SOCIETÀ ITALIANA IPERTENSIONE ARTERIOSA (SIIA)
SOCIETÀ ITALIANA DI MEDICINA INTERNA (SIMI)
SOCIETÀ ITALIANA PER LO STUDIO DELL'ATEROSCLEROSI (SISA)

Spring Meeting Giovani Ricercatori SIIA, SIMI, SISA

NOVITÀ NELLO STUDIO DELL'ATEROSCLEROSI E DELLE SUE COMPLICANZE

Roma, 7-8 Aprile 2017

COMUNICAZIONI GIOVANI RICERCATORI

Comitato organizzatore:
Rosa Maria Bruno (SIIA), Marianna Maranghi (SISA),Giacomo Pucci (SIMI)

Comitato scientifico:
Marco Busnelli (SISA), Laura Guarino (SIIA), Giuseppe Mandraffino (SISA), Martino Pengo (SIIA), Miriam Pinna (SIMI), Eliezer Joseph Tassone (SIMI)

La seconda edizione dello "Spring Meeting Giovani Ricercatori" si è svolta quest'anno a Roma il 7 e 8 Aprile, con il coinvolgimento di oltre 60 giovani ricercatori afferenti da 3 diverse società scientifiche: la Società Italiana per lo Studio dell'Aterosclerosi (SISA), la Società Italiana Ipertensione Arteriosa (SIIA) e la Società Italiana di Medicina Interna (SIMI).
Il meeting, attraverso il confronto e la discussione di lavori scientifici presentati all'interno di sessioni dedicate, è riuscito nell'intento di favorire l'interazione tra i giovani soci delle società scientifiche coinvolte. Durante le due giornate di lavoro, sono state presentati oltre 30 comunicazioni orali e presentati altrettanti poster, in cui tutti i partecipanti al meeting (supportati da travel grant forniti dalle 3 società) hanno avuto la possibilità di presentare i risultati dei propri lavori di ricerca clinica o di base.
Le tematiche scientifiche affrontate, specchio del diverso background delle società coinvolte, sono state molteplici e si è spaziato dalla ricerca di base a quella clinica e traslazionale. Sono state attivamente partecipate le sessioni su immunologia e biologia vascolare e su nuovi fattori di rischio cardiovascolare, come ad esempio i regolatori di funzione mitocondriale. Non sono poi mancati gli studi clinici nelle sessioni sulle nuove prospettive diagnostiche e terapeutiche. Il meeting si è svolto un clima di serena e sempre attiva discussione da parte dei giovani ricercatori, particolarmente stimolati dal diverso background scientifico dei partecipanti.

COMUNICAZIONI ORALI

CHRONIC STRESS AND HYPERTENSION: THE ROLE OF GENDER

D. Guarino[1,2], C. Bachi[1], R. Gherardini[1], E. Duranti[1], M. Nannipieri[1], S. Taddei[1], R.M. Bruno[1]
[1]Dpt Clinical and Experimental Medicine, University of Pisa;
[2]Scuola Superiore Sant'Anna, Pisa

Aim. psychosocial factors have been linked to cardiovascular diseases and hypertension. Chronic stress might have different cardiovascular consequences in men and women.
Our aim was to investigate the impact of perceived chronic stress on blood pressure (BP) control in men and women attending for the first time a visit in a Hypertension Outpatient Clinic.

Materials and Methods. 128 individuals (62 women and 66 men) were enrolled. The following parameters were acquired: medical history, socioeconomic status, anthropometric parameters (height, weight, waist circumference), office BP, standardized questionnaires evaluating quality of life (SF-12, 12-item Short Form Health Survey), chronic stress, anxiety and depression (DASS21 - Depression Anxiety Stress Scales) and work-related stress (ERI - Effort-reward Imbalance and DCSQ - Demands-Control-Support Questionnaire).
Results. Women tended to be older than men (61.7±12.6 vs 57.6±12.6 years; p=0.07). Women had similar systolic BP than men (143.8±15.5 vs 142.01±18.7 mmHg; p=0.57) but lower diastolic BP (79.8±8.5vs 85.9±8.6 mmHg; p=0.0003) and BMI [26.8(24.9-29.4) vs 25.0 (23.1-27.6) kg/m^2; p=0.01). Diastolic BP was higher in individuals with moderate-to-severe chronic stress (evaluated by DASS21 questionnaire)

than in those with mild or no stress (85.7±7.7 vs 81.7±8.2 mmHg, p=0.023). Interestingly, diastolic BP was increased in the presence of chronic stress in women (p=0.014), who experienced higher stress scores than men (13.2±8.3 vs 9.4±7.6, p=0.02). In multivariate logistic analysis, adjusted for age, sex, and socioeconomic status, chronic stress was associated with an increased diastolic BP (ß=3.8; p=0.048). Sex tended to be an effect modifier in the relationship between chronic stress and diastolic BP (interaction term sex*stress: ß=-5.89; p=0.09), indicating a greater effect of stress on diastolic BP in women. No significant relationship was found between quality of life, work-related stress and mood disorders and BP control.

Conclusion. Perceived chronic stress per se is independently associated to higher diastolic BP values, particularly in women.

ROLE OF SPHINGOSINE-1- PHOSPHATE (S1P) AND ITS RECEPTOR S1P3 IN THE ANTIATHEROGENIC PROCESS OF REVERSE CHOLESTEROL TRASPORT (RCT)

S. Battista[1], I. Zanotti[1], D. Greco[1], L.B. Giva[4,6], M.P. Adorni[1], G.D. Norata[5], M. Simoni[4], F. Bernini[1], J.R. Nofer[3], F. Poti[2,6]
[1]Department of Food and Drug, University of Parma;
[2]Department of Medicine and Surgery, Unit of Neurosciences, University of Parma;
[3]Center for Laboratory Medicine, University Hospital Münster, Germany;
[4]Department of Biomedical, Metabolic and Neural Sciences, Unit of Endocrinology, University of Modena and Reggio Emilia, Modena;
[5]Department of Pharmacological and Biomolecular Sciences, University of Milan and Centro Studi Aterosclerosi, Milano;
[6]AUSL of Modena, Modena

Aim. S1P plays a crucial role in atherosclerotic disease, even though the molecular mechanisms underlying its anti-atherosclerotic function are partially unknown. The aim of this study was to demonstrate that the anti-atherogenic activity of S1P was related at least in part to the modulation of lipid metabolism. In detail, we aimed to evaluate the role of S1P3 receptor in the RCT process, using mice, characterized by the overexpression of S1P3 only in macrophages (S1P3-Lyz mice).

Methods. RCT in vivo was measured through a radiolabelled technique in C57BL/6 mice: control group (n=7) received murine peritoneal macrophages (MPM) from C57BL/6 mice, whereas S1P3 group (n=7) received MPM from S1P3-Lyz mice. MPM *Abcg1* gene expression was measured through a real-time PCR assay. *Abcg1* protein expression was evaluated through western blotting analysis. The cholesterol efflux of C57BL/6 and S1P3 macrophages was evaluated through a standardised radiolabelled technique, using different mouse plasma concentrations (0.1%, 2% v/v) and HDL (12.5 mg/ml) as cholesterol acceptors.

Results. RCT in vivo was higher in S1P3 group compared to control group, as demonstrated from the higher radioactivity found in plasma (0.99%±0.32 vs. 0.60%±0.12; p<0.05), liver (2.66%±0.41 vs 1.99%±0.35; p<0.01) and faeces (0.99%±0.19 vs 0.66%±0.10; p<0.01). In addition, S1P3-MPM showed an increased protein and gene expression of Abcg1 in comparison with C57BL/6-MPM. According with these results, cholesterol efflux to mouse plasma was higher in S1P3-MPM compared to C57BL/6-MPM (3.18%±0.32 vs 2.05%±0.53; p<0.05). Similarly, in acetylated LDL-loaded MPM, S1P3-MPM cholesterol efflux was higher compared to C57BL/6-MPM, both to HDL (8.47%±0.63 vs 5.93%±0.46; p<0.001) and to mouse plasma (8.04%±0.43 vs 10.49±1.2 and 22.99%±1.2 vs 37.09%±5.43, to 0.1% and 2% respectively).

Conclusions. these results evidence that the antiatherogenic function of endogenous S1P is also due to the modulation of lipid metabolism, through the interaction with the macrophage S1P3 receptor.

LEPTIN AND RESISTIN AFFECT PCSK9 AND DE NOVO LIPOGENESIS THROUGH THE INVOLVEMENT OF STAT3

M. Botta[1], C. Macchi[1], S. Marchianò[1], E. Giorgio[1], P. Magni[1], A. Corsini[1,2], N. Ferri[3], M. Ruscica[1]
[1]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano; [2]Multimedica IRCCS, Milano; [3]Dipartimento di Scienze del Farmaco, Università degli Studi di Padova

Aim. Clinical, genetic and experimental evidence indicate that proprotein convertase subtilisin/kexin 9 (PCSK9) may be either a cause or an effect of metabolic syndrome. We have demonstrated that PCSK9 is regulated by pro-inflammatory cytokine TNF-α in a SOCS3-dependent manner (Ruscica *et al.*, JBC, 2016). The present work aimed to further extend this observation and studied the effects of inflammatory adipokines, namely, leptin and resistin on *de novo* lipogenesis and PCSK9 expression

Methods. Human hepatocellular liver carcinoma cell line (HepG2), HepG2 transiently overexpressing $PCSK9^{D374Y}$ (gain-of-function), HepG2 stably overexpressing PCSK9 ($HepG2^{PCSK9}$) and luciferase reported promoter activities assay were used as *in vitro* tools. qPCR, ELISA and luciferase reporter assays, together with siRNA directed to STAT3 and PCSK9, were used.

Results. A 48-h treatment with leptin and resistin (both 50 ng/mL) induced both PCSK9 mRNA expression (2.0±0.22 and 3.5±0.8 fold, respectively) and protein secretion (+15±2.4% and +18±3.4%, respectively) in HepG2 cells. These effects were completely inhibited by transfection with siRNA anti-STAT3. Leptin and resistin induced expression of mediators involved in *de novo* lipogenesis, namely, apolipoprotein B, sterol regulatory element-binding (SRE) protein 1 (SREBP1), stearoyl-CoA desaturase-1 (SCD-1), fatty acid synthase (FAS) and microsomal triglyceride transfer protein (MTP). Transient ($PCSK9^{D374Y}$) and stable ($HepG2^{PCSK9}$) PCSK9 overexpression led to an activation of *de novo* lipogenesis genes and siRNA anti-STAT3 or anti-PCSK9 completely abolished the effects driven by leptin and resistin. To further corroborate the link among leptin, resistin, PCSK9 and *de novo* lipogenesis, a 48-h leptin and resistin treatment showed an increment in the luciferase activity of human PCSK9 promoter (30±5.2% for both). These effects were completely abolished when a SRE mutation were inserted in the promoter region of PCSK9.

Conclusions. Inflammatory adipokines up-regulate (i) PCSK9 in a STAT3-fashion and (ii) *de novo* lipogenesis in a STAT3-manner.

URIC ACID AND ANGIOTENSIN II ADDITIVELY PROMOTE INNATE IMMUNITY AND INFLAMMATION IN HUMAN RENAL TUBULAR CELLS

F. Viazzi, D. Verzola, S. Milanesi, B. Bonino, F. Cappadona, E.L. Parodi, A. Murugavel, R. Pontremoli, G. Garibotto
DIMI, University of Genoa and IRCCS Azienda Ospedale Università San Martino-IST, Genova

Aim. Renal tubular cells are involved in several mechanisms of innate immunity and they express TLRs and different pro-inflammatory cytokines. Hyperuricemia is a possible mediator of inflammation and tubule-interstitial damage while the mechanisms are not well-defined. Angiotensin II (AII) is a vasoconstrictive peptide with some inflammatory effects but there still exists few data with regard to the mechanism of action of AII-mediated kidney injury.
The aim of this study is to evaluate the activation of innate immunity and the presence of inflammation induced by uric acid (UA) and angiotensin II (AII) in human renal tubular cells (hTCs) in colture (HK-2).
Methods. HK-2 were incubated for 0-5 hours with UA and AII. HK-2 were pre-treated with an antagonist of TLR4 (Tak 242), Valsartan or Losartan. In every case the genic expression of TLR4, MCP1 and NOX4 was quantified with rtPCR, TLR4 protein was evaluated with western-blot.
Results. The incubation of HK-2 either with UA or with AII determines an increased expression of TLR4 ($p<0.05$) and production of pro-inflammatory cytokines as MCP-1 ($p<0.05$) and pro-oxidants as NOX4 ($p<0.05$). When HK-2 are incubated with UA and AII the effects showed to be synergic. Pre-treating HK-2 with an inhibitor of TLR4 (Tak242) attenuates the expression of MCP-1 and NOX4 mRNA induced both by UA and AII. Pre-treatment with valsartan attenuated all the affects we described as induced by AII but not those we described to be promoted by UA. At variance, pre-treatment with losartan which inhibits UA internalization, attenuates the expression of TLR4, MCP-1 and NOX4 in cells treated with UA, AII and both.
Conclusions. This study shows as pro-inflammatory pathways and mechanisms of cellular damage are induced in an additive manner by UA and AII and are mediated by TLR4 in HK-2 hTC. These effects are prevented by antagonizing TLR4, AII receptor and the internalization of UA. Our results can help to clarify the interplay between RAAS activation, hyperuricemia and innate immunity in the development of tubular damage and confirm that the internalization of UA is necessary to promote his toxic effects.

ENVIRONMENTAL CHRONIC STRESS INDUCES ABNORMAL MEGAKARYOPOIESIS PREDISPOSING TO THROMBOSIS: PROTECTIVE EFFECTS OF APOCYNIN

L. Sandrini[1], A. Ieraci[1], P. Amadio[2], M. Popoli[1], E. Tremoli[2], S.S. Barbieri[2]
[1]Università degli Studi di Milano; [2]Centro Cardiologico Monzino, IRCCS, Milano

Aim. Environmental chronic stress (ECS) has been identified as a trigger of acute coronary syndromes (ACS). Changes in redox balance, enhanced reactive oxygen species (ROS) production, and platelet hyper-reactivity could be detected in both ECS and ACS. However, the mechanisms by which ECS predisposes to thrombosis are not well known yet.
We analysed the impact of ECS on megakaryopoiesis and platelet activation in a mouse model and tested the effect of Apocynin, an inhibitor of NADPH oxidase, in this experimental setting.
Methods. Apocynin at 2.4 mg/ml was administrated in drinking water for 4 days. Forced swimming for 4 days (5 min twice/day) was used to induce ECS and FeCl3 arterial injury as model to assess thrombosis. Megakaryocytes and platelets were characterized by flow cytometry.
Results. We show that ECS increases the number of BM megakaryocytes (MKs) and affected circulating platelets. MKs of stressed mice show an advanced maturation state (e.g. expression of CD42d), and an enhanced ability to produce ROS. Interestingly, a higher number of large and reticulated platelets with marked functional activation (e.g. integrin $a_{IIb}b3$ and P-selectin expression, and platelet/leukocyte aggregates) is detected after ECS. Apocynin treatment decreases the total number of MKs and prevents their ability to generate ROS without affecting the percentage of $CD42d^+$ cells.
Finally, Apocynin reduces the hyper-activation of platelets and the enhanced susceptibility to $FeCl_3$-induced arterial thrombosis in stressed mice.
Conclusion. Apocynin treatment, reducing ROS generation in MKs, restores the physiological bone marrow megakaryopoiesis and platelet behaviour, and it prevents the effect of chronic stress on atherothrombosis. These data suggest a potential use of NADPH oxidase inhibitors in the occurrence of thrombosis associated with chronic stress. Studies in human will verify the clinical impact of these findings.

PLASMA PHOSPHOLIPID FATTY ACID COMPOSITION, LIPID OXIDATION AND INFLAMMATORY PARAMETERS IN A GROUP OF FISHERMEN

P. Bigolin, C. Panzavolta, P. Scarinzi, A. Antonucci, R. Marin, D. Vianello, G. Pagin, S. Bertocco, L. Previato, A. Boscolo[1], G. Gessoni[1], S. Valverde[1], A. Boscolo Bariga[1], E. Manzato, S. Zambon, F. Fabris, A. Zambon
Department of Medicine, University of Padova; [1]Laboratory for clinical analysis, Hospital of Chioggia

Aim. Dietary fish intake influences plasma phospholipid fatty acids. Few data are available on the role of habitual fish consumption at low latitude sea. We evaluate the potential relationships between sea food dietary consumption and plasma phospholipid fatty acid composition, lipid profile, lipid oxidation and inflammatory parameters in a group of fishermen living in Chioggia, in the Northern Adriatic.
Methods. Anthropometric and biochemical data, lipid concentrations, phospholipid fatty acid percentage composition, apolipoprotein B and oxidized LDL (ox-LDL) in fasting plasma samples were determined in 208 subjects (age mean ±SD 46.6±11.0 years). A food questionnaire was recorded.
Results. Subjects were divided into three groups according to habitual fish consumption (0-1, 2-3 fish meals per week, ≥1 fish meal per day). Plasma phospholipid percentage composition of n-6 polyunsaturated fatty acids (PUFA) decreased from the lowest to the highest fish-consumption group (37.19±2.50%

vs 36.05±2.62% vs 35.59±2.63%, p=0.002 ANOVA) while n-3 PUFA increased (4.52±1.13 vs 5.24±1.52 vs 5.32±1.74, p=0.001 ANOVA), mainly eicosapentaenoic (EPA) and docosahexaenoic (DHA). Analysing the whole group, white blood cells count (WBC) was related to total monounsaturated fatty acids (r=0.152, p=0.029), and WBC and interleukin-6 were related to n-3 PUFA (r=-0.245, p=0.000; and r=0.168, p=0.016). Ox-LDL were related to stearic (r=0.243, p=0.008), dihomogammalinolenic (r=0.264, p=0.004) and DHA (r=0.206, p=0.024), and inversely related to monounsaturated fatty acid (r=-0.229, p=0.012), mainly oleic and eicosanoic.

Conclusions. The results offer clear evidence that fish consumption is reflected by the plasma phospholipid percentage composition of n-3 PUFA, and literature data support that n-3 PUFA have hypotriglyceridemic effect and may have anti-inflammatory properties. Since Ox-LDL are related to some n-6 PUFA, saturated and monounsaturated fatty acids, and inversely to n-3 PUFA, we suppose that habitual fish intake might have both pro and anti-atherogenic effects on lipid profile, probably depending on the different fish species.

ROLE OF PCSK9 (PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9) BEYOND LDLR TARGETING: FOCUS ON GLUCOSE METABOLISM

G. Balzarotti[1], M. Ruscica[1], E. Di Cairano[1], C. Perego[1], A.L. Catapano[1, 3], G.D. Norata[1, 2]
[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano;
[2]Centro SISA per lo Studio dell'Aterosclerosi, Ospedale Bassini, Cinisello Balsamo, Milano;
[3]IRCCS Multimedica Milano

Background. PCSK9 is mainly synthesized and secreted by the liver and regulates the levels of circulating LDL-C by enhancing the degradation of the hepatic LDLR. Pre clinical and some clinical evidences, however, suggest a biological role for PCSK9 in extra-hepatic organs specifically on adipose tissue and pancreas. In this study we addressed to clarify the role of PCSK9 on glucose metabolism.

Methods and Results. WT and PCSK9 KO mice were fed for 20 weeks with a High Fat Diet (HFD) or Standard Fat Diet (SFD). Glucose Tolerance Test (GTT) revealed significant higher glycemia in PCSK9 KO mice compared to WT (AUC +25%±11% SFD; +40%±9% HFD, p<0,05), with no differences in the Insulin Tolerance Test (ITT) with both diets. The absence of PCSK9 led to a significant reduction in plasma insulin levels (4,3±0,6 ng/ml vs 3,2±0,2 ng/ml) and an increased insulin content in pancreas (104±7 ng/ml vs 138±12 ng/ml). The histological analysis revealed a significant difference in the size of Langerhans islets and insulin-positive areas between PCSK9 KO and WT. Interestingly, there were no differences in GTT, ITT, plasma and pancreatic insulin levels between PCSK9/LDLR DKO and LDLR KO mice. Moreover, preliminary data obtained in liver-selective PCSK9 KO mice, (a Cre-LoxP system), suggest that circulating PCSK9 is not dependent for the effect observed.

Conclusion. PCSK9 deficiency affect glucose homeostasis and insulin secretion. The results obtained in the double KO mice suggest that this impaired glucose metabolism could be due to the effect of the protein on pancreatic LDLR.

PCSK9 INDUCES A PRO-INFLAMMATORY RESPONSE IN MACROPHAGES

C. Ricci[2], [2]M. Ruscica, M. Camera[2,3], L. Rossetti[3], C. Macchi[2], A. Colciago[2], I. Zanotti[4], M.P. Adorni[4], F. Bernini[4], A. Corsini[2,5], N. Ferri[1]
[1]Dipartimento di Scienze del Farmaco, Università degli Studi di Padova; [2]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano; [3]Centro Cardiologico Monzino, Milano; [4]Dipartimento di Farmacia, Università di Parma; [5]Multimedica IRCCS, Milano

PCSK9 plays an important role in cholesterol metabolism, by inducing hepatic LDL receptor degradation. In our laboratory, we demonstrated that PCSK9 also degrade macrophages LDLR in vitro. Dwivedi et al. observed that PCSK9 deficiency confers protection against inflammation, while its overexpression exacerbates inflammation in early sepsis in mice. Moreover, Atheroremo IVUS study results show a direct correlation between PCSK9 and coronary plaque inflammation.

In the present work, we investigate the possible pro-inflammatory activity of PCSK9 on human macrophages.

We treated human and THP-1-derived macrophages for 24h with increasing concentrations of PCSK9 (0.25, 0.5, 1.0 and 2.5 µg/ml). We first evaluated pro-inflammatory cytokines mRNA levels: in response to 2.5 µg/ml of PCSK9, all the tested cytokines and chemokines undergo to a strong increase (IL6: 200.5±13.9 fold; TNF-α: 80.04±13 fold; MCP-1: 17±6.8 fold; MIP2α: 42.57±0.01 fold; IL-1β: 9.7±1.2 fold). Through ELISA assay, we also observed a significantly higher amount of IL-6 protein in the conditioned media. In order to understand if PCSK9 pro-inflammatory activity is LDLR dependent, we isolated bone marrow macrophages (BMM) from C57BL/6 WT and LDLR KO mice and then stimulated them with 2.5 µg/ml of PCSK9. WT BMM showed, as human macrophages, a strong induction of TNF-α mRNA levels (35.37±2.72 fold), while in LDLR KO, TNF-α increase was significantly reduced (5.44±0.29 fold), even if not totally abolished. PCSK9 is transcriptionally regulated by SREBPs pathway. Proinflammatory cytokines activate the same pathway by interacting both with STAT and SREBP cleavage-activating protein (SCAP). By western blot analysis, we observed a significant increase in SCAP protein levels in both human and THP-1 derived macrophages in response to the treatment with 2.5 µg/ml of PCSK9.

Taken together, these results suggest that PCSK9 can behave like a pro-inflammatory cytokine, by interacting with the STAT/SREBP/SCAP intracellular pathway and that this pro-inflammatory activity is LDLR dependent.

IMPACT OF A 29 SNPS-BASED GENETIC RISK SCORE FOR HYPERTENSION ON AORTIC DISEASE.

A.Tagetti[1], T. Ohlsson[2], G. Engström[2], G. Smith[2], P. Minuz[1], O. Melander[2] and C. Fava[1,2]
[1]Department of Medicine, University of Verona, Section of General Medicine and Hypertension, Verona;
[2]Department of Clinical Sciences, Lund University, University Hospital of Malmö, Sweden

Aim. A genetic risk score (GRS) based on 29 SNPs associated with high blood pressure (BP) was shown to be associated with future development of hypertension, stroke and cardio-

vascular events. Aim of the present study is to evaluate the impact of this polygenetic BP component on the incidence of aortic disease, namely aortic dissection (AD), thoracic aorta aneurysm (TAA), abdominal aorta aneurysm (AAA), including possible events (either rupture or need of surgical correction).
Methods. More than 27,000 people in the Swedish Malmo Diet and Cancer Study had at least 24 valid SNPs and were followed up for a median of more than 18 years. The number of BP elevating alleles of each SNPs, weighted by their effect size in the discovery studies, was summed into a BP-GRS.
Results. In Cox regression models, adjusted for traditional cardiovascular risk factors (TRF) including hypertension, we found a significant associations of the BP-GRS, prospectively, with incident TAA (hazard ratio 1.37; 95% confidence interval (CI) 1.055–1.619 comparing the third vs. first tertile; P.0.017) but not with either AAA or aortic dissection. Calibration, discrimination and reclassification analyses did not show any improvement in prediction using the BP-GRS in addition to the model which used only the TRF.
Conclusions. A GRS for hypertension associates with TAA suggesting a link between genetics determinant of BP and aortic disease. The effect size is small but the addition of more SNPs to the GRS could improve its discriminatory capability.

HIGHER PULSE PRESSURE AND RISK FOR CARDIOVASCULAR EVENTS IN YOUNG AND OLD PATIENTS WITH ESSENTIAL HYPERTENSION

C. Mancusi, M.A. Losi, A. d'Amato, G. De Stefano, G. Albani, R. Izzo, N. De Luca, B. Trimarco, G. de Simone
Hypertension Research Center Federico II University Hospital, Roma

Aim. In current guidelines for hypertension, increased pulse pressure (PP) is considered a marker of target organ damage (TOD) in elderly patients, which associated with increased cardiovascular (CV) events. However, whether high PP influences prognosis also in younger adults, similar to what is reported in older subjects, is unknown. Our aim is to evaluate the prognostic impact of increased PP on incident CV events in young or middle aged and elderly hypertensive patients, participants of the Campania Salute Network (CSN) registry.
Methods. Treated hypertensive participants from CSN (n=9480) without prevalent coronary or cerebrovascular disease, with ejection fraction >50%, no valve disease and no more than stage III Chronic Kidney Disease were followed for a median follow-up of 49 months for the occurrence of major CV events (MACE, fatal and non-fatal stroke or myocardial infarction, and atrial fibrillation). Patients were classified as young or middle aged adult if age <65 yrs and elderly adults if age ≥65 yrs. Increased PP was defined as ≥60 mmHg, based on current guidelines, echocardiographic LVH defined using prognostically validated values (LV mass index >50 g/m$^{2.7}$ in men and >47 g/m$^{2.7}$ in women). Concentric geometry was defined by relative wall thickness ≥0.43. Patients were categorized into 4 groups according to age-stratum and presence of high PP:Elderly adults with normal PP (EANPP, n=550), Elderly adults with high PP (EAHPP n=853), Younger adult with normal PP (YANPP n=5348) andYoung adult with high PP (YAHPP n=2729), Outcome was compared in Cox regression analysis, reported as hazard rate (HR) and 95% confidence interval (CI).
Results. EAHPP were older and exhibited higher prevalence of diabetes, LVH and concentric geometry than EANPP (all p<0.05). Similarly, YAHPP were older, more frequently women, and had higher prevalence of diabetes, LVH and concentric geometry (all p<0.05). In Cox regression analysis, controlling for age, sex, prevalent diabetes, presence of LVH and concentric LV geometry, high PP substantially increased the risk of MACE in younger patients (HR 1.53 [95% CI 1.18-1.96], p=0.0001) but not the elderly group (HR 1.17 [95% CI 0.76-1.78], p=0.474).
Conclusions. High PP remains a potent marker of target organ damage predicting CV events in young and middle-aged hypertensive patients, even independently of LV geometry. In elderly patients the prognostic effect of high PP appears largely associated with abnormal LV geometry.

DUTCH LIPID CLINIC NETWORK SCORE IN ITALIAN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA: DATA FROM THE LIPIGEN STUDY

M. Casula[1], L. Chiodo[1], K. Garlaschelli[2], F. Galimberti[1]; M. Averna[3], M. Arca[4], S. Bertolini[5], P. Tarugi[6], S. Calandra[7], A.L. Catapano[1,8] on behalf of the LIPIGEN Group*
[1]Epidemiology and Preventive Pharmacology Centre (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan; [2]Center for the Study of Atherosclerosis, E. Bassini Hospital, Cinisello Balsamo, Milano; [3]Biomedical Department of Internal Medicine and Specialistics (DIBIMIS), University of Palermo; [4]Department of Internal Medicine and Medical Specialties, Sapienza University, Rome; [5]Department of Internal Medicine, University of Genoa; [6]Department of Life Sciences, University of Modena and Reggio Emilia, Modena; [7]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena; [8]IRCCS MultiMedica, Sesto S. Giovanni, Milano

Aims. This analysis was aimed to describe, according to the Dutch Lipid Clinic Network (DCLN) criteria, the clinical and genetic features of the FH population enrolled in the LIPIGEN (LIpid TransPort Disorders Italian GEnetic Network) study.
Methods. LIPIGEN study collects anamnestic, biochemical, and genetic data of a cohort of FH subjects under a follow up of a lipid clinic network throughout Italy. The network centres work in concert, following shared protocols according to the recommendations of major national and international scientific societies.
Results. LIPIGEN network includes more than 3400 FH patients. Based on clinical and biochemical DCLN criteria, 56.1% of patients reported DCLN score ≥6: in particular, 23.6% had score between 6 and 8 (probable diagnosis of FH) and 32.5% had score ≥9 (definite diagnosis). The prevalence of family history of premature coronary events and hypercholesterolemia (calculated on the total number of cases in which such information was available, about 3 subjects of 5), was 38.3% and 89.6%, respectively. The personal history of early coronary or cerebral/peripheral vascular events showed a prevalence of 11.3% and 9.6%, while the presence of tendon xanthomas or non-senile corneal arc (the latter reported in just over a third of subjects) was observed in 21.8% and 8.8%, respectively. The prevalence of untreated LDL-C levels >325 mg/dL was 20.3%, and of LDL-C levels >250 mg/dL was 56.2%. In subjects with score ≥6 mutations were detected in 92.0% of the subjects.

More than 98% of subjects with genetic diagnosis had mutations of LDL receptor (LDLR) gene.
Conclusion. In the Italian context, the DCLN score is of a great help for guiding the clinical diagnosis and the identification of patients in which the genetic analysis should be performed.

***LIPIGEN Group:** Marcello Arca (Roma), Maurizio Averna (Palermo), Stefano Bertolini (Genova), Claudio Borghi (Bologna), Adriana Branchi (Milano), Laura Calabresi (Milano), Maurizio Cassader (Torino), Alberico L. Catapano (Milano), Luigi Cattin (Trieste), Francesco Giorgino (Bari), Arcangelo Iannuzzi (Napoli), Lorenzo Iughetti (Modena), Graziana Lupattelli (Perugia), Enzo Manzato (Padova), Andrea Mezzetti (Chieti), Roberto Miccoli (Pisa), Paolo Pintus (Cagliari), Luca Puccetti (Siena), Enrica Riva (Milano), Paolo Rubba (Napoli), Carlo Sabbà (Bari), Tiziana Sampietro (Pisa), Riccardo Sarzani (Ancona), Mariella Trovati (Torino), Giovanni B. Vigna (Ferrara), Josè Pablo Werba (Milano) and Maria Grazia Zenti (Verona)
*www.sisa.it/index.php?class=Comp&className=Content&op=Show&param=cid,1000.

RIGHT ATRIUM MODIFICATIONS IN PATIENTS WITH WELL CONTROLLED HYPERTENSION

S. Cicco, M. Portaluri, A. Cirulli, A. Vacca, G. Ranieri
Centro Ipertensione Arteriosa, Clinica Medica "G. Baccelli", Dept. of Internal Medicine and Clinical Oncology, University of Bari Medical School

In hypertensives left heart remodeling is normally described. Recently some authors had focused a possible right ventricle remodeling. Aim of this study was to analyze if patients with right atrium enlargement addresses a specific condition in hypertensive.
We analyzed 313 well controlled hypertensives (147 M; 59,5±9,8 years) who underwent to a heart ultrasound. 17,6% of them were smokers and 37,8% assumed statins.
Mean PaPs was 16,5±11,9 mmHg with a tricuspid regurgitation velocity (TRV) 1,46±0,93 m/s. Right Atrium (RA) area was bigger than 18 cm^2 (InRA) in 28,8%. InRA patients did not have PaPs nor TRV statistically different to those evaluated in non RA enlargement (nRA). InRA had an increased Left Ventricle Mass (LVM; InRA 237,2±4,5 vs nRA 212,0±4,0 gr; p=0.0002), LVMi (InRA 125,0±2,8 vs nRA 119,0±1,8 gr/m^2; p=0.05), TAPSE (InRA 27,7±0,3 vs nRA 26,4±0,2 mm; p=0.05), as well as aortic root dimensions (InRA 34,2±0,4 vs nRA 31,8±0,3 mm; p<0.0001) and Left Atrium Volume (LAV; InRA 71,5±2,3 vs nRA 57,0±1,3 ml; p<0.0001; LAVi: InRA 37,4±1,3 vs nRA 32,3±0,7 ml/m^2; p=0.0003). Atherosclerosis Cardiovascular Risk (ASCVD) resulted increased in InRA despite it was not significant (InRA 10,7±7,6 vs nRA 9,9±9,2%; ns).
In InRA patients ASCVD was directly related to PaPs (p=0.021), TRV (p=0.0089), Creatinine (p=0.001) and uric acid (p=0.048) while inversely to eGFR (p<0.0001). Also in nRA ASCVD is related to Creatinine, eGFR and uric acid (p<0.0001 each), but not to PaPs or TRV. Moreover, PAS is not related to ASCVD in InRA, while is directly related in nRA (p<0.0001).
In conclusion RA enlargement should be an expression of a more severe organ involvement. Further studies are necessary to assess whether this finding should be an independent prognostic factor.

DYSLIPIDEMIA IN HYPERTENSIVE PATIENTS STUDIED BY ABPM IN A HYPERTENSION CENTRE

F. Spannella,G. Cocci, F. Giulietti, P. Balietti, R. Sarzani
Internal Medicine and Geriatrics, Hypertension Excellence Centre of the ESH, IRCCS-INRCA, UNIVPM

Aim. Hypertension and dyslipidemia are two of the most relevant modifiable cardiovascular risk (CVR) factors. Aim: to evaluate lipid profile and lipid control in a large unselected hypertensive population.
Methods. Retrospective study on 1440 hypertensives referred to our Hypertension Centre between 2010 and 2015 and studied by ABPM. Dyslipidemia was defined by presence of at least one of: total cholesterol (TC) ≥200 mg/dl, calculated LDL cholesterol (LDLc) higher than recommended based on individual CVR, HDLc <40mg/dl in men and <50 mg/dl in women, triglycerides (TG) ≥150 mg/dl.
Results. Mean age: 55.9±13.4 years. Male: 823 (57.2%). Overweight/obese: 68%. Smokers: 18.1%. Diabetics: 10.7%. Dyslipidemia: 82.6%. Patients with peripheral arterial disease (PAD): 23.7%. Mean eGFR: 75.0±15.6 ml/min/1.73 m^2. Mean TC: 205.9±39.7 mg/dl. Mean HDLc: 51.8±13.7 mg/dl. Median TG: 111 mg/dl (82-157). Mean LDLc: 129.2±34.5 mg/dl. Drug therapy: 19% (statins 87.6%, ezetimibe 8.2%, others 4.2%). At target LDLc: 39% (all patients), 42.9% (treated). Females were less controlled, despite the same treatment rate with lipid-lowering drugs. Hypertensive diabetics not treated with lipid-lowering drugs were 56.4% and treated diabetics were less controlled. Patients with PAD not treated with lipid-lowering drugs were 78.3%. TG>=150 mg/dl: 28% (all patients), 33% (treated patients). Low HDLc (according to sex): 28.3%. Statins frequencies: simvastatin (38.9%), atorvastatin (31.7%), rosuvastatin (15.0%), pravastatin (11.9%), others (2.5%). High intensity statins were taken only by 2% of treated patients. The ABPM values controlled by therapy were 29.9% and patients with both controlled hypertension and LDLc were only 12%.
Conclusions. Our data show an extremely poor management of dyslipidemia in hypertensives referred to a Hypertension Centre. Even patients at high CVR were often untreated with lipid-lowering drugs. Whenever treated, they received low-medium intensity statins, reaching low rate of therapeutic goals, similarly to untreated patients. A better attention and management of dyslipidemia in hypertensives is urgently needed to improve CV prevention.

DETECTING FAMILIAL HYPERCHOLESTEROLEMIA BY SCREENING SERUM LIPID LEVELS IN HOSPITAL CLINICAL PRACTICE: CLINICAL AND GENETIC ASPECTS

R. Platania, R. Scicali, A. Di Pino, V. Ferrara, G. Purrazzo, A. Giannone, S. Piro, A.M. Rabuazzo, F. Purrello
Department of Clinical and Experimental Medicine, University of Catania

Background and Aims. Familial hypercholesterolemia (FH) is the most frequent monogenetic disorder characterized by elevated plasma levels of low density lipoprotein (LDL) cholesterol and premature atherosclerotic cardiovas-

cular disease (CVD). However, FH patients are underdiagnosed and undertreated. So, public cholesterol screening is essential to find individuals with FH and prevent cardiovascular disease in these patients. In this study, we aim to investigate the percentage of patients with FH in an hospital cholesterol screening program and the characterization of their cardiovascular risk.
Methods A total of 1575 LDL cholesterols were screened. Finally, 56 suspected FH patients (DCLN score ≥ 4) were selected to perform genetic analysis. The diagnosis of FH was supposed using the Dutch Lipid Clinic Network (DLCN) criteria and confirmed by pathogenic genetic variants presence. Familial Hypercholesterolemia Genetic Analysis (SEQPRO LIPO) detected variants associated with Autosomal Dominant Hypercholesterolemia (ADH) and Autosomal Recessive Hypercholesterolemia (ARH). To confirm probably or possibly pathogenic status of the novels genetic variants, the in silico prediction of the LDLR, APOB and PCSK9 genes missense mutations effect was performed using PolyPhen-2 and SIFT Human Protein refined SIFT. Mean common carotid intima media thickness (IMT) were assessed using consensus criteria in subjects without history of cardiovascular disease (CVD).
Results. The detection rate of FH was 1.64% (one in 61). All FH patients had a pathogenic genetic variants. FH patients had higher mean IMT than non-exposed subjects (0.73±0.14 vs. 0.68±0.1mm, $p<0.05$). In addition, in a multiple linear regression, IMT was associated with corneal arcus and/or tendon xanthomas presence ($p<0.01$) and age ($p<0.01$).
Conclusions. In clinical practice, detecting FH patients by screening serum lipid levels is useful to find new cases of FH. In addtion FH patients in primary prevention had higher mean IMT than non-FH subjects. Finally, corneal arcus and/or tendon xanthomas presence is associated with early carotid atherosclerotic injury in subject with higher LDL value.

BIGLYCAN EXPRESSION IN CARDIOVASCULAR RISK CONDITIONS: ARTERIAL HYPERTENSION AND CIGARETTE SMOKING

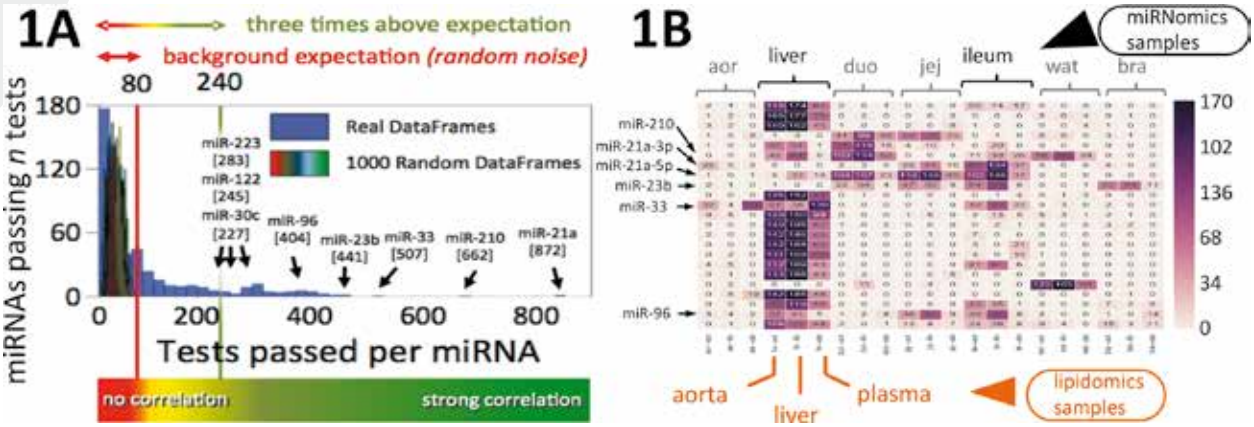
M. Scuruchi, C.O. Aragona, A. Lo Gullo, V. Cairo, F. Savarino, A. Saitta, G. Mandraffino
Department of Clinical and Experimental Medicine, University of Messina

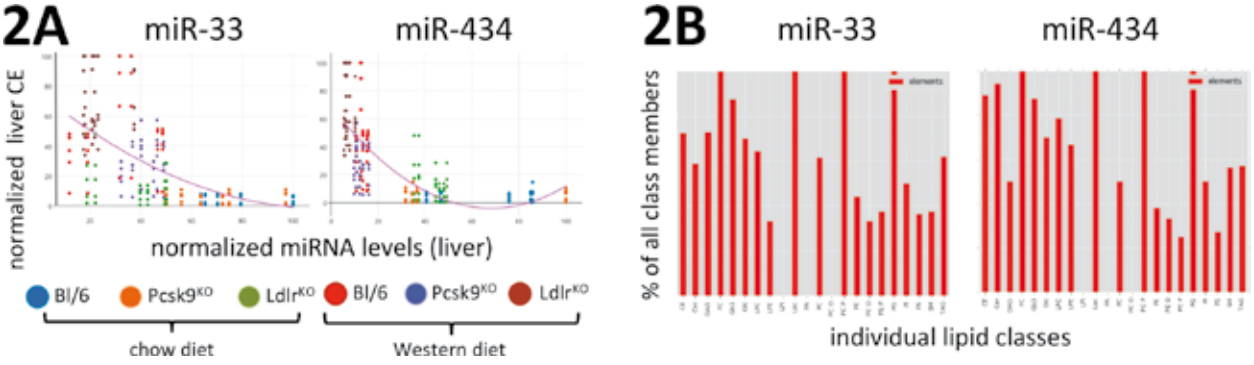
Background. Biglycan (BGN), a small leucine rich proteoglycan, plays a pivotal role in initiating the deposition of lipids in the arterial subintimal space by its ability to bind and retain apoB-containing lipoprotein, including LDL, VLDL and IDL. Moreover, BGN acts as an endogenous ligand of Toll-like receptors mediating innate immunity and inflammation.
Patients, Methods and Results. Evaluating patients affected by arterial hypertension (AH) we found that BGN expression is increased, and that Angiotensin II can mediate BGN expression. The treatment by an Angiotensin receptor blocker, Losartan, administrated at standard dose of 50 mg/day, significantly reduced BGN expression with respect to baseline. IL-6, TNF-alpha, CRP, and fibrinogen were also significantly reduced. These results suggest that Losartan can reduce BGN expression in monocytes from AH patients and that the effects of AngII on BGN expression in monocytes may be modulated, in part, by an Angiotensin receptor blocker.
We also investigate whether cigarette smoking (CS) may enhance monocyte BGN in subjects without additional cardiovascular risk factors (CVRFs). Fibrinogen Il-6, CRP, carotid-femoral pulse wave velocity (cf-PWV) and intima media thickness (cIMT) were also evaluated. BGN was increased in young smokers, as compared to controls, and appears associated with increased fibrinogen, CRP, and IL-6, lower HDL-C, and altered AS and cIMT. Last, we evaluated whether monocyte BGN may decrease after smoke cessation. Anthropometrics, laboratory profile, cf-PWV, cIMT, and BGN were evaluated during active CS, and 12 months after smoke cessation.
We found that BGN, IL-6, CRP, fibrinogen, HDL-C, and cf-PWV were significantly improved as compared to baseline. These data show that BGN expression may be reversibly induced by CS, and also by AH.
Conclusions. Overall, these data suggest that BGN represents a link between proatherogenic status induced by CS or AH and the development and progression of vascular damage.

INTEGRATED HIGH-THROUGHPUT MIRNOMICS AND LIPIDOMICS OF WILD-TYPE, PCSK9 AND LDLR KNOCKOUT MICE AS A TOOL TO DISSECT MIRNA TO MOLECULAR LIPID LEVELS CORRELATIONS

S. Manzini[1], M. Busnelli[1], M. Hilvo[3], M. Chiara[2], C. Parolini[1], F. Dellera[1], G. Sara Ganzetti[1], D.S. Horner[2], R. Laaksonen[3], G. Chiesa[1]
[1]Department of Biomolecular and Pharmacological Sciences, University of Milan; [2]Department of Biosciences, University of Milan; [3]Zora Biosciences Oy, Finland

Aim. Pcsk9 and LDL receptor knockout mice are two sides of the same coin, showing profound changes in lipid metabolism related to wild-type mice. We leveraged on these different genotypes to reconcile differences in miRNA expression and lipid levels.
Methods. Wild type, Pcsk9- and Ldlr-knockout mice were fed both chow and Western diets for 16 weeks. MicroRNAs were high-throughput sequenced in liver, aorta, white adipose tissue, duodenum, jejunum, ileum and brain; 387 molecular lipid species were quantified by high-throughput mass-spectrometry in liver, aorta and plasma. An algorithm to reconcile and integrate the two datasets was developed; background correlations were calculated against 1,000 randomized datasets.
Results. Expression levels for each miRNA were tested for correlation with each lipid measurement, permuting all possible combinations of samples. ~150 miRNAs showed more correlations with lipid values than those expected from background expectation; 48 miRNAs showed at least thrice (*Fig. 1A*). We found that liver and small intestine (ileum, especially) are the tissues where the number of such correlations are higher (*Fig. 1B*). The analysis could identify established miRNAs related to lipid metabolism (like miR-33, miR-210 and miR- 21a) and also find novel miRNAs with similar association patterns. For example, hepatic miR-33 associates strongly with cholesteryl esters levels in the liver, as does previously unre-





ported miR-434 (*Fig. 2A*). The distribution of their correlation also shows similarities (*Fig. 2B)*. As an additional proof of reliability, when considering miRNA values in one organ correlated to lipid values in one sample, all lipid in each class show the same positive or negative correlation with respect of each miRNA, as expected from consistent and non-random interactions.
Conclusions. This joint analysis of - omics datasets provided results in accordance with previous discoveries, and suggested possibly novel miRNA/lipid relationships that "classical" miRNA target searches would miss.

SCREENING FOR SLEEP DISORDERS AND THEIR RELATIONSHIP WITH THE ONSET OF TARGET ORGAN DAMAGE IN ADULT HYPERTENSIVE PATIENTS

V. Bisogni, M.F. Pengo, G. Maiolino, C. Berton, G. Rossitto, M. Cesari, T.M. Seccia, G.P. Rossi
Clinica dell'Ipertensione Arteriosa - DIMED, Department of Internal Medicine, University of Padova

Aim. Prolonged short sleep duration and poor quality of sleep can lead to arterial hypertension and increase cardiovascular risk (1). Hypertensive patients with concomitant obstructive sleep apnoea (OSA) show higher prevalence of left ventricular hypertrophy, increased relative wall thickness, and microalbuminuria excretion, indicating cardiac and renal organ damage (OD), respectively (2, 3). Nevertheless data about the relationship between other sleep disorders and the onset of hypertensive target OD are still missing. We hypothesised that adult hypertensive patients at risk of the three most common sleep disorders (OSA, insomnia, and restless leg syndrome (RLS) are more prone to develop cardiac and renal OD.
Methods We enrolled 283 patients between 18 and 60 years old. All subjects underwent microalbuminuria and glomerular filtration rate measurements, as markers of hypertensive kidney disease. A subgroup of 128 subjects was assessed for left ventricular remodelling by transthoracic echocardiography. We submitted to all participants questionnaires to evaluate the risk of insomnia (Insomnia Severity Index), OSA (STOP-Bang), and RLS (Index of Restless Leg Syndrome Severity-IRLS).
Results 163 males and 120 females, with mean age 43.3±9.2 years, body mass index 26.1±4.6 kg/m^2, mean office systolic blood pressure 143±16 mmHg and mean diastolic blood pressure 92±10 mmHg, have been studied. Patients with high risk of OSA (STOP-Bang≥4) showed an increased left ventricular mass index (125.4±45.3 *vs* 103.1±23.2 g/m^2, p<0.001), left atrium enlargement (27.9±4.7 *vs* 25.1±5.1 ml/m^2, p<0.01), and diastolic dysfunction (E/e' 10.9±3.4 *vs* 8.4±2.5). Subjects with IRLS>0 presented higher values of interventricular septum (12.0±2.3 *vs* 11.2±2.0 mm, p=0.05). We did not found any significant correlation with renal OD.
Conclusions High risk of sleep disorders, in particular OSA and RLS, in adult with arterial hypertension seems to be associated with cardiac organ damage.

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TARGETED SEQUENCING OF APOC3, GCKR, LIPA, PPP1R3B, NCAN, LYPLAL1 AND TM6SF2 GENES IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE (NAFLD).

A. Di Costanzo[1], F. Belardinilli[2], D. Bailetti[3], M. Sponziello[1], L. D'Erasmo[1], L. Polimeni[1], F. Baratta[1,4], M. Del Ben[1], G. Giannini[2], F. Angelico[5], M. Arca[1]
Departments of Internal Medicine and Medical Specialties[1], Molecular Medicine[2], Experimental Medicine[3], Anatomical, Histological, Forensic Medicine and Ortopedics[4], Public Health and Infectious Disease[5], La Sapienza University of Rome

Aim. GWAs have identified many loci associated with NAFLD. However, the definitive confirmation of these genetic associations may derive from the demonstration of the enrichment of genetic variants in the genes of interest. To this aim, we characterize the enrichment of non-synonymous (NS) variants in *APOC3*, *GCKR*, *LIPA*, *PPP1R3b*, *NCAN*, *LYPLAL1* and *TM6SF2* genes in NAFLD patients compared to controls.
Methods. A total of 362 subjects, 183 with ultrasound-defined NAFLD and 179 controls, were re-sequenced by NGS techniques employing the Ion Torrent PGM platform.
Results. Overall, we identified 194 sequence variants within the selected genes, 54 of which were NS. Compared to controls, NAFLD patients showed higher frequencies of rs1260326 (L446P) in *GCKR* and rs58542926 (E167K) in *TM6SF2*. Conversely, rs1051338 (T16P) in *LIPA* showed lower frequency in NAFLD (all $P<0.05$). After adjustment for traditional NAFLD risk factors, carriers of *TM6SF2* EK+KK and *GCKR* PP genotypes had a significantly increased risk of NAFLD (OR, 4.5; 95%CI, 1.6-12.6, $P=0.007$; OR, 1.9; 95%CI, 1.1-3.4; $P=0.038$; respectively). Interestingly, carriers of *LIPA* PP genotype showed a negative correlation with NAFLD, suggesting a possible protective effect of this variant (OR=0.3, 95%CI, 0.1-1.1, $P=0.054$). When the cumulative frequencies of all NS variants within each gene were considered, only the *TM6SF2* emerged as gene conferring a significantly increased risk of NAFLD (OR, 2.9; 95%CI, 1.3-6.6; P=0.015 in the adjusted model).
Conclusions. Our data confirm the major role of *TM6SF2* as genetic predictor of NAFLD risk and suggest the usefulness of NGS technology to further characterize the genetic burden in complex inherited disorders.

EFFECTOR MEMORY T CELLS PREDICT ATHEROSCLEROSIS PROGRESSION AND CARDIOVASCULAR EVENTS OVER 4 YEARS FOLLOW-UP

A. Baragetti[1,2], K. Garlaschelli[1], F. Bonacina[2], L. Grigore[1], E. Ammirati[5], A.L. Catapano[2,3], G.D. Norata[2,4]
[1]Centro per lo Studio dell'Aterosclerosi, Ospedale Bassini Cinisello Balsamo, Milano;
[2]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano;
[3]IRCCS Multimedica, Sesto San Giovanni, Milano;
[4]The Blizard Institute, Barts and The London School of Medicine & Dentistry Queen's Mary University, London, United Kingdom;
[5]Niguarda Ca' Granda Hospital, Milano

Aim. Experimental models demonstrate that adaptive T-cell response is promoted during atherogenesis. Aim of the study is to see whether expanded circulating T effector memory (CD4+TEM) (CD3+CD4+CD45RA–CD45RO+CCR7–) subtypes predict long term atherosclerosis progression and incidence of cardiovascular events in the general population.
Methods. We analyzed subsets of circulating CD4+T cells by flow cytometry (markers: CD3/CD4/CD45RO/CD45RA/CCR7/CCR5/CXCR3/HLA-DR) in peripheral blood from 163 (79 women and 84 men, 58 (43-65) years-old) subjects from the general population (PLIC Study), followed for up to 4 years. Anthropometric parameters, pharmacological history and incidence of cardiovascular events (CVE) during the observational period were collected; biochemistry and lipid parameters were measured. Ultrasound common carotid artery Intima-Media Thickness (CCA-IMT) was determined at both visits to measure its annual progression; carotid plaques (and their number for each subject) were detected.
Results. T-cell subsets were differently correlated with cardio-metabolic risk factors; in particular CD4+TEM were increased in obese, in dyslipidemic subjects and in smokers while opposite trend were observed for T naïve (CD3+CD4+CD45RA+CD45RO-CCR7+) subtypes. No effect of pharmacological treatment was observed.
CCA-IMT progression was 0.008 (-0.003-0.021) mm/year and 14 incident CVE were reported during the observational period.
Basal CD4+HLADR+TEM (percentage out of CD4+ subsets) predicted faster CCA-IMT progression, adjusting for confounders (BMI, LDL lipoprotein cholesterol, triglycerides). CD4+TEM subsets (CD4+HLADR+TEM in particular) increased with number of carotid plaques for each subject, independently from confounders.
In parallel, percentage of CD4+CCR5+TEM was higher in subjects who developed incident CVE during observation (8.9% vs 6.5% out of CD4+ T subsets compared to those who did not developed events, p=0.033).
Conclusions. Circulating CD4+TEM cells are associated with cardio-metabolic parameters, with faster subclinical atherosclerosis and increased incidence of CVE in the general population. Further analyses unveiling cellular aspects will contribute to understand the inflammatory pathogenesis of atherosclerosis.

ASSOCIATION BETWEEN NECK CIRCUMFERENCE, AORTIC AND CAROTID STIFFNESS IN HEALTHY ADOLECENTS. THE MACISTE STUDY

F. Battista[1,2], E. Bianchini[3], F. Faita[3], V. Gemignani[3], M. D'Abbondanza[1], G. Pucci[1,2]
[1]Department of Medicine, University of Perugia;
[2]Unit of Internal Medicine, Terni University Hospital, Terni;
[3]Institute of Clinical Physiology, CNR, Pisa

Aim. obesity has a negative impact on arterial distensibility. Neck circumference (NC) is a marker of upper body adiposity and unfavourable metabolic profile among young subjects. The association between NC and arterial stiffness is unknown. We evaluated the association between NC and other measures of adiposity, with carotid-femoral pulse wave velocity (cf-PWV) and carotid stiffness (CS) in healthy adolescents.
Methods. 431 individuals (mean age 16.9±1.4y, SBP/DBP 124/67±11/7 mmHg) attending the Donatelli High School in Terni, Italy, were evaluated. Cf-PWV was measured with applanation tonometry (SphygmoCor device, subtracted distance). CS was measured with Carotid Studio (Quipu): a contour tracking algorithm was applied to B-mode longitudinal scans of common carotid artery to obtain diameter changes, stroke change in lumen area (ΔA) and lumen area (A). The cross-sectional distensibility coefficient (DC=ΔA / (A*PP)) was converted (Bramwell-Hill equation) into a carotid stiffness parameter (CS=(DC*ρ)-1/2, ρ=blood density) with same measurement units of PWV. Carotid waveform was calibrated to brachial MAP/DBP. Brachial MAP was derived from brachial tonometry calibrated to brachial SBP/DBP.
Results. average NC was 33±3 cm, cf-PWV was 4.9±0.8 m/s, CS 4.3±0.6 m/s. NC, as well as other measures of adiposity (BMI, BMI z-score, waist and hip circumferences, waist-hip ratio, waist-height ratio), showed some degrees of association with both cf-PWV and CS. The association between NC and CS was the only association which remained significant after adjustement for age, sex and MAP (partial R=0.13, p<0.01), and remained significant also after further adjustment for BMI (partial R=0.10, p=0.03).
Conclusions. in healthy adolescents, NC and other measures of global and local adiposity showed significant associations with cf-PWV and CS. The association between NC and CS was the only correlation independent from age, sex, MAP and BMI. Both local and systemic factors may be involved in explaining this relationship. NC may help in identifying adolescents at increased CS.

Table - *Table - Bivariate associations between indexes of adiposity and measures of arterial stiffness (*p<0.05, **p<0.01).*

	Carotid-femoral PWV	Carotid stiffness
BMI	0.12**	0.21**
BMI z-score	0.07	0.18**
Waist circumference	0.17**	0.20**
Hip circumference	0.11*	0.17**
Waist/hip ratio	0.13**	0.12*
Waist/height ratio	0.08	0.11*
Neck circumference	0.25**	0.35**

OCULAR FUNDUS PHOTOGRAPHY WITH A SMARTPHONE DEVICE IN ACUTE HYPERTENSION

F. Bertacchini, A. Paini, M. Salvetti, C. Agabiti Rosei, D. Stassaldi, C. Aggiusti, M. Riviera, F. Semeraro, A. Russo, M.L. Muiesan
Clinica Medica, Università di Brescia, ASST Spedali Civili di Brescia

Aim. The ocular fundus (FO) examination is infrequently and poorly performed in the emergency department (ED) clinical settings, placing patients at risk for missed diagnosis of hypertensive emergencies. Aim: to investigate the feasibility of the FO photography with a smartphone small optical device (D-Eye; J Ophtalmol. 2015) in a ED setting and to compare it to a traditional FO examination.
Design and Method. The study included 41 consecutive patients (mean age 69±16 years, 50% women) presenting to an hospital ED with an acute increase in blood pressure (SBP >180 and/or DBP >100 mmHg). When admitted to the ED all patients had mydriatic FO examination obtained by an Emergency physician (EP) using both a traditional ophtalmoscope and a commercially available FO smartphone device (D-Eye, Si14 S.p.A., Padova). All FO images and videos recorded with the D-Eye system were analysed by 2 independent expert (ophthalmologist) and inexpert (EP) observers. A quantitative score of hemorrages, exudates and/or papillary edema was used (0 absent, 1 early, 2 moderate, 3 severe, 4 very severe). The Cohen K coefficient (Ki) was used to assess the inter-observer concordance index.
Results. Six patients had headache, 6 had focal neurologic symptoms, and 4 had acute visual changes. The mean duration of FO examination was 130±39 and 74±31 seconds for traditional ophtalmoscopy and for smartphone D-Eye, respectively. No relevant abnormalities of their FO were detected by traditional ophthalmoscopy, performed by the EP, while a signifcant number of abnormal FO findings were detected by the use of the D-eye device in 17 and 19 patients by the EP and ophthalmologist, respectively. The Ki value ranged from 0,66 to 0,77 (good concordance) for the assessment of hemorrages and exudates, and from 0,89 to 0,90 (optimal concordance) for the evaluation of presence and severity of papilledema.
Conclusions. Our results show that a new small smartphone device (D-Eye) may be feasible in an ED setting for the fundoscopic examination, detecting a signifcant number of abnormal FO. The reliability of relevant FO abnormalities seems to be superior in respect to traditional fundoscopy.

STEATOMETER: A NEW US-BASED SYSTEM FOR THE ASSESSMENT OF LIVER FAT CONTENT

N. Di Lascio[1,2], C. Avigo[2], A. Salvati[3], M. Mancini[4], M. Ragucci[4], N. Martini[5], D. Chiappino[5], L. Ghiadoni[6], F. Bonino[6], M.R. Brunetto[3], F. Faita[2]
[1]Institute of Life Science, Scuola Superiore Sant'Anna, Pisa;
[2]IFC-CNR, Pisa; [3]AOUP Pisa, Pisa; [4]IBB-CNR, Napoli;
[5]Tuscany Foundation G. Monasterio, Massa; [6]University of Pisa

Fatty liver is becoming a global epidemic and represents a risk factor for cardiovascular disease. For this reason, reliable, non-invasive, cheap and non-ionizing measurement systems are needed. Aim of this study was to introduce the Steatometer for assessing liver fat content based on ultrasound (US) images analysis.
Thirty subjects (41.9±9.6years, 65%males) were examined. Ste-

atometer values were obtained acquiring US images with a standard equipment in right inter/subcostal views and processing them to calculate 5 parameters (hepatic-renal ratio (HR_ratio), hepatic-portal-vein ratio (HPV_ratio), attenuation-rate (AR), diaphragm (DV) and portal-vein-wall visualization (PVwall_vis)); the overall score (Steato-score) was obtained as their linear combination. Furthermore, 122 features were obtained, for each patient, from the image texture analysis. Magnetic Resonance Spectroscopy measures of the percentage fat content were obtained using a clinical 3T scanner (MRS_fat); a 5% cut-off for MRS_fat was employed to differentiate between steatosis absence or presence. Feature selection algorithms were employed to choose the more informative parameters between standard and texture-derived indexes, which were then used for the training (5-fold cross-validation) of an automatic classifier. Receiver-Operating Characteristic (ROC) curves were obtained in order to assess the overall accuracy of Steato-score as well as that of the classifier approach.
HR_ratio, AR, DV, PVwall_vis and Steato-score were significantly correlated with MRS_fat (R=0.41-0.65). For the Steato-score the area under the ROC (AUROC) was 72%, specificity 58% and sensitivity 89%. HR_ratio, AR, DV, PVwall_vis and 4 different texture features were selected by the selector; the Quadratic Discriminant classifier was trained giving AUROC, specificity and sensitivity of 100%.
The Steatometer represents a valid approach for non-invasive, non-ionizing quantification of fatty liver applicable to any standard US scanner and can be useful for screening and monitoring fatty liver in both clinical trials and practice. The system diagnostic performance is improved when considering parameters derived by texture analysis.

CIRCULATING MONOCYTE SUBPOPULATION PROFILING IN PRE-CLINICAL AND CLINICAL ATHEROSCLEROSIS

V. Bianconi, M.R. Mannarino, F. Bagaglia, M. Fiorini, M. Pirro
Università degli Studi di Perugia, Dipartimento di Medicina, Medicina Interna

Aim. Monocytes play a central role in the progression of atherosclerotic vascular disease. We wanted to estimate the influence of circulating sub-populations of monocytes on markers of preclinical and clinical atherosclerosis.
Methods. In 43 dyslipidemic patients without clinical atherosclerosis, 19 patients diagnosed with carotid atherosclerotic plaque and 32 healthy controls, we measured, by flow cytometry, the number of mature circulating monocyte subpopulations, including $CD14^{++}/CD16^{-}$, $CD14^{++}/CD16^{+}$ and $CD14^{+}/CD16^{++}$ cells, referred to as classical monocytes, intermediate monocytes and non-classical monocytes respectively. Each patient underwent a measurement of aortic Pulse Wave Velocity (PWV), brachial Flow Mediated Dilation (FMD) and carotid intima-media thickness (IMT) or plaque. Ultrasound estimation of visceral fat area (VFA) and clinical, metabolic and laboratory parameters were also obtained.
Results. The number of non-classical monocytes $CD14^{+}/CD16^{++}$ was significantly higher in patients with carotid plaque, compared to dyslipidemic patients and controls ($p<0.05$). The number of $CD14^{+}/CD16^{++}$ positively correlated with aortic PWV (rho=0.25; p=0.02) and VFA (rho=0.35; p=0.02), while negatively correlated with FMD (rho=-0.26; p=0.02). VFA was an independent predictor of $CD14^{+}/CD16^{++}$ count (β= 0.36; p=0.01). $CD14^{+}/CD16^{++}$ count was an independent predictor of aortic PWV (β=0.25; p=0.01). We did not find significant difference in classical and intermediate monocyte count between study groups.
Conclusions. Our study shows that the number of circulating blood non-classical monocytes is associated with the presence of atherosclerosis plaques, markers of preclinical atherosclerosis and visceral adiposity. These findings suggest that non-classical monocytes may play a crucial role in the development and progression atherosclerosis.

HBA1C INCREASE IS ASSOCIATED WITH HIGHER CORONARY AND PERIPHERAL ATHEROSCLEROTIC BURDEN IN NON DIABETIC PATIENTS

R. Scicali[1], P. Giral[2,4], A. Gallo[2,3], A. Di Pino[1], A.M. Rabuazzo[1], F. Purrello[1], P. Cluzel[3,5], A. Redheuil[3,5], E. Bruckert[2,4], D. Rosenbaum[2,3]
[1]*Department of Clinical and Experimental Medicine, University of Catania;*
[2]*Cardiovascular Prevention Unit, of Metabolism and Endocrinology Service; Paris Hospital Public Assistance, Pitié-Salpêtrière Hospital Group, Pierre et Marie Curie University, Paris, France;*
[3]*Sorbonne Universités, UPMC Univ Paris 06, INSERM 1146, - CNRS 7371, Laboratoire d'imagerie Biomédicale, Paris, France;*
[4]*Dyslipoproteinemia and Atherosclerosis Research Unit, UMRS 939, National Institute for Health and Medical Research (INSERM) and Pierre et Marie Curie University (UPMC - Paris VI), Paris, France;*
[5] *Département d'imagerie cardiovasculaire et de radiologie interventionnelle, Pôle Imagerie-Groupe Hospitalier Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, France*

Background and Aims. Prediabetes is associated with an increased risk of developing diabetes and cardiovascular disease. Our objective was to examine the cardiovascular (CV) risk profile of non-diabetic patients with and without prediabetes according to HbA1c using macroangiopathic imaging biomarkers.
Methods. Our population consisted in 272 non diabetic patients aged between 40 and 70 years, with a normal fasting plasma glucose (FPG < 5.6 mmol/L) and at least 1 CV risk factor. Exclusion criteria were prior history of CV disease or clinical evidence of advanced renal disease. Prediabetes was defined as an HbA1c value of 5.7-6.4%. Coronary artery calcium (CAC) score as well as mean common carotid intima media thickness (IMT) and plaque presence were assessed using consensus criteria.
Results. CAC score was higher in the prediabetes group compared to non prediabetic subjects (131.7±295.6 vs 62.4±178.8 AU, $p<0.001$). Prediabetic subjects had higher mean IMT than non-exposed (0.77±0.14 vs 0.61±0.15 mm, $p<0.001$). The proportion of prediabetic patients with CAC = 0 was significantly lower compared to non-exposed (35% vs 63%, $p<0.01$). In opposite, the proportion of patients with a CAC>400 was significantly higher in the prediabetes group (10% vs 3%, $p<0.05$). Moreover, carotid plaques were significantly more present in patients with prediabetes than in the normoglycemic subjects ($p<0.01$). In a multiple linear regression, IMT was associated with HbA1c continuous levels ($p<0.001$). Also, logistic regression showed that higher HbA1c levels were as-

sociated with CAC and carotid plaques presence (p for trend for all <0.001)
Conclusions. Among patients with normal fasting glucose, HbA1c increase is associated with higher coronary and peripheral atherosclerotic burden in non-diabetic patients.

LOW ADVANCED GLYCATION END PRODUCT DIET THE LIPID AND INFLAMMATORY PROFILES OF PREDIABETIC SUBJECTS

Giannone, W. Currenti, A. Di Pino, R. Scicali, V. Ferrara, G. Purrazzo, S. Piro, F. Purrello, A.M. Rabuazzo
Department of Clinical and Experimental Medicine, University of Catania

Background and Aims. Prediabetes is associated with risk for cardiovascular disease, and the first step in its management emphasizes lifestyle and diet modifications; however, modern diets are high in advanced glycation end products (dAGEs), derived from processing methods that exert a pivotal role in promoting atherosclerotic risk. We studied the effect of low vs standard dAGE diets (L-dAGEs vs S-dAGEs) on lipid profile, inflammation, and cardiovascular risk in prediabetic subjects.
Methods. A 24-week randomized dietary intervention was conducted on 62 prediabetic subjects. We evaluated lipid profile, endogenous secretory receptors for AGEs, high-sensitivity C-reactive protein, arterial stiffness, and intima-media thickness.
Results. After 24 weeks, patients with L-dAGEs showed a significant reduction of total cholesterol, apolipoprotein B, and low-density lipoprotein compared with controls (5.26±1.09 vs 5.53±0.87 mmol/L, p<0.05; 0.77±0.25 vs 1.16±0.13 mmol/L, p<0.05; and 3.53±0.93 vs 3.68 ± 0.7 mmol/L, p<0.05); with respect to baseline, high-sensitivity C-reactive protein levels were significantly reduced in the L-dAGEs group (0.21 [0.11-0.69] vs 0.12 [0.08-0.48] mg/dL, p<0.05) but not in the S-dAGEs group. Endogenous secretory receptor for AGEs was similar in both the groups at baseline and at the 24-week follow-up. With respect to baseline, L-dAGE patients showed a significative reduction of intima-media thickness (0.77 [0.73-0.81] vs 0.73 [0.70-0.75] mm, p<0.05). We did not observe the same reduction in S-dAGEs. No difference in arterial stiffness was found from baseline to follow-up in both the groups.
Conclusions. L-dAGEs improved the lipid and inflammatory profiles of prediabetic subjects and seemed to reduce atherosclerotic burden compared with a standard diet. Further studies are needed to recommend this dietary regimen for prevention of cardiovascular risk in prediabetes.

NUTRACEUTICAL-POT IN PATIENTS WITH PERIPHERAL ARTERIAL OCCLUSIVE DISEASE AND STATINS INTOLERANCE: MORE THAN A CHANCE

S. Quartuccio[1], E. Imbalzano[1], G. Lizio[1], G. Trapani[1], R. Pardeo[1], G. Mandraffino[1], A. Saitta[1]
[1]Clinical and Experimental Medicine, University of Messina

Aim. Peripheral Arterial Occlusive Disease is a clinical and very common manifestation of atherosclerosis in occidental countries. The main objective of these patients is the reduction of cholesterol levels (LDL-c). This objective improves claudicatio and reduce heart-associated events.
We aimed evaluating the efficacy, the safety and the tolerance of a nutraceutical compound in PAOD patients which, due to an intolerance or to a volunteer refuse, weren't in treatment with any statin. We tested the hypothesis of a possible improvement of exercise performances in claudicatio patients intaking an integrator with multiple anti-atherosclerotic principles.
Methods. LDL-c level and treadmill test performance were evaluated on PAOD patients intaking this nutraceutical product were compared to patients in treatment with a placebo at T0 and at T6. The nutraceutical integrator contained: Omega-3: 651 mg; Monascus Purpureus: 417 mg; Policosanol: 10 mg; CoQ10: 10 mg; Resveratrol: 10 mg; Vitamine B6: 3 mg; Vitamine B12: 2.5 mcg, Folic Acid 300 mcg.
68 patiens were enroled and were divided in 2 groups (A group = nutraceutical, B group = placebo).
The study had 3 phases: patient selection, screening part and patient evaluation under blind-treatment. Eligible patients had to be older than 40 years and had suffered from claudicatio for more than 6 months.
Results. A Group patients had a progressive and significative reduction of serum levels of total and LDL cholesterol and triglycerides than B group (p<0,001). A Group had also better PWV values than control group. Treadmill performance was significantly improved in patients belonging to A group than B group patients.
Conclusions. PAOD patients well tolerated nutraceutical administration and the treatment resulted being secure and effective. Whether it isn't possible using a statin in PAOD patients, a nutraceutical formulation it has to be considered as a valid alternative.

EFFICACY OF LOMITAPIDE IN THE TREATMENT OF FAMILIAL HOMOZYGOUS HYPERCHOLESTEROLEMIA: RESULTS OF A REAL WORLD CLINICAL EXPERIENCE IN ITALY

L. D'Erasmo[1], A.B. Cefalù[2], D. Noto[2], A. Giammanco[2], M. Averna[2], P. Pintus[3], P. Medde[3], G.B. Vigna[4], C. Sirtori[5], L. Calabresi[5], C. Pavanello[5], M. Bucci[6], C. Sabbà[7], P. Suppressa[7], F. Natale[8], P. Calabrò[8], T. Sampietro[9], F. Bigazzi[9], F. Sbrana[9], K. Bonomo[10], F. Sileo[11], M. Arca[1]
[1]Department of Internal Medicine and Clinical Specialties, Policlinico Umberto I, "Sapienza" University of Rome; [2]Dipartimento Biomedico di Medicina Interna e Specialistica, Università di Palermo; [3]Centro per le Malattie Dismetaboliche e l'Arteriosclerosi, Dipartimento Internistico, Cagliari; [4]Medical Department, Azienda Ospedaliera-Universitaria di Ferrara; [5]Center E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, and Dyslipidemia Center, Niguarda Hospital, Milan; [6]Geriatric Clinic, European Center of Excellence on Atherosclerosis, Hypertension and Dyslipidemia, University of Chieti, Chieti, Italy, [7]Department of Interdisciplinary Medicine, Geriatric Unit and Rare Disease Center, "Aldo Moro", University of Bari, Bari; [8]Cardiology Department, Second University of Naples, Ospedale dei Colli, Naples; [9]U.O. Lipoapheresis and Center for Inherited Dyslipidemias, Fondazione Toscana Gabriele Monasterio, Pisa, [10]Division of Internal Medicine, San Luigi Gonzaga Hospital Orbassano, Torino; [11]Divisione Endocrinologica, Ospedali Riuniti, Bergamo

Introduction. Homozygous familial hypercholesterolaemia (HoFH) is a rare form of inherited dyslipidemia resistant to

conventional cholesterol-lowering medications so that lipoprotein apheresis (LA) is usually required. Lomitapide has been approved for the treatment of HoFH. The objective of this study was to evaluate the benefits of lomitapide in HoFH patients followed in the usual clinical care setting.
Methods. Clinical and biochemical data were retrospectively collected in 15 HoFH patients (10 with mutations in *LDLR* and 5 in *LDLRAP1* gene) treated for at least 6 months with lomitapide in addiction to conventional lipid lowering therapies (LLT) in different Lipid Clinic across Italy.
Results. The mean follow-up period was 32.3±29.7 months. During background therapies, HoFH patients showed a mean LDL-C level of 426.0±204.0 mg/dl. The addition of lomitapide at the average dosage of 19 mg/day lowered LDL-C levels by 68.2±24.8%. At last visit, 60% of patients showed LDL-C<100 mg/dl and 46.6% <70 mg/dl. During follow-up, 8 over 10 patients receiving LA (80%) stopped this treatment due to marked LDL-C reduction. A wide range (13-95%) of individual LDL-C reduction was observed, but this was not related to genotype. During follow-up, 53.3% of patients reported at least one episode of diarrhoea, but none was referred as severe; none had liver transaminase >5 X ULN or had to stop treatment due to side effects. A subset of patients was evaluated by liver ultrasound and fibroscan (n=5) or nuclear magnetic resonance with spectroscopy (MRS) (n=1) not showing clinical evidence of liver damage.
Conclusion. In this real world experience, lomitapide was confirmed to be a very powerful cholesterol-lowering agent in HoFH showing a good safety profile.

EFFECT OF VITAMIN D REPLACEMENT ON SERUM LIPOPROTEIN FUNCTIONALITY IN HEALTHY PRE-MENOPAUSAL WOMEN

C. Marchi[1], D. Greco[1], D. Kocyigit[2], M. P. Adorni[1], N. Ronda[1], F. Bernini[1], K. M. Gurses[3], SH. Oguz[4], A. Gurlek[4], L. Tokgözoglu[2], F. Zimetti[1]
[1]Dipartimento di Scienze degli Alimenti e del Farmaco, Università degli Studi di Parma;
[2]Department of Cardiology, Hacettepe University Faculty of Medicine, Ankara, Turkey; [3]Department of Cardiology, Konya Training and Research Hospital, Konya, Turkey; [4]Department of Internal Medicine, Section of Endocrinology and Metabolism, Hacettepe University Faculty of Medicine, Ankara, Turkey

Aim. Low vitamin D (vitD) status has been linked to increased cardiovascular (CV) risk. Serum HDL cholesterol efflux capacity (CEC) is a metric of HDL functionality that is inversely correlated to CV risk, independently of HDL-cholesterol (HDL-C) plasma levels. In the present work we evaluated the effect of vitD replacement on:) HDL CEC; 3) serum cholesterol loading capacity (CLC), as index of its pro-atherogenic potential.
Material and Methods. Healthy pre-menopausal women (n=31) with vitD levels <20 ng/mL, status defined as vitamin D deficient, were enrolled for the study and scheduled for vitD replacement. Flow-mediated dilatation (FMD) and pulse wave velocity (PWV) were measured by standard techniques as markers of subclinical atherosclerosis**.** HDL CEC was assessed by radioisotopic technique while serum Serum CLC was measured by a fluorimetric assay. Sera adipokines levels were analysed by ELISA kit.
Results. No differences were found at baseline and after replacement in total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride levels. Markers of subclinical atherosclerosis were significantly improved after vitD replacement with an increased in FMD (10,32%± 1,2 vs 10,01% ±1,2 p<0.0001) and a reduction of PWV (5,4 m/s ±0,8 vs 5,6 m/s ±0,9 p<0.0001) compared to baseline. Total and ABCA1-mediated CEC were increased after vitD replacement compared to baseline (+19,44% p=0.003 and +70,8% p=0.0008 respectively), while no difference was found in ABCG1-mediated CEC. Serum CLC was significantly decreased after vitD replacement as compared to baseline (-13,35% p=0.02). After vitD replacement, adiponectin concentrations increased (+50,56% p<0.0001) while resistin decreased (-24,33% p<0.0001); moreover after vitD replacement, changes of resistin levels were inversely associated with total and ABCA1- mediated CEC (r=-0,326 p=0.07 and r=-0,469 p=0.009 respectively).
Conclusions. VitD status influences circulating lipoprotein functions relevant for atherosclerosis in healthy pre-menopausal women which may be involved in the association between vitD status and CV risk.

COFFEE CONSUMPTION AND RISK OF HYPERTENSION: A DOSE-RESPONSE META-ANALYSIS OF PROSPECTIVE STUDIES

L. D'Elia, E. La Fata, F. Galletti, P. Strazzullo
Department of Clinical Medicine and Surgery, ESH Excellence Center of Hypertension, "Federico II" University of Naples Medical School, Naples

Aim. Recently, a large prospective study provided additional information concerning the debated possible association between habitual coffee consumption and risk of hypertension (HP). Therefore, we updated the state of knowledge on this issue by carrying out a comprehensive new systematic review of the literature and a meta-analysis of the available relevant studies.
Methods. We performed a systematic search for prospective studies on general population, published without language restrictions (1966-May 2016). A random-effects dose-response meta-analysis was conducted to combine study specific relative risks (RRs) and 95% confidence intervals.
Results. Four studies (196,256 participants, 41,184 diagnosis of HP) met the inclusion criteria. Coffee intake was assessed by dietary questionnaire. Dose-response meta-analysis showed a non-linear relationship between coffee consumption and risk of HP (P for non-linearity <0.001). Whereas, compared with non-drinkers, the habitual drinking of one or two cups of coffee per day was not associated with risk of HP, a significantly protective effect of coffee consumption was found starting from the consumption of 3 cups of coffee per day (RR=0.97, 95% CI=0.94 to 0.99), and was confirmed for greater consumption.
Conclusions. The results of this analysis indicate that habitual moderate coffee intake is not associated with higher risk of HP in the general population and that in fact a non-linear inverse dose-response relationship occurs between coffee consumption and risk of HP.

LYSOSOMIAL ACID LIPASE ACTIVITY INCREASE AFTER A VERY LOW CARBOHYDRATE KETOGENIC DIET

L. Calzini[1], M.A. Ricci[2], A.R. Roscini[3], D. Siepi[4], G. Lupattelli[5], G. Tozzi[6], G. Daviddi[7], S. Ministrini[8]
[1]University of Study of Perugia, Internal Medicine; [2]University of Study of Perugia, Internal Medicine; [3]S. Maria della Misericordia Hospital, Internal Medicine; [4]University of Study of Perugia, Internal Medicine; [5]University of Study of Perugia, Internal Medicine; [6]"Bambin Gesù" Pediatric Hospital, Dipartment of Neurosciences; [7]University of Study of Perugia, Internal Medicine; [8]University of Study of Perugia, Internal Medicine

Background. Lysosomal Acid Lipase (LAL) is an enzyme involved in lipid metabolism. Cholesterol ester Storage Disease (CESD) is a late onset phenotype that may present in childhood or later in life with serum lipid abnormalities, hepatosplenomegaly and/or elevated liver enzymes. LAL deficiency has also been suggested as an under-recognized cause of dyslipidaemia and non-alcholic fatty liver disease (NAFLD). So far, no studies have assessed LAL activity in morbid obesity.
Aims. The aim of the study was to test the LAL activity in obese patients and to observe the modification of the enzyme activity after the weight loss. The reduction of body weight was obtained through a very low carbohydrate ketogenic diet (VLCKD) lasting 20-25 days. LAL activity was tested before and after the diet.
Methods. A VLCKD was administered for 25 days to eight morbid obese patients (BMI 44,96±8,16 kg/m^2, age 53±10); the following parameters were evaluated before and after diet; glyco-lipidic pattern, NAFLD fibrosis score, abdominal ultrasonography scanning (liver steatosis degree and visceral fat area -VFA) and LAL-activity (dried blood spot -DBS- technique using the inhibitors Lalistat 2), (Whatman grade 903 Schleicher & Schuell). All blood samples were taken afer 12-hour fast.
A group of healthy normal weight subjects (age 43±13, BMI 22,8±2,6 kg/m^2) was also included in the study.
Results. In obese patients LAL activity was lower in obese comparing to healthy controls (1,09±0,36 vs 1,19±0,49 nmol/spot/h, p<0,001); after VLCKD, BMI was reduced (from 44,96±8,16 to 42,51 ± 8,6 nmol/spot/h; p≤0,001). Most of our patient also experienced a significant improvement of livers steatosis
LAL activity increses after VLCKD, with a significant mean increment of 26,05% (1,09±0,36 vs 1,29±0,38; p≤0,001).
Conclusions. Our data show for the first time that morbid obese subjects have a reduced activity of LAL and that weight loss induced by VLKCD increases the activity of the enzyme.

POSTER MODERATI

MICROBIOTA COMPOSITION AFFECTS LIPID METABOLISM AND INTESTINAL HOMEOSTASIS

M. Busnelli[1], S. Manzini[1], A. Bruneau[2], A. Boukadiri[3], C. Philippe[2], P. Gérard[2], G. Chiesa[1]
[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano;
[2]Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France;
[3]INRA, UMR1313 Génétique Animale et Biologie Intégrative, Jouy-en-Josas, France

Aim. Protein malnutrition is characterized by stunted growth, hepatic steatosis and a damaged gut mucosal architecture, associated with impaired mucosal permeability and inflammation. This study was aimed at determining whether different microbiota can limit the devastating effects of a protein-deficient diet by modulating energy extraction from food.
Methods. Germ-free 5-week-old C57Bl/6 mice were maintained in separate, sterile isolators and colonized with different microbiota obtained from Specific-Pathogen-Free C57BL/6 mice, fed for 5 weeks chow diet (CD), high-fat diet (HFD), or low-protein diet (LPD). After receiving the microbiota, mice were fed a low-protein diet for 8 weeks.
Results.
1) The CD microbiota-recipient mice had a reduced concentration of plasma phospholipids and were more prone to develop microvesicular hepatic steatosis without anomalous extracellular matrix deposition or increased development of inflammatory foci.
2) The HFD microbiota-recipient mice showed a reduced length of duodenal and ileal villi and shortened crypts, both in the ileum and in the colon. These histological modifications were accompanied by a robustly upregulated expression of Duox2 and Duoxa2, indicative of a perturbed intestinal mucosal homeostasis.
3) The LPD microbiota-recipient mice had an increased plasma concentration of HDL-cholesterol.

Conclusions. Different microbiota are able to modulate plasma lipid levels and the accumulation of lipids in the liver parenchyma leading to steatosis. Additionally, the HFD-shaped microbiota severely modifies the histological architecture of the gut and the intestinal expression of genes indicative of an increased microbiota/mucosa interaction, reflecting an altered microbiota composition or a defective host defense mechanism.

APOLIPOPROTEIN E ORCHESTRATES ADAPTIVE IMMUNE RESPONSE BY MODULATING THE CROSSTALK BETWEEN SYSTEMIC AND IMMUNE CELLS LIPID METABOLISM

F. Bonacina[1], D. Coe[2], G. Wang[2], A. Baragetti[1,3], K. Garlaschelli[2], F. Pellegatta[2], L. Grigore[2], A.L. Catapano[1,4], F. Marelli-Berg[2], G.D. Norata[1,2,3]
[1]Department of Pharmacological and Biomolecular Sciences, University of Milan; [2]William Harvey Research Institute, Queen Mary University, London, UK; [3]Centro SISA, Ospedale Bassini, Cinisello Balsamo, Milano; [4]IRCSS Multimedica, Milano

Aim. Activation of adaptive immunity characterized by T effector memory cell (T_{EM}) expansion and reduced T naïve cells (T_N) is an hallmark of atherosclerosis. Aim of this work is to investigate how dyslipidemia associated with apolipoprotein E (ApoE) deficiency affects adaptive immune response.
Methods. Phenotypical, *in vivo* and *in vitro* functional characterization through flow cytometry was performed in ApoE KO and WT littermates and human carriers od ApoE isoforms.
Results. ApoE deficiency resulted in increased levels of T_{EM} (blood:2.89±0.35 vs 8.74±1.93, p<0.05; lymphnodes: 10.88±1.36 vs 16.3±1.14, p<0.05; spleen: 21.84±3.6 vs 34.88±3.3, p<0.05), an increased proliferation of CD4Tcells (mitotic index: 31810±792.2 vs 44620±1683, p<0.01) and a faster rejection following skin graft allotransplantation (SGA, p<0.01). This phenotype was the consequence of myeloid-derived ApoE as WT recipients transplanted with ApoEKO bone marrow presented a reduced graft survival after SGA compared to ApoEKO transplanted with WT BM (p<0.05). Among myeloid-derived cells, ApoE deficiency was associated with an enhanced ability of DCs to trigger allogenic Tcell proliferation compared to WT DCs (mitotic index: 27150±4759 vs 57230±4530, p<0.01) but no difference in Tcells proliferation induced by allogenic DCs (mitotic index:7967±1506 vs 6507±1035, p=n.s.). DCs were significantly increased in the spleen of ApoE KO mice ($1.3x10^6$±$0.14x10^6$ vs $2.23x10^6$±$0.22x10^6$, p<0.01) and characterized by accumulation of cholesterol and oxysterols.
In humans, circulating T_{EM} cells were significantly correlated with cholesterol levels independent of other cardio-metabolic risk factors. Furthermore, carriers of apoE4 isoform (ε4/3, ε4/4) showed increased T_{EM} levels compared to apoE2 (ε2/2,2/3) and apoE3(ε3/3) carriers (13.69±4.0% vs 10.28±4.9% and 11.05±4.4%, p<0.01 and p<0.05). Finally, following mixed lymphocyte reaction, ApoE4 carriers presented increased T_{EM} polarization compared to ApoE2 and ApoE3 carriers.
Conclusion. our data suggest that DCs-derived ApoE orchestrates the activation of DCs-Tcell axis via the control of cholesterol and oxysterol availability in DCs thus connecting systemic vs immune cell lipid metabolism.

DEPLETION IN LPA-I:A-II PARTICLES ENHANCES HDL-MEDIATED ENDOTHELIAL PROTECTION IN FAMILIAL LCAT DEFICIENCY

A. Ossoli[1], M. Gomaraschi[1], S. Castelnuovo[3], S. Simonelli[1], C. Ravanello[1], G. Balzarotti[1], M. Arca[4], A. Di Costanzo[4], T. Sampietro[5], G. Vaudo[6], D. Baldassarre[7], F. Veglia[7], G. Franceschini[2], L. Calabresi[1]
[1]Centro E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari
[2]Section of Chemical and Biomolecular Sciences, DeFENS, Università degli Studi di Milano; [3] Center for Dyslipidemias, Ospedale Niguarda Ca' Granda, Milano;
[4]Atherosclerosis Center, Department of Internal Medicine and Allied Sciences, Sapienza University of Rome;
[5]Institute of Clinical Physiology, CNR, Pisa;
[6]Department of Medicine, University of Perugia;
[7]Monzino Cardiologic Institute, IRCCS, Milano

Aim. objective of the study was to evaluate the vasoprotective effects of HDL isolated from carriers of LCAT deficiency, which are characterized by a selective depletion of LpAI:A-II particles and predominance of pre-β migrating HDL.
Methods. HDL were isolated from LCAT deficient carriers and tested in vitro for their capacity to promote NO production and to inhibit VCAM-1 expression in cultured endothelial cells.
Results. HDL from carriers were more effective than control HDL in promoting eNOS activation with a gene-dose dependent effect (PTrend=0.048). As a consequence, NO production induced by HDL from carriers was significantly higher than that promoted by control HDL (1.63±0.24 vs 1.34±0.07 fold, P=0.031). HDL from carriers were also more effective than control HDL in inhibiting the expression of VCAM-1 (homozygotes, 65.0±8.6%; heterozygotes, 53.1±7.2%; controls, 44.4±4.1%; PTrend=0.0003). The increased efficiency of carrier HDL was likely due to the depletion in LpA-I:A-II particles. The in vitro findings might explain why carriers of LCAT deficiency showed flow-mediated vasodilation and plasma soluble cell adhesion molecule concentrations comparable to controls, despite low HDL-C levels.
Conclusion. These results indicate that selective depletion of apoA-II-containing HDL, as observed in carriers of LCAT deficiency, leads to an increased capacity of HDL to stimulate endothelial NO production, suggesting that changes in HDL apolipoprotein composition may be the target of therapeutic interventions designed to improve HDL functionality.

PROSTACYCLIN PREVENTS DEEP VEIN THROMBOSIS IN COX-2 KNOCKOUT MICE

P. Amadio[1], E. Tarantino[1], L. Sandrini[2], E. Tremoli[1], S.S. Barbieri[1]
[1]IRCCS Centro Cardiologico Monzino, Milano; [2]DiSFeB-Università degli Studi di Milano

Background. Venous thrombosis is a common condition that often leads to pulmonary thromboembolism and death. Cyclooxygenase (COX)-1 and -2, catalyzing prostanoids from arachidonic acid, play a critical role in the occurrence of thrombotic events.
Aim. To investigate the molecular mechanisms underlying venous thrombosis (VT) focusing on the impact of COX-2 deletion.
Methods. Thrombi were induced by inferior vena cava ligation (IVCL); thromboelastomethry, mass weight, histology and venous ultrasonography approaches were used to assess the effect of COX-2 ablation in mice ($COX\text{-}2^{-/-}$). Tissue factor (TF), Annexin A2 (ANXA2) and S100A10 were analysed in venous thrombi and in peritoneal macrophages (PM) isolated from WT and $COX\text{-}2^{-/-}$ mice.
Results. COX-2 deletion predisposes to VT as suggested by greater clot firmness and clot elasticity, by higher plasma levels of functional fibrinogen, factor VIII and PAI-1 activity, and proved by bigger thrombi detected after IVCL compared to WT mice. $COX\text{-}2^{-/-}$ thrombi have greater fibrin content, higher number of $F4/80^+$, TF^+ and $ANXA2^+$ cells, and lower $S100A10^+$ cells. Remarkably, monocyte depletion reduced thrombus size in mutant mice, suggesting a key role of $COX\text{-}2^{-/-}$ monocytes in this experimental setting. Interestingly, COX-2 deletion increased ANXA2, reduced S100A10, promoted assembly of

ANXA2/p50NF-kB complex and its nuclear accumulation, and induced TF in PM, whereas ANXA2 silencing decreased dramatically TF.
Finally, treatment with a stable analogue of prostacyclin, Carbaprostacyclin, prevented VT formation in mutant mice, reduced the ANXA2 binding to p50NF-kB subunit and its nuclear trafficking, and decreased TF in COX-2$^{-/-}$ PM.
Conclusion. The increased activation of haemostatic system observed in COX-2$^{-/-}$ mice may partly explain their predisposition to thrombosis. In addition, COX-2 deletion promotes macrophage TF activity by nuclear accumulation of ANXA2 sustaining venous thrombus growth, which suggests a new role for ANXA2 in venous thrombosis.

IDENTIFICATION AND CHARACTERIZATION OF A LARGE DELETION INCLUDING THE LIPOPROTEIN LIPASE GENE IN A FAMILY WITH SEVERE HYPETRYGLICERIDEMIA

C. Giacobbe[1,2], M.D. Di Taranto[2], C. Pagano[3], G. Marotta[3], P. Rubba[3], G. Fortunato[1,2]
[1]CEINGE S.C.a r.l. Biotecnologie Avanzate, Napoli;
[2]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli;
[3]Dipartimento di Medicina Clinica e Chirurgia, Università degli Studi di Napoli Federico II, Napoli

Aim. Familial Severe Hypertriglyceridemia (HTG) is a rare autosomal recessive disorder characterized by high plasma levels of triglycerides (TG>10 mmol/L), eruptive xanthomas, lipaemia retinalis and recurrent pancreatitis, with a population prevalence of approximately one in a million. HTG is caused by mutations in five different genes: Lipoprotein lipase (LPL), Apolipoprotein A-V (APOA5), Apolipoprotein C-II (APOC2), Glycosyl-phosphatidyl-inositol-anchored HDL-binding protein (GPIHBP1), and Lipase maturation factor-1 (LMF1). We aim to study a 32 years old woman with a diagnosis of severe HTG (TG>20 mmol/L) and several episodes of pancreatitis.
Patient, Materials and Methods. After genomic DNA extraction from peripheral blood samples, the 5 genes associated with HTG, were amplified by PCR and directly sequenced. SALSA MLPA kit (MRC-Holland) was used to identify large rearrangements in the LPL gene and the array-Comparative Genomic Hybridization (array CGH) was used to define the deletion boundaries.
Results. The patient is a compound heterozygote for 2 mutations in the LPL gene: c.755T>C (p.Ile252Thr) in the exon 5 and a large deletion of entire gene. The c.755T>C mutation was previously described as causative of lipoprotein lipase deficiency, while the second mutation leads to the absence of protein. For the first time, the deletion of the entire gene was better defined by array CGH indicating a deletion of 650Kbp including four genes: SH2D4A, CSGALNACT1, INTS10, LPL. By a cascade screening, the patient's brother with maximum TG of 15.4 mmol/L, without previous pancreatitis, was analyzed and resulted to be a compound heterozygote carrying the same mutations of his sister. It was finally evaluated the LPL mass level, necessary for the gene therapy treatment with "*Glybera*".
Conclusions. The genetic analysis confirmed the diagnosis of severe HTG and allowed to identify and treat our patient with gene therapy.

THE PLACENTAL GROWTH FACTOR (PLGF) HAS A CRUCIAL ROLE IN HYPERTENSION INDUCED BY DESOSSICORTICOSTERONE ACETATE (DOCA)-SALT, MEDIATING THE ACTIVATION OF IMMUNITY IN THE SPLEEN

M. Perrotta[1], G. Cifelli[1], S. Fardella[1], R. Iacobucci[1], F. Mastroiacovo[1], F. Pallante[1], V. Fardella[1], L. Carnevale[1], G. Lembo[1,2], D. Carnevale[1,2].
[1]Dipartimento di Angiocardioneurologia e Medicina Traslazionale, IRCCS Neuromed, Pozzilli, Isernia;
[2]Dipartimento di Medicina Molecolare, "Sapienza" Università di Roma

Some hypertensive patients show lower levels of renin, associated to sodium-sensitivity. In experimental models, this condition can be induced by a chronic administration of deoxycorticosterone acetate combined with a high sodium content diet (DOCA-salt). Indeed, chronic DOCA-salt causes an elevation of arterial blood pressure consequently to an impairment of RAS peripherally; whereas more interesting, the brain RAS is activated by this treatment.
Previously, we demonstrated that the placental growth factor (PlGF) mediates the interaction between the sympathetic nervous-and-the immune system activation, needed to recruitment of T lymphocytes into target organs of hypertension.
It is interesting to explore the involvement of PlGF in the regulation of adaptive immunity in DOCA-salt hypertension. Therefore, in order to evaluate the role of PlGF, KO and respective WT mice were subjected to DOCA-salt for 21 days or placebo as control and monitored by both tail cuff and radiotelemetry measurements, showing that KO mice were significantly protected from the blood pressure increase caused by this hypertensive stimulus. Then, we evaluated whether the DOCA-salt can activate the PlGF expression in the spleen, finding that PlGF is significantly induced in the marginal zone by immunofluorescence analysis. Additionally, by surgical removal of the coeliac ganglion, we demonstrated that PlGF expression depends on the integrity of DOCA-salt activated drive. To assess how this neuroimmune regulation could affect the immune responses on target organs of hypertension, we conducted flow cytometry and immunohistochemistry analyses to evaluate the presence of T lymphocytes into kidneys, finding a significant reduction in PlGF KO mice after DOCA-salt. The reduction of renal fibrosis in PlGF KO mice upon DOCA-salt, compared to WT mice, showed protection from parenchymal renal damage.
These data candidate PlGF as a mediator of the pathway responsible for the activation of immune splenic T cells, that is crucial for the hypertensive response to DOCA-salt.

ROLE OF MICRORNA-155 IN THE PATHOGENESIS AND TREATMENT OF PSORIATIC ARTHRITIS

Toro, U. Ianni, E. Celletti, E. Sabatini, D. De Cesare, F. Cipollone, M. Bucci
Department of Medicine and Science of Aging, School of Medicine and Health Sciences, University of Chieti-Pescara; Ce.S.I. - Me.T., Chieti

Aim. MicroRNAs (miRNAs) are small non-coding RNA sequences capable of binding to specific mRNAs. Dysregulation of their function causes altered gene transcription. Psoriatic arthritis (AP) is a chronic inflammatory arthropathy often related to "dysregulated" levels of miRNAs. The miRNA-155 (miR-155) is an example of multi-functional miRNA responsible for controlling pathological processes that contribute to the development of the PA. Understanding the role of miR-155 may give us new insight on the pathogenesis of the disease as well as open new therapeutic prospective. The aim of the study was to evaluate serum levels of miR-155 at the beginning of the biological therapy and after 6 months of treatment.
Methods. We collected plasma samples from 9 patients with diagnosis of PA (CASPAR criteria). These patients require the introduction of a biological drug (TNF-inhibitors: Infliximab, Etanenercept, Adalimumab). The first sampling was performed prior to starting treatment with biologic drug; a second sampling was done after six months to evaluate the changes induced by the on-going therapy.
Results. Complete data include only the 3 patients who completed the therapeutic window of 6 months. The reassessment was done using a validated score (CDAI SCORE) to determine the degree of illness and to get a clinical predictive parameter capable of anticipating the lab result. All patients showed significant clinical improvement with a reduction in the baseline score and this data correlates with significantly reduced levels of miR-155 observed in response to treatment in patients who had completed the six-monthly cycle.
Conclusions. These results, even if partial and preliminary, suggest that miRNAs represent not only an important player in the pathogenesis of the disease, but also a potential therapeutic target. Correct those miRNAs expressed in an aberrant manner could stop the process by acting on gene expression of several elements involved in the development of the disease.

ANTI-ATHEROSCLEROTIC EFFECTS OF NUTRACEUTICAL COMPOUND "OLEACTIV®" IN HYPERCHOLESTEROLEMIC MODEL OF HASMTER

A.Piemontese[1], S. Battista[1], C. Romain[2], F. Bernini[1], J. Cases[3], I. Zanotti[1]
[1]Department of Doof and Drug, University of Parma; [2]R&D Project Manager, Fytexia Company, Vendres, France, [3]Scientific Director, Fytexia Company, Vendres, France

Aim. In the last decades, the use of nutraceutical compounds is spreading in different therapeutic fields, such as in the treatment of atherosclerosis. Aim of this study is to evaluate the effects of the Oleactiv® compound on atherosclerotic plaque development, lipid and lipoprotein profile in hypercholesterolemic hamsters. Oleactiv® is a nutraceutical compound patented by Fytexia Company. Experimentations are part of an industrial project, and Oleactiv® composition is secreted.
Material and Methods. 30 *Golden syrian* hamsters were divided in 3 groups. Standard group (STD group), fed with normolipidemic diet, received daily vehicle for 12 weeks. The remaining groups, fed with a hypercholesterolemic diet (2g/kg of cholesterol), received daily vehicle (CTRL group), or Oleactiv® (OLA group). After sacrifice, the aortic arch was dissected and stained with Oil-Red-O to evaluate aortic fatty streaks. In addition, plasmatic HDL, total cholesterol (TC), non-HDLc, triglycerides (TG) were analyzed. We evaluated total efflux, ABCA1-mediated efflux and passive diffusion efflux using a radiolabelled technique in murine macrophages J774.
Results. OLA administration induced a significant decrease of foam cells infiltration (-1.9% of aortic total area), indicating a significant reduction of lipid deposits by -69% ($P<0.0001$) of Aortic Fatty Streak Area compared to CTRL group. Hamsters of Ola group showed decrease of TC (-1,04 mmol/L), non-HDLc (-1,14 mmol/L) and a significant decrease of TG (-1,21 mmol/L) vs CTRL group. Conversely, HDL-C levels are similar in two groups. Interestingly, plasma of OLA group showed an higher passive diffusion in comparison with CTRL group (28,44%, $p<0,05$). In addition, ABCA1-mediated efflux of sera treated with Oleactiv® was increased vs CTRL group (14,5%). Overall, OLA induced a significant increase of total Cholesterol Efflux Capacity (17,33%, $p<0,05$) vs CTRL group.
Conclusions. Results show that the administration of Oleactiv® reduces atheroma development and has positive effects on lipid profile of an animal model more akin to human, compared to other animal models. In addition, the increased CEC, due to an increased ABCA1-mediated efflux by Oleactiv®, may be a potential anti-atherosclerotic mechanism, underlying the atheroma reduction observed.

ASSOCIATION BETWEEN APOE RS7412 POLYMORPHISM AND CAROTID ATHEROSCLEROSIS IN THE ELDERLY

Y. Ferro[1], R.M. Mancina[2], G. Gorgone[3], A. Fava[3], E. Mazza[1], S. Rotundo[3], M. Moraca[3], S. Romeo[1,2], A. Pujia[1], T. Montalcini[4]
[1]Department of Medical and Surgical Science, Nutrition Unit, University Magna Grecia, Catanzaro;
[2]Department of Molecular and Clinical Medicine, University of Gothenburg, Sweden;
[3]Nutrition Unit, University Magna Grecia, Catanzaro;
[4]Department of Clinical and Experimental Medicine, Nutrition Unit, University Magna Grecia, Catanzaro

Aim. Aging increases the risk of cerebrovascular diseases (CVD), but not all older people develop these diseases. To date a number of risk factors have been identified, but still many others could be identified. The two single nucleotide polymorphisms (SNPs), rs7412 and rs429358, that collectively form the ε2, ε3, and ε4 alleles of apolipoprotein E (ApoE) are genetic risk factors for dementia, Alzheimer's disease, dyslipidemia and coronary atherosclerosis. Even though most CVD occurs in elderly, there are few studies of the effects of these polymorphisms on CVD in these people. The aim of this study is to evaluate the link between ApoE-SNPs and carotid atherosclerosis in Italian elderly.
Methods. We enrolled consecutively 94 Caucasian elderly, of both gender, free of CVD and dementia. We performed ultrasonography of the carotid arteries and genotyped for the two main ApoE-SNPs (rs7412 and rs429358), both located in the fourth exon of the ApoE.

Logistic regression analysis - Factors associated with Carotid atherosclerosis

					C.I. 95%	
Dependent variable Carotid plaque	**B**	**SE**	β	**P Value**		
Model I					LL	UL
BMI	0.16	0.79	1.17	0.042	1.00	1.37
ApoE rs7412-CC	2.11	0.88	0.12	0.017	0.02	0.68
Model II					LL	UL
ApoE rs7412-CC	2.20	0.88	0.11	0.013	0.01	0.63
Model III					LL	UL
DBP	-0.08	0.03	0.92	0.011	0.86	0.98
CIMT	6.85	2.34	951	0.003	9.57	945
ApoE rs7412-CC	1.82	0.77	0.16	0.019	0.03	0.73

Note. Excluded variables-model I: gender, smokers, age, SBP, DBP, glucose; LDL-Cholesterol; model II: gender, smokers, hypertension, age, BMI, glucose, LDL-Cholesterol; model III: gender, SBP, BAD; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; CIMT = carotid intima-media thickness; CI = confidence interval; LL = lower limit; UL = upper limit.

Results. The mean age was 69±4 years; the mean BMI was 28±4 kg/m^2, MMSE 24±8, ADAS-Cog 16±7 and 68% of the subjects were female. The prevalence of carotid plaque was 71%. Subjects with ApoE rs7412 C/C genotype had a higher prevalence of carotid plaque than those with ApoE rs7412 C/T + T/T genotype (75% vs 40%, p=0.030; respectively). In logistic regression, analysis carotid plaque was significantly associated with BMI, diastolic blood pressure, CIMT and C/C homozygotes, while was not associated with ApoE rs429358 SNP.
Conclusions. This study may provide evidences of an association between the presence of carotid atherosclerotic plaque and ApoE rs7412 SNP in Italian free-living elderly and explain the high risk found for CVD in a sub group of elderly.

LDLR, PCSK9, AND LDLRAP1 MUTATIONS IN THE SAME PATIENT IN A FAMILIAL HYPERCHOLESTEROLEMIA (FH) FAMILY

G. Cocci, F. Spannella, F. Giulietti, P. Balietti, R. Sarzani
Internal Medicine and Geriatrics, Hypertension Excellence Centre of the ESH, IRCCS-INRCA, UNIVPM

Aim. Mutations in four identified genes cause familial hypercholesterolemia (FH). We report a case with multiple mutations on 3 of the 4 genes involved in FH (LDLR, PCSK9 and LDLRAP1) in the same subject.
Methods. Genetic variants (substitutions, indels or copy number variations) associated with autosomal dominant hypercholesterolemia (ADH) and autosomal recessive hypercholesterolemia (due to LDLRAP1 mutations) were detected using SEQPRO Lipo genetic test (Progenika Biopharma) in the contest of the LIPIGEN project.
Results. A 12 years-old female, hospitalized in the Department of Cardiac Surgery, had LDLc=332 mg/dl. The DUTCH Lipid Score was 9, diagnostic for FH. We found 4 mutations: 3 mutations inherited from her father (with LDLc=283 mg/dl) and 1 de novo mutation. No pathogenic mutations were detected in the mother. There were 2 mutations in the LDL receptor (LDLR) in different positions: 1 possible pathogenic variant p.Ser670Leu (exon 14) and 1 probable pathogenic variant p.Ala(-9)_Ala(-3) del (exon 1). However, for both mutations there is no full validation of their pathogenicity. We also found 1 mutation in PCSK9 p.Ala443Thr (exon 8) with loss of function of the gene, which is associated with hypocholesterolemia. The fourth was a heterozygous de novo mutation in LDLRAP1 p.Thr218Ile (exon 7).
Conclusion. To the best of our knowledge, this is the first case carrying 4 mutations in 3 of the 4 FH known genes. Up to now, the pathogenicity of the LDLR mutations found in our patients is still not validated. LDLRAP1 heterozygous mutation alone is not sufficient condition to develop the disease. Therefore, the high LDLc levels, despite the protective PCSK9 mutation, may reinforce the pathogenic role of these LDLR mutations, especially of the probable pathogenic variant p.Ala(-9)_Ala(-3) del (exon 1), in this peculiar genetic context.

SUBCLINICAL ATHEROSCLEROSIS AND FETUIN-A PLASMA LEVELS IN ESSENTIAL HYPERTENSIVE PATIENTS

M. Guarneri, L. Guarino, C. Pugliares, G. Mulè, F. Vaccaro, C. Luna, S. Cottone
U.O. di Nefrologia ed Ipertensione, Dipartimento Biomedico di Medicina Interna e Specialistica, Università degli Studi di Palermo

Aim. The intima-media thickness (IMT) is considered as a surrogate marker for atherosclerotic disease. The aim of this study was to analyze the relationship of carotid IMT with fetuin-A in patients with essential hypertension (EH) and normal renal function.
Methods. The plasma levels of fetuin-A, interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α) and the biomarker of oxidative stress 8-iso-PGF2alpha were assayed in samples from 105 untreated EH patients. Carotid IMT measurements were also performed. EH was studied overall and after dividing in EH with IMT ≥ and o <0.9 mm.
Results. All of the biomarkers were significantly different between the two subgroups, in particular, the fetuin-A level was lower in the patients with an IMT ≥0.9 mm. In the overall group, the linear analysis of correlation demonstrated that the IMT was significantly inversely correlated with the fetuin-A level (r=-0.40, P<0.0001) and directly with TNF-a (r=0.39, P<0.0001), IL-6 (r=0.38, P<0.0001) and 8-iso-PGF2alpha (r=0.356, P<0.0003). The multiple regression analysis performed that assigned IMT as a dependent variable showed that fetuin-A (β=-0.268, P<0.0001) was independently correlated with the IMT. Receiver-operator curves demonstrated that fetuin-A levels have a predictive power of IMT>0.9 mm (AUC (area under the curve) 0.738, P<0.0001).
Conclusions. Our results suggest that in EH, fetuin-A is associated with the IMT independently of oxidative stress and renal function, thus predicting increases in the IMT.

FOKI AND BSMI VITAMIN D RECEPTOR GENE POLYMORPHISMS AND ESSENTIAL ARTERIAL HYPERTENSION

S. Cottone[1], L. Guarino[1], C. Scazzone[2], C. Pugliares[1], M. Guarneri[1], G. Mulè[1]
[1]U.O. di Nefrologia ed Ipertensione, Dipartimento Biomedico di Medicina Interna e Specialistica, Università degli Studi di Palermo;
[2]Dipartimento di Biopatologia e Biotecnologie Mediche e Forensi, Università degli Studi di Palermo

Aim. Our aims were to analyze the relationship between 25-hydroxyvitamin D (25[OH]D) plasma levels and clinical and ambulatory blood pressure (BP) values, and to identify any possible association between hypertension and FokI and BsmI vitamin D receptor (VDR) gene polymorphisms in essential hypertensive patients.
Methods. Seventy-one essential hypertensive patients and seventy-two controls, 18-75 years old, were enrolled. Patients underwent clinical BP, 24-h ambulatory BP monitoring, 25[OH]D and plasma renin activity (PRA) evaluations. In both groups we studied VDR gene polymorphisms, FokI and BsmI by PCR-RFLP.
Results. We observed a significant negative correlation between 25[OH]D and 24h systolic BP (r=-0.277, p=0.043). This correlation persisted in backward stepwise multivariate analyses (β=-0.337; p=0.022) including as covariates first age, gender, body mass index, glomerular filtration rate estimated (eGFR) by MDRD equation, and secondly PRA. We did not observe statistically significant correlation between 25[OH] D and PRA. When we compared anthropometric, clinical and bio-humoral parameters among patients with different VDR (FokI and BsmI) genotypes, we found a significant difference of clinic diastolic BP values among the three FokI genotypes (p=0.018). Significantly higher diastolic BP values in patients with ff FokI genotype compared with patients with Ff FokI genotype (p=0.002) were disclosed through the Mann-Whitney U test. Lastly any association between a specific genotype or allele and hypertension or PRA wasn't found when we compared allelic frequencies and genotype distribution between patients and controls.
Conclusions. Our findings confirm the relation between 25[OH]D and BP values in essential hypertensive patients and they suggest that FokI and BmsI VDR polymorphisms is not associated either with hypertension or with PRA.

OMEGA-6 FATTY ACIDS IN ERYTHROCYTE MEMBRANE ARE INVERSELY ASSOCIATED WITH SEVERAL FEATURES OF THE METABOLIC SYNDROME IN A GROUP OF OBESE CHILDREN

S. Bonafini[1], A. Tagetti[1], A. Mantovani[1], A. Donato[1], A. Dalbeni[1], C. Maffeis[2], F. Antoniazzi[2], P. Minuz[1], C. Fava[1]
[1]University of Verona, Department of Medicine, Verona;
[2]University of Verona, Department of Surgery, Dentistry, Paediatrics and Gynaecology, Verona

Objective. Overweight and obesity lead to the clustering of cardiovascular (CV) risk factors and the metabolic syndrome (MetS) not only in adults but also in children and are often accompanied by non-alcoholic fatty liver disease. Quality of dietary fat, beyond the quantity, can influence CV risk profile.
Design and Method. This observational study aimed to evaluate the associations of individual CV risk factors, characterizing the MetS, with erythrocyte membrane FA, in a group of obese children.
Results. We enrolled 70 children (BMI =29.4±4.4 Kg/m^2; percentile of BMI =98.0±1.7), aged 5-17 years. Mean content of Omega-3 FA was low (Omega-3 Index =4.7±0.8%). Omega-3 FA were not associated with MetS characteristics, whereas omega-6 FA, in particular arachidonic acid (AA), were inversely associated with several features of MetS: AA resulted inversely correlated with waist circumference (r_S=-0.352), waist/hip ratio (r_S=-0.311), Waist/height ratio (r_S=-0.248), triglycerides (r_S=-0.366), fasting insulin (r_S=-0.337), 24-hour-SBP (r_S=-0.313), daytime-SBP (r_S = -0.267), nighttime-SBP (r_S=-0,245) and nighttime DBP (r_S=-0.344). Contrarily, total amount of saturated FA (SFA) and specifically, palmitic acid, correlated positively with waist circumference (r_S=0.254), waist/hip ratio (r_S=0.247), total cholesterol (r_S=0.258), triglycerides (r_S = 0.373) and fasting insulin (r_S=0.287).
Thirty-five children (50%) had hepatic steatosis. Fatty Liver Index (FLI), a predictive score of steatosis, was directly correlated to SFA (r_S=0.479), palmitic acid (r_S=0.515) and inversely to omega-6 FA (r_S=-0.435) and AA (r_S=-0.472). AA was inversely correlated with ALT (r_S=-0.331) and palmitic acid directly with GGT (r_S=0.339).
Conclusions Omega-6 FA, and especially AA, may be protective toward CV risk factors featuring the MetS and also to indexes of hepatic steatosis in obese children, whereas SFA seems to exert opposite effects.

ANALYSIS OF CHILDREN AND ADOLESCENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

I. Minicocci, PhD[1], S. Pozzessere, MD, PhD[1], C. Prisco, PhD[1], A. Montali, BSc[1], A. di Costanzo, PhD[1], E. Martino, *MD[2], F. Martino, MD[2], M, Arca, MD[1]*
[1]Department of Internal Medicine and Allied Sciences;
[2]Department of Pediatrics, Sapienza University of Rome

Aim. To evaluate the effectiveness of criteria based on child-parent assessment in predicting familial hypercholesterolemia (FH)-causative mutations in unselected children with hypercholesterolemia.
Methods. LDLR, APOB and PCSK9 genes were sequenced in 78 children and adolescents (mean age 8.4±3.7 years) with clinically diagnosed FH. The presence of polygenic hypercholesterolemia was further evaluated by genotyping 6 low-density lipoprotein cholesterol (LDL-C)-raising single-nucleotide polymorphisms.
Results Thirty-nine children (50.0%) were found to carry LDLR mutant alleles but none with APOB or PCSK9 mutant alleles. Overall, 27 different LDLR mutations were identified, and 2 were novel. Children carrying mutations showed higher LDL-C (215.2±52.7 mg/dL vs 181.0±44.6 mg/dL, P<.001) and apolipoprotein B levels (131.6±38.3 mg/dL vs 100.3±30.0 mg/dL, P<.004), compared with noncarriers. A LDL-C of ~190 mg/dL was the optimal value to discriminate children

with and without LDLR mutations. When different diagnostic criteria were compared, those proposed by the European Atherosclerosis Society showed a reasonable balance between sensitivity and specificity in the identification of LDLR mutations. In children without mutation, the FH phenotype was not caused by the aggregation of LDL-C raising single-nucleotide polymorphisms.
Conclusions In unselected children with hypercholesterolemia, LDL-C levels >190 mg/dL and a positive family history of hypercholesterolemia appeared to be the most reliable criteria for detecting FH. As 50% of children with suspected FH did not carry FH-causing mutations, genetic testing should be considered.

ASSOCIATION BETWEEN AMBULATORY ARTERIAL STIFFNESS INDEX, MARKERS OF BLOOD PRESSURE VARIABILITY AND INDICES OF SUBCLINICAL VASCULAR DAMAGE IN OBESE CHILDREN.

A. Tagetti[1], S. Bonafini[1], P. Minuz[1], C. Fava[1]
General Medicine and Hypertension Unit, Department of Medicine, University of Verona

Aim. Ambulatory Arterial Stiffness Index (AASI) and symmetric AASI (sAASI) have been proposed as indices of arterial stiffness easily obtained by 24-hour ambulatory blood pressure monitoring (ABPM). Moreover, ABPM allows the analysis of indices of BP variability such as day and night SD, nocturnal BP dipping, weighted 24-h SD (wSD), average real variability (ARV). The relationship between these indices and other markers of vascular subclinical damage has seldom been evaluated in children. Aim of the present study was to address this issue in a sample of obese children.
Methods. 45 obese children (27 males,18 females), were included. Children underwent vascular measurements, including:
1) office and 24-hour ambulatory BP;
2) brachial flow-mediated dilatation (FMD), carotid intima media thickness (cIMT), and distensibility (cDC) measured using ultrasound;
3) systemic arterial stiffness (SIDVP) measured using digital volume pulse analysis. From ABPM (if at least 70% of the programmed BP measurements were correctly recorded), we calculate AASI, sAASI, ARV, SD, SD, systolic and diastolic dipping and wSD.

Results. ARV showed a significant correlation with SIDVP (r=0.379; p=0.023), index of systemic stiffness. AASI but not sAASI correlated with FMD (r=0.361; p=0.031), marker of endothelial function. In the population divided in hypertensive (n=11)/normotensive (n=34), on the basis of office BP values above 95th percentile according to sex and age, ARV was associated with SIDVP only in normotensive (r=0.446; p=0.015) but not in hypertensive children ($r_{spearman}$=0.000; p=1). In normotensive, z score-BMI was correlated with both sAASI and wSD (respectively 0.340; p=0.049 and 0.423; p=0.014), wSD correlated with FMD (r=0.384; p=0.048); in hypertensive children, ARV correlated with FMD (r=0.828; p=0.011; $r_{spearman}$=0.738; p=0.037). No indices of BP variability correlated with cIMT or cDC.
Conclusions. BP variability, in particular ARV, shows a correlation with systemic but not local vascular stiffness in a sample of obese children, suggesting a relation between daily BP variability and arterial elastic properties. Some other relations between BP variability and endothelial function, were detectable only in the subgroups of children divided according to hypertensive status. Further studies, especially perspective ones, are needed to clarify the pathophysiological significance of these relations.

EARLY MICROVASCULAR MODIFICATIONS AND ALTERATION OF NITRIC OXID HOMEOSTASIS IN METASTATIC RENAL CELL CARCINOMA PATIENTS TREATED WITH VEGF-INHIBITORS

A. Dalbeni[1], F. Famà[1], C. Ciccarese[2], M. Bevilacqua[1], G. Tortora[2], P. Minuz[1], F. Massari[2], R. Iacovelli[2], C. Fava[1]
[1]Department of Medicine, General Medicine and Hypertension Unit, University of Verona;
[2]Department of Medicine, Oncology Unit, University of Verona

Aims. Cardiovascular adverse effects and development of hypertension have been described in patients with several types of cancer treated with VEGF-inhibitors. The mechanism of angiogenesis inhibitors-related hypertension is still debated. The aim of this work was to study the effect of anti-angiogenic drugs on arterial blood pressure, endothelial function and microvascular structure in patients with metastatic renal cell carcinoma.
Methods. we investigated 19 patients (16 males and 3 females, medium age 59±10) at the beginning of anti-angiogenic therapy (T0), one month (T1), three months (T2) and six months (T3) later. Patients underwent office and home arterial blood pressure measurement, Flow Mediated Dilatation (FMD), dosage of urine nitrates, carotid artery compliance by ultrasonography and nailfold capillaroscopy (NC).
Results. At T1 8 patients showed a clinically significant increase in blood pressure (systolic blood pressure T1-T0≥10 mmHg ordyastolic ≥5 mmHg). Nitrate concentration in urine at T0 was lower in the group showing an increase in blood pressure at T1 than in the group showing no increase at the same time (nitrate/creatinine T0 62,96±30,80 uM/mmol vs. 147,29±81,64 uM/mmol; p-value=0,01). At T2 and T3 FMD was lower in the group showing an increase in blood pressure at T1 than in the group showing no increase at T1 (Δ-FMD T2-T0 -7,33±6,09 vs 2,35±8,60; p-value=0,03; Δ-FMD T3-T0-7,80±7,62 vs -0,55±4,30; p-value=0,03). At T0 none of the patients presented morphological abnormalities at NC while at T1 52,6% of patients developed NC changes, at T2 70,6% and at T3 81,2%, with no clear correlation with increase in blood pressure. At T3 68,7% of subjects showed stable disease or disease remission with imaging studies (CT), without correlation with increased blood pressure or changes in NC.
Conclusion. Our findings are consistent with an early effect of anti-angiogenic drugs in modifying microvascular structure and with an increase in arterial blood pressure by potential role of nitric oxide. Further analysis in larger patient groups are needed to confirm our data.

PREVALENCE OF RESISTANT ARTERIAL HYPERTENSION AND SECONDARY CAUSES IN A COHORT OF HYPERTENSIVE PATIENTS: THE RESULTS OF A SPECIALIZED CENTER OF HYPERTENSION

A. Concistrè[1] L. Petramala[1], F. Olmati[1], M. Galassi[1], G. De Toma[2], C. Letizia[1]
[1]Specialized Center of Secondary Hypertension, Department of Internal Medicine and Medical Specialties, and [2]Department of Surgery "Pietro Valdoni", Policlinico "Umberto I", University of Rome "Sapienza", Rome

Background. Despite the growing number of clinical studies in the past decade, the prevalence of resistant hypertension (RHT) still remains unknown. Aim of the study was to investigate in a large cohort of hypertensive patients the prevalence of RHT. Moreover, we sought to recognize the prevalence of secondary causes below RHT.
Material and Methods We enrolled a series of 3.685 consecutive hypertensive patients who were referred to our Specialized center of Secondary Hypertension (SH). All patients underwent complete physical examination, laboratory tests, screening for SH with hormone analysis. Ambulatory Blood Pressure Monitoring (ABPM) was performed to exclude white-coat hypertension. Further, we used the Epworth Sleepness Scale Questionnaire and performed nocturnal polysomnography to discover any obstructive sleep apnea syndrome (OSA).
Results In 3.685 hypertensive patients, 232 patients (5.8%) fulfilled criteria for RHT. In these RHT patients, 91 (39%) had a SH. 56 (61%) patients had a primary aldosteronism (PA), 22 (24%) had OSA, 7 (7.7%) had an hypercortisolism (SC), and 5 (5.5%) had a renovascular hypertension (RVH). Only one patient had adrenal pheocromocytoma (PHEO).
Conclusions We suggest with our abundant data that an accurate definition and investigation into RHT is needed. First, we recommend ABPM to all patients at diagnosis. Second, all patients must be screened for SH, such as adrenal hypertension, OSA and RVH, especially those who are apparently resistant to polypharmacologic treatment. Once detected, they must be appropriately treated and cured.

CORRELATION BETWEEN CAROTID ATHEROSCLEROSIS AND CORONARY ARTERY DISEASE: A RETROSPECTIVE STUDY OF 1067 PATIENTS

D. Migliorino, A. Mignano, G. Polizzi, S. Evola, G. Novo, E. Corrado, S. Novo
Chair and Division of Cardiology, Centre for the Early Diagnosis of Preclinical and Multifocal Atherosclerosis, Reference Regional Centre for the Diagnosis and Care of Heart Failure, Department of Internal Medicine and Cardiovascular Diseases, University Hospital "Paolo Giaccone" of Palermo

Aim. The aim of our study was to evaluate the association of carotid atherosclerosis evaluated by Doppler ultrasound with the severity of coronary artery disease (CAD) in patients with typical chest pain undergoing diagnostic coronary angiography during hospitalization.
Methods. we studied retrospectively 1067 patients admitted to our Cardiology Unit for chest pain that underwent to coronary angiography. According to ESC Guidelines on CV prevention we considered carotid arteries as normal if intima-media thickening <0,9 mm, with intima media thickness if >0.9 and <1,5 mm, with plaque when protrusion into the arterial lumen was >1,5 mm. During the coronary angiography we considered one, two or three vessel disease if coronary vessels had stenosis >50%.
Results. carotid ultrasound examination showed a 81% prevalence of asymptomatic carotid plaques, whereas coronary angiography showed that 12% of patients had normal coronary arteries. The detection of carotid plaque was predominantly associated with the presence of angiographically diseased coronary arteries in 72,8% of patients (p=0,0015). Particularly, the presence of a carotid plaque with a diameter >2,5 mm (p<0,0001) was associated with a higher prevalence of coronary artery disease. Dimensions of carotid plaques were significantly correlated with the complexity of coronary artery disease calculated by Syntax score (p<0,0001). Moreover bilateral carotid atherosclerosis was associated with coronary atherosclerosis too (p<0,0001). Besides the detection of carotid atherosclerosis was strongly correlated with the coronary artery disease itself (overall p=0,006).
Conclusions. we believe that the evaluation of Doppler ultrasound of carotid arteries might provide the clinician additional informations about the global cardiovascular risk of the patients with typical chest pain and negative markers of acute coronary syndrome. Moreover, the presence of carotid atherosclerotic plaque may be predictive of coronary atherosclerosis and its severity.

HYPERPARATHYROIDISM AND HYPOVITAMINOSIS D BEYOND BONE MINERAL METABOLISM: A POSSIBLE CARDIOVASCULAR RISK FACTOR IN MORBID OBESE.

S. Ministrini[1], M.A. Ricci[1], G. Daviddi[1], M. Scavizzi[1], A. Spinozzi[1], M. Boni[4], M.T. Paganelli[4], A.M. Scarponi[2], G. Lupattelli[1]
[1]Internal Medicine, Department of Medicine, Università degli Studi di Perugia;
[2]Internal Medicine, "S.M. della Misericordia" Hospital, Perugia;
[3]General Surgery, "S.M. della Misericordia" Hospital, Perugia;
[4]General Surgery, "S. Giovanni Battista" Hospital, Foligno (Perugia)

Aim. obesity is a risk factor for hypovitaminosis D. Many pathophysiological mechanisms have been claimed to explain the link between obesity and hypovitaminosis D, such as sequestration of vitamin D in the exceeding fat tissue. Hyperparathyroidism is a consequence of vitamin D deficiency and hyperparathyroidism has been claimed as a possible risk factor for vascular damage. The aim of the study was to evaluate a possible association among iPTH, vitamin D and early vascular impairment in morbid obesity.
Methods. We evaluated the levels of 25(OH)vitamin D and iPTH in a cohort of 150 morbid obese patients with a BMI >35 kg/sqm and stable normal levels of calcemia and eGFR.
Results. ninety-seven percent of patients had levels of 25(OH) vitamin D <30 ng/mL and 72% of them had levels of iPTH ≥65 pg/mL. Dividing our population according to the median level of BMI (44 kg/sqm), we found that patients

with a BMI above median level had also significantly higher levels of iPTH (p=.021) with no significant difference in 25(OH) vitamin D levels. Performing univariate correlations, we confirmed a significant positive correlation between iPTH, BMI (rho=.176, p=.030) and waist circumference (rho=.189, p=.021); we found also a significant positive correlation between iPTH and HOMA index (rho=.222, p=.007), systolic blood pressure (rho=.202, p=.013) and carotid IMT (rho=.179, p=.032). At the stepwise regression analysis age, 25(OH) vitamin D and waist circumference were the independent predictors of iPTH.
Conclusions. our data confirm that hypovitaminosis D and hyperparathyroidism are common metabolic disorders in obese patients, although other factors than hypovitaminosis D could be responsible for hyperparathyroidism. Furthermore, levels of iPTH correlate with markers of metabolic and vascular damage, supporting the hypothesis that hyperparathyroidism could be an additional risk factor for cardiovascular disease in morbid obese.

MARKES OF CARDIOVASCULAR RISK IN WOMEN SUFFERING FROM GESTIATIONAL HYPERTENSION

R. Carbonara[1]; F. Giardinelli[1]; F. Clemente[1], A. Paccone[1];
L. Zaccaro[1]; R. Nitti[1]; A. Mallardi[1]; C. Carbonara[2], A. Ciavarella[2],
Vulpis[2]; M.M. Ciccone[1]
[1]Sezione di Malattie dell'Apparato Cardiovascolare, Dipartimento di Emergenza e Trapianti d'Organo (DETO), Università di Bari;
[2]U.O. Medicina Interna Ospedaliera, Policlinico di Bari

Aim. Hypertensive disorders complicate about 10% of pregnancies. The endothelium is negatively influenced by hypertension and its dysfunction, assessed by FMD (Flow Mediated Dilation), can be considered a very sensitive marker of early cardiovascular damage. The objective of this study was to evaluate the presence, in a group of patients with gestational high blood pressure, of early markers of cardiovascular damage.
Methods. 14 patients diagnosed with gestational hypertension underwent: 2D-transthoracic echocardiogram; carotid IMT (intima-media thickness) measurements; brachial FMD (b-FMD) evaluation and measurements of Renal Resistance Index (IRR). These parameters have been assessed at the First Trimester (t0) and the Second Trimester (t1) and compared. A p value <0.05 has been considered as statistical significant.
Results. Significant differences were found in the antero-posterior left atrium diameter (t0: 36,6±3,93 mm vs t1: 38,1±3,0 mm; p=0,033); end-diastolic left ventricle diameter (t0: 43,8±4,37 mm vs t1: 46,4±3,34 mm; p= 0,046); left iso-volumetric releasing time (IVRT) (t0: 73,1±19,8 ms vs t1: 104±21,8 ms; p=0,006); right IVRT (t0: 96,6±18,8 ms vs t1: 123±14,9 ms; p=0,007); pulmonary artery systolic pressure (PAPs) (t0: 21,4±3,81 mmHg vs t1: 27,1±3,64 mmHg; p=0,012) and b-FMD (t0: 6,23±0,992% vs t1: 5,38±0,518%; p=0,013). No significant differences were found for what concern carotid IMT (IMTdx: p=0,446; IMTsx: p=0,861) and IRR (IRRdx: p=0,793; IRRsx: p=1.0).
Conclusions. The increase in cardiac chamber diameters is a clear marker of fluid overload; the increase of IVRT (left and right) is indicative of diastolic dysfunction. The most innovative aspect is the significant reduction of FMD in the second trimester: it may demonstrate as gestational hypertension is associated with a significant endothelial dysfunction. In addition, FMD changes precede modifications of carotid IMT and IRR: this confirms that the non-invasive assessment of endothelial function is a useful early marker of atherosclerosis and cardiovascular damage.

ASSESSMENT OF CARDIOVASCULAR RISK IN ADULT PATIENTS SUFFERING FROM MILD OSAS: FLOW MEDIATED DILATION AS EARLY MARKER OF ENDOTHELIAL DAMAGE.

R. Carbonara[1], F. Giardinelli[1], A. Ciavarella[2], C. Carbonara[3],
F. Clemente[1], A. Paccone[1], R. Nitti[1], A. Girasoli[1], V. Vulpis[2],
M.M. Ciccone[1]
[1]Department of University Cardiology, University Hospital of Bari;
[2]Department of Internal Medicine, University Hospital of Bari;
[3]Department of Geriatry, University Hospital of Bari

Aim. Strong correlations were found between respiratory alterations in OSAS and several markers of cardiovascular damages. This study investigates the relationship among mild OSAS, endothelial function and intima-media thickness (IMT). We focused on an early stage of OSAS.
Methods. 138 patients were divided into 3 groups (46 patients each): severe OSAS, mild OSAS and a control group of simple snorers. We collected anthropometric parameters, cardiovascular risk factors and all patients underwent a noninvasive assessment of endothelial function (flow mediated dilation, FMD) and vascular anatomy (carotid IMT). ANOVA and Chi-Square tests were performed. A p value <0.05 was considered significant.
Results. There were no differences in gender, diabetes, dyslipidemia and hypertension when the 3 groups were compared. Severe OSAS group showed greater values of age, BMI, IMT and lower value of FMD when compared both to mild OSAS and to the controls. Moreover mild OSAS, while not showing difference in age and IMT, demonstrated lower FMD values (p<0.001) when compared to controls.
Conclusion. FMD decreases very precociously. This is confirmed by its sensible reduction not only in severe OSAS but also in the earliest stages. FMD can be considered a marker earlier than IMT in mild OSAS.

VASCULAR FUNCTION AND MYOCARDIAL PERFORMANCE INDEXES IN CHILDREN BORN SMALL FOR GESTATIONAL AGE

R. Carbonara[1], R. Nitti[1], F. Giardinelli[1], P. Scicchitano[1]
A. Paccone[1], F. Clemente[1], A. Girasoli[1], F. Cortese[1], M.F. Faienza[2],
P. Giordano[2], V. Vulpis[3], M.M. Ciccone[1]
[1]U.O. Cardiologia Universitaria, Azienda Ospedaliero-Universitaria Policlinico Di Bari; [2]U.O. Pediatria Universitaria, Azienda Ospedaliero-Universitaria Policlinico di Bari;
[3]U.O. Medicina Interna Ospedaliera "Pende", Policlinico di Bari

Aim. Small-for-gestational-age (SGA) children have increased cardiovascular risk, but the mediating factors are poorly understood. We hypothesized that birth size could affect cardiovascular system since childhood in absence of other risk

factors. We investigated endothelial and myocardial function in SGA children with regular catch up growth.
Methods. Biochemical markers, blood pressure, flow-mediated vasodilation [FMD], common carotid intima-media thickness [cIMT], antero-posterior diameter of the infrarenal abdominal aorta [APAO] and echocardiographic parameters of left and right ventricular function were studied in 27 SGA and 25 appropriate for gestational age (AGA).
Results. SGA subjects showed a higher homeostasis model assessment index than controls (2.61±1.27 vs 1.56±0.40, p=0.01), higher values of cIMT (0.51±0.04 mm vs 0.45±0.07 mm, p=0.007) and APAO (1.31±1.35 cm vs 1.30±0.16 cm, p=0.005), and lower FMD (10.11±4.17% vs 12.34±4.28, p=0.04) than controls. The echocardiographic evaluations demonstrated that SGA had higher values of TEI index both at left and right ventricles than controls (p=0.001). A reduced systolic function of the right ventricle was also observed in SGA subjects.
Conclusions. SGA subjects showed vascular morphological and functional abnormalities respect to AGA, which increase their cardiovascular risk profile. Furthermore, a subtle cardiac alteration in both right and left ventricle functions can be outlined in SGA patients as compared to AGA.

CORRELATES OF CAROTID ORGAN DAMAGE IN PEOPLE LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS-1

F. Viazzi, B.A. Bonino, F. Cappadona, M. Mirabella, L. Taramasso, N. Riccardi, A. Di Biagio
DIMI, University of Genoa and IRCCS Azienda Ospedale Università San Martino-IST, Genova

Aim. People living with human immunodeficiency virus-1 (PLHIV) are at high risk of developing cardiovascular disease and target-organ damage due to HIV-1 infection itself, prolonged combination antiretroviral therapy (cART) and traditional cardiovascular risk factor like age, gender and smoking.
The aim of our study is to investigate prevalence and correlates of carotid -organ damage (COD and of increased renal resistive index (RRI), among HIV-1-infected patients (PLHIV) and uninfected hypertensive patients (HT-non HIV).
Method. PLHIV aged >40 years and virologically suppressed were matched with pair-age, gender and body mass index (BMI) HT-non HIV. Patients on antihypertensive treatment were excluded. All patients were evaluated for COD defined as carotid plaque or intima media thickness (cIMT) >0.9 mm by ultrasonography (US). RRI were evaluated at the interlobar arterioles by Doppler-US. Data were analysed throughout X^2 test, analysis of variance and logistic regression.
Results. Fifty-nine PLHIV were enrolled (71% men) and matched with 59 HT-non HIV. No differences were found in cIMT values (p=0.827) and in the prevalence of COD between PLHIV and HT-non HIV (36% vs 38%, p=0.79). Among PLHIVs, those found to be hypertensive (n=8, 14%) had significantly higher prevalence of COD (46% vs 21%, P< 0.05) and higher cIMT (0.747±0.104 vs 0.654±0.100 mm, p=0.0185) as compared to those normotensive. Patients with COD were older (p=0.004) and more frequently current smokers (p=0.022). At the logistic regression analysis, COD was significantly related to age (p=0.04, 95% CI 1.0-1.1) and smoke, current (p=0.178, 95% CI 1.2-12.8) or previous (p=0.04, 95% CI 1.0-7.2). Mean RRI were identical for both HIV-1 infected and uninfected patients (0.60, SD±0.05 and 0.60, SD±0.04, respectively, p=0.996).
Conclusions. In our study COD was associated to hypertension, older age and smoke, but not to HIV serostatus itself, confirming the major importance of traditional risk factors and the need of risk assessment and cardiovascular prevention measures in PLHIV.

CHRONIC STRESS AND HYPERTENSION: THE ROLE OF GENDER

D. Guarino[1,2], C. Bachi[1], R. Gherardini[1], E. Duranti[1], M. Nannipieri[1], S. Taddei[1], R.M. Bruno[1]
[1]Dpt Clinical and Experimental Medicine, University of Pisa; [2]Scuola Superiore Sant'Anna, Pisa

Aim. Psychosocial factors have been linked to cardiovascular diseases and hypertension. Chronic stress might have different cardiovascular consequences in men and women.
Our aim was to investigate the impact of perceived chronic stress on blood pressure (BP) control in men and women attending for the first time a visit in a Hypertension Outpatient Clinic.
Materials and Methods. 128 individuals (62 women and 66 men) were enrolled. The following parameters were acquired: medical history, socioeconomic status, anthropometric parameters (height, weight, waist circumference), office BP, standardized questionnaires evaluating quality of life (SF-12, 12-item Short Form Health Survey), chronic stress, anxiety and depression (DASS21 - Depression Anxiety Stress Scales) and work-related stress (ERI - Effort-reward Imbalance and DCSQ - Demands-Control-Support Questionnaire).
Results. Women tended to be older than men (61.7±12.6 vs 57.6±12.6 years; p=0.07). Women had similar systolic BP than men (143.8±15.5 vs 142.01±18.7 mmHg; p=0.57) but lower diastolic BP (79.8±8.5 vs 85.9±8.6 mmHg; p =0.0003) and BMI [26.8(24.9-29.4) vs 25.0(23.1-27.6) kg/m^2; p=0.01). Diastolic BP was higher in individuals with moderate-to-severe chronic stress (evaluated by DASS21 questionnaire) than in those with mild or no stress (85.7±7.7 vs 81.7±8.2 mmHg, p=0.023). Interestingly, diastolic BP was increased in the presence of chronic stress in women (p=0.014), who experienced higher stress scores than men (13.2±8.3 vs 9.4±7.6, p=0.02). In multivariate logistic analysis, adjusted for age, sex, and socioeconomic status, chronic stress was associated with an increased diastolic BP (ß=3.8; p=0.048). Sex tended to be an effect modifier in the relationship between chronic stress and diastolic BP (interaction term sex*stress: ß=-5.89; p=0.09), indicating a greater effect of stress on diastolic BP in women. No significant relationship was found between quality of life, work-related stress and mood disorders and BP control.
Conclusion. Perceived chronic stress per se is independently associated to higher diastolic BP values, particularly in women.

ADHERENCE, ADVERSE EVENT AND EFFICACY OF INCRETIN BASED THERAPIES IN TYPE 2 DIABETIC SUBJECTS: EXPERIENCE FROM A TERTIARY OUTPATIENT REFERRAL CENTER

S. Garofalo, F. Brancato, F. Prandi, P. Coletta D. Pergolini, A. Carnovale, M. Santilli, F. Fozzi, E. Sussolano, M. Mastronicola, M. Maranghi
Sapienza Università di Roma, Dipartimento di Medicina Interna e Specialità Mediche, Roma

Aim. The primary objective of the study was to evaluate adherence and adverse events of incretin based therapy in patients with type 2 diabetes (T2DM) at 6 and 12 months of follow-up in a tertiary outpatient referral Center (Sapienza University, Centro di Diabetologia e Prevenzione Cardiovascolare).
The secondary objective was to evaluate efficacy (A1c reduction) of incretin based therapies during the follow-up.
Method.s We consecutively recorded data of T2DM subjects starting incretin based therapy in November 2014 and with complete follow-up data at 6 and 12 months. At each visit blood tests, anthropometric variables, presence of adverse events (defined as nausea, hypoglycemia, abdominal pain, cardiovascular events, subcutaneous nodules), adherence to theraphies were recorded. DPPIV inhibitors prescribed were Sitagliptin, Vildagliptin, Alogliptin, Linagliptin, Saxagliptin and GLP1 analogues were Exenatide twice a day, Liraglutide and Lixisenatide twice a day. Data are expressed as mean ±SD or median and min- max for continuous variables and frequencies for categorical ones. SPSS version 20.0 was used for statistical analysis.
Results. We consecutively recorded data form 67 subjects with complete follow-up at 6 and 12 months. The majority of subjects were obese male, in primary prevention (mean age of 65 y.o, 10 years of diabetes duration). They were treated with insulin sensitizer (80%), oral hypoglycemic drugs (32%) and basal insulin (44%). They were started on DPPIV therapy (73.1%) and GLP1 analogues (26.9%).
After 6 months only 2 subjects discontinued incretin based therapies due to inefficacy (increase in A1c) and adverse events (abdominal pain). Furthermore, 9% of subjects changed drug type (6 from DPPIV to GLP1 analogues; and 1 form GLP1 analogue to DPPIV inhibitor). After 12 months of follow up 10 subjects (14.9%) discontinued incretin based therapies: 7 for A1c increase, 1 for nausea and gastro-intestinal side effects and 1 for subcutaneous nodules. One subject changed drug from DPPIV inhibitor to GLP1 analogue.
After 6 and 12 months A1c significantly decrease from baseline (A1c baseline 7.9%±0.5 to A1c 6 months 7.5%±0.9. and A1c 12 months 7.5%±0.8).
Conclusions. In our cohort 15.3% of subjects discontinued incretin based therapy due to inefficacy. Incretin based therapy were well tolerated in terms of side effects. Prolonged and large studies are necessary in order to establish long term adherence to incretin based therapies.

ADHERENCE TO PCSK9 INHIBITORS IN HIGH CARDIOVASCULAR RISK PATIENTS IN REAL-WORLD SETTING: RESULTS FROM A SINGLE-CENTER EXPERIENCE AND COMPARISON WITH STATIN THERAPY

F. Gragnano, C. Concilio, A. Cesaro, M. Crisci, S. Sperlongano, F. Fimiani, F. Natale, E. Pezzullo, R. Calabrò, M.a Russo, P. Calabrò
University of Campania "Luigi Vanvitelli", Division of Cardiology, Department of Cardio-thoracic and Respiratory Sciences, Naples

Aim. In real world setting there is an unmet need in adherence to statin therapy. Nonadherence is associated with increased cardiovascular risk. PCSK9 inhibitors have proved efficacy in reducing LDL-C. Considering their dosing regimen (biweekly or monthly) and low rate of adverse effects, PCSK9 inhibitors may increase patients' adherence and reduce discontinuation rate to lipid-lowering therapy. In literature, data on adherence to PCSK9 inhibitors in "real-world" setting have not been reported. Our aim was to measure level of adherence to PCSK9 inhibitors in high risk "real-world" patients and to compare results with adherence to statin. In addition, we evaluate adherence to statin in patients on PCSK9 inhibitors.
Methods. We retrospectively analyzed 102 patients with previous MI. Patients were divided in two groups: 34 patients in "PCSK9 inhibitors group" (evolocumab or alirocumab), and 68 patients in "statin group". Adherence to therapy was measured using pen count for PCSK9 inhibitors, and pill count and/or 4-items Morisky scale for statin. We considered "fully adherent" patients with adherence ≥80% or a total score of 0, "partially adherent" with adherence >40% and <80% or a score of 1 to 2, and "nonadherent" with adherence ≤40% or a score of 3 to 4.
Results. Level of adherence to PCSK9 inhibitors was higher than statin (79.4% vs 30.9% "fully adherent", p=0.75). In addition, the percentage of patients "fully adherent" to statin therapy resulted higher in PCSK9 group than in statin group (51.8% vs 30.9%; p=0.15).
Conclusions. In real-world setting, PCSK9 inhibitors may reveal higher level of adherence than statin. The use of PCSK9 inhibitors may also positively influence patients' adherence to statin. In our study, non-statistically significant results were probably due to low number of patients. Further studies with more numerous populations are needed to confirm our findings.

RELATIONSHIP BETWEEN LIPOPROTEIN(A) AND INSULIN LEVELS DURING PREGNANCY IN NOT DIABETIC WOMEN. PRELIMINARY DATA

C.O. Aragona, V. Cairo, F. Mamone, M. Scuruchi, L. Giunta, A. Di Benedetto, S. Loddo, E. Imbalzano, A. Saitta, G. Mandraffino
Department of Clinical and Experimental Medicine, University of Messina

Background and Aims. Lipoprotein(a) [Lp(a)] is a circulating lipoprotein composed of apo(a) covalently bound

to apoB100. Lp(a) is a recognized independent risk factor for cardiovascular disease (CVD). However, Lp(a) levels were found lower in diabetics with respect to the general population. It has been suggested that hyperinsulinemia may decrease Lp(a) concentrations. Pregnancy is a physiological life time characterized by glucose and lipid profile abnormalities. In this study we investigate any association between insulin and Lp(a) levels during pregnancy in not diabetic women.
Methods. We evaluated insulin levels and lipid profile, including Lp(a), in women between the 24th and the 28th gestational weeks who present a negative oral glucose tollerance test. Women selected (age 33.6±.5) had not CV or metabolic risk factors.
Results. Lp(a) was 34±27, Plasma fasting insulin 9.5±7.9. We found an inverse correlation between Lp(a) and plasma insulin (r -0.461, p=0.004), finding no correlation with other study parameters (weight, lipid profile, age, glucose). Dependence analysis showed an inverse association only between insulin and Lp(a) (B -0.460, p=0.012).
Conclusions. Our study suggests that during pregnancy Lp(a) levels are inversely related to insulin levels and show an inverse trend to other lipid fractions. These results encourage us to extend the study to the pastpartum period, in order to evaluate whether the insulin levels reduction may influence Lp(a) also in the inverse way.

ADD- PROTECTION: AN HOME CARE PROGRAM FOR HYPERTENSIVE PATIENTS WITH HIGH CARDIOVASCULARRISK

A. Valeria Pascale[1], R. Finelli[1], V. Visco[1], I. Matula[1], M. Ciccarelli[1], G. Iaccarino[1]
[1]Dipartimento di Medicina e Chirurgia, Università degli Studi di Salerno

Introduction. In Italy less than 40% of hypertensive patients that receive an antihypertensive treatment have a good control pressure (<140/90 mmHg). Among the most common causes is poor adherence to therapy, in particular in polytreated patients.
Methods. We selected among patients who access the Outpatient Clinic for hypertension at the AOU San Giovanni e Ruggi in Salerno, those with poor BP control despite optimal drug therapy and with declared poor adherence to therapy. We recruited them in an home care program, funded by Salerno local health authority, including a weekly nurse access, for four weeks, and telemonitoring through 3G connected devices of systolic (SBP) and diastolic (DBP) blood pressure, heart rate (HR) and body weight measuring and Oxygen Saturation. Each patient was instructed to scheduled self-assesment of those parameters that were collected on the Local Health Authority Server of Salerno. The compliance was verified weekly by the nurse through drug blister count.
Results. From the data collected on the Local Health Authority Server we observed a significant improvement of SBP (before 165,57 mmHg versus after 142,29 mmHg; *p*value 0.005) and as much a reduction of mean blood pressure (MBP) from 110,8 mmHg to 96,4 mmHg (*p*value 0.003).
Conclusion. The presence of nurse at patient's home and the assets of medical devices 3G connected by which the patients can communicate in every moment with and their specialist doctors, improve patient's compliance and enhance blood pressure profile. This suggest that strategies of ICT based home care might represent a real breakthrough in the management of hypertensive patients, indeed, to reach the therapeutic goals it's not only important the optimization of pharmacological strategies, but also define a proper management of the patient, which increases awareness and adherence to therapy.

CARDIO-METABOLIC PROFILE IN A COHORT FROM LOMBARDY REGION: THE PLIC STUDY

F. Galimberti[1], L. Chiodo[1], M. Casula[1], A. Baragetti[2], L. Grigore[2], F. Pellegatta[2], G.D. Norata[2], E. Tragni[1], A.L. Catapano[1,2,3]
[1]Epidemiology and Preventive Pharmacology Centre (SEFAP), Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano;
[2]SISA Center for the Study of Atherosclerosis, Bassini Hospital, Cinisello Balsamo, Milano;
[3]IRCCS Multimedica Hospital, Sesto San Giovanni, Milano

Aim. This study aimed to describe the profile of the cardio-metabolic risk factors and the intima-media thickness (IMT) of the carotid artery in a cohort of adult subjects, with the purpose of identifying subjects with pre-clinical atherosclerosis or at high cardiovascular risk.
Methods. PLIC study (studio sulla Progressione delle Lesioni Intimali Carotidee) is a single-centre, observational study, with a mean follow-up of 11 years. In each visit, data about personal and familial pathological history, lifestyle habits, clinical parameters, drug therapies, and lipid and glycaemic profiles were collected.
Results. The sample included 1445 patients; 21.6% fulfilled the criteria for the diagnosis of MetS in visit 1. This prevalence reached 25.2% in visit 4. In each visit, the most prevalent determinant was high blood pressure (95.5% of MetS patients in visit 1). Prevalence of patients with high glucose values increased during the study (from 18.8% in visit 1 to 30.6% in visit 4); the same trend was observed for waist circumference (from 34.1% to 44.6%), while the prevalence of determinants related to HDL-c and triglycerides decreased during follow-up (from 23.9% to 11.8% and from 19.6% to 14.1%, respectively). At visit 1, 72.2% of the sample had IMT values less than 0.9 mm, while an atheromatous lesion was detected in 22.4% of the sample (as focal plaque and/or IMT values ≥1.3 mm). Among subjects with IMT values <0.9 mm in visit 1, 40.4% progressed to plaque.
The Global Cardiovascular Risk score (mean±SD) in the total sample in visit 1 was 2.00%±2.20%, significantly increasing in time (2.53%±1.90% in visit 4).
Conclusions. We provide a detailed description of the prevalence of cardiovascular risk factors in the PLIC population. Further investigations are needed to quantify the role of cardio-metabolic risk factors in determining cardiovascular risk and to confirm their usefulness in stratifying patients at intermediate risk.

REAL LIFE EXPERIENCE WITH PCSK9 INHIBITORS FOR THE MANAGEMENT OF HYPERCHOLESTEROLEMIC SUBJECTS AT HIGH CARDIOVASCULAR RISK IN PALERMO

A. Giammanco, A.B. Cefalù, R. Spina, V. Ingrassia, C. Scrimali, V. Valenti, C.M. Barbagallo, A. Ganci, D. Noto, A. Cardella, G.I. Altieri, F. Fayer, M.R. Averna
Dipartimento Biomedico di Medicina Interna e Specialistica (DIBIMIS), University of Palermo

Background. Monoclonal antibodies anti-PCSK9 (Alirocumab and Evolocumab) have been recently approved by Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of patients with familial hypercholesterolemia (FH) and for secondary prevention in subjects with high cardiovascular (CV) risk on top of standard lipid lowering therapy (LLT). Several clinical trials have demonstrated the efficacy and safety of these PCSK9 inhibitors.
Objectives and Methods. The aim of this study was to evaluate the efficacy and safety of Alirocumab and Evolocumab in 20 patients (11 FH and 9 High CV Risk). 10 subjects (6 FH and 4 High CV Risk) were treated with Alirocumab (75 mg or 150 mg based on LDL-C at baseline) and 10 subjects (5 FH +5 HCR) obtained Evolocumab (140 mg). Patients have self administered monoclonal antibodies subcutaneously every two weeks. Clinical and biochemical parameters, anthropometric measures, medical history, background LLT and side effects were evaluated over a period of 8 months.
Results. Patients treated with Alirocumab and Evolocumab exhibit a substantial and sustained reduction of the LDL-C plasma levels by a mean of more than 50% (LDL-C decrease variability between 46.2% and 68.4%) and LDL-C targets <100 mg/dl or <70 mg/dl were reached in all subjects. No significant change was observed in liver function tests. Injection site local reactions such skin erythema, pain, swelling and bruise were reported in 20% of patients but these adverse events progressively reduced over the subsequent drug administrations. No flu-like symptoms have been reported.
Conclusions. This study confirms the efficacy and safety of treatment with Alirocumab and Evolocumab both in FH and high CV risk patients.

THE ROLE OF ANKLE BRACHIAL INDEX IN THE EVALUATION OF HYPERTENSIVE PATIENT'S ORGAN DAMAGE

R. Finelli[1], A.V. Pascale[1], V. Visco[1], R. Giannotti[1], D. Fabbricatore[1], M. Ciccarelli[1], G. Iaccarino[1]
[1]Dipartimento di Medicina e Chirurgia, Università degli Studi di Salerno

The early identification of target organ damage (TOD) is very important for the stratification of the hypertensive patient's cardiovascular risk. The Ankle-Brachial Index (ABI) has recently been reassessed for the definition of death risk, that increases with the reduction of ABI under the cut-off value of 1.10. Nevertheless, its relevance to predict TOD is less explored. We evaluated the ABI efficacy as predictor of cardiac TOD in the hypertensive population of the Outpatient Clinic of Hypertension of AOU San Giovanni and Ruggi of Salerno. ABI was calculated using automatic oscillometric-technology pressure recorder (Microlife). The size of the ventricle was evaluated according to the Devereux formula and indexed for body surface area. We examined *173* patients, of which 70 females and 103 males (average age 62.92±0.86) treated for essential hypertension (average PAS 134 ±1,76; average PAD 79,56±1,08), with no history of myocardial infarction, diabetes, stroke or PAD (ABI<0,9). In this population, ABI directly correlates with both the left ventricular diameter (R^2:0,04; F=5,7, P<0,02) and the indexed mass (R^2:0,06; F=7,9; P<0,01). When dividing the population in quartiles according to the ABI, patients in the 4th quartile (ABI>1,41) presented a larger cardiac size then the rest of the population (LVMI: 148±37 vs 124±33 g/m^2, p<0.02). This association holds true even after correction for confounders such as sex, bmi, diabetes and dyslipidemia. Our study shows the association between ABI and left ventricular hypertrophy. We propose that an ABI>1,4 can be considered a fast and valid tool for the stratification of hypertensive patient's organ damage.

EMERGING CONCEPTS IN ACUTE HEART FAILURE: FROM THE PATHOPHYSIOLOGY TO THE CLINICAL CASE BASED APPROACH

A.G. Solimando[1,2#], A. Argentiero[3#], A. Ruckdeschel[2], M. Bittrich[2], A. Schneider[4], R. Sbrojavacca[5], A. Vacca[1], G. Fritz[6], H. Einsele[2]
[#]These authors contributed equally
[1]Department of Biomedical Sciences and Human Oncology, Section of Internal Medicine "G. Baccelli", University of Bari Medical School, Bari;
[2]Department of Internal Medicine II, Hematology and Medical Oncology, University Medical Center Würzburg, Germany;
[3]Department of Biomedical Sciences and Human Oncology, Section of Medical Oncology, University of Bari Medical School, Bari;
[4]Department of Internal Medicine I, Emergency Medicine and Intensive Care, University Medical Center Würzburg, Germany;
[5]Department of Medicine, Emergency Medicine Unit, University Hospital "Santa Maria della Misericordia", Udine;
[6]Department of Intensive Care and Anesthesiology, Heart Center Brandenburg, Bernau, Germany

Aim (state background and specific object of study): Acute heart failure (AHF) represents a heterogeneous clinical syndrome comprising new or worsening signs and symptoms on a background of stable chronic heart failure (HF), as well as new-onset HF. In either clinical picture, urgent care is crucial. The aim of the present study is to provide practical, evidence-based guidelines for the diagnosis of AHF to define a rational basis for a patient-oriented approach to treatment of AHF.
Methods: we evaluated twenty acute heart failure patients. We stratified them evaluating blood pressure, EF, symptoms of acute decompensated heart failure, RX, ECG.
Results: in our small patients cohort, in-hospital mortality as well as 60- to 90-day mortality and rehospitalization are increased in the hypotensive patient. Although counterintuitive, it seems to be the case that in AHF, higher systolic blood pressure (SBP) is associated with a better prognosis.
Conclusions: Acute heart failure is recognized as an important public health problem. Given the variety of clinical scenario and importance of immediate treatment, stratifying patient subgroups on a pathophysiologic base can help direct appropriate therapy.

We also confirmed the clinical effectiveness of current clinical stratification of AHF patients in order to better approach this diffuse medical condition.

C REACTIVE PROTEIN INCREASE IS ASSOCIATED WITH HIGH CORONARY AND PERIPHERAL ATHEROSCLEROTIC BURDEN IN VISCERAL OBESITY SUBJECTS

V. Ferrara, R. Scicali, A. Di Pino, R. Platania, G. Purrazzo, A. Giannone, S. Piro, A.M. Rabuazzo, F. Purrello
Department of Clinical and Experimental Medicine, University of Catania

Background and Aims. The association of visceral obesity with an increased risk of developing cardiovascular disease is unclear. In these subjects, inflammation plays a central role in developing atherosclerosis. Our objective was to examine the cardiovascular (CV) risk profile of visceral obesity patients with and without inflammation according to high sensitivity C reactive protein (CRP), using macroangiopathic imaging biomarkers.
Methods. Our population consisted of 140 patients aged between 40 and 70 years, with a waist circumference ≥88 for women and ≥102 for men and at least 1 CV risk factor. Exclusion criteria were prior history of CV disease or clinical evidence of advanced renal disease. Inflammation was defined as a CRP value ≥2. Coronary artery calcium (CAC) score as well as mean common carotid intima media thickness (IMT) were assessed using consensus criteria.
Results. CAC score was higher in inflammation group compared to non-exposed group (60.74±159.57 vs 50.74±179.68 AU, $p<0.05$). Visceral obesity subjects with inflammation had higher mean IMT than non-exposed subjects (0.62±0.18 vs. 0.56±0.11 mm, $p<0.05$). In addition, in a multiple linear regression, CAC was associated with inflammation ($p<0.05$) and age ($p<0.001$).
Conclusions. Among patients with visceral obesity, CRP increase is associated with higher coronary and peripheral atherosclerotic burden in visceral obesity patients.

ANTI-PROPROTEIN CONVERTASE SUBTILISIN-KEXIN TYPE 9 MONOCLONAL ANTIBODIES: BEYOND LIPID PROFILE

G. Mandraffino*, C.O. Aragona*, F. Savarino#, V. Cairo, G. Trapani, S. Quartuccio, F. Mamone, M. Scuruchi, A. Saitta, E. Imbalzano
**the first two authors equally contributed to the work*
Department of Clinical and Experimental Medicine, University of Messina

Background. Patients with familial hypercholesterolemia (FH) present early signs of vascular inflammation and damage. Monoclonal antibodies that inhibit proprotein convertase subtilisin-kexin type 9 (PCSK9), have emerged as a new class of drugs that effectively lower LDL cholesterol levels.
Purpose. We investigate whether after six months of treatment with anti-PCSK9 monoclonal antibodies (alirocumab or evolocumab) we can find any improvement of pro-atherogenic profile and arterial stiffness (AS) in patients affected by FH already in treatment with the maximally tolerated statin therapy.
Methods. We enrolled 18 people not at target LDL-C values. At enrollment and 6 months later we evaluated anthropometrics, laboratory profile, pulse wave velocity (PWV) and carotid intima-media thickness (cIMT).
Results. After 6-months of treatment we found a significant decrease of inflammatory markers (hs-CRP: -43.5%; Fibrinogen: -17.8%), LDL-C and lipoprotein(a) levels (respectively -63.1% and -35.1%).
PWV (-9.3%) appeared to be improved; cIMT remained unchanged. PWV reduction appeared to be correlated with inflammatory markers and LDL-C reduction. However, ΔPWV appeared to be not dependent on ΔLDL-C or Δfibrinogen by the multiple regression analysis.
Conclusion. After 6 months of treatment with monoclonal antibodies anti-PCSK9 the levels of CRP, Fibrinogen, LDL-C, and Lp(a), as well AS indices, are significantly improved as compared to baseline. A treatment with anti-PCSK9 monoclonal antibodies may improve significantly the arterial stiffness and the proatherogenic profile in patients affected by FH who have not achieved the expected LDL-C target with the maximally tolerated statin therapy.