective than fenofibric acid in stimulating LAL activity in FA-loaded hepatocytes. All agonists increased mitochondrial mass, decreased ROS production and inflammation.
Conclusions. PPAR agonists reduce lipid accumulation in hepatocytes by promoting autophagy, lipid hydrolysis in the lysosomes and FA oxidation in the mitochondria. LAL activation plays a key role in PPAR-mediated hydrolysis of intracellular lipids. Agonists of PPAR gamma and delta, and the dual alpha/gamma agonist were more effective than alpha agonist in promoting LAL activation and decreasing oxidative stress. These data suggest that the pharmacological modulation of LAL should be explored in the management of steatosis.

IMPACT OF HDL LEVELS AND DIETARY CHOLINE CONTENT ON LIPID METABOLISM AND ATHEROSCLEROSIS DEVELOPMENT IN MICE

A. Colombo[1], M. Busnelli[1], S. Manzini[1], E. Franchi[1], M.G. Rivera[2], J. Kirwan[2], G. Chiesa[1]
[1]Università degli Studi di Milano, Dipartimento di Scienze Farmacologiche e Biomolecolari, Milano;
[2]Max Delbrück Center for Molecular Medicine, BIH Core Facility Metabolomics, Berlin, Germany

Aim. Epidemiological studies have shown that plasma levels of apoA-I/HDL and TMAO, a product of dietary choline metabolism, correlate with cardiovascular risk. In view of their role in cholesterol metabolism, the study was aimed at investigating, in athero-prone mouse models, a possible interaction between HDL and TMAO in atherosclerosis development.
Methods. Mice with extremely low plasma HDL levels, deficient for both murine apoA-I and apoE (DKO), were compared with mice characterized by elevated HDL, deficient for both murine apoA-I and apoE, but overexpressing human apoA-I (DKO/hA-I). Standard rodent diets, with different choline content (0.09% or 1.2%), were administered to 10-week-old female mice of both genotypes for 16 weeks. Atherosclerosis development was quantified at the aortic sinus. Targeted plasma metabolomics was performed, gene expression was analyzed by qPCR in liver, duodenum, jejunum, ileum.
Results. With both diets, DKO mice developed much larger plaques than DKO/hA-I mice. High-choline diet increased plasma TMAO levels in both genotypes. Interestingly, a worsening of plaque development by high choline diet occurred in DKO/hA-I mice only. Plasma metabolomics indicated that choline supplementation, only in the presence of HDL (in DKO/hA-I mice), increased the concentration of several lipid metabolites belonging to the ceramide, glycoceramide and sphingomyelin classes. High-choline diet increased the hepatic gene expression of Fmo1 and Fmo2 in DKO/hA-I, whereas the expression of Scarb1 was higher in DKO/hA-I compared to DKO mice. Gene expression of Abca1, Abcg5, Abcg8, Cd36, Npc1l1, Mttp, Srebf2 and of Slc10a2 in intestinal segments was not different between genotypes and was not modified by the dietary choline intake.
Conclusions. High choline diet increased plasma TMAO concentration in both genotypes, but affected atherosclerosis development, plasma metabolome and hepatic gene expression only in high HDL mice. Different levels of apoA-I/HDL did not affect the intestinal expression of genes playing a relevant role in lipid metabolism.