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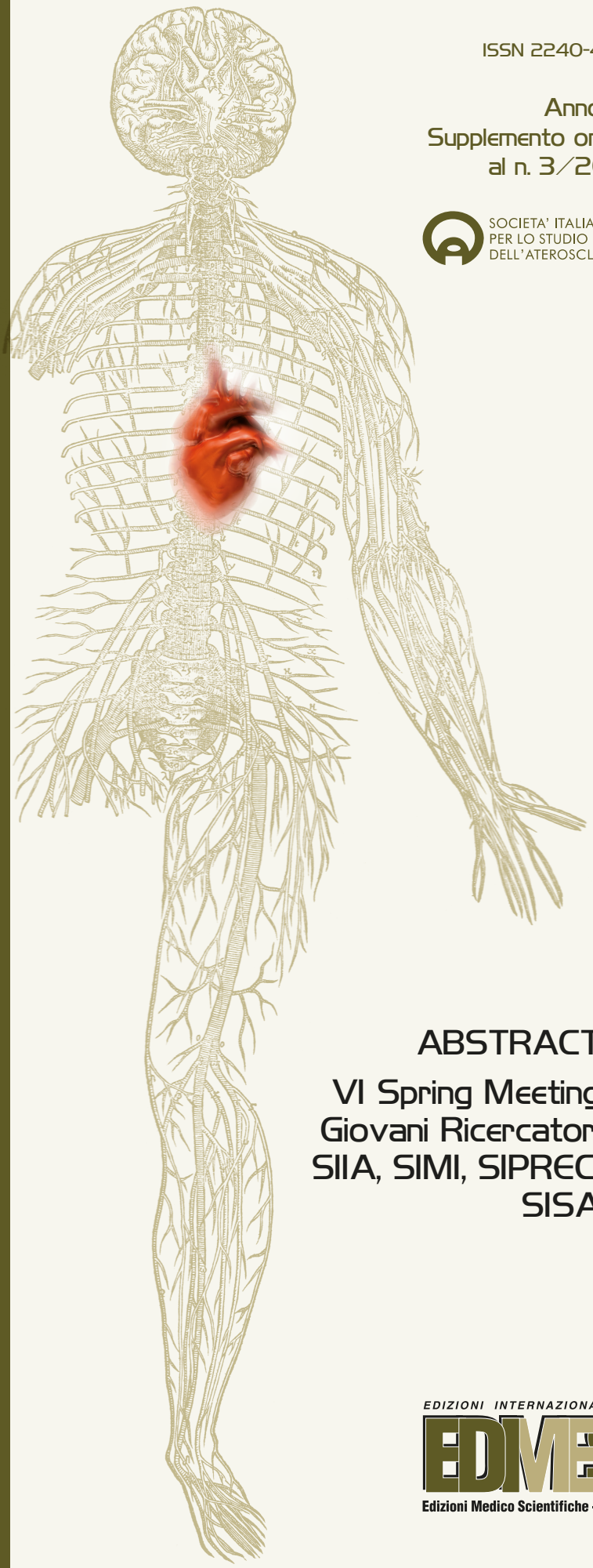

ABSTRACT

VI 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 2021

Next Gen Researchers Meetings on Metabolic and Cardiovascular Disease

21-22 Maggio 2021

Nelle giornate del 21-22 Maggio 2021 si è svolta la sesta edizione dello Spring Meeting Giovani Ricercatori, organizzato, con il supporto immancabile ed essenziale delle rispettive società, dai gruppi giovani della SISA (Società Italiana per lo Studio dell'Aterosclerosi), SIMI (Società Italiana Medicina Interna), SIIA (Società Italiana Ipertensione Arteriosa, e che quest'anno, per la prima volta, ha coinvolto anche il gruppo giovane della SIPREC (Società Italiana per la Prevenzione Cardiovascolare).

Per il secondo anno consecutivo, in seguito alle restrizioni legate alla pandemia COVID-19, il meeting si è tenuto in versione virtuale. Tuttavia l'entusiasmo per il congresso si è mantenuto molto alto, come mostrato dai numerosi abstract ricevuti (62 in totale) che sono poi stati selezionati come presentazioni orali o poster. Nell'intrinseco spirito innovativo di questo meeting, il programma si è arricchito dell'utilizzo di una piattaforma digitale in cui gli autori hanno potuto condividere un breve video di 3 minuti di presentazione del proprio lavoro. Tale piattaforma (accessibile a questo indirizzo https://padlet.com/FondazioneSisa/SPRING2021_SIIA_SIMI_SIPEC_SISA) ha favorito lo scambio e la creazione di networking tra i partecipanti, con discussioni "on-line" durante l'evento e anche "off-line" nei periodi prima e dopo il congresso. Per favorire ulteriormente l'interazione, la comunicazione e lo scambio interpersonale tra i partecipanti (inevitabilmente limitata in questo periodo di "social distancing") si sono svolti anche diversi momenti di "Coffee-break/Networking chill-out rooms" che sono stati ampiamente seguiti e che hanno permesso ai partecipanti di interagire tra loro e con i moderatori o relatori su invito. Il successo di queste "stanze virtuali di networking" è sottolineato dalle numerose idee progettuali nate in tale contesto.

Non sono mancate le "classiche letture" su invito che hanno visto cinque giovani ma esperti relatori fornire diverse letture. Questi gli argomenti trattati: "Lo scompenso cardiaco: la tempesta perfetta fra sistemi neurormonali, stress ossidativo e disfunzione mitocondriale" (Giovanna Gallo, Università La Sapienza - Roma), "Disautonomia: come riconoscerla diagnosticarla e trattarla nel paziente iperteso" (Francesca Saladini, Università degli Studi di Padova), "SARS-CoV-2 impairs electrical and mechanical function of human pluripotent stem cell-derived car-

diomyocytes" (Silvia Marchianò University of Washington - Seattle), "Impatto della dieta chetogenica sulla malattia cardiometabolica" (Mikiko Watanabe, Università la Sapienza – Roma) e "Sex and Gender in Cardiovascular Disease: Have you ever considered the possibilities?" (Valeria Raparelli, Università degli Studi di Ferrara). Queste letture sono poi state seguite da 25 comunicazioni orali selezionate tra gli abstract inviati dai partecipanti.
Da diversi anni lo Spring meeting pone grande attenzione alla tematica dello sviluppo della carriera dei giovani ricercatori. In tale area si è svolta una tavola rotonda dal titolo "Sviluppo carriera: all'estero sì o no?" con l'intervento di diversi giovani ricercatori e presentazioni dedicate a dirimere i dubbi sull'utilità, difficoltà e opportunità dell'intraprendere un periodo di formazione e lavoro all'estero. In tale rotonda è stato presentato (da Giacomo Pucci e Francesca Saladini) anche il progetto "Vascage Net", che rappresenta un esempio di rete nata proprio per favorire gli scambi internazionali di ricerca in ambito cardiovascolare.
Nel complesso l'edizione virtuale dello Spring Meeting ha visto la partecipazione attiva di 97 ricercatori appartenenti alle quattro società. Nelle due giornate live, si sono collegate 108 persone ("accessi unici") nella giornata del venerdì e 98 nella giornata del sabato. Le relazioni e le comunicazioni orali del meeting sono state registrare e rese disponibili, per chi non avesse avuto modo di partecipare alle sessioni live (sono trovabili sul sito www.sisa.it, nella sezione gruppo giovani Spring Meeting 2021).

Anche quest'anno il meeting è stato apprezzato, ricevendo numerosi feedback positivi, e il giudizio degli organizzatori è stato senza dubbio positivo. Seppur limitato dal format virtuale, il meeting è riuscito nei suoi intenti principali, ossia di dare visibilità e opportunità ai giovani ricercatori per confrontarsi e crescere, creando nuove reti e potenziali collaborazioni future. L'appuntamento è quindi allo Spring Meeting 2022 con la più viva speranza, che con il completamento della campagna vaccinale, sarà possibile svolgere un meeting in presenza.

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

COMUNICAZIONI ORALI

ENDOTHELIAL FUNCTION IMPROVEMENT IN PATIENTS WITH DIABETES MELLITUS AND CHRONIC HEART FAILURE IN TREATMENT WITH SGLT2 INHIBITORS

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Aim. The prevalence of patients with concomitant heart failure (HF) and diabetes mellitus (DM) continues to increase with the general aging of the population. T2DM frequently causes macrovascular and/or microvascular pathologic changes. Recently a new class of antidiabetic drugs, the SGLT2 inhibitors, have been demonstrated to reduce cardiovascular mortality and heart failure hospitalizations. The use of SGLT2 was associated in previous studies with an improved vascular function. We therefore sought to evaluate possible changes in endothelial function assessed by flow-mediated dilation (FMD) in patients with CHF and diabetes shifting their therapy to SGLT2 inhibitors.
Methods. Seventy-eight consecutive outpatients with CHF and T2DM were screened in the Daunia Heart Failure Registry. Thirty-eight of them were enrolled and followed up between May 2019 and February 2020. Enrollment criteria included HbA1c ≥6.5%, eGFR ≥60 mL/min/1.73 m^2, age >18 years old and LVEF ≤50% with CHF. Exclusion criteria included previous amputation surgery and recurrent urinary tract infections.
Medical history, heart rate, systolic blood pressure, Body Mass Index, NYHA functional class and medications were recorded and monitored. All patients underwent blood analysis, ECG and evaluation of endothelial function reserve assessed by FMD in an ambulatory setting, under resting conditions, at the beginning and after 3 months of therapy with SGLT2 inhibitors.
Results. Thirty-eight consecutive outpatients with CHF and T2DM (mean age 67±7,60 years, male: 87%) were enrolled in the study. Twenty-one of them started the treatment with SGLT2 inhibitors, while the remaining seventeen continued their original therapy. After 3 months follow up, the patients who started therapy with SGLT2i showed an improvement in endothelial function versus control group (18,60±6,478% vs 10,29± 3,4331 p<0,000552). Changes in FMD values were not significant in patients who did not changed T2DM therapy.
Conclusions. The beginning of therapy with SGLT2 inhibitors in patients with CHF and T2DM was associated in an observational non randomized study with an improved endothelial function.

ROLE OF VENTRICULAR-ARTERIAL (VAC) COUPLING IN THE PATHOPHYSIOLOGY OF HYPERTENSION

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Introduction. Left ventricular arterial coupling (VAC) is the ratio between arterial (Ea) and ventricular elastance (Ev). Potential changes of these parameters in hypertension (HTN)-mediated organ damage, namely left ventricular (LV) hypertrophy, has never been assessed.
Aim. To estimate the values of Ea, Ev and VAC in adult outpatients with hypertension stratified according to different left ventricular (LV) geometries.
Methods. We analysed data from adult outpatients who were referred to our Hypertension Unit, University of Rome Sapienza, Sant'Andrea Hospital, Rome (IT), for BP evaluation and cardiovascular risk assessment. Parameters for non-invasive evaluation of VAC, including LV ejection fraction [LVEF], stroke volume [SV], pre-ejection time [PET], and total ejection time [TET], were also assessed. All BP measurements were performed according to international guidelines. Patients were stratified into four groups according to relative wall thickness (RWT >0.43) and LV mass indexed by body surface area (BSA) >95 g/m^2 in women and 115 g/m^2 in men, as follows: normal LV geometry (A), LV remodelling (B), eccentric (C) and concentric (D) LV hypertrophy.
Results. We included an overall population sample of 295 adult outpatients without overt CV diseases (37.3% female, mean age 54.3±14.7 years, clinic BP 145.1±19.5/88.0±12.3 mmHg, 24-hour BP 135.2±16.4/85.0±12.3 mmHg), among whom 52.9% in group A, 14.2% in group B, 20.7% in group C, and 12.2% in group D. We observed significantly higher values of Ea (1.90±0.6 vs.1.55±0.4; P<0.001) and Ev (2.48±0.9 vs. 2.07±0.6; P<0.001) in patients with RWT>=0.43 than in those with RWT<0.43, whilst no significant difference was observed for VAC between groups (0.78±0.2 vs. 0.79±0.2; P=0.546). Patients in group B (2.08±0.7) and D (1.74±0.6) showed significantly higher levels of Ea than those in group A (1.67±0.4) and C (1.33±0.56; ANOVA P<0.001). Also, patients in group B (2.76±0.9) and D (2.20±0.8) had higher Ev than those in group A (2.22±0.7) and C (1.77±0.5; ANOVA P<0.001). Ea and Ev showed a strong mutual correlation (r=0.753; P<0.001) and both were positively corelated with RWT (Ea: r=0.397; P<0.001; Ev: r=0.378; P<0.001) and inversely corelated with LVM (Ea: r=-0.349; P<0.001; Ev: r=-0.349; P<0.001), LVMi (Ea: r=-0.301; P<0.001; Ev: r=-0.349; P<0.001) and LVMh^2.7 (Ea: r=-0.226; P<0.001; Ev: r=-0.276; P<0.001). Of note, VAC showed no significant differences among LV geometries (P=0.499), but significant positive correlations with LVM (P<0.001 for all comparisons).
Conclusions. In this sample of adult outpatients without overt CV diseases, increased LVM was associated with reduced Ea and Ev; in order to maintain an effective LV performance, VAC remained below the normal threshold, independently by changes in LV geometries. VAC assessment might be considered in the diagnostic work-up of essential HTN, to improve the prognostic accuracy and therapeutic outcomes.

PREVALENCE AND PREDICTORS OF SUBCLINICAL ATRIAL FIBRILLATION IN MULTIMORBID OLDER ADULTS HOSPITALIZED FOR ACUTE MEDICAL CONDITIONS: AN EXPLORATORY RESEARCH

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Aim. Subclinical atrial fibrillation (SCAF) is associated with an increased risk of clinical AF, ischemic stroke, major adverse cardiovascular events and death. Most of the data regarding SCAF derive from long-term studies on adult outpatients with known heart disease and implanted devices. We investigated the prevalence and predictors of SCAF in multimorbid older adults hospitalized for acute medical conditions.
Methods. We performed a study on 60 older patients (aged 80+) consecutively admitted to our Internal Medicine and Geriatrics Department for acute medical conditions, with no history of clinical AF. Portable ECG monitoring was placed within the first 12 hours from admission and ECG recording lasted for 5 days.
Results. Mean age: 85.7±4.9 years. Most patients (87.9%) had a high burden of comorbidities. All enrolled patients had a CHA2DS2-VASc score ≥3. A SCAF was detected in 16 patients (26.7%) and 11 patients (18.4%) had at least a SCAF episode lasting 6 minutes or more. Median SCAF burden was 3.7% (0.4-6%). Maximum heart rate during SCAF episodes was 183.3±45.4 bpm. We found no significant predictors of SCAF within the clinical, laboratory and echocardiographic parameters available. Regarding ECG parameters, patients with SCAF had both a higher number/24 h and burden of supraventricular ectopic beats (SVEBs), and a higher burden of ventricular ectopic beats. Patients with ≥2004 SVEBs/2 4h (3° tertile) had a 6-fold higher prevalence of SCAF than patients with <411 SVEBs/24 h (1° tertile) [OR 6.2 (1.1-34.7), p=0.038]. Time to first SCAF episode was within 3 days of ECG recording in all enrolled patients.
Conclusion. Our study shows how SCAF is prevalent in older multimorbid patients hospitalized for acute medical conditions, with possible relevant clinical implications, in terms of both AF risk factors control and prevention of AF-related complications. Our findings could foster larger multicenter studies in similar patients.

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

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Aim. To date, no effective pharmacological therapy has proven to halt or delay the progression of calcific aortic valve disease (CAVD). We aimed to investigate the effects of the activation of Formyl Peptide Receptor 2 (FPR2), a known receptor for lipoxins, resolvins and anti-inflammatory molecules, on interstitial aortic valve cells (VICs) calcification.
Methods. A clone of primary VICs seeded on culture plates or type I collagen scaffolds was treated with lipopolysaccharide (LPS, 500 ng/mL) for 12 days to acquire a pro-calcific profile, with or without the concomitant supplementation of three synthetic FPR2 agonists (MMK1, 50 μM; Ac2-26, 3.2 μM or BML-111, 1 μM). Alkaline phosphatase (ALP) activity and calcium deposition were determined through colorimetric assays. Proteins and RNA were extracted for western blotting and gene expression analyses (RT-PCR). Immunohistochemistry was used to investigate the expression of FPR2 on human pathological and healthy valves.
Results. FPR2 expression increased in pathological valves compared to normal tissue. VICs treated with LPS showed increased ALP activity and inflammatory activation, together with higher FPR2 expression. The treatment of cultured VICs with FPR2 agonists (BML-111, MMK1 and Ac2-26) reduced the overexpression of ALP ($p<0.05$) and of inflammatory cytokines (such as IL6, $p<0.05$) induced by LPS. The same treatments were effective in reducing the deposition of calcium in collagen scaffolds ($p<0.05$).
Conclusions. The receptor FPR2 is overexpressed under pathological conditions and its selective activation is associated with reduced pro-calcific and pro-inflammatory differentiation of VICs. These promising data offer new insights for a novel therapeutic strategy for CAVD.

CORONARY ATHEROSCLEROSIS AND SYSTEMIC INFLAMMATION IN HDL DEFICIENT MICE

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

PLASMA PCSK9 INCREASES UPON ANTI-PCSK9 MONOCLONAL ANTIBODIES TREATMENT IN HYPERCHOLESTEROLEMIC PATIENTS: INVESTIGATION OF THE BASIC MOLECULAR MECHANISMS

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Aim. Monoclonal antibodies anti-PCSK9 block the function of PCSK9 but determine a significant increase of its plasma levels, an effect that can be determined by
1) an increased synthesis or
2) a reduced clearance by the liver.
Methods. We have measured total PCSK9 plasma levels, by ELISA assay, in FH patients or in severly hypercholesterolemic patients with no evident mutations in any FH-related
1) genes at baseline and under treatment with mAbs anti-PCSK9 (cohort A, n=26),
2) few days after mAbs injection and 2 weeks later (group B, n=31).
In vitro human hepatocarcinoma cell line (Huh7) was incubated with mAbs and total PCSK9 (ELISA and Western Blotting) and LDLR (Western Blotting) were determined. mRNA levels of *PCSK9* and *LDLR* were measured by RT-qPCR and SREBP2 transcriptional activation was evaluated by the means of *PCSK9*-Luciferase reporter gene.
Results. In the first cohort of patients (A) baseline plasma levels of total PCSK9 was 458.8±297.4 ng/mL and increased to 1533.3±333.5 ng/mL in response to mAbs anti-PCSK9 (+3.3-fold, $p<0.0001$). In the second cohort of patients (B) we observed levels of PCSK9 equal to 1702.0±164.9 ng/mL upon mAbs injection, which declined to 1360.0±344.8 ng/mL (-21%, $p<0.0001$) after 2 weeks post-injection.
Huh7 were incubated with simvastatin (40 µM), evolocumab (10 µg/mL) and alirocumab (10 µg/mL) for 4, 24 and 48 h. Simvastatin induced both the LDLR and PCSK9 in a time-dependent manner with maximal effect at 48 h (2.15 and 10.8-fold for LDLR and PCSK9, respectively, $p<0.001$ *vs* control 4h). mAbs anti-PCSK9 induced the LDLR already after 4 h incubation (+46% and +43% for evolocumab and alirocumab, respectively, $p<0.05$ *vs* control 4h). On the contrary, evolocumab and alirocumab strongly reduced both intracellular and extracellular PCSK9 at 48 h, as determined by western blot and ELISA assays. While a slight, even if not significant, decrease in *PCSK9* mRNA levels were observed both upon 4h and 24 h of treatments, compared to the basal condition, not statistically significant differencies in SREBP2 activity was observed between control and mAbs treatments, suggesting that mAbs do not affect *PCSK9* transcriptional activation.
Conclusion. Our *in vitro* results suggest that alirocumab and evolocumab increase PCSK9 plasma levels by reducing its hepatic clearance rather than affecting its transcriptional activation. Our ongoing studies on 3-month-old wild-type male C57BL/6 mice will provide further insights on the subject.

EVALUATION OF DIASTOLIC PARAMETERS AND FUNCTION IN ADULT OUTPATIENTS WITH DIFFERENT HYPERTENSION PHENOTYPES

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Introduction. Essential hypertension (HTN) is a chronic disease often associated with different degrees of diastolic dysfunction (DD), including impaired relaxation (IR), pseudo-normal (PNF) and restrictive (RF) filling. Prevalence and clinical characteristics according to different HTN phenotypes have not been fully elucidated.
Aim. To analyse DD parameters in a large cohort of outpatients stratified according to HTN phenotypes, including normotension (NT), white-coat HT (WHT), masked HT (MHT), and sustained hypertension (SHT).
Methods. We extracted data from a medical database of adult outpatients who were referred to the Hypertension Unit, Sant'Andrea Hospital, Rome (IT), including anthropometric data, CV risk factors and comorbidities, presence or absence of antihypertensive therapy. Patients with valid clinic and 24-hour ambulatory BP levels and concomitant (±10 days) echocardiographic assessment of LV geometry and function have been stratified into 4 HTN groups. LV systolic and diastolic properties have been assessed in each patient; in particular, DD was classified as follows: grade 1 (IR: E/A <0.8, E/e′ ≤8), grade 2 (PNF: E/A 0.8–1.5, E/e′ 9–12), and grade 3 (RF: E/A ≥2, E/e′ ≥13).
Results. We included an overall sample of 289 (%) adult outpatients among whom 51 (17.6%) with NT, 75 (26%) with WCHT, 29 (10%) with MHT, 134 (46.4%) with SHT. Prevalence of IR was significantly higher in MHT and SHT patients (P<0.005), whereas PNF was more frequently observed in MHT patients (P=0.064) than other groups. No significant differences were found among HTN phenotypes for LV mass (P=0.227) and LV systolic parameters, including LV ejection fraction (P=0.435), fractional shortening (P=0.390), cardiac output (P=0.080) and index (P=0.761). Among DD parameters, ratio E/e' resulted significantly higher in WCHT and SHT patients (P=0.047) compared to other groups; no significant differences were found for E/A (P=0.408) and Em/Am (P=0.722) ratio among groups, though MHT showed higher values of these parameters.
Conclusions. MHT patients showed higher prevalence of DD compared to WCHT and NT, thus supporting the potential clinical implication of this HTN phenotype in promoting incidence of cardiovascular events.

THE EFFECT OF INHIBITION OF CGMP-DEPENDENT PROTEIN KINASE (PKG) ON ANTICONTRACTILE FUNCTION OF PERIVASCULAR ADIPOSE TISSUE

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Aim. Perivascular adipose tissue (PVAT) has an anticontractile effect which is lost in obesity through hypoxia, inflammation and oxidative stress. Previous studies demonstrated that cGMP-dependent protein kinase (PKG) plays a key role in regulating PVAT function. We investigated the functional responses of small resistance arteries with and without PVAT in normotensive subjects and obese patients, testing the effects of DT-2, (a peptidic highly selective inhibitor of PKG) on contractile response.
Method. 9 normotensive severely obese patients (Obese) and 6 hypertensive severely obese patients (Obese-Hyper) undergoing bariatric surgery were investigated, together with 7 normotensive lean subjects (CTRL) undergoing an elective surgical intervention. Subcutaneous small resistance arteries were dissected and mounted on a wire myograph. A concentration-response curve to noradrenaline (NA, from 10-9 to 10-5 Mol/l) was evaluated in vessels with PVAT (WF) and in vessels in which PVAT was removed (NoF). Concentration response to NA was repeated in small arteries WF and NoF in the presence of DT-2 (125 nM) in normoxic and hypoxic (95% N2/5%CO2 for 30') conditions.
Results. In normoxic conditions, presence of PVAT reduced contractile response to NA in small arteries from CTRL (ANOVA p=0.04). The anticontractile effect of PVAT completely disappeared in Obese and Obese-Hyper patients. In CTRL preincubation with DT-2 increased contractile responses to NA in arteries WF (ANOVA p=0,04). This was more evident during hypoxic conditions. No significant effect was observed in arteries WF and NoF from Obese and Obese-Hyper patients after incubation of DT-2, in normoxic and hypoxic conditions.
Conclusion. The anticontractile effect of PVAT is present in normotensive subjects and lost after inhibition of PKG signaling. The absence of effect after preincubation of DT-2 on arteries WF from Obese and Obese-Hyper patients suggests a dysregulation of PKG signaling pathways in these groups, which seems essential for its downstream dilator function in small resistance arteries.

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

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Aim. Altered lipid metabolism and increased neuroinflammation are associated with Alzheimer's disease (AD) pathogenesis. As in plasma, in the brain the PCSK9 protein may degrade the receptors that, interacting with apolipoprotein E-containing HDL like particles (apoE-HDL), promote the uptake of astrocyte-derived cholesterol by neurons, essential process to guarantee neuronal functions. In addition, PCSK9 has shown a pro-inflammatory effect in macrophages, microglial-like cells. The aim of the study was to investigate PCSK9 involvement in AD by evaluating its influence on lipid metabolism and neuroinflammation in human cell models of astrocytes and neurons.
Methods. The following cell models have been utilized: human astrocytoma cells (U-373) exposed to exogenous human recombinant PCSK9; human neuroblastoma SH-SY5Y cells, retrovirally transduced to overexpress human PCSK9. Cells have been exposed also to fibrils of Aβ (1 μM) to mimic an AD-like condition. The effect of PCSK9 has been evaluated on: cholesterol synthesis, efflux and uptake by radioisotopic and fluorimetric assays; gene and protein expression assessed by qRT-PCR and Western Blot analyses, respectively; the interaction between fluorescent apoE and living cells visualized by confocal microscopy.
Results. In U-373 astrocytoma cells, incubation with PCSK9 significantly increased endogenous cholesterol synthesis in a dose dependent manner ($p<0.05$ for 5 μg/ml and $p<0.01$ for 10μg/ml), either in the absence or presence of Aβ, and reduced LDLR and apoER2 expression. Overall, the intracellular cholesterol content in the presence of PCSK9 is significantly reduced ($p<0.5$) and worsened by incubation with Aβ ($p<0.001$). In the same cells stimulated with LXR/RXR agonists, PCSK9 did not have any effect on cholesterol efflux to both apoE and apoE-HDL, while treatment with Aβ significantly reduced cholesterol efflux to apoE ($p<0.001$). In PCSK9-overexpressing SH-SY5Y neuroblastoma cells, the uptake of [^{3}H]-cholesterol-labeled apoE-HDL was significantly reduced ($p<0.001$). PCSK9 overexpression also reduced the interaction between fluorescein labelled-apoE and neuroblastoma cells. Moreover, in the same cells, the expression of the apoER2 and of the LDLR was significantly reduced ($p<0.05$). In U-373, PCSK9 worsened the inflammatory response induced by Aβ, further increasing the gene expression of IL-1β and TNF-α ($p<0.05$).
Conclusion. Altogether, the results suggest an influence of PCSK9 on cerebral cholesterol metabolism and neuroinflammation, with a possible deleterious role in AD pathogenesis.

CHARACTERIZATION OF PLASMA AND CEREBROSPINAL FLUID LIPOPROTEINS IN ALZHEIMER'S DISEASE

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Aim. Several epidemiological evidences suggest an inverse association between plasma HDL-C levels and the risk of developing Alzheimer's disease (AD). The mechanism by which plasma HDL could influence the pathogenesis and progression of AD remains still unknown. It has been postulated a direct effect of plasma HDL on brain cholesterol homeostasis, due to the ability of specific HDL subclasses to cross the blood-brain barrier. Despite the emerging and consistent evidences of plasma HDL-C levels involvement, a qualitative analysis of plasma and brain HDL is still lacking. In this study HDL subclasses have been characterized in plasma and cerebrospinal fluid (CSF) of AD patients.
Methods. Blood and CSF samples of 20 (10M/10F) AD patients have been collected and plasma separated by low-speed centrifugation at 4° C. HDL subclasses have been characterized in plasma and CSF by non-denaturing two-dimensional (2D)-electrophoresis, followed by immunodetection with polyclonal antibodies against human apoE and apoA-I.
Results. Plasma HDL subclass distribution in AD patients is similar to that described for healthy controls. Three different classes of apoA-I-containing HDL are detected; they comprise small pre-β HDL, and α HDL_2 and HDL_3. Curiously CSF apoE-containing lipoproteins showed only α-migration particles in all samples. CSF apoA-I-containing lipoproteins of AD patients are characterized by an high amount of α-HDL and interestingly pre-α migrating apoA-I-containing lipoproteins have been detected in 6 CSF samples.
Conclusions. Since it is known that apoA-I mRNA is not present in central nervous system, these results confirm the hypothesis of cholesterol delivery from plasma-to-brain. The qualitative characterization of HDL together with their quantitative determination could help to better understand the influence of lipoproteins in the pathogenesis and progression of AD.

IMPACT OF STATIN USE ON DEMENTIA AND ALZHEIMER'S DISEASE INCIDENCE: A SYSTEMATIC REVIEW AND META-ANALYSIS OF OBSERVATIONAL STUDIES

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Aims. Previous studies have suggested an impact of statins use on cognitive decline and dementia, but the relationship between statins and cognitive impairment remains elusive. We conducted a meta-analysis of observational studies to investigate the impact of statin use on Alzheimer's disease and dementia incidence.
Methods. This meta-analysis was conducted according to the PRISMA reporting guidelines. PubMed, Cochrane, and EMBASE were searched since inception to January 2021. Inclusion criteria were:
1) observational studies (cohort or case-control studies);
2) adult subjects;
3) statin use compared to non-use;
4) reporting an adjusted estimate (such as odds ratio/risk ratio/hazard ratio) and 95% confidence intervals (CI) for Alzheimer's disease (AD) and/or dementia incidence as outcomes.

Estimates from original studies were pooled and compared using restricted maximum-likelihood random-effect model. Measures of effects were reported as odds ratio (OR) and 95% CI.
Results. A total of 46 studies (38 cohort studies and 8 case-control studies) met the criteria for the analysis. Specifically, the analysis of 36 studies showed a significant risk reduction of 20% for dementia (OR 0.80 [95%CI, 0.75 to 0.86]), whereas 21 studies showed a risk reduction of 32% for AD (OR 0.68 [95%CI, 0.56 to 0.81]). Stratified analysis according to the study design (case-control or cohort studies) confirmed the risk reduction associated with statin use. Dementia risk reduction was observed in both men and women (OR 0.86 [0.81-0.92] and 0.86 [0.81-0.92], respectively). Our analysis observed similar risks for lipophilic and hydrophilic statins (OR 0.83 [0.76-0.90] and 0.80 [0.71-0.89] for dementia; OR 0.61 [0.46-0.81] and 0.59 [0.43-0.82] for Alzheimer's disease).
Conclusions. This meta-analysis of observational studies suggests a preventive effect of statin use on Alzheimer's disease and dementia risk. Better-designed studies are needed to draw unequivocal conclusions about the effect of statins on cognitive function.

ENDOTHELIAL DYSFUNCTION PREDICTS WORSE PROGNOSIS IN HOSPITALIZED PATIENTS WITH COVID-19

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Aim. Endothelial injury can be induced by coronavirus disease 2019 (COVID-19) and seems to exert a crucial pathogenic role in its most severe clinical manifestations. We aimed to investigate the association between endothelial function and in-hospital prognosis of COVID-19 patients.
Methods. Brachial artery flow-mediated dilation (bFMD), a non-invasive measure of endothelial function, was assesses in hospitalized COVID-19 patients within 48 hours since hospital admission. The association between bFMD and the composite endpoint of intensive care unit (ICU) admission/in-hospital death was explored using univariable and multivariable analyses.
Results. Two hundred patients were enrolled. Significantly lower bFMD values emerged in COVID-19 patients with either radiographic signs of pneumonia, respiratory distress, or the need for non-invasive ventilation, as compared to those without ($p<0.001$, $p=0.034$, and $p=0.044$). Sixty-three (32%) patients met the composite endpoint of ICU admission/in-hospital death. Patients with bFMD <4% (the median value) had a significantly higher risk for ICU admission/in-hospital death as compared to those with bFMD ≥4% ($p=0.021$). At Cox regression analysis bFMD was independently associated with a 2.262-fold (95% CI 1.223-4.184) increased risk of ICU admission/in-hospital death.
Conclusions. Endothelial dysfunction, as assessed by bFMD, may be clinically useful in the prognostic stratification of COVID-19 patients upon hospital admission.

SKIN CAPILLARY ALTERATIONS IN PATIENTS WITH ACUTE SARS-CoV-2 INFECTION

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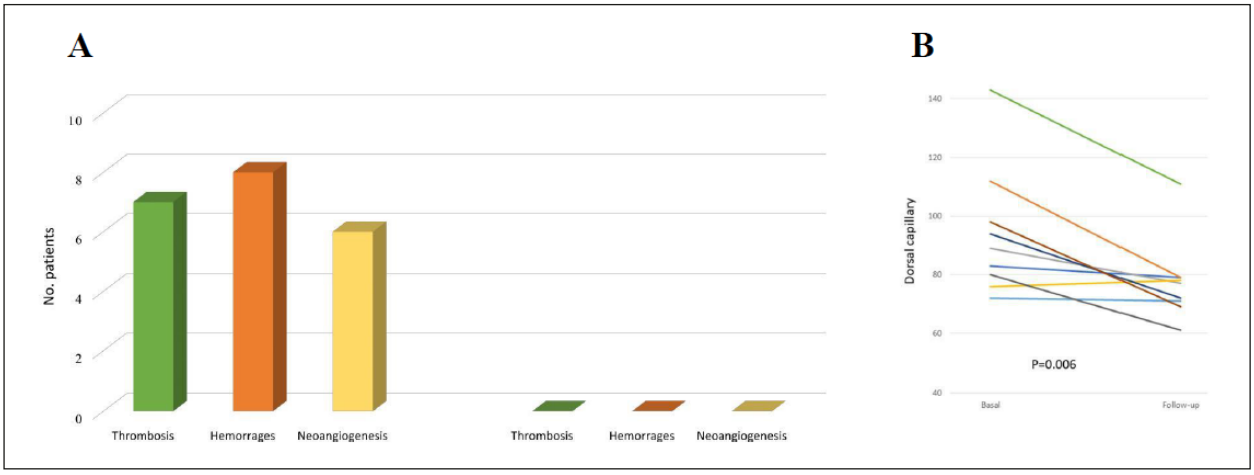
Aim. Acute SARS-CoV2 infection is associated with endothelial dysfunction and "endothelitis" which might explain systemic microvascular impairment. The presence of endothelial damage can promote vasoconstriction with consequent organ ischemia, inflammation, tissue edema, and a procoagulant state leading to increase in the incidence of cardio- and cerebro-vascular events. Microvascular thrombosis has been demonstrated in post-mortem autopsy of COVID-19 patients. However, no data are available about skin capillary alterations in these patients.
Method. We evaluated skin microvascular alterations in 22 patients admitted to Internal Medicine Department at Spedali Civili Hospital in Brescia and tested positive for a SARS-CoV-2 nasopharyngeal swab admission. Basal capillary density was performed by capillaroscopy bedside in the nailfold and the dorsum of the 4th finger. Capillaroscopy was repeated after 3 months from hospitalization for acute infection. Blood chemistry parameters and inflammatory markers were obtained at baseline and at the follow up.
Results. Patients with COVID-19 showed skin microvascular complications such as thrombosis, microhemorrhages, and neoangiogenesis which were not detected after 3 months from the discharge (Fig. 1A). A significant negative correlation between CRP and basal capillary density was observed in patients with acute Sars-CoV-2 infection ($p<0.05$). A positive correlation between basal capillary density and lymphocyte number was detected. Moreover, a significant reduction of basal capillary density in the dorsum was observed after 3 months from the acute infection (Fig. 1B).
Conclusion. This is the first *in vivo* evidence of skin microvascular complications in patients with acute Sars-CoV-2 infection which supports the presence of endothelial dysfunction, inflammation, and thrombosis. Capillary alterations may reflect systemic vascular effects of viral infection. Moreover, a reduction of basal capillary density was observed after three months from acute infection probably due to acute inflammation and hypoxia which might have induced both vasodilation and angiogenesis.

Figure 1. Combined figure showing: patients complications at baseline and at 3 months (panel A); dorsal capillary number changes during the follow up period (panel B).



IMPACT OF CHRONIC KIDNEY DISEASE IN PATIENTS HOSPITALIZED FOR COVID-19 ACCORDING TO DIABETES STATUS

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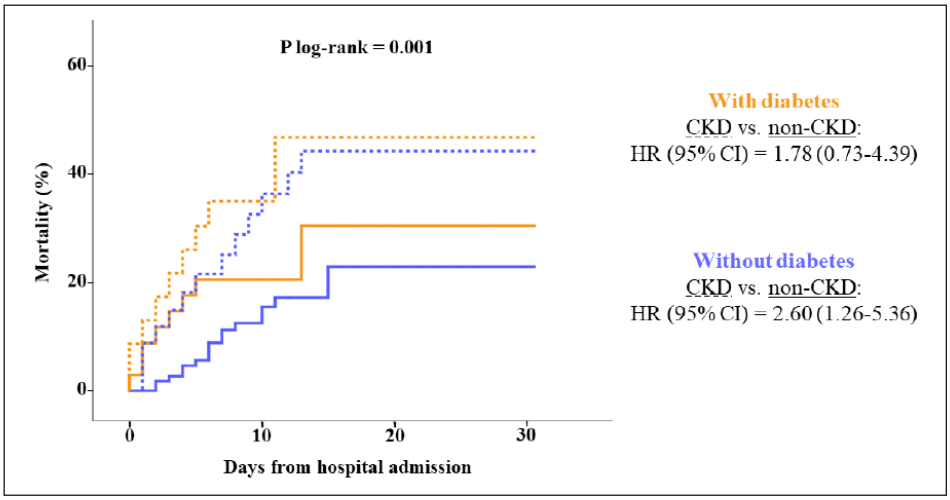
Aim. Chronic kidney disease (CKD) and diabetes mellitus have been recognized among several other comorbidities associated with poorer outcome in COVID-19 patients, with potential even worse prognosis if co-present. In this study we evaluated whether CKD impacts on clinical outcome of diabetic and non-diabetic patients hospitalized for COVID-19.
Method. We retrospectively analyzed medical record of a cohort of patients hospitalized for COVID-19 between February and April 2020 in the Internal Medicine Department at Montichiari Hospital, Brescia. All patients tested positive for a SARS-CoV-2 nasopharyngeal swab at admission and showed signs of pneumonia and respiratory insufficiency. CKD was defined as creatinine clearance <60 ml/min calculated through CKD-EPI formula. Primary endpoint was all-cause mortality at 30 days after admission.
Results. A total of 205 patients were included. 57 (27.8%) were diabetics at admission and 23 (40.3%) of these had CKD. Baseline characteristics, as shown in Table 1, did not differ among diabetic patients with or without renal insufficiency, while among non-diabetic patients we found that a higher percentage of patients with CKD showed hypertension, other cardiovascular disease (p<0.001, respectively) and were on treatment with ARBs (p=0.033). Kaplan-Meier curves for mortality are depicted in Figure 1. CKD confers significant higher risk of mortality only among non-diabetic patients. After adjustment for age and sex, a significant impact of CKD was no more appreciable in both groups.
Conclusion. Our results confirm that CKD and diabetes worsen prognosis of patients hospitalized for COVID-19. A significant increased risk of mortality was driven by CKD presence among non-diabetes without reaching statistical significance among diabetics. The confirmation at multivariate analysis was missed.

Table 1. Baseline characteristics according to the presence of CKD among diabetes and non-diabetes patients

Variable	Without Diabetes (n=148)			With Diabetes (n=57)		
	No-CKD (n=113)	CKD (n=35)	p-value	No-CKD (n=34)	CKD (n=23)	p-value
Age, years (±SD)	66.4 (±14.2)	79.3 (±8.8)	<0.001	71.4 (±15.7)	79.7 (±6.6)	0.021
Male, %	65.5	74.3	0.411	67.6	60.9	0.778
Hypertension %	46.0	88.6	0.000	79.4	87.0	0.724
Other CV disease %	20.4	60.0	0.000	47.1	60.9	0.419
Obesity, %	17.6	20.8	0.768	26.1	35.7	0.713
ACE-inhibitors, %	21.4	12.3	0.443	31.3	17.4	0.350
ARBs, %	14.3	32.3	0.033	25.0	34.8	0.550
Lymphocyte, $*10^3/mm^3$ (±SD)	1.31 (±2.52)	1.14 (±1.37)	0.729	1.25 (±0.78)	1.04 (±0.46)	0.300
Hemoglobin, g/dl (±SD)	12.8 (±1.7)	13.2 (±1.7)	0.213	12.1 (±2.2)	12.2 (±1.9)	0.864
CRP, mg/l (±SD)	89.8 (±74.9)	133. (±96.6)	0.006	103.9 (±60.4)	106.9 (±53.2)	0.846
Ferritin, ng/ml (±SD)	928 (±1232)	1387 (±1973)	0.362	1049 (±1005)	521 (±249)	0.225

CKD=chronic kidney disease; CV= cardiovascular; ACE=angiotensin converting enzyme; ARB=angiotensin receptor blockers; CRP=c-reactive protein; SD=standard deviation.

Figure 1. Kaplan—Meier curves for mortality and Cox regression estimated hazard ratio (HR) with relative 95% confidence interval (CI) in our population stratified according to diabetes and CKD.



REDUCTIONS OF PLASMA TOTAL CHOLESTEROL AND LOW-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS BETWEEN PRE- AND POST-INFECTION ARE ASSOCIATED WITH COVID-19 SEVERITY AT HOSPITAL ADMISSION

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Aim. Several studies have described the association between lipid profile at the onset of COVID-19 and infection prognosis as well as the association between changes in lipid profile during the clinical course of COVID-19 and infection outcomes. However, a gap exists on the understanding of the clinical significance of changes between pre- and post-COVID-19 plasma lipids. This study aimed to determine the variations of lipid profile due to COVID-19 and their association with disease severity.
Methods. Two hundred hospitalized patients with COVID-19 were retrospectively enrolled. Inclusion criteria were:
1) age ≥18 years;
2) a standard lipid profile [total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG)] prior to COVID-19;
3) a standard lipid profile within 48 hours since hospital admission due to COVID-19.

The association of lipid changes between pre- and post-infection and COVID-19 severity at hospital admission (according to the National Institute of Health classification) was assessed through univariable and multivariable analyses.
Results. TC, LDL-C and HDL-C levels obtained before COVID-19 were significantly higher than those measured upon hospitalization ($p<0.001$ for all comparisons). No significant change was found in TG levels ($p>0.05$). Absolute reductions of TC and LDL-C differed significantly according to COVID-19 severity ($p=0.033$ and $p=0.038$), being greater in patients with severe COVID-19 as compared to those with non-severe COVID-19 ($p=0.019$ and $p=0.008$). The associations between either TC reduction or LDL-C reduction and severe COVID-19 remained significant after adjustment for confounders (comorbidities, concomitant therapies, inflammatory biomarkers, time interval between lipid profiles) (OR 1.009, CI 1.001-1.018, $p=0.035$ and OR 1.013, CI 1.001-1.024, $p=0.029$).
Conclusions. A significant decrease of TC and LDL-C levels occurs in COVID-19 patients, which is greater with increasing severity of clinical manifestations. COVID-19-related mechanisms beyond pre-existing conditions and systemic inflammatory response during infection might be involved in these lipid changes.

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

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Aim. ANGPTL3 and ANGPTL4 are regulators of lipolysis and thereby participate to substrate energy disposal, since bariatric surgery produce a profound impact on energy metabolism, this research aimed to determine whether this treatment affects circulating levels of these two ANGPTLs.
Methods. Serum levels of ANGPTL3 and ANGPTL4, together with parameters of lipid and glucose metabolism, were measured at baseline and 1-year after bariatric surgery in 42 obese patients submitted to Roux-en-Y gastric bypass (RYGB, n=27) or bilio-pancreatic diversion (BPD, n=15).
Results. ANGPTL3 levels, showed a significant rise 1-year after surgery in BPD [from 225±20 to 300±15; $p=0.003$], while no significant change was observed in RYGB patients. A direct association between ANGPTL3 levels and M value was observed in both groups both before and after surgery. In the BPD group only, ANGPTL3 was directly associated with free fatty acids (FFA) and inversely with total bile acid (TBA) [$p<0.02$]. ANGPTL4 levels, were reduced in all groups after surgery [$p<0,05$] and its levels were inversely related to M-value.
Discussion. Plasma levels of ANGPTL4 were consistently affected by bariatric surgery while those of ANGPTL3 depended on type of surgical intervention. While levels of ANGPTL4 are closely determined by adipose tissue trophism and grade of insulin resistance, ANGPTL3 regulation appeared to be related to insulin sensitivity, levels of circulating FFA and total bile acids.
Conclusion. While decrease in ANGPTL4 levels is considered to be a consequence of reduction of fat mass and amelioration of insulin sensitivity, ANGPTL3 change suggest that chronic nutrient loss and malabsorption associated to BPD might be counterbalanced by increased liver production of ANGPTL3. Thus, opening a new view on the complex regulation of ANGPTL3 secretion which involve also some step of hepatic cholesterol metabolism.

PROTECTIVE EFFECT OF PHYSICAL ACTIVITY ON MORTALITY IN OLDER ADULTS WITH ADVANCED CHRONIC HEART FAILURE

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Aim. The objective of this study was to evaluate the effect on mortality of self-reported physical activity evaluated by the physical activity scale for the elderly (PASE) in elderly patients with advanced heartfailure enrolled in a cardiac rehabilitation unit after heart failure decompensation (NYHA class IIIB).
Methods. The study prospectively enrolled 314 elderly patients (≥65 years) with heart failure in NYHA class IIIB (symptomatic with a recent history of dyspnoea at rest) consecutively admitted to cardiac rehabilitation between January 2010 and July 2011. Comprehensive geriatric assessment was performed. Physical activity was evaluated by PASE and stratified in tertiles (0–15, 16–75 and >75). Mortality was collected from September to October 2015 in 300 patients.
Results. The mean age was 74.5±6.1 (range 65-89); 74.7% were men, 132 patients (44.0%) died during the follow-up (44.1±20.7 months). Univariate analysis shows that physical activity level conducted before heart failure decompensation was inversely related to mortality (from 76.0% to 8.2%, P=0.000). Multivariate analysis confirms that the PASE score predicts mortality independently of several demographic and clinical variables (hazard rate 0.987, 95% confidence interval (CI) 0.980-0.994, P=0.000). Notably, when considering PASE 0-15 versus 16-75 score and PASE 0-15 versus>75 score, the hazard rate is 4.06 (95% CI 1.67-9.84, P<0.001) and 7.25 (95% CI 2.7-19.5, P<0.001), respectively.
Conclusions. Physical activity level evaluated by the PASE score is inversely related to mortality in elderly patients with advanced heart failure confirming the reduction of mortality exerted by moderate physical activity in such patients.

THE CAUSAL EFFECT OF ADIPOSITY MEASURES ON BLOOD PRESSURE TRAITS IN TWO URBAN SWEDISH COHORTS: A MENDELIAN RANDOMIZATION STUDY

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Aim. Different adiposity traits may be causally related to hypertension in different ways. By using genetic variants as randomly allocated proxies for studying the effect of modifying adiposity traits, the Mendelian randomization approach can be used to investigate this.
Methods. In this study, we used four different genetic risk scores (GRSs: GRS-BMI565, GRS-WHR324, GRS-VAT208, GRS-BF81) including hundreds of single nucleotide polymorphisms (SNPs) associated with Body Mass Index (BMI), Waist to Hip Ratio (WHR), Visceral Adipose Tissue (VAT), and Body Fat (BF), respectively, using risk alleles and weight from largest available GWAS meta-analyses. These were applied as instrumental variables (IVs) in Mendelian Randomization analyses. Two Swedish urban-based cohort studies, the Malmö Diet and Cancer (MDC) and the Malmö Preventive Projects (MPP; subjects evaluated both at baseline and after an average follow-up of 23±4.7 years), were used to obtain genetic association estimates with blood pressure (BP).
Results. In both the MPP and MDC, with the exception of that for BF, all the GRSs were significantly associated with systolic BP and diastolic BP, but with different magnitude.: In particular, each standard deviation (SD) increase in the GRS-WHR324 doubled the likelihood of hypertension prevalence at MDC or MPP baseline exam. However, only the GRS-BMI565 was significantly associated to BP and with hypertension incidence during 23.6±4.3 years of follow-up in MPP.
Conclusions. In conclusion, we have confirmed a causal link between genetically mediated adiposity, especially WHR and BMI, and BP-related traits including hypertension prevalence and, for the first time to our knowledge, hypertension incidence. The differences in magnitude between these associations might suggest different mechanisms by which different adiposity traits mediate their effect on BP/hypertension and consequently may indicate that tailored interventions are needed to reduce cardiovascular risk.

NON-ALCOHOLIC FATTY LIVER DISEASE AND SUBCLINICAL ATHEROSCLEROSIS IN A POPULATION AFFECTED BY FAMILIAL HYPERCHOLESTEROLEMIA

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Aims. There are no data on the association between non-alcoholic fatty liver disease (NAFLD) and subclinical atherosclerosis in people affected by Familial Hypercholesterolemia (FH). This study investigates the influence of NAFLD on subclinical atherosclerosis and arterial stiffness in an asymptomatic FH population.
Methods. A total of 169 consecutive asymptomatic young individuals affected by genetically defined Heterozygous FH (HeFH) with no prior history of cardiovascular disease, diabetes or secondary steatosis were enrolled and underwent carotid/femoral ultrasonography, cardiac Computed Tomography (CT), PWV. NAFLD was assessed by CT, defined as liver/spleen density ratio <1 or liver density <40 HU. Carotid, femoral and coronary atherosclerotic burden were assessed through Doppler ultrasound and calcium score. Arterial stiffness was evaluated through SpygmoCor® pulse wave analysis and velocity.
Results. Of the study participants 22 (12,4%) had CT-diagnosed NAFLD. Individuals with NAFLD exhibited a significant increase in CAC score (metti il valore medianop =0,011). After adjustment for cardiovascular risk factors, CRP and type of mutation, HeFH subjects with NAFLD had a higher risk of presenting a CAC score >100 (OR 8.45 [95% CI 1.75-40.8]; p=0.006). ApoB mutation was another independent predictor of NAFLD in this population (5,99; 95% CI 1,44-25,04; p=0.0014). No difference were found for carotid intima-media thickness (p=0,91), carotid plaque (p=0,35), and PWV (p=0,34).
Conclusion. NAFLD constituted an aggravating marker of cardiovascular risk in asymptomatic FH. Genotype-phenotype interaction could be a driver for this metabolic overrisk. Further studies are needed to confirm the impact of NAFLD in cardiovascular outcomes in asymptomatic HeFH.

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

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Aim. In a significant proportion of individuals clinically diagnosed with familial hypercholesterolemia (FH), where a disease-causing mutation was not found, hypercholesterolemia could have a polygenic base. We aimed to describe the distribution of a polygenic risk score, comprised of 12 LDL-C raising variants (LDLc-score), in subjects clinically diagnosed with FH.
Methods. The analysis was performed in patients enrolled in the LIPIGEN study, an observational, multicentre study collecting data on FH subjects. Adult individuals were divided in mutation-positive FH patients (FH/M+) and mutation-negative FH patients (FH/M).
Results. A total of 846 FH/M+ patients (females 54.74%, mean age 42.31±15.01 years) and 627 FH/M- patients (females 53.76%, mean age 49.55±13.55 years) were identified. FH/M- patients had lower mean levels of pre-treatment LDL-C (217.19±55.85 vs 270.44±68.30 mg/dL, p-value <.0001) than FH/M+ group, and a significant lower prevalence of tendinous xanthomata (4.31% vs 17.49%, p-value <.0001). The mean value (±SD) of the LDLc-score was 1.00 (±0.18) in FH/M- patients and 0.94 (±0.20) in FH/M+ group (p-value <.0001). In the ROC analysis, the AUC for recognizing subjects characterized by a polygenic hypercholesterolemia was 0.60 (95% CI 0.57-0.63), with a sensitivity and specificity being 79% and 34% at 0.90 as a cut-off value. Higher mean LDLc-score levels were observed amongst FH/M- having pre-treatment LDL-C levels between 150-350 mg/dL (150-249 mg/dL: 1.02 vs 0.91, p-value <.0001; 250-349 mg/dL: 1.02 vs 0.95, p-value =0.0001). A positive correlation between LDLc-score and pre-treatment LDL-C levels was observed among FH/M- subjects (r=0.12, p-value =0.002), even more marked among FH/M+ patients (r=0.16, p-value <.0001).
Conclusions. This analysis confirmed the role of polymorphisms in modulating LDL-C levels, even in subjects with a monogenic mutation, suggesting a decisive impact of non-genetic factors. More data is needed to support the use of the polygenic score in the diagnosis of FH and in the prediction of cardiovascular risk.

EFFECTS OF GENDER AFFIRMING HORMONE THERAPY ON CHOLESTEROL EFFLUX CAPACITY AND ON SERUM LOADING CAPACITY IN TRANSGENDER INDIVIDUALS

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Aim. The main proposed atheroprotective function of HDL lays on their role to promote macrophage cholesterol efflux (cholesterol efflux capacity, CEC). In addition, the serum capacity to load macrophages with cholesterol (cholesterol loading capacity, CLC) represents an index of pro-atherogenic potential. The present study was aimed to evaluate the effects of gender affirming hormone therapy (HT) on HDL-CEC and on serum CLC within transgender individuals.

HDL-CEC and serum CLC have been evaluated in 15 trans women treated with estradiol and cyproterone acetate and in 15 trans men treated with testosterone at baseline and after 12 months of HT.

Methods. Total HDL-CEC from macrophages and its major contributors, the ATP-binding cassette transporters (ABC) A1 and ABCG1 HDL-CEC and HDL-CEC by aqueous diffusion were determined by a radioisotopic assay. CLC was evaluated in human THP-1 macrophages by a fluorimetric assay.

Results. In trans women, estradiol levels were raised (+200%; p=0.013) whereas LH and testosterone significantly reduced. HT reduced total cholesterol (-10.7%; p=0.0017), HDL-C (-14.3%; p=0.0024) and LDL-C (-10.9%; p=0.0058). Total HDL-CEC decreased (-10,8%; p <0.001) with a specific decrement in CEC mediated by the ABCA1 (-23,8%; p<0.001) and AD (-4.8%; <0.01). In trans men, while estradiol levels did not change, testosterone markedly increased (+97%; p<0.0001), whereas luteinizing hormone (LH) decreased significantly (-64%; p=0.049) after HT. Total cholesterol and LDL-C were not affected by testosterone treatment, whilst TG were raised (+11.76%; p=0.0078) and HDL-C reduced (-19.6%; p=0.0103). Concerning HDL-CEC, only the aqueous diffusion (AD) process was lowered (-9.8%; p<0.01), an effect directly correlated with HDL-C changes (r=0.6242; p=0.0002). ABCG1 HDL-CEC did not change in either group. Serum CLC was not modified by HT in both groups.

Conclusions. Total HDL-CEC decreased during HT in trans women, with a specific reduction in ABCA1 CEC. This finding might contribute to a higher CVD risk.

SUPERVISED AND UNSUPERVISED LEARNING TO DEFINE THE CARDIOVASCULAR RISK OF PATIENTS ACCORDING TO AN EXTRACELLULAR VESICLE MOLECULAR SIGNATURE

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Aim. Secreted extracellular vesicles (EVs) are membrane-bound nanoparticles released from cells. Since their content reflect the specific signatures of cellular activation and injury, EVs display a strong potential as biomarkers in the cardiovascular (CV) field. We aimed at dissecting a specific EV signature able to stratify patients according to their CV risk and likelihood to develop fatal CV events.

Methods. A total of 404 patients were included in the analysis. For each subject, we evaluated several CV risk indicators (age, sex, BMI, hypertension, hyperlipidemia, diabetes, coronary artery disease, chronic heart failure, chronic kidney disease, smoking habit, organ damage) and the likelihood of fatal CV events at 10 years, according to the SCORE charts of the European Society of Cardiology. Serum EVs were isolated by immuno-capture and analyzed for the expression of 37 EV surface antigens by flow cytometry. Unsupervised and supervised learning algorithms were applied for clustering patients according to CV risk.

Results. Based on expression levels of EV antigens, unsupervised learning classified patients into three clusters (cluster I, 288 patients; cluster II, 86 patients; cluster III, 30 patients). Prevalence of hypertension, diabetes, chronic heart failure and organ damage (defined as left ventricular hypertrophy and/or microalbuminuria) progressively increases from cluster I to cluster III, with an average 6.9-fold increase. Several EV antigens, including markers from platelets (CD41b-CD42a-CD62P), leukocytes (CD1c-CD2-CD3-CD4-CD8-CD14-CD19-CD20-CD25-CD40-CD45-CD69-CD86), and endothelium (CD31-CD105) were independently associated to the CV risk indicators and correlated to age, blood pressure, glucometabolic profile, renal function, and SCORE risk. EV specific signature obtained by supervised learning allowed the accurate classification of patients according to their 10-year risk of future CV events, as estimated with the SCORE risk charts.

Conclusions. EV profiling, obtainable from minimally-invasive blood sampling, may be integrated into CV risk stratification, displaying a potential role in the tailored management of these patients.

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

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Aim. Long non-coding (lnc) RNAs are potent regulators of many pathophysiological processes including atherosclerosis. Using RNAseq profiling of the intima lesions of LDLR-/- mice, our group identified for the first time a macrophage-specific lncRNA and called it MAARS (Macrophage Associated Atherosclerosis lncRNA Sequence), as its expression was strongly increased during atherosclerosis progression and its *in vivo* silencing reduced atheroma formation. As a possible mechanism, we explored the possibility of a direct effect of MAARS on macrophage cholesterol handling.
Methods. Bone marrow monocytes were isolated from C57bl/6 mice and differentiated to BMDM (bone marrow-derived macrophages) with mMCSF (mouse macrophage-colony stimulating factor). BMDM were silenced using Lipofectamine and customized GapmeRs for MAARS or negative control. MAARS knockdown efficacy was verified through RT-qPCR. Cholesterol synthesis and efflux were evaluated though a radioisotopic technique, while macrophage cholesterol uptake through a fluorimetric assay.
Results. MAARS expression in BMDM was significantly reduced after the silencing with MAARS GapmeRs compared to that with control GapmeRs (-72%; $p<0.0001$). MAARS silencing in acLDL-loaded BMDM induced an increased cholesterol efflux to HDL ($p<0.01$), ApoA-I ($p<0.001$) and serum from normolipidemic subjects ($p<0.05$) compared to control BMDM. MAARS knockdown-BMDM exposed to acLDL and hypercholesterolemic human serum showed reduced cholesterol uptake compared to control BMDM ($p<0.05$). Finally, MAARS knockdown-BMDM displayed a reduced cholesterol synthesis compared to control BMDM ($p<0.01$).
Conclusions. Altogether, these results suggest that MAARS might modulate total intracellular and membrane macrophage cholesterol content, as demonstrated by the increased cholesterol efflux, decreased cholesterol uptake and decreased cholesterol synthesis following the in vitro MAARS silencing. Thus, the impact of MAARS on macrophage cholesterol handling may partially explain its proaterogenicity; ongoing studies will explore the underlying molecular pathways.

HYPERURICEMIA PREVALENCE IN HEALTHY SUBJECTS AND ITS RELATIONSHIP WITH CARDIOVASCULAR TARGET ORGAN DAMAGE

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Background. Heterogeneous results have been obtained in the relationship between Uric Acid (UA) and Target Organ Damage (TOD).
Purpose. In the present study we sought to assess the prevalence of hyperuricemia in healthy subjects as well as the role of UA in determining TOD. We evaluated vascular, cardiac and renal TODs in the whole population as well as sub-grouped by gender.
Methods. 379 blood donors participated at the present analysis. TOD was evaluated as Pulse Wave Velocity (PWV), Left Ventricular Mass Index (LVMI) carotid Intima-Media Thickness (IMT) and Glomerular Filtration Rate (GFR). Hyperuricemia was defined with the classic cut-off (>7.0 in men and >6.0 mg/dL in women) but also with a most recently defined one (5.6 mg/dL for both sex).
Results. Hyperuricemia was present in 6.3% of the whole population (7.3% males, 2.8% females) considering the classic cut-off, while, with the recently identified one, it was present in 28.2% of the whole population (37.3% males, 4.7% females). Despite all the evaluated TODs significantly correlated with UA, linear multivariate regression analysis showed that none of them, except for GFR, displayed UA as a significant covariate. Similar figures were found also when both correlation and linear regression analyses were repeated in the two genders separately.
Conclusions. Hyperuricemia is an important problem also in healthy subjects and its prevalence could further increase if lower cut-off will be used. In this specific population, UA is statistically associated only with renal impairment while this was not the case for cardiac and vascular damage.

EFFECTS OF DIRECT ACTING ANTIVIRAL AGENTS ON LIPID AND GLUCOSE PROFILE IN HEPATITIS C VIRUS PATIENTS WITH TYPE 2 DIABETES: A REAL LIFE ITALIAN EXPERIENCE

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Background and Ams. Hepatitis C virus (HCV) is associated with an increased risk of type 2 Diabetes Mellitus (T2DM) and cardiovascular (CV) diseases. The impact of HCV eradication on metabolic profile in diabetic patients treated with DAAs is not well defined. The aim of our study was to evaluate the effects of DAAs therapy on lipid and glucose profile in a cohort of diabetic patients with different liver fibrosis stage.
Methods. 131 HCV T2DM patients with sustained virological response (SVR) to DAAs were enrolled. T2DM was diagnosed according to WHO criteria. Assessment of cholesterol profile and glucose profile before and after treatment (12 months) with DAAs were measured. Liver fibrosis stage was assessed by FibroScan.
Results. Diabetic patients were subdivided according to liver fibrosis stage in low-moderate (F1-F3) (46.6%) and cirrhotic (F4) (53.4%). At baseline, no significant differences were found in lipid and glucose profiles in subgroup analysis according to liver fibrosis, HCV genotype and CV risk factors. At 12 months after treatment, liver function and stiffness improved; total cholesterol and LDL cholesterol (but not triglycerides and HDL) significantly increased irrespective of liver fibrosis grade, baseline anthropometric, clinical and virological profile. The glycaemic control was shown to clinically and significantly ameliorate only in patients with cirrhosis (glycated haemoglobin mmol/L -1.0 (-4.0; 1.0) in F1-3 vs -5.2 (-7.5;2.3) in F4, p=0.038).
Conclusion. Our results confirm that HCV eradication in diabetic patients is associated with a better glycaemic control and worsening lipid profile that could impact on future CV events. A careful global monitoring of CV risk factors in all diabetic patients before and after HCV eradication is needed.

ABSTRACT SELEZIONATI

ACCELERATED ATHEROSCLEROSIS IN SYSTEMIC LUPUS ERYTHEMATOSUS: A STUDY IN A COHORT OF 47 PATIENTS

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Introduction. Vasculitis is a primary feature of Systemic Lupus Erythematosus pathophysiology. Nowadays, it is clear that, besides vasculitis, vasculopathy due to accelerated atherosclerosis plays a major role in the morbidity and mortality of SLE patients. What we present here is a cross sectional study investigating the prevalence and association of vasculopathy in our SLE patients, especially focusing to the integer point version of the SLE cardiovascular risk equation (iSLECRE) index.
Materials and Methods. We selected a cohort of 47 patients (6 males, 41 females) suffering from SLE, and admitted to our Internal Medicine Unit from 2014 to 2019. We obtained the past clinical history and lab tests of the last follow up visit. Moreover, we calculated the iSLECRE to assess correlation with atherosclerotic vasculopathy. Vasculopathy was defined by means of ultrasound and Doppler examination, or in the presence of history of prior stroke or myocardial infarction. We also obtained the value of the aortic stiffness index.
Results. The mean age of our sample's patients was 48,8 yrs., of whom 68% had some form of vasculopathy. The differential analysis of the baseline characteristics related to the presence or absence of vasculopathy showed a higher prevalence of classic (smoke, diabetes, hypertension) and SLE-related (antiphospholipid syndrome, anti-DNA antibodies positivity, duration of steroids usage) cardiovascular risk factors in the vasculopathy group. Moreover, the aortic stiffness index value showed correlation to disease activity, being significantly higher in the high activity group, as assessed by the SELENA-SLEDAI activity index. The iSLECRE value proved good as a diagnostic score for the presence of vasculopathy (even subclinical), having an AUROC of 78%, with a sensitivity of 82% and specificity of 71% at the best cut-off of 7,5.
Conclusions. This study shows the importance of the interplay between classical and immunological cardiovascular risk factors in the pathogenesis of early atherosclerosis in SLE. Given its importance in end-organ damage in SLE patients and the well known efficacy of primary and secondary prophylaxis in reducing the cardiovascular disease burden, the iSLECRE could be a fundamental screening test in driving diagnostic effort to uncover and treat early cardiovascular pathology in the daily management of SLE patients

THE ASSOCIATION BETWEEN NEUTROPHIL TO LYMPHOCYTE RATIO AND ENDOTHELIAL DYSFUNCTION IN PEOPLE LIVING WITH HIV ON STABLE ANTIRETROVIRAL THERAPY

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Aim. Residual inflammation is thought to promote endothelial dysfunction and atherosclerotic cardiovascular disease (ASCVD) risk among people living with HIV (PLWH) receiving antiretroviral therapy (ART). Whether the neutrophil to lymphocyte ratio (NLR), a putative marker of systemic inflammation, may be associated with endothelial dysfunction has not been investigated in PLWH on stable ART.
Methods. In this cross-sectional study, 210 PLWH (mean age 49 years, 79% males) on long-term ART (median ART duration 8 years) were enrolled. The association between NLR and brachial flow-mediated dilation (bFMD), was tested by univariable and multivariable analyses.
Results. A curvilinear association was observed between LG-NLR and LG-bFMD (R square=0.034, p=0,027), with LG-bFMD decreasing significantly with increasing LG-NLR values only in PLWH with high NLR (≥1,47, median NLR) (r=-0,369, p<0,001). However, among PLWH with high NLR, the most favorable Youden index describing the association between NLR and low bFMD (≤4.55, median bFMD) was 0,35 (for an NLR value of 2,20), thus suggesting poor prediction accuracy (55% sensitivity and 80% specificity).
Conclusions. Although there is a significant inverse association between NLR and bFMD among long-term ART-treated PLWH with high NLR, NLR has a low discriminatory ability towards endothelial dysfunction in this category of patients.

LYSOSOMAL ACID LIPASE IMPACTS DENDRITIC CELLS MATURATION

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Aim. Lysosomal acid lipase (LAL) hydrolyzes cholesterol esters and triglycerides in the lysosome. LAL-mediated lipid catabolism provides free cholesterol and fatty acids which contribute to cellular metabolic reprogramming. Indeed, in immune cells, LAL was shown to be critical for the polarization of macrophages toward pro-resolutive phenotype and the generation of memory T cell pools. Aim of this project is to investigate the role of LAL during dendritic cells development and maturation.
Methods. Dendritic cells (DCs), were differentiated from PB-MC-derived monocytes of healthy donors, following incubation with GM-CSF and IL-4 for 6 days. Then LPS was added for 14 hours to induce DCs maturation in the presence or absence of Lalistat, a selective LAL inhibitor.
Immunophenotyping of DCs was performed by flow cytometry paralleled by the evaluation of LAL activity during DCs maturation.
Results. While LAL expression and enzymatic activity increased during DCs differentiation (+70%, p<0.01), DCs maturation induced by LPS incubation led to an unchanged LAL expression concomitantly with a decreased LAL activity (-26%). Moreover, LAL activity inhibition by Lalistat upon DCs maturation resulted in a reduced abundance of lysosomes (-46%) and a decreased expression of a DCs maturation markers, namely the co-stimulatory receptor CD80 (-58%, p<0.05).
Conclusions. Lysosomal acid lipase is a checkpoint in lysosomal lipid homeostasis and, in immune cells, it could represent a link between lipid metabolism and immunometabolic reprogramming during DCs differentiation and maturation. Ongoing studies are now aimed at elucidating the immunometabolic effect of targeting LAL during DCs maturation.

ASGR1 DEFICIENCY IMPACTS GLYCAN SIGNATURE IN INNATE AND ADAPTIVE IMMUNE CELLS DURING OBESITY

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Aim. Altered glycosylation is a well-established feature in many inflammatory diseases. Adaptations in glycan patterns are associated with pathogen recognition, modulation of the immune system, control of immune cell homeostasis and inflammation. Our aim was to investigate the glycosylation signature in innate and adaptive immune cells during obesity in ASGR1$^{-/-}$ mice.
Methods. ASGR1$^{-/-}$ mice and WT littermates were fed in a high-fat diet (45% Kcal fat) for 20 weeks. Glucose metabolism (glucose-tolerance test (GTT), insulin-tolerance test (ITT)) was checked at 5 and 20 weeks. Blood and adipose tissue immunophenotyping were performed at 20 weeks by flow cytometry. Lectin staining was used to discriminate Galactose (Gal), Sialic acid (SIA) α2,3 and α2,6-linked glycoproteins within monocytes, macrophages and neutrophils.
Results. ASGR1$^{-/-}$ mice presented a similar GTT, and ITT response compared to WT as were circulating monocytes and neutrophils counts (monocytes ASGR1$^{-/-}$ 633.25 cell/ul ±6.01, WT 655.3±8.09; neutrophiles ASGR$^{-/-}$ 586.25 cells/ul±6.45, WT 652.27±8.05). Lower infiltrating monocytes and macrophages were observed in adipose tissue in ASGR1$^{-/-}$ compared to WT (monocytes ASGR1$^{-/-}$ 63861.54cell/g±108.52, WT 77527.34±70.00; macrophages 53692.95 cell/g±91.55, WT 71091.71±65.87). Monocytes and macrophages in adipose tissue of ASGR1$^{-/-}$ mice presented increased expression of Gal and α2,6-linked Sia (monocytes Gal ASGR1$^{-/-}$ MFI 2051.17±5.21, WT 1709.6±8.07, α2,6-linked Sia ASGR1$^{-/-}$ MFI 5206.83±38.03,WT 1214.8±24.17,$p<0.05$; macrophages Gal ASGR1$^{-/-}$ MFI 5343.00±10.36,WT 4303.8±7.96, $p<0.05$, α2,6-linked Sia ASGR1$^{-/-}$ MFI 33736.67±60.58,WT 10161.4±55.40,$p<0.05$).
Conclusions. Our data suggest that glycan signature in innate immune cells is influenced during obesity in ASGR1$^{-/-}$ mice. Whether Gal and Sia ratios observed in innate immune cells can reflect a different immune activation status is under investigation.

IMPAIRED FATTY ACID SYNTHESIS AFFECTS THE ACTIVATION OF IMMUNOSUPPRESSIVE T REGULATORY CELLS: ROLE OF THE STEROL REGULATORY ELEMENT BINDING FACTOR-1C

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Aim. Reprogramming of cellular metabolism represents an integral part of T lymphocyte differentiation and activation. The immunosuppressive T regulatory cells (Tregs) adapt their metabolic machinery to accomplish cellular function: while fatty acids oxidation (FAO) sustains their maintenance and suppressive function, glycolysis is used for migration. Here we aimed at depicting the contribution of FA metabolism on Treg biology, exploring the role of SREBP1c, the master regulator of FA synthesis.
Methods. A detailed immunophenotyping through flow cytometry and in vitro assays were performed in Tregs isolated from SREBP1c KO and WT littermates.
Results. SREBP1c KO mice present significantly decreased Treg levels in both lymphoid (thymus, LNs and spleen) and non- (blood, liver and peritoneal lavage, PL), paralleled to a reduction in markers of suppressive function (CD25, Helios, PD-1) and different expression of chemokine receptors CCR7 and CXCR3 respectively in LNs and PL. Functionally, KO Treg were less suppressive in vitro, migrated more toward CCL19 and CXCL10 chemokines and display a reduced vitality. mTORC1 activation (pS6 expression) was increased, suggesting a shift toward glycolytic flux in KO compared to WT Treg.
Conclusions. Tregs from SREBP1c KO mice showed an increased migration rather than a suppressive function, suggesting that FA synthesis controls Treg functionality. Our data indicate that SREBP1c is a metabolic checkpoint coupling FA synthesis to Treg survival and suppressive function, suggesting that strategies able to target FA metabolism could modulate immunosuppressive response.

STUDY OF THE ROLE OF THE INTERLEUKIN-1 RECEPTOR/TOLL LIKE RECEPTOR TIR8/SIGIRR IN EXPERIMENTAL ATHEROSCLEROSIS

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Aim. TIR8/SIGIRR is a negative regulator of the excessive activation mediated by ILRs and TLRs agonism and thus is a key regulator of inflammation. Aim of this work was to study the role of TIR8 in atherosclerosis.
Methods. 8 weeks old-LDLR KO and TIR8/LDLR double KO (DKO) male mice were administrated standard diet (STD) or cholesterol-enriched diet (WTD) for 12 weeks. The impact on the pathogenesis was evaluated through plasma lipid profiling, extensive immunophenotyping and histological analysis of the atherosclerotic plaques.
Results. TIR8 deficiency in STD-fed LDLR KO mice impacts circulating immune cells distribution: T lymphocytes distribution decreased (-29%, $p<0.001$) and that of B cells increased (+14%, $p<0.05$) when compared to LDLR KO mice, as well as increased percentage of mature Natural Killer cells (+13%, $p<0.0001$) was observed, as already described in the TIR8 KO mouse model. When fed with cholesterol rich diet for 12 weeks to induce atherosclerosis, similar shifts in the percentage of T and B lymphocytes and Natural Killer cells, were observed. In addition, circulating levels of monocytes increased in DKO mice compared to LDL-R KO mice (mean 1464 vs 910 cells/ul, $p<0.05$). Although the immune profile revealed significant changes, atherosclerotic plaque area or stability are not affected. Similarly, no differences in plasma lipid profile were observed.
Conclusions. TIR8 deficiency in LDLR KO mice increases NKs and monocytes blood levels compared to LDL-R KO mice. Alterations in the distribution of these immune populations, however, do not impact the development of atherosclerosis.

CARDIAC MONITORING OF VENTRICULAR DYSFUNCTION DURING TRASTUZUMAB THERAPY IN METASTATIC BREAST CANCER

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Aim. Trastuzumab therapy has dramatically changed breast cancer prognosis. Consensus documents recommend a close cardiac imaging monitoring during therapy, every 3 months, especially in metastatic breast cancer (MBC). The objective of this study is to describe the cardiovascular toxicity profile of trastuzumab in patients with MBC to understand how to improve cardiovascular monitoring.
Methods. We retrospectively studied MBC patients scheduled for trastuzumab therapy (2001-2018). All patients underwent a baseline evaluation and monitoring during therapy. Cardiotoxicity (CTOX) was defined as symptomatic heart failure (HF) or asymptomatic decrease in left ventricular ejection fraction (LVEF) >10% from baseline and <53%. Patients who developed CTOX were referred to the cardio-oncology clinic.
Results. Ninety-two women were included, mean age 61 years (±14.43), median follow-up 42.5 months IQR (26-74). Fourteen percent developed CTOX: two HF with preserved LVEF, three HF with reduced LVEF (mean EF 44%±3.61), and eight asymptomatic decreased in LVEF (mean EF 45.7%±4.89). Eighty-one percent of CTOX cases occurred within the first 4 years and on median of 31 months from trastuzumab initiation. Fifty-three patients died from disease progression but none due to cardiac events.
Conclusions. In MBC patients, trastuzumab-mediated CTOX occurs more frequently during the first 4 years. These data should be considered to optimize follow-up protocols with a less restricted timing, based on individual patients risk profiles, after the fourth year of therapy and under a close clinical monitoring by the cardio-oncology team.

IRON DEFICIENCY IN ACUTE HEART FAILURE: A REAL-LIFE SURVEY ON IN-HOSPITAL DIAGNOSIS AND TREATMENT AND LONG-TERM OUTPATIENT FOLLOW UP

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Aim - Background. Iron deficiency (ID) is common in heart failure (HF) and is associated with worse outcomes. Iron supplementation is seldom administered in chronic HF in clinical practice. Real-life data about the long-term impact of iron supplementation in acute decompensated heart failure (ADHF) are insufficient.
Aim - Object of study. To evaluate the effect of in-hospital and subsequent out-of-hospital iron supplementation on mortality and readmission rate on a long-term follow-up.
Methods. We observed all patients with ADHF admitted to our Internal Medicine Unit between October 2019 and March 2020. Clinical data, laboratory values, and outcomes were collected. In patients screened for ID, a long-term follow-up interview was performed.
Results. 104 patients were admitted to our Medical Unit for ADHF. 14 patients were excluded for missing data on iron status. A total of 90 patients (median age 84, 36% female) were included. Hypertension (n=72, 80%) and atrial fibrillation (n=45, 50%) were the most common comorbidities. ID was identified in 72 subjects (80%), 55 of whom (76%) were treated with ferric carboxymaltose. Target dose was reached in 13(23,6%).
We interviewed by phone 80 patients (89%), after a median of 427 days (IQR 405-466). During follow-up, in-hospital supplemented patients had a similar mortality and readmission rate in comparison to non-supplemented ones. Iron status was evaluated in 31 patients (38%), and 24(29%) were supplemented. In the 56 patients non-supplemented during follow-up, 12 died. On the contrary, all outpatients in whom iron was administered survived (p=0.014). Similar readmission rate was observed in supplemented vs non-supplemented patients.
Conclusions. In this real-life survey no differences in readmission rate and mortality were registered in supplemented vs non-supplemented inpatients at more than 1 year follow-up. Conversely, a significant reduction in mortality was observed in those outpatients who received iron supplementation during follow-up.
In order to improve clinical outcomes of patients with ADHF, outpatient ID treatment is mandatory.

A MULTISTEP STRATEGY TO DEAL WITH HEART FAILURE

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Aim. Different devices have been investigated to help in identifying early decompensation events in patients with heart failure (HF), reducing hospital admissions. In this report, we present a step-by-step approach to treat HF.
Case summary. A 68-year-old man with HFrEF was enrolled in our HF Clinic from October 2017. The patient's comorbidities included CKD, dyslipidaemia, COPD, hypertension, AF and obesity. The patient had a non-ischemic dilated heart disease; despite the optimal medical therapy (OMT), from 2004 to 2015, he had suffered more than 20 hospitalizations for exacerbation of HF with a progressive reduction of EF. In 2015 for the evidence of EF<35%, he had biventricular ICD implantation. In 2017, severe mitral regurgitation was treated by positioning of MitraClip; at discharge, the patient was enrolled in our departmental HF Clinic. At enrolment, echocardiogram showed a dilated LV with EF29% and PASP40 mmHg.
From October 2017 to May 2019, the patient went through numerous hospitalizations, despite OMT and introduction of sacubitril/valsartan; subsequently, was adopted a strategy of levosimendan infusions guided by CardioMEMS. In June 2019 CardioMEMS was implanted; if the cardiologist detected a trend towards increasing diastolic pulmonary artery (PAd) pressures, the patient was contacted, and his medications adjusted. When home therapeutic changes were not sufficient, the patient was contacted for hospitalization and infusion of levosimendan. From June 2019 to January 2021, the patient had only 5 hospitalizations scheduled for levosimendan infusion. In January 2021, following a progressive shortening of the time between hospitalizations for levosimendan infusions, the patient attended for Optimizer Smart device implantation for CMM therapy. PAP values on monitoring in the 30days following discharge were significantly lower than in the previous hospitalization (PAPs33.67±2,92 vs 40,6±3,37 mmHg, PAPd14,5±2,01 vs 22,5±2,53 mmHg, PAPm22,87±2,20 vs 30,9±2,99 mmHg, HR60,93±1,53 vs 80,83±3,66 bpm; p<0.0001).
Conclusions. The CardioMEMS is an implantable device positioned in the pulmonary artery able to detect higher cardiac filling pressures, estimated to rise more than 2 weeks before the onset of symptomatic clinical congestion. In this report, we present the first patient experience with levosimendan infusion led by CardioMEMS and the subsequent Optimizer Smart device implantation. Our case supports a step-by-step approach to treat heart problems, with the help of innovative devices.

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

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Background and Aim. PCSK9 controls the expression of several receptors of the LDL-R family and also of CD36 by favoring their degradation in the lysosome. When overexpressed in cardiomyocytes, these receptors of the LDL-R family favor lipid accumulation and cardiac dysfunction. This project aims at testing whether PCSK9 impacts lipoprotein receptors expression and cardiac mitochondrial metabolism.
Methods. Two-months old WT and PCSK9 KO male mice underwent echocardiographic analysis Mitochondrial respiration was investigated in cardiac tissue under resting conditions and following maximal coupling and uncoupling conditions. This was followed by metabolomic analysis and proteomic analysis.
Results. PCSK9 deficiency results in increased thickness of the left ventricular wall but with preserved Ejection Fraction compare to WT. This observation was coupled to a reduced performance in the treadmill test that is further associated with reduced Oxygen consumption in the heart paralleled by a reduced expression of key proteins of complex 1, 2 and 3 of the electron transport chain subunits in PCSK9 KO mice compared to controls. PCSK9 deficiency in the heart led to intracardiac cholesterol accumulation. The reduced flux of fatty acid oxidation and of Krebs cycle and the increase in lactate dehydrogenase, suggested a shift from fatty acid oxidation toward anaerobic glycolysis in PCSK9 KO hearts.
Conclusion/Discussion. PCSK9 deficiency results in a morphological and metabolic alteration in the heart that develops Heart Failure with preserved ejection Fraction.

HEART RATE IN PATIENTS WITH SARS-CoV-2 INFECTION: PREVALENCE OF HIGH VALUES AT DISCHARGE AND RELATIONSHIP WITH SEVERE DISEASE

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Aim. Despite the arrhythmic risk related to COronaVIrus-related Disease (COVID) is still under evaluation, the most common arrhythmia associated with infection is sinus tachycardia. It is not known if high Heart Rate (HR) in COVID is simply a marker of an important systemic response to sepsis or if its persistence could be related to a long-term autonomic dysfunction.The aim of our work is to assess the prevalence of elevated HR at discharge in patients hospitalized for COVID-19 and to evaluate the variables associated with it.
Methods. We enrolled 701 cases of SARS-CoV2 infection admitted in our hospital after February 21st and discharged within July 23rd 2020. We collected data on clinical history, vital signs, laboratory tests and pharmacological treatment. Severe disease was defined as the need for Intensive Care Unit (ICU) admission and/or mechanical ventilation.
Results. Median age was 59 years (IQR 49, 74), and male was the prevalent gender (60.1%). 84.7% of the subjects showed a SARS-CoV-2 related pneumonia, and 13.4% resulted in a severe disease. Mean HR at admission was 90±18 bpm with a mean variation of 10 bpm to discharge. Only 5.5% of subjects presented HR >100 bpm at discharge; they were younger (54 – IQR 37-65 vs 60 – IQR 50-74, years, p=0.004) and more frequently had a severe disease (36.8 vs 11.8%, p<0.001) with a longer hospital stay (21-IQR 10.5-34 vs 15 IQR 9-24, days, p=0.036) and a wider use of tocilizumab during their recovery (21.2 vs 9.9%, p=0.055).
Furthermore, they presented higher HR (95 – IQR 80-104 vs 88 – IQR 77-101, bpm, p=0.037) and body temperature (37.2 – IQR 36.5-38 vs 36.7 – IQR 36.1-37.6, °C, p=0.044) at admission as well as higher C-reactive protein (8.4 – IQR 4.7-13.3 vs 4.3 – IQR 1.4-9.3, mg/dL, p=0.002). Significant predictors of discharge HR at multivariate model were admission HR (ß=0.17, 95% CI 0.11;0.22, p<0.001), haemoglobin (ß=-0.64, 95% CI -1.19; -0.09, p=0.023) and severe disease (ß=8.42, 95% CI 5.39;11.45, p<0.001).
Conclusions. High HR at discharge in COVID-19 patients is not such a frequent problem, but when it occurs it seems strongly related to the evidence of a severe course of disease.

USEFULNESS OF THE CORPORATE WELLNESS PROJECTS IN PRIMARY PREVENTION AT THE POPULATION LEVEL: A STUDY ON THE PREVALENCE, AWARENESS AND CONTROL OF HYPERTENSION IN THE FERRARI COMPANY

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The aim of our study was to assess the hypertension prevalence, awareness and control in a company population of apparently healthy employees.
From January 2017 to December 2019 our cross-sectional study included a total sample of 2058 individuals, 1784 males (87%) and 274 females (13%), with a mean age of 38±9 years. The subjects were selected among those at the first entrance of the Ferrari *"Formula Benessere"* corporate wellness programme, organized by the *Med-Ex* company on a voluntary basis at the Ferrari factory in Maranello (Modena, Italy).
BP categories were defined according to the 2018 European Guidelines. Hypertension was defined as SBP ≥140 mmHg or DBP ≥90 mmHg or self-reported use of antihypertensive medication at the time of the visit or for the last 15 days. Awareness was defined as the knowledge of being hypertensive. BP control was identified as BP level <130 mmHg for SBP and <80 mm Hg for DBP, according to the targets recommended by European and US Guidelines. Hypertension prevalence was calculated in the overall population, awareness and treatment were calculated in the hypertensive sample, whereas control of hypertension was calculated among treated hypertensive patients.
All participants were divided into three groups based on age decades: Group 1 including 1177 individuals aged <40 years (57%), Group 2 including 627 participants aged 40-50 years (30%) and Group 3 consisting of 254 individuals aged >50 years (13%).
Two-hundred and sixty-one individuals (12.7%) had resting BP levels corresponding to the definition of high-normal BP (SBP 130-139 mmHg or DBP 85-89 mmHg). One-hundred and forty individuals (6.8%) had rest SBP values ≥140 mmHg or DBP values ≥90 mmHg. Among these 140 subjects, 99 (70.8%) had grade 1 hypertension (SBP 140–159 mmHg or DBP 90-99 mmHg) and 41 (29.2%) had grade 2 hypertension (SBP 160–179 mmHg or DBP 100-109 mmHg). Among individuals with BP ≥140/90 mmHg, the percentage of grade 2 hypertension was higher in younger subjects: 38.6% in Group 1 (17 out of 44), 29.4% in Group 2 (15 out of 51) and 20% in Group 3 (9 out of 45).
In the overall population (n=2058), 259 subjects were affected by hypertension with rates for awareness and antihypertensive treatment of 51% and 45.9%, respectively. Only 67 individuals, accounting for the 57% of treated hypertensive patients, achieved an adequate BP control.
As expected, prevalence of hypertension increased with age (5% in Group 1, 16.5% in Group 2 and 37% in Group 3, p<0.001), as well as awareness (28.3% in Group 1, 56.9% in Group 2 and 61% in Group 3, p<0.001) and use of BP-lowering medications (27.8% in Group 1, 51.4% in Group 2 and 52.1% in Group 3, p<0.001). Among subjects with resting grade 2 hypertension, only a minority (17%) received a BP-lowering therapy, the percentage being even lower among younger subjects (11.7% in Group 1, 13.3% in Group 2 and 33% in Group 3).
Our study has shown that awareness, treatment and control of hypertension is still unsatisfactory in a real-life setting of young-adults, needing strict follow-up programs and periodic re-assessment of estimated CV risk. Our results support the role of the corporate wellness projects as an essential tool in identifying apparently healthy young individuals with an inadequate control of CV risk factors, such as hypertension, anticipating therapeutic strategies and reducing clinical inertia.

CVDS IN WOMEN: THE NEXT CHALLENGE

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Gender Medicine is the field of medicine that focuses on the impact of the gender on human physiology, pathophysiology and clinical features of diseases. Atherosclerotic cardiovascular disease remains the leading cause of premature death worldwide. It is not widely appreciated that cardiovascular disease is the main cause of premature death in women.
As life expectancy increases and it does so particularly in women, the proportion of women with cardiovascular diseases shows an important rise. Coronary heart disease is still considered to be a male disease and it is likely to be under-diagnosed in women since symptoms are different in women and diagnostic tests may be less specific or sensitive. An additional cardiovascular risk factor in women is menopause. Although part of cardiovascular risk after menopause is due to detrimental effect of age, oestrogenic deprivation plays a major role in the pathophysiology of atherosclerotic disease. It causes endothelial dysfunction and adrenergic stimulation which produce systemic arterial hypertension and it is correlate with visceral obesity and inflammation. Diabetes causes 2-3 fold increase of cardiovascular risk in men and 3-7 fold in women. Waist circumference and waist to hip ratio are independent predictors of ischemic heart disease in women.
Of the heart transplants performed yearly, approximately 25% are received by women. Woman more often than man refused a transplant and are much younger than men at the time of transplantation. If ischemic heart disease in men looks like severe epicardial coronary artery disease, in women, often, there is a coronary microvascular disease.
In our hospital, we collect the number of admission in Cardiology department in one year (from January 1st to December 31th 2020). We found a number of 866 men and 467 women.
Are the women healthier than man or do the women need more clinical attention? We have to focus on it.

QUALITY OF LIFE IN PRIMARY ALDOSTERONISM

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Aim. Previous reports suggested that patients with primary aldosteronism (PA) have impaired quality of life (QOL) compared to the general population, in both unilateral and bilateral forms. However, a direct comparison with patients affected by essential hypertension (EH) has never been performed, nor a comparison between specific treatment for PA and general antihypertensive treatment. We thus designed a prospective observational study to compare the QOL of patients affected by PA to the QOL of patients affected by EH, before and after treatment.
Methods. We prospectively enrolled 70 patients with PA and 70 patients with EH, carefully matched for age, sex, blood pressure levels and intensity of anti-hypertensive treatment. We assessed QOL at baseline and after 2 and 6 months of specific treatment for PA (laparoscopic adrenalectomy or mineralocorticoid receptor antagonists) or 6 months after optimization of medical therapy for patients with EH.
Results. QOL of patients with PA was significantly impaired compared with the general healthy population, but not significantly different from QOL of matched patients with EH. Both unilateral adrenalectomy and medical treatment for PA allowed an improvement of QOL in patients with PA, that was more pronounced after surgery. Optimization of antihypertensive treatment (without MR antagonists) allowed a significant reduction of blood pressure levels, but only a minimal improvement in one of eight domains, in patients with EH.
Conclusions. Patients with PA have impaired QOL, which is likely caused by the effects of intensive anti-hypertensive treatment and uncontrolled blood pressure. Surgical and medical treatment significantly improves QOL in patients with PA, by improvement of blood pressure control and reduction of anti-hypertensive treatment after surgical treatment.

BODY WEIGTH AND LIPID PROFILE IN HYPERTENSIVE PATIENTS DURING COVID-19 LOCKDOWN

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Background. In Italy the lockdown was imposed in March 2020 to contrast and contain the spread of the new COVID-19. During lockdown people were forced to stay in their homes which has resulted in change in physical activity, dietary pattern and psychological status of the individuals. The main objective of this study is to evaluate the impact of this constraint on blood pressure control, body weight, lipid profile and fasting glucose in hypertensive patients.
Methods. This observational study included 72 hypertensive patients visited at Hypertension Research Center of Federico II University Hospital in Naples between May and July 2020. BMI, lipid profile, blood glucose and office blood pressure were collected for subsequent comparison with last available visit before lockdown date.
Results. After 1st lockdown period no significant change in Body weight, lipid profil and blood glucose was found compared to previous visits while a significant reduction in systolic and diastolic values was observed $p<0.05$).
Conclusion. The pandemic induced some challenging lifestyle habits, many of these that were changed favorably during quarantine are well - known modifiable risk factors for hypertension and might be associated with the reduction of BP.

REDUCED CARDIORESPIRATORY FITNESS IN YOUNG HYPERTENSIVES. ROLE OF MASS EXERCISE STRESS TEST IN THE FERRARI CORPORATE POPULATION

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The aim of our study was to evaluate the clinical relevance of mass exercise stress testing (EST) in a company healthy population in evaluating the relationship between exercise performance parameters, expressed as cardiorespiratory fitness (CRF), an exaggerated blood pressure (BP) response to exercise and other characteristics of the subjects (age, body mass index, body fat percentage, baseline heart rate - HR).
From January 2017 to December 2019 our cross-sectional study included a total sample of 2058 individuals, 1784 males (87%) and 274 females (13%), with a mean age of 38±9 years. The subjects were selected among those at the first entrance of the Ferrari *"Formula Benessere"* corporate wellness program, organized by the *Med-Ex* company on a voluntary basis at the Ferrari factory in Maranello (Modena).
The study group included a total sample of 2058 individuals, 1784 males (87%), 274 females (13%), with a mean age of 38±9 years. All participants were divided into three groups based on age: Group 1 including 1177 individuals aged <40 years (57%), Group 2 including 627 participants aged 40-50 years (30%), Group 3 consisting of 254 individuals aged >50 years (13%).
The distribution ranges for peak SBP and DBP varies from 120 to 210 mmHg and from 60 to 110 mmHg, respectively. One-hundred and twenty-five subjects (6%) showed peak BP values above these cut offs, showing an exaggerated exercise BP response. Out of these 125, 77 subjects (62%) had normal resting values.
We also divided our healthy population in groups according to cardiorespiratory fitness (CRF): optimal CRF (n= 1183) defined as pVO_2 >100% of predicted, normal CRF (n=613) corresponding to pVO_2 of 85-99% of predicted, mildly reduced CRF (n=115) with a pVO_2 of 70-84% of predicted and moderately reduced CRF (n=147) identified as pVO_2<70% of predicted.
Individuals with elevated resting BP ≥130/85 mmHg (n=401), including those with high-normal BP (n=261), grade 1 (n=99) and grade 2 (n=41) hypertension (no one had baseline grade 3 hypertension) showed a worse cardiorespiratory fitness than those with normal BP levels (<130/85 mmHg), also after the adjustment for age, sex, BMI, smoking habits, antihypertensive treatment, peak SBP and DBP. Among subjects with elevated resting BP levels, performance capacity was inversely related to BP values, those individuals with grade 2 hypertension showing the worst performance capacity compared to both those with grade 1 hypertension and high-normal BP. Furthermore, the percentage of subjects who presented a reduced exercise performance increased in parallel with BP, mild and moderate CRF accounting for 18% and 29% of grade 2 hypertensive subjects, respectively.
According to these results, the inclusion of mass EST in the protocol increases the diagnostic refining of hypertension in the general population, both in providing the still unclear normal ranges of BP response to exercise (also in the better definition of exaggerated BP), and in evaluating the relationship between the early abnormal values of exercise BP parameters and reduced cardiorespiratory fitness, as further CV risk index.

LONG-TERM FUNCTIONAL CAPACITY AND PERIPHERAL ARTERIAL FUNCTION IN PATIENTS WITH CORRECTED AORTIC COARCTATION

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Aim. Patients with Aortic Coarctation (CoA) are at risk of hypertension during life. Peripheral arterial function in patients with CoA can be evaluated by measuring systolic ankle BP and the ankle brachial index (ABI) both at rest and after exercise. Furthermore, blood pressure (BP) during exercise is a predictor of future hypertension. This study aims to evaluate long-term functional capacity, exercise blood pressure and peripheral arterial function at rest and after exercise in patients with surgically well-repaired CoA.
Methods. 48 patients that underwent to a follow up visit on average 13.8±3.1 years after surgical CoA repair were enrolled. Patients underwent to a maximal cardiopulmonary exercise test (CPET). Brachial BP was measured at rest, during exercise and the recovery phase, ankle BP at rest and after exercise by using ascoltatory method. ABI was calculated at rest and after exercise.
Results. Median (IQR) age was 13 (11-16) years, 68.8% males. Maximum oxygen consumption and CPET parameters were in the range of normality. SBP during exercise was not exaggerated on average, nor at submaximal exercise [120 (110-130) mmHg], neither at exercise peak [152.5 (132.5-180) mmHg]. No clinically significant differences between BP in left and right arms were observed at rest [-9.5 (-15.5; -1.5) mmHg]. Median percentage of change of ABI after exercise was -21.6 (-32.1; -12.2)% and median variation of ankle SBP after exercise was equal to -5 (-23; 2.5) mmHg. 26,8% of patients showed a reduced peripheral arterial pressure both at rest and after exercise, 24,4% only at rest and 29,3% only after exercise.
Conclusions. In patients with surgically well-repaired CoA, long-term functional capacity and blood pressure profile during exercise seems not to be worsened respect normal values. Indices of reduced peripheral arterial function were present in 80,5% of patients and in a third of this cases the alteration was visible only after exercise.

PREVALENCE OF TARGET ORGAN DAMAGE IN SUBJECTS WITH HIGH-NORMAL BLOOD PRESSURE WITHOUT KNOWN HYPERTENSION AS WELL AS CARDIOVASCULAR AND KIDNEY DISEASE

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Background and Aim. Purpose of our study was to assess the prevalence of Target Organe Damage (TOD) in healthy subjects with high-normal Blood Pressure (BP) comparing them with subjects with BP values that are considered normal (<130/85 mmHg) or indicative of hypertension (≥140/90 mmHg).

Methods and Results. 755 otherwise healthy subjects participated at the present analysis. TOD was evaluated as Pulse Wave Velocity (PWV), Left Ventricular Mass Index (LVMI) and carotid Intima-Media Thickness (IMT) and plaque. When subjects were classified according to BP levels we found that high-normal BP group showed intermediate values of PWV and LMVI with higher value of IMT. This corresponds to intermediate prevalence of arterial stiffness (PWV>10 m/s: 3.4% vs 2.3% for normal and 9.6% for high BP groups, p=0.0014), Left Ventricular Hypertrophy - LVH (32.4% vs 25.7% for normal and 46.6% for high BP groups, p=0.0001) while there were no differences for IMT>0.9 or carotid plaque. At multivariable analysis the odds of having a PWV>10 m/s (OR=1.75, 95% C.I. 0.59-5.16), an IMT>0.9 mm (OR=1.81, 95% C.I. 0.60-5.00) or a LVH (OR=1.1, 95% C.I. 0.72-1.67) in the high-normal group resulted not different to the normal group.

Conclusions. In our otherwise healthy population, high-normal BP values were not related to aortic, carotid or cardiac TOD.

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

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Aim. Familial Hypercholesterolemia (FH), the major coronary risk factor, is in turn the best clinical model to study endothelial dysfunction with special attention to the regulation of vascular endothelium dependent tone. In FH high plasma cholesterol per se may be a sufficient stimulus to upregulate endothelial adhesiveness, phenomenon that can be acutely "restored", by Lipoprotein Apheresis (LA).

Methods. The Cold Pressor Test (CPT) is a validated test, which induces systemic stress involving immersion of an individual's hand in ice water (normally temperature between 0-5 °C for a period of 4 minutes), is used to measure cardiac microvascular function because modifies vascular tone and increased blood flow in the epicardial coronary arteries via sympathetic activation mainly through endothelial release of nitroxide in normal endothelium.

The aim of the present work is to evaluate the behavior of coronary flow velocity reserve (CFR) during sympathetic stimulus induced by CPT before and after LA.

Results. We enrolled 12 patients (mean age 57±6 years, male 75%) with FH and ischemic heart disease on maximally tolerated lipid lowering therapy and chronic LA (median inter-apheresis interval of 14 [10-16] days). No relevant comorbidity was present in any patient. CPT was performed immediately before and within 48 hours to LA procedure.

CFR showed a significant increase (from 1.34±0.22 to 1.59±0.33; p<0.05) after LA treatment. No adverse event was reported during the test.

Conclusion. This study, as performed in a small number of patients, show that a single LA procedure can increase a CFR measured by CPT. This diagnostic exam stands as a non-invasive, cheapest method that mimic a physiological effect, like cold exposure, is validated test to measure cardiac microvascular function.

Have more methods to measure CFR has importance because myocardial blood flow should represent the ultimate target of any lipid lowering therapy.

OVEREXPRESSION OF PCSK9 IN HUMAN SMOOTH MUSCLE CELLS RENDERS EXTRACELLULAR VESICLES ATHEROGENIC

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Background and Aims. EVs contribute to the pathogenesis of atherosclerotic cardiovascular diseases (ASCVD) by promoting inflammation and thrombosis. EVs carry proteins, lipids, RNAs and/or microRNAs that function as a cell-to-cell communication.
Aim. To unravel the impact of EVs derived from human smooth muscle cells overexpressing PCSK9 ($hSMC^{PCSK9}$) on the inflammatory pathways and migratory capacity of human monocytes THP-1 and derived-THP-1 macrophages.
Methods.
a) cell lines: hSMC, hSMCPCSK9, human THP-1 and human THP-derived-macrophages;
b) EV isolation and characterization (Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscopy (TEM), Flow Cytometry analysis (FC));
c) gene (qPCR) and protein expressions (Western Blot) and cell migration. Overall, cells have been always treated for 24 hours with EVs.

Results. The isolated EVs contain both exosomes and microvesicles as assessed by the expression of specific markers, *e.g.,* alix and integrin. They express CD63 and CD9, markers of exosomes. NTA showed no differences in concentration and size of EVs derived from hSMCs overexpressing or not PCSK9 ($2*10^{10}$ particles/ml vs $1.64*10^{10}$ particles/ml and 141 nm *vs* 153.5 nm, respectively). TEM showed that EVs from both cell lines displayed a similar rounded morphology and size. EVs isolated from $hSMC^{PCSK9}$ carry PCSK9 protein.
In THP-1 and THP-1-derived-macrophages, 24-h exposure to EVs-derived-$hSMC^{PCSK9}$ activated an inflammatory cascade: CCL2, interleukin (IL)-1 β, IL-6, and IL-8. In these two cell models, the protein expression of inflammatory pathways of signal transducer and activator of transcript 3 (pSTAT3) was increased and the protein expression of suppressor of cytokine signaling (SOCS3) was decreased upon exposure to EVs isolated from $hSMC^{PCSK9}$. The migratory capacity of THP-1 monocytes was raised when cells were incubated with EVs isolated from $hSMC^{PCSK9}$ and exposed to CCL2 as chemotactic agent.
Conclusions. These findings are in line with our previous evidence reporting PCSK9 to play a feed-forward inflammatory loop in the context of atheroma formation.

CAROTID LOCAL STIFFNESS: CHANGES DURING A SIX-YEARS FOLLOW UP IN A GENERAL POPULATION OF NORTHERN ITALY

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Background. Local carotid stiffness (Carotid PWV) is an independent predictor of cardiovascular events but few data are available on the progression of Carotid stiffness over time.
The aim of the present longitudinal study was to analyze the progression of local carotid stiffness over a 6-year period in a general population in Northern Italy (Vobarno Study).
Methods. 123 subjects, age 55±4 years (42% males, hypertension in 43% at baseline visit), underwent a baseline (BL) and a follow up (FU) visit, 6.2±0.5 years apart. In all subjects laboratory examinations, measurements of blood pressure (BP) and of Carotid PWV (using an echotracking approach) were performed at BL and at FU. Progression rate of Carotid PWV (Δ_Carotid_PWV/Year) was defined as (Carotid PWV at FU-Carotid PWV at BL)/years of FU.
Results. In the overall population, Carotid PWV increased from 6.39±1.41 at BL to 6.79±1.18 m/s at FU ($p<0.05$). Δ_Carotid_PWV/Year was inversely related to BP (mean BP: $r=-0.193$, $p<0.05$; pulse pressure: $r=-0.1.81$, $p<0.05$) and to Carotid PWV at BL ($r=-0.669$, $p<0.001$). No other correlation was observed between Δ_Carotid_PWV/Year and demographic or laboratory parameters at BL. Δ_Carotid_PWV/Year was also related to changes over time in BP values (ΔSystolicBP: $r=0.291$, $p<0.01$; ΔDiastolicBP: $r=0.266$, $p<0.01$; ΔMeanBP: $r=0.310$, $p<0.0001$).
At multivariate analysis the variables independently related to the annual progression rate of Carotid PWV were Carotid PWV and mean BP at BL (beta -0.68, $p<0.0001$, and beta 0.20, $p<0.05$, respectively) and changes in mean BP during follow-up (beta 0.18, $p<0.05$).
Conclusions. In a general population sample in Northern Italy the main determinants of the increase in local carotid stiffness during a 6 years FU were local carotid stiffness and mean BP at BL and change in mean BP over time.

ENHANCED PREDICTION OF SUBCLINICAL CAROTID ATHEROSCLEROSIS BY MARKERS OF IMMUNE-INFLAMMATION AND CELLULAR AGING

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Aim. Leukocyte Telomere Length (LTL) is an index of cellular aging. LTL shortening (TS) physiologically occurs over time but faster TS has been still linked to increased risk of atherosclerosis, even during its subclinical stages. We recently identified a set of proteomic circulating immune-inflammatory markers predicting the occurrence of Subclinical Carotid Atherosclerosis (SCA) independently from classical risk factors (CVRFs). Is over time predicted by the same immune-inflammatory markers predicting SCA occurrence, on top of CVRFs?
Methods. 368 circulating proteins were measured in 328 subjects from the general population followed for six-years. LTL was measured at basal visit and after follow-up by qPCR-based protocols (as telomere-to-reference gene expression ratio) using genomic DNA from lympho-monocytes. SCA was defined by ultrasound (Intima-Media Thickness and analysis of echolucency of atheromas at basal visit and after follow-up).
Results. Subjects were divided by median TS identifying those with faster TS (n=223, -0.13 (-0.06 - -0.23) T/S reduction/year) vs those with slower TS (n=105, -0.06 (-0.09 - -0.03) T/S reduction/year). In the first group 31 subjects, while only 5 in the second one, developed SCA over follow-up. Faster TS was predicted by a set of 21 proteins, while SCA occurrence was predicted by 35 different proteins. Only three proteins were in common (Gal9, TIE1 and TNFSF10A). Sensitivity analyses (ROC curves) showed that adding these independent proteomic sets improved the predictive power for both for TS and for the occurrence of SCA (AUC 0.735 (0.675-0.795) in the first case and AUC 0.868 (0.805-0.932) in the second).
Conclusions. TS and SCA occurrence over time appears predicted by different proteomic sets, independently from CVRFs. Larger numbers of subjects with increased risk of atherosclerosis are warranted to validate these findings and whether TS is more likely reflecting intracellular mechanisms of lympho-monocytes activation might by an alternative possibility.

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

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

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

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Background and Aims. Hypercholesterolemia and cigarette smoke (CS) are main risk factors for cardiovascular disease. Cholesterol-loading of smooth muscle cells (SMCs) causes a phenotypic switch resulting in less-differentiated cells that lack SMC markers but have an increased inflammatory profile, consequent to a downregulation of the myocardin axis in a Kruppel-like factor 4 (Klf4)-dependent manner. Whole cigarette smoke is made up of over 7000 chemical components distributed between the condensate (CSC) and gas phases. Cigarette smoke aqueous extract (AE) contains water soluble components present in both the condensate and gas phases. Alternative next generation tobacco products (such as E-cigarettes (E-cig)) are being developed as
less toxic cigarettes. To characterize the role of CS on SMC behavior, we studied the effects of AEs (from traditional cigarette (TC) and E-cig) on SMC phenotypic modulation and inflammatory response.
Methods. Human aortic SMCs (HSMCs) were incubated for 48 hours with AEs and gene/protein
expression analyzed by real-time PCR and western blot analysis.
Results. E-cig extract promoted the expression of contractile markers (α-actin, calponin and SM22) compared to TC. Interestingly, E-cig is the more potent in inducing a faster SMC migration and proliferation rate compared to traditional cigarette. With regards to inflammatory markers, TC and E-cig showed different effects. TC significantly increases the expression of all pro-inflammatory markers tested (IL-1beta, IL-6, IL-8 and MMPs), while E-cig reduces all these markers.
Conclusions. AEs obtained from a traditional cigarette and E-cig, have completely different effects on SMC plasticity. The E-cig extract stimulate the expression of contractile SMC-related genes. At the same time, E-cig is the more potent in inducing SMC proliferation and migration activity, causing a complete reclosure (100%) of the cell monolayer after 20 hours from the scratch, much faster than control or TC-treated SMCs. With regards to inflammatory markers, TC significantly increases the expression of all pro-inflammatory markers tested (IL-1beta, IL-6, IL-8 and MMPs), while E-cig reduces their expression.

ASSOCIATION BETWEEN ADIPOSITY AND CORONARY ARTERY DISEASE: A MENDELIAN RANDOMIZATION MEDIATION ANALYSIS

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Aims. Increased adiposity is associated with an increased risk of coronary artery disease (CAD). Whether adiposity has a direct causal effect on the risk of CAD, or whether the effect of adiposity on CAD is mediated indirectly through the effect of adiposity on other risk factors is unknown.
Methods. We constructed a body mass index (BMI) instrumental variable genetic score composed of 96 single-nucleotide polymorphisms strongly associated with BMI. We used this instrument to conduct a multivariable Mendelian randomization (MR) mediation analysis to estimate the direct and indirect effects of BMI on CAD. The study included individual level data on 445,774 participants including 23,032 cases of CAD and 28,563 cases of type II diabetes (T2D); and summary data on up to 877,643 participants including 63,746 cases of CAD and 26,676 cases of T2D.
Results. Genetic predisposition to higher BMI was significantly associated with CAD. A one unit increase in BMI was associated with a 7% increased odds of CAD (OR: 1.07; 95% CI:1.05-1.09, p=1.6E-10), a 25% increased odds of T2D (OR: 1.25, 95%CI:1.23-1.28, p=2.2E-131); and 0.34 mmHg increase in SBP (95% CI: 0.25-0.42, p=8.5E-15). A one unit increase in BMI was also associated with a 0.02 mmol/L increase in TG (95% CI: 0.02-0.03, p=4.9E-21), but not with increased apoB levels. In MR mediation analyses, 80.0% of the effect of BMI on the risk of CAD was mediated through BMI induced increases in SBP (29.6%) and T2D risk (50.4%).
Conclusions. Our results suggest a casual association between lifetime exposure to high BMI and higher risk of CAD. However, the vast majority of the effect of increased BMI on the risk of CAD appears to be mediated indirectly through the effect of BMI on SBP and T2D.

THERAPEUTICS TARGET ACHIEVEMENT DURING A CARDIAC REHABILITATIVE CYCLE: THE NIGUARDA HOSPITAL EXPERIENCE

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Background. The Cardiac Rehabilitation is a dynamic and composite process aimed at both endorsing clinical stability of patients with heart disease and reducing psychophysical disabilities resulting from the disease as well as promoting the full labour recovery. The final purpose is to improve patients' life quality and survival, by decreasing the risk of further cardiovascular events.
Purpose. This study aims to assess the achievement of blood pressure targets, heart rate and lipid target at the completion of the outpatient cardiac rehabilitation cycle for patients with ischemic heart disease.
Methods. A retrospective study on Niguarda outpatient Cardiac Rehabilitation has been carried out on the population that has completed the cycle between January 2012 and December 2019. The Blood Pressure therapeutics targets analysed were of SBP<140 mmHg and DBP<90 mmHg for patients rehabilitated before August 2018, and of SBP<130 mmHg and DBP<80 mmHg afterwards, in accordance with the new ESC hypertension guidelines. The heart rate <65 bpm has been considered the therapeutic target. The attainment of LDL therapeutic target for patients rehabilitated after August 2019 has been considered optimal for values lower than 55 mg/dL, in accordance with the last Guidelines on Dyslipidaemias, while previously, a value lower than 70mg/dL has been considered to be appropriate.
Results. A total of 882 patients has been analysed (710 men and 172 women). BP target achievement increase during rehabilitation (from 85,9% to 93,5%, p<0,001). Heart rate did not significantly change between the beginning and the conclusion (from 62,0% to 62,1%, p=0,958).
52,7% of patients reached the LDL target at the end of rehabilitation against only 20,3% of patients at the beginning (p<0,001).
Conclusions: a cardiac rehabilitation cycle provided an improvement in clinical cardiac parameters as well as a significant achievement of LDL therapeutic targets.

THE ROLE OF URIC ACID IN FUNCTIONAL RECOVERY AFTER CARDIAC REHABILITATION

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Background. Uric Acid (UA) is one of the emerging cardiovascular risk factors, however, in the literature there is still a dispute on the real pathogenic role.
Purpose. Aim of our study was to evaluate if UA could have a role as a determining factor of functional recovery after a cardiac rehabilitation's program. Secondary purpose was to determine whether uric acid's levels were linked to systolic and diastolic function of the left ventricle.
Methods. We retrospectively enrolled 319 patients (255 men and 64 women) who took part at the cardiac rehabilitation program at Niguarda Hospital in Milan between January 2015 and December 2019. Functional status was evaluated through 6-minute Walking Test (6mWT) both at baseline and at the end of rehabilitation while UA was measured only at the beginning and echocardiography was performed only at the end (evaluating Left Ventricular Ejection Fraction - LVEF - and diastolic function). Hyperuricemia was defined as an UA higher than 6 mg/dL for women and 7 mg/dL for men.
Results. Mean age was 62,6±10 years, male percentage was 79,9%, baseline UA was 5,6±1,4 mg/dL and baseline 6-MWT distance covered was 456,5±102,3 mt. After the 4 weeks of cardiological rehabilitation program an increase of 117,9±76,1 mt (D6mWT) was observed. Correcting for confounding factors, no correlation was established between baseline UA levels and initial or final 6mWT nor D6mWT as well as with FEVS or the presence of a diastolic disfunction.
Conclusions. UA at baseline in cardiovascular rehabilitation seems not to influence short- term results both as functional improvement or cardiac systolic and diastolic function.

ASSESSMENT OF THE PRO- OR ANTI-INFLAMMATORY PROPERTIES OF DIET IN THE GENERAL POPULATION OF THE PLIC STUDY

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Aim. The atherogenic effect of elevated intake of saturated fat, carbohydrate, and sugar on the risk of Cardiovascular Diseases (CVDs) has been questioned by large epidemiological trials. Beyond their metabolic impact, macro-and micro-nutrients exert anti- or pro-inflammatory effects and whether this balance independently predicts risk of CVDs could surmise a tool for future personalized dietary approaches in primary prevention.
Methods. Clinical, anthropometric, dietary, and blood measures of 250 subjects (from the PLIC study) in primary prevention for CVDs were collected. Seven-days food records were used to determine the balance between pro-and anti-inflammatory nutrient intake by calculating the Energy Adjusted-Dietary Inflammatory Index (EA-DII), as mean of nutrient intakes over the week (Week-EA-DII) or, as sum of nutrient intakes during each day of the week (Daily-EA-DII). We verified the correlation between Week-EA-DII and Daily-EA-DII with Subclinical Carotid Athersocleoris (SCA), a marker of pre-clinical CVD, by combining the presence/absence of focal carotid atherosclerosis (-/+SCA) and intima-media thickening (-/+IMT).
Results. Mean Week-EA-DII was 0.11±1.66, 128 subjects consumed a more anti-inflammatory-balanced diet (EA-DII<0) while 122 subjects consumed a more pro-inflammatory-balanced diet (EA-DII>0). Mean Daily-EA-DII was 0.65±1.41, and 77 subjects presented an EA-DII<0 while 173 had an EA-DII>0.
Both Week-EA-DII and Daily-EA-DII were numerically, but not statistically, different in subjects with SCA (n=145, 58.0%) vs those without SCA (n=105, 42.0%), respectively 0.21±1.71 vs 0.03±1.63 (p=0.407) and 0.55±1.46 vs 0.80±1.32 (p=0.172). Higher hs-CRP was associated with higher Week-EA-DII in subjects with –SCA/-IMT and with higher Daily-EA-DII among those with +IMT/-SCA.
Conclusions. The evaluation of the balance between the pro/anti-inflammatory effects of nutrients remains an aspect that requires further investigation, with the intriguing possibility to cluster subjects at higher CVD risk, who are exposed to pro-inflammatory dietary patterns over time and for whom personalized dietary interventions can be considered.

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

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Aim. Liver steatosis and fibrosis are associated with an increased atherosclerotic cardiovascular disease (ASCVD) risk. We aim at determining the prevalence and the predictors of liver steatosis and fibrosis, and at evaluating whether they are associated with increased prevalence of ASCVD in familial hypercholesterolemia (FH) patients.
Methods. 180 adults (men/women 92/88, median age 48) with a clinical and/or molecular diagnosis of FH, within the LIPIGEN Project, underwent comprehensive evaluation including assessment of liver steatosis by abdominal ultrasound. In 154 patients the Fibrosis-4 (Fib-4) index was measured (values >1.30 were considered at risk of advanced liver fibrosis)
Results. The prevalence of liver steatosis was 31.7%. Patients with liver steatosis were significantly more likely to have visceral adiposity, dysglycemia, high blood pressure and metabolic syndrome (MetS) than those without liver steatosis. They also showed significantly higher age, BMI, waist circumference, blood pressure, triglycerides, HbA1c, uric acid, AST, ALT and GGT and lower HDL-cholesterol than their counterpart without liver steatosis. Median Fib-4 values were 1.04 and 33.8% patients were at risk of advanced liver fibrosis. Fib-4 was significantly and positively correlated with age, waist circumference, blood pressure, HbA1c, number of factors of the MetS, AST and GGT. Accordingly, patients at risk of advanced liver fibrosis were significantly more likely to have dysglycemia, high blood pressure and MetS than patients not at risk. At univariate logistic regression analysis, liver steatosis and the risk of advanced liver fibrosis were significantly associated with ASCVD. However, after adjustments for sex, age, smoking status, untreated LDL-cholesterol and metabolic comorbidities, the significant associations between liver steatosis, liver fibrosis and ASCVD went lost.
Conclusions. Liver steatosis and fibrosis, mainly attributable to metabolic dysfunction-associated fatty liver disease, are common in FH patients and are associated with metabolic comorbidities and ASCVD. However, the associations between liver steatosis, liver fibrosis and ASCVD seem to be largely mediated by metabolic comorbidities.

IMPACT OF MEDITERRANEAN DIET ON INFLAMMATORY BIOMARKERS, MACES AND GLYCEMIC CONTROL IN PATIENTS WITH POLYVASCULAR ATHEROSCLEROTIC DISEASE

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Background. Adherence to the Mediterranean diet has been promoted as a key player in cardiovascular disease prevention. Therefore, we aimed to assess whether there is a relationship among adherence to the Mediterranean diet (MD), inflammatory, lipid and glycemic patients' profile, and incidence of Major Adverse Cardiovascular Events (MACEs) in a cohort of patients suffering from Polyvascular Atherosclerotic Disease (PAD), characterized by a more aggressive and prognostically negative atherosclerotic load compared with patients with one-district disease.
Methods. We evaluated 107 patients, aged ≥45 years, with a diagnosis of PAD defined as the simultaneous involvement of at least two vascular districts. Within this cohort, adherence to the MD was estimated through the Mediterranean Diet Score (MDS). Moreover, during the follow-up period, we analyzed glycemic and lipid profiles, C-Reactive Protein (CRP), Platelet to Lymphocyte ratio (PLR) and Neutrophil to Lymphocyte ratio (NLR) levels.
Results. Improved glycemic and LDL cholesterol levels were observed in high-adherence group compared with patients with low-adherence to the MD (106±25 mg/dl versus 167±105 mg/dl, $p<0.001$, and 81±2 mg/dl versus 98±13 mg/dl, $p=0.0049$, respectively). Both CRP and PLR measurements were significantly lower in patients demonstrating high adherence compared with those with poor adherence to the MD (CRP 3±5 mg/dl and PLR 132±57, $p=0.0045$ and CRP 8±9 mg/dl and PLR 192±92, $p=0.008$, respectively). During follow-up, with a mean of 35±11 months, 17 MACEs (16%) occurred. In particular, fatal events occurred exclusively in the group of patients with poor adherence to the MD (58%), among which event-free survival reached only 37% compared to 87% in the moderate-adherence group and 70% in the high-adherence group ($p<0.001$).
Conclusions. This study provides strong evidence regarding the relevance of MD as a secondary preventive tool in patients suffering from PAD. Indeed, high adherence to this dietary pattern is effective in improving outcomes of inflammation and glycemic control and probably may significantly improve long-term prognosis of these high-risk patients.

ANGPTL3 AFFECTS LIPOLYSIS BY INTERFERING WITH THE CROSSTALK BETWEEN BETA-ADRENERGIC AND INSULIN PATHWAYS IN 3T3-L1 CELLS

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Aim. Loss-of-function (LOF) mutations in the ANGPTL3 gene in humans revealed a new lipid phenotype called familial combined hypolipidemia (FHBL2). ANGPLT3 protein is known to be involved in the inhibitory regulation of extracellular lipases (LPL and HL), and its absence increases the lipolytic degradation of TG-rich lipoproteins (VLDL and HDL). Accordingly, reduced levels of circulating free fatty acids (FFA) characterize the FHBL2, suggesting that ANGPTL3 plays a role in regulating the FFA metabolism. Our previous data have shown that ANGPTL3 facilitates β-adrenergic-dependent lipolysis in adipocytes. ANGPTL3 treated cells showed high levels of the phosphorylated form of ERK1/2 and PKA and HSL, downstream the cAMP-dependent signaling pathway. Our purpose is to understand if ANGPTL3 can be involved in insulin resistance development to improve the knowledge of molecular mechanisms involving lipolysis in adipose tissue.
Methods. Using 3T3-L1 cells differentiated in adipocytes treated with β-agonist Isoproterenol or ANGPTL3 or both, the levels of the phosphorylated form of the main kinases of the MAP pathway (AMPK, AKT, P38) and the involvement lipolysis and insulin targets, IRS1 and HSL were evaluated by western blot and qRT-PCR. Moreover, we investigated the IRS1 sub-cellular localization using immunofluorescence.
Results. The preliminary results showed higher levels of ERK phosphorylated form after ANGPTL3 treated 3T3-L1 adipocytes. The AKT phosphorylation analysis revealed reduced levels of phosphorylation of AKT in the same cells. Moreover, the ANGPTL3 treatment showed higher levels of HSL (ser660) phosphorylated form and increased lipolytic process. Moreover, IRS1 was localized in the cytoplasm.
Conclusion. We can conclude that the treatment with ANGPTL3 increases the FFA release in adipocytes counteracting the insulin inhibitory action against β-adrenergic-dependent lipolysis starting from the switch of the intracellular signal in favor of ERK arm of MAPK pathway.

PRELIMINARY EVIDENCE FOR THE IMPLEMENTATION OF DIAGNOSTIC CRITERIA IN CHILDREN AND ADOLESCENTS AFFECTED BY FAMILIAL HYPERCHOLESTEROLEMIA

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Aim. The early identification of familial hypercholesterolemia (FH) in childhood is made challenging by the lack of validated diagnostic criteria and by the usually less severe phenotype in first decades of life. We aimed to compare the Dutch Lipid Clinic Network (DLCN) criteria in genetically-confirmed adult and paediatric individuals affected by FH.
Methods. From the LIPIGEN (LIpid transPort disorders Italian GEnetic Network) study, we selected 1204 (≥18 years) and 798 (<18 years) genetically-confirmed FH patients. DLCN parameters and proportion of missing data were compared between the two groups.
Specific LDL-cholesterol (LDL-c) cut-off for children/adolescents (based on percentile distribution of LDL-c cut-off in DLCN score among adults) and data about premature coronary heart disease (CHD) in second-degree family members were evaluated.
Results. According to the original DLCN score, probable/definite FH (score ≥6) was identified in 67.1% of adults, but only in 26.9% of children/adolescents. In this latter group, the percentage increased to 52.1% using specific LDL-c classes. The lower prevalence of typical FH features in children/adolescents vs adults was confirmed: tendon xanthoma was retrieved in 2.9% vs 17.5% and arcus cornealis in 1.7% vs 17.5%, respectively. No children presented clinical history of premature CHD or cerebral/peripheral vascular disease (in adults, percentages were 12.9% and 4.5%, respectively). Data about premature CHD in first-degree family members were missing for 4.7% of adults and 14.2% of children/adolescents. This prevalence was 43.9% in adults and only 22.0% in children, increasing to more than half of subjects when considered also second-degree relatives (data available for 229 individuals <18y), but without relevant changes in the proportion of probable/definite FH.
Conclusions. In children/adolescents, the DLCN score is clearly less informative than in adults. A re-modulation of the scoring is necessary to improve the detection rate.

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

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Aims. Familial hypercholesterolemia (FH) is associated with increased risk of atherosclerotic cardiovascular disease (ASCVD). The risk of chronic kidney disease (CKD), a potential manifestation of peripheral ASCVD, is largely unknown. We evaluated the prevalence of CKD and the factors associated with kidney damage in adult FH patients.
Methods. 270 patients (men/women 132/138; median age 49) with clinical suspicion of FH within the LIPIGEN Project underwent comprehensive evaluation, including glomerular filtration rate estimation (eGFR) using CKD-EPI equation. eGFR values <90 or 60ml/min/1.73 m^2 were considered as mildly or moderately decreased, respectively. In 180 patients, urine albumin-to-creatinine ratio (ACR) was also measured (values >30 mg/g considered as moderately increased).
Results. 15.6% of patients had a history of ASCVD. Among 244 patients with available genetic test, 73.8% were carriers of a "pathogenic/likely pathogenic" mutation in FH-causing genes. Median BMI was 25.1 kg/m^2 and the prevalence of arterial hypertension and type-2 diabetes mellitus was 23.3% and 3.0%, respectively. 30% of patients presented at least a mild decrease in eGFR; only 1.9% had a moderate decline in kidney function. eGFR values were significantly and negatively correlated with female sex, age, BMI, waist circumference, blood pressure, triglycerides and HbA1c levels. 6.1% of patients showed moderately increased ACR levels. ACR was significantly and positively associated with female sex, blood pressure and metabolic syndrome. However, there were not associations between eGFR values or ACR with FH-related variables. We found significant and negative associations between eGFR, carotid intima-media thickness and prevalence of carotid plaques. Moreover, FH patients with a history of ASCVD had significantly lower eGFR values and a higher prevalence of increased ACR than those without ASCVD.
Conclusions. Kidney function is normal or mildly altered in the majority of our cohort of FH patients and is not associated with FH-related variables but with cardio-metabolic comorbidities. The pathophysiological mechanisms of CKD in FH may extend beyond LDLc burden.

EVIDENCE OF NOVEL APOB VARIANTS AS A CAUSE OF FAMILIAL HYPERCHOLESTEROLAEMIA

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Aim. *APOB* mutations are a rare cause of familial hypercholesterolaemia (FH). Most of *APOB* variants remain of uncertain significance (USV) for the absence of functional studies. We aim to characterize rare variants in *APOB* identified in a patient with a clinical suspect of FH.
Materials and Methods. We report a 63-years-old female with a clinical suspect of FH.
Patient was screened by next-generation sequencing (NGS). No clearly pathogenic variants of FH were found, although rare variants were identified in *APOB* gene confirmed by Sanger sequencing. These variants were absent from databases of mutations (HGMD professional) and according to the ACMG guidelines can be classified as USV. Family segregation study was performed. Patient plasma was used to characterize the APOB variants by: proliferation assay of U937 cells by incubating the patient's LDL in the culture medium, evaluation of LDL uptake in hepatoma cells labelling the patient's LDL with FITC and affinity assay of the patient's LDL for LDLR measuring the EC50 by solid-phase immunoassay.
Results. NGS analysis revealed the presence at heterozygous state of rare variants in the exon 21 and 26 of *APOB* gene: c.3220G>A - p.(Gly1074Arg), c.10031A>T - p.(Lys3344Ile) and c.11087T>C - (p.Ile3696Thr). According to bioinformatics predictions, two variants resulted as probably damaging and one as benign. Segregation study showed that the variants are on the same allele. The U937 proliferation assay revealed a decrease in cell growth (less than 65% of wild-type).
Results were confirmed by the decrease in the uptake of patient's LDL (less than 25%) and also in the affinity of the patient's LDL for LDLR (EC50 approximately four times greater than wild-type).
Conclusions. The two rare variants in the APOB gene affect the protein function being causative of FH. Variants' segregation does not allow to establish the contribution of each variant on the functional alteration.

PCSK9 ASSOCIATION WITH LOW-DENSITY LIPOPROTEINS: EFFECTS OF ANTI-PCSK9 MABS IN PATIENTS WITH HIGH CHOLESTEROL

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Aim. Lowering plasma low-density lipoprotein cholesterol (LDL-C) levels results in the reduction of cardiovascular risk. Proprotein convertase subtilisin-kexin type 9 (PCSK9) increases LDL-C levels in plasma by enhancing low-density lipoprotein receptor (LDLR) degradation. Monoclonal antibodies (mAbs) targeting PCSK9 inhibit PCSK9 activity, impacting positively on cardiovascular events. PCSK9 in known to associate with LDL in plasma. The aim of our study was to address whether anti-PCSK9 mAbs therapy interferes with the PCSK9-LDL association.
Methods. Lipoprotein (LP) fractions were isolated from plasma of mAbs treated subjects (n=12) using Iodixanol gradient (IGr) ultracentrifugation. The PCSK9 and cholesterol content of each fraction was quantified with ELISA and clinical-grade colorimetric assay, respectively. The LP fractions were also studied for their protein content and electrophoretic behaviour.
Results. Plasma PCSK9 levels increased after therapy from 420±151 ng/mL to 4053±1077 ng/mL, while LDL cholesterol levels decreased from 156±79 mg/dL to 65±44 mg/dL. PCSK9 associated with a specific LDL subfraction before and after anti-PCSK9 mAbs therapy. No significant changes in the percent of association were found after therapy, with an individual variation between 1% to 20% of total PCSK9. The absolute amount of bound PCSK9 increased 18±9 fold.
Conclusions. After anti-PCSK9 mAb treatment, the PCSK9-LDL association remains, even if the LDL level are dramatically reduced as a consequence of therapy. The LDL subclass that shows enhanced affinity for PCSK9, as well as the physiological significance of the PCSK9-LDL association, needs to be further investigated.

TREATMENT OF PATIENTS WITH SEVERE HYPERCHOLESTEROLEMIA: A META-ANALYSIS OF MIPOMERSEN, LOMITAPIDE AND INCLISIRAN

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Aim. Many dyslipidemic patients fail to achieve the therapeutic target recommended by 2019 ESC/EAS Guidelines, especially those affected by severe forms of hypercholesterolemia. Thus, new therapeutic strategies are urgently demanded. In this meta-analysis, we aimed at evaluating the efficacy of three approved drugs: inclisiran, mipomersen, and lomitapide.

Methods. Pubmed/MEDLINE, EMBASE, Web of Science, and ClinicalTrial.gov were searched for randomized controlled clinical trials from inception to October 2020. The primary outcome was the effect on LDL-cholesterol (LDL-C) levels, the secondary outcomes were variations in other lipid parameters. Data were expressed as mean difference between percentage reductions in the active group and in the control group (standard therapy or placebo alone).

Results. A total of 21 studies (5 for inclisiran, 13 for mipomersen, and 3 for lomitapide) were included in the analysis. Lomitapide (2.5-10 mg), when compared to placebo/standard therapy alone, determined an additional decrease in LDL-C of 32.82% (95%-CI: -57.24; -8.39), total cholesterol (TC) of 20.87% (-31.70; -10.04), and HDL-cholesterol (HDL-C) of 11.44% (-17.90; -5.37), but not in triglycerides (TG) levels. Regarding mipomersen (200 mg) it was observed an additional 30.30% reduction (-35.61; -24.39) for LDL-C, a 24.88% (-28.64; -21.07) additional reduction for TC, -22.49% (-30.53; -14.46) for TG, and -20.56% (-27.90; -13.23) for lipoprotein (a) (Lp(a)). No significant effects on HDL-C were observed. Inclisiran (284-300 mg) determined an additional lowering in LDL-C of 45.55% (-50.46; -40.63), TC of 30.91% (-33.04; -28.78), TG of 12.35% (-22.64; -2.06) and Lp(a) of 18.01% (-27.40; -8.63), while increased HDL-C by 6.74% (1.66; 11.83).

Conclusions. All three therapies show considerable efficacy in reducing LDL-C levels and other lipid parameters, and are indicated for patients with severe hypercholesterolemia, such as those on a genetic basis. The differences in overall effect may be attributable to the patients selected in the studies (familial hypercholesterolemia vs severe HC).

IMPACT OF BEMPEDOIC ACID ON LDL-C TARGET ACHIEVEMENT IN A REAL-WORLD POPULATION

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Aim. Bempedoic acid is a first-in-class agent that inhibits the enzyme adenosine triphosphate (ATP)-citrate lyase. In clinical trials, bempedoic acid in combination with statins and/or ezetimibe significantly reduced LDL-C. To date, this drug is not yet marketed. The aim of this study is to evaluate the impact of bempedoic acid on achieving the LDL-C target in patients who do not reach their lipid goal, simulating the percentage reduction shown in the studies.

Methods. For this analysis, we considered patients at low, moderate, high and very-high cardiovascular risk enrolled at a tertiary center in southern Italy, and collected 1-year data on lipid profile. The primary objective of the study is to generate data about proportion of patients reaching target at 1-year follow-up and the proportion of patients who would reach target with hypothetical application of the percentage reduction due bempedoic acid according to background lipid-lowering therapy. We stratified patients into 3 groups with different percentage reductions in LDL-C:

1) only ezetimibe or very low statin (-20.7% with bempedoic acid);
2) only statin, low-moderate and high intensity (-36.2% with bempedoic acid and ezetimibe fixed combination);
3) statin+ezetimibe (-16.1% with bempedoic acid).

Results. 260 patients completed 1-year follow-up. The mean age was 66.7 (±11.8) years and male patients were mostly present (71.9%). 200 (76.9%) patients were at very-high cardiovascular risk. At baseline 62 (23.8%) patients achieved the LDL-C target for their risk profile and at 1-year follow-up the target is achieved in 103 (39.6%) patients (23.8% vs 39.6%, p<0.001). Simulating the effect of bempedoic acid or its fixed combination with ezetimibe on the non-target group at 1-year follow-up, 220 patients achieved target vs 103 patients (84.6% vs 39.6%, p<0.001).

Conclusions. Bempedoic acid could be a novel oral therapeutic opportunity for reaching the LDL-C therapeutic target in all cardiovascular risk classes.