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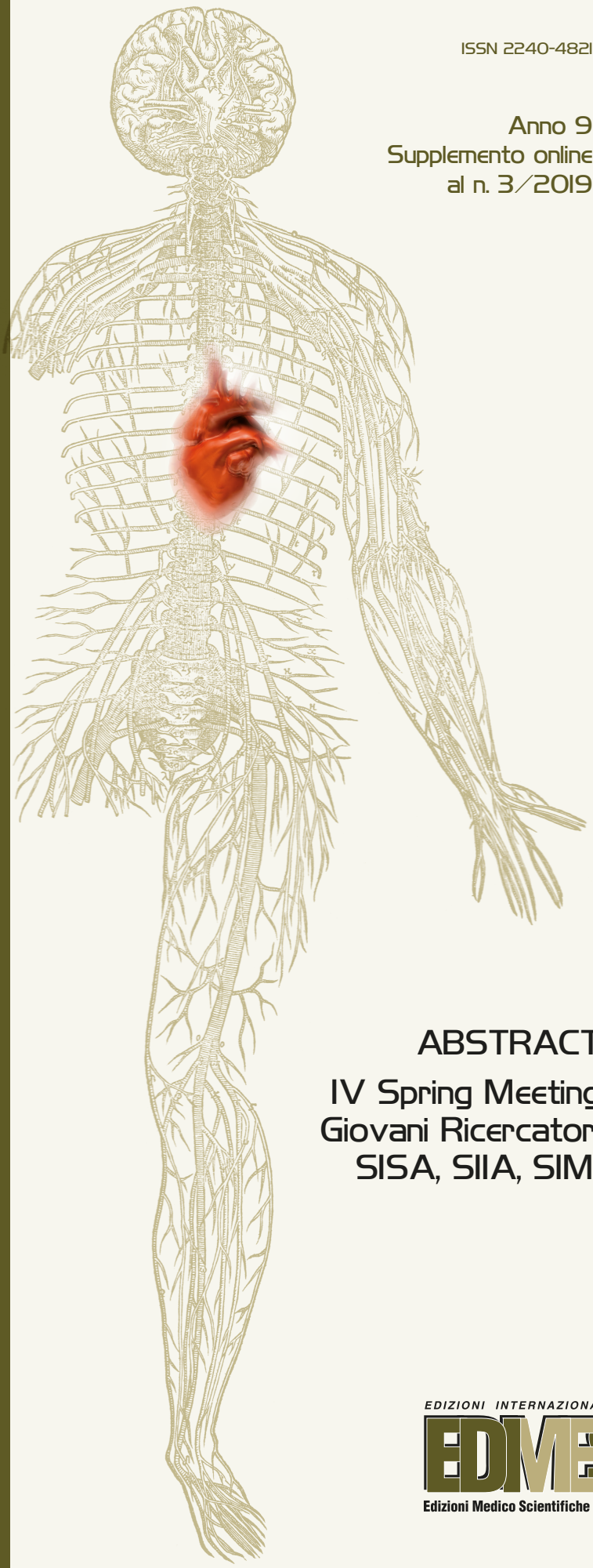

ABSTRACT

IV Spring Meeting
Giovani Ricercatori
SISA, SIIA, SIMI

EDIZIONI INTERNAZIONALI srl
EDIMES
Edizioni Medico Scientifiche - Pavia

SOCIETÀ ITALIANA PER LO STUDIO DELLA ATEROSCLEROSI (SISA)

IV Spring Meeting Giovani Ricercatori SISA, SIIA, SIMI

Report Spring Meeting Nazionale 2019 – Giovani ricercatori Next Gen SIIA SIMI SISA "Novità e updates sulla prevezione e cura della malattia cardiovascolare"

Dal 28 Febbraio 2019 al 2 Marzo 2019 si è svolto, a Rimini, il 4° Spring Meeting dei Giovani Ricercatori SIIA, SIMI e SISA.

Giunto alla quarta edizione anche quest'anno il Meeting è stato caratterizzato da una ampia e particolarmente attiva partecipazione dei giovani ricercatori provenienti da tutte e tre le società scientifiche. Sono pervenuti 72 abstract, valutati in cieco dal comitato scientifico, e distribuiti in 30 comunicazioni orali e 42 comunicazioni poster con moderazione.

Quest'anno, grazie alla maggior durata del meeting rispetto alle precedenti edizioni e al lavoro del comitato organizzatore (Marianna Maranghi e Mario Luca Morieri per la SISA, Martino Pengo e Francesca Saladini per la SIIA, Elena Buzzetti ed Emilia Donnarumma per la SIMI) sono state aggiunte al programma scientifico diverse sessioni innovative. Il programma si è arricchito infatti della realizzazione di tre workshop che hanno permesso di sviluppare, anche attraverso il lavoro in piccoli gruppi, aspetti importanti sia dal punto di vista della ricerca che dal punto di vista professionale per i giovani ricercatori. È stata ripetuta, dopo l'esperienza positiva dello scorso anno, la tavola rotonda di presentazione di progetti scientifici da parte delle tre società scientifiche. Inoltre, grazie alla disponibilità di ecografi on-site, sono state organizzate delle sessioni pratiche di ecografia in cui i partecipanti hanno appreso come eseguire diverse misurazioni utili non solo dal punto di vista clinico ma anche dei progetti di ricerca. Non sono poi mancate, secondo il format ormai consolidato e ben funzionante negli anni, le letture introduttive alle 6 sessioni delle comunicazioni orali.

Il primo workshop si è incentrato sull'importanza della divulgazione scientifica, preceduto dalla lettura del Prof. Ivano Eberini "Social network - un nuovo paradigma di divulgazione anche per la scienza" questa attività ha permesso di sottolineare l'importanza di una corretta e adeguata comunicazione dei risultati della ricerca, non solo sulle classiche piattaforme scientifiche, bensì anche attraverso strumenti in grado di raggiungere un pubblico più ampio.
Il secondo workshop, è stato introdotto dalla lettura del biostatistico Davide Soranna su "Come riconoscere i veri punti deboli degli studi scientifici", attraverso lavori di gruppo questo workshop ha permesso di apprendere come valutare la validità statistica e quindi scientifica dei lavori pubblicati, fornendo anche strumenti essenziali ai giovani ricercatori su come progettare al meglio i propri studi.

L'ultimo workshop, "Uno sguardo al percorso formativo e professionale", introdotto dalla lettura Dott.ssa Chiara De Girolamo "Lo sviluppo della carriera: accademia e non solo" ha permesso di sviluppare insieme a tutti i partecipanti quali sono alcuni punti chiave da considerare per favorire una pianificazione consapevole della propria carriera professionale, aspetto di essenziale importanza per i giovani ricercatori.

Nella tavola rotonda sui "forthcoming project: condivisione di progetti di ricerca tra le tre società", sono stati presentati questi diversi progetti dai giovani ricercatori delle diverse società,
per la SISA:
- *Ruolo dell'ecografia tendinea nell'ipercolesterolemia familiare (F. Nascimbeni)*
- *ILNA Study (E. Molina)*
- *Caratterizzazione del profilo rischio CV nelle donne con FH (M. Maranghi)*
- *Prevalence and Predictors of Poor response to PCSK9-inhibitors (M.L. Morieri)*

Per la SIIA:
- *Unattended Blood pressure and Target organ damage: update 2019 (C. Mancusi)*
- *Common mistake in home blood pressure measurement: a survey (C. Mancusi)*

Per la SIMI
- *SIMID (E. Buzzetti)*
- *Sottostudio REPOSI (A. Cimellaro)*
- *SIMINUTRO (S. Cicco)*

Come negli anni precedenti, prima di ognuna delle sessioni orali si sono tenute le letture a cura di 6 invited speakers
- *Malattie infettive, HIV e rischio cardiovascolare • Massimo R. Mannarino*
- *Fibrillazione Atriale: il profilo di rischio oltre gli eventi tromboembolici • Marco Proietti*
- *Impatto delle differenze etniche sullo sviluppo dell'aterosclerosi • Luca Faconti*
- *Diagnostica ecografica nella sindrome aortica acuta • Peiman Nazerian*
- *Differenze di sesso nel metabolismo lipidico e lipoproteico • Sara della Torre*
- *Cross-talk heart-liver: NAFLD e rischio cardiovascolare • Alessandro Mantovani*

Complessivamente hanno partecipato al meeting 97 persone di cui 25 tra relatori, moderatori, comitato organizzatore e comitato scientifico e 72 iscritti.
43 partecipanti risultano iscritti alla SISA, 25 alla SIIA, 20 alla SIMI, 7 a SIMI e SISA e 2 a tutte e tre le società.

Il successo del meeting è stato confermato da molteplici punti di vista: tutte le sessioni sono state attivamente partecipate e sempre caratterizzate da una vivace discussione in tutte le presentazioni; i feedback raccolti on-site hanno confermato la soddisfazione dei partecipanti e degli organizzatori. Inoltre, nel desiderio di una valutazione più quantitativa e misurabile, dopo il meeting è stato inviato un questionario di gradimento. Hanno risposto ad oggi in 31 partecipanti (7 SIMI, 4 SIIA, 20 SISA). Alla richiesta di dare un giudizio generale sul meeting 17 partecipanti si sono dichiarati molto soddisfatti, 13 soddisfatti, 1 poco soddisfatto, nessuno per nulla soddisfatto.
L'86% ha dato una risposta positiva riguardo la maggior durata del meeting, con oltre il 90% dei soggetti che ha valutato positivamente l'organizzazione dei workshop che hanno ricevuto un punteggio (da 1 a 10) pari o superiore ad 8 in più dell'80% delle risposte.

COMUNICAZIONI ORALI

INCREASED PLASMATIC NETS BY-PRODUCTS IN PATIENTS IN SEVERE OBESITY

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Background and aims: neutrophil extracellular traps (NETs) are DNAs products involved in immune process. Obesity through a low-grade chronic inflammation determines neutrophil activation, but it is still unclear its role in NETs formation. Here we analyzed the NETs levels in healthy and morbid obese, their association with anthropometric and glyco-metabolic parameters and their changes after bariatric surgery.
Methods: we enrolled 73 patients with morbid obesity (BMI 40 kg/m^2 or 35 kg/m^2 + comorbidity) eligible to sleeve gastrectomy and a control group of 32 healthy subjects. We evaluated anthropometric parameters, peripheral blood pressure, biochemical and serum analysis at the enrollment and at twelve months after surgery. Plasmatic levels of MPO-DNA complexes were assessed by ELISA.
Results: NETs levels were higher in obese than in control group ($p<0.001$) and correlated with the main anthropometric variable (BMI, waist, hip), glyco-metabolic variables and systolic blood pressure. NETs trend after intervention was uneven. The reduction of NETs correlated with the entity of reduction of BMI ($\rho=0.416$, $p<0.05$), visceral fat area ($\rho=0.351$, $p<0.05$), and glycemia ($\rho=0.495$, $p<0.001$). In medical history of patients in whom NETs increased, we observed a higher number of thromboembolic and atherosclerotic events.
Conclusion: severe obesity is associated with increased generation of NETs, which in turn could influence the patients' systemic inflammatory state. Weight loss and in particular loss of adipose tissue after bariatric surgery does not in itself correct NET's dysregulated production. Finally, patients in whom NETs accumulation persists after surgery are probably those at the highest risk of cardiovascular events.

LOW HDL-CHOLESTEROL LEVELS ARE ASSOCIATED WITH A DECREASED MONOCYTE ACTIVITY AND INFLAMMATION IN CARRIERS OF LCAT MUTATION

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Aim: Epidemiological studies have shown an inverse relationship between severely low HDL-cholesterol levels and CHD. Besides the capacity in promoting cholesterol efflux, HDL can contribute to atheroprotection through a role in the modulation of the immune response. Among immune cells, monocytes can contribute in creating a pro-inflammatory milieu that supports the atherosclerotic process. In this study we aim to evaluate the effect of genetic HDL deficiency, specifically determined by mutations in *LCAT* gene, on monocyte immunophenotype and function.
Methods: Monocytes were isolated from fresh blood in carriers of LCAT mutations (n=12) and from controls (n=9) matched for age, sex and BMI. Ex vivo analysis was performed with fluorescence-activated cell sorter analysis, inflammatory stimulation and transendothelial migration assays.
Results: While no difference in the monocyte subset distribution was found between carriers and controls, expression of the integrin CD11c was nearly half in mutant LCAT (ΔMFI 220±56 *vs.* 415±38 P=0.042) and monocytes displayed a reduction in migratory capacity compared to control (39.7±1.92 *vs.* 48.0±1.83 %of migrated monocytes P=0.006). Monocytes from carriers proved to be less efficient in producing IL-8 and IL-1β upon 24-hour stimulation with LPS and Pam3Cys, and shown a decreased production of MCP-1 at baseline. All the effects were gene-dose dependent. No difference in circulating levels of hsCRP, MCP-1, TNF-α and IL-6 was appreciated. Comparison with ABCA1 and APOA1 mutation carriers display differences in monocyte phenotype and function.
Conclusion: Genetic HDL deficiency due to LCAT gene mutations is associated with a dampened immune response of monocytes, despite the low plasma HDL-C levels. These findings contribute to our understanding of the increased atheroprotective role of HDL often seen in this disease.

BENEFICIAL EFFECTS OF DAAS (DIRECT-ACTING ANTIVIRALS) ON CARDIAC FUNCTION AND STRUCTURE IN HEPATITIS C (HCV) PATIENTS WITH LOW-MILD LIVER FIBROSIS

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Introduction: Hepatitis C virus **(**HCV)-related chronic infection has been associated with a higher incidence of cardiovascular (CV) diseases. Systemic chronic inflammation, specific viral cytotoxicity on either the endothelium or the myocardium or an unexpectedly high prevalence of CV risk factors in HCV patients, may represent etiological factors. A reduced right and left ventricular function and morphology were found in patients affected by HCV hepatitis in a few studies but the causality of the association is still debated.
Methods: Eighty-six non-obese and non-diabetic HCV-patients (59.5±12.0y; males 52%) with fibroscan-documented low-mild liver fibrosis were eligible for virus eradication with DAAs (Direct-Acting Antivirals), and were included. Fifty-six were compared with 52 control subjects matched for age, sex and CV risk factors at baseline. A transthoracic echocardiography was performed at baseline (T0) in all participants and repeated in all HCV patients after successful eradication (6 months later, T1). HS TnT (High-Sensitive TroponinT), NT-proBNP (N-terminal Btype natriuretic peptide), TNF-α (Tumor Necrosis Factor α) and IL-10 (Interleukin-10) concentrations on serum were measured at baseline and at T1.
Results: At baseline, no differences in ejection fraction, MAPSE, E/A ratio and indexed Left Ventricular Mass (LVMi) were detected whereas Relative-wall Thickness (RWT) was higher in HCV patients as compared to matched controls. Right indexed atrial volume, basal ventricular diameter and pulmonary pressure were higher in HCV participants. After virus eradication, left atrial volume, tele-diastolic diameter and LVMi were lower as compared to baseline. Indexed right atrial volume, basal ventricular diameter, TAPSE, pulmonary arterial pressure and vena cava diameter decreased significantly after treatment. A statistical significant decrease of TNF-α was shown at T1, while HS-TnT, NT-proBNP and IL-10 did not changed compared to baseline.
Conclusion: The study shows a concentric remodelling of left ventricle along with structural modifications in the right sections along with a higher pulmonary pressure in HCV subjects. Treatments with DAAs is associated with regression of most cardiac alterationsindicating a direct involvement of the HCV virus and chronic inflammationin cardiac alterations, reversible after etiological treatment.

EFFECTS OF ANTIRETROVIRAL THERAPY ON PROPROTEIN CONVERTASE SUBTILISIN/KEXIN 9: FOCUS ON LIPIDS, INFLAMMATION AND IMMUNOVIROLOGICAL PARAMETERS

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Aim: Plasma levels of proprotein convertase subtilisin/kexin type 9 (PCSK9), a major regulator of cholesterol metabolism, show a trend toward an increase in HIV+ patients compared to uninfected controls. We investigated the impact of antiretroviral therapy (ART) on plasma PCSK9 levels and its relation with plasma lipids, immunovirological control and inflammation.
Methods: We enrolled 80 HIV+ ART-naïve patients and 30 uninfected controls. At baseline and at 3, 6 and 12 months after ART initiation plasma PCSK9 levels, lipids, high-sensitivity C-reactive protein (hs-CRP), CD4 T cell count and HIV-1 RNA viral load were measured.
Results: Baseline PCSK9 levels were significantly higher in HIV+ patients compared to uninfected controls. In HIV+ ART-naïve patients PCSK9 levels were associated with CD4 T cell count (rho=-0.45, $p<0.001$), and HIV-1 RNA viral load (rho=0.49, $p<0.001$), whereas no significant association was found with low-density lipoprotein (LDL) cholesterol. ART-mediated immunovirological control was paralleled by significant increases in plasma total, LDL and high-density lipoprotein (HDL) cholesterol, and lipoprotein(a) levels and decreases in plasma PCSK9 and hsCRP levels. The reduction of PCSK9 levels was independently predicted by baseline CD4 T cell count and CD4 T cell count recovery.
Conclusion: ART initiation is associated with a progressive PCSK9 reduction that is mirrored by a concomitant attenuation of low-grade inflammation and a complex constellation of lipid changes. The CD4 T cell count recovery after ART initiation is the most relevant factor explaining the ART-mediated PCSK9 reduction.

SPHINGOSINE 1-PHOSPHATE (S1P)/S1P1 AXIS AS A MODULATOR OF MACROPHAGE FUNCTIONS

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Aim: Several studies documented that HDLs act as a carrier of bioactive lipids such as sphingosine1-phosphate (S1P) and raised the possibility that S1P contributes to atheroprotective effects exerted by these lipoproteins, mainly via the binding with its specific receptors. The molecular mechanisms underlying these putative effects are still unknown. The aim of this study was to investigate the role of S1P, in particular the S1P/S1P-receptor 1 (S1P1) axis, in modulating macrophage functions relevant to the pathogenesis of atherosclerosis, in a conditional knock-in mouse model overexpressing S1P1 in myeloid lineage.
Methods: LDL-receptor-deficient mice (Ldl-r-/-) were transplanted with bone marrow of S1P1/LysCre or control mice (n=12/group) and placed on a Western type diet (0.5% of cholesterol, 21% of fat) for 12 weeks. Afterwards, heart with proximal aorta and artery brachiocephalic were dissected and embedded in Tissue-Tek OCT. Serial cross-sections were collected, stained with Oil-Red-O for lipids and counterstained with haematoxylin or labelled with CD68.
The functional macrophage phenotype was evaluated by examining surface markers of macrophage polarization and differential gene expression by flow cytometry and qPCR techniques, respectively. The inflammatory response was investigated by determining the secretion of cyto/chemokines from serum and macrophages through qPCR and ELISA.
Results: Morphological studies showed a significant reduction of atherosclerotic lesion formation, both in the aortic root and brachiocephalic artery, -50% and -75% respectively, in S1P1/LysCre mice compared to Ctrl. This regression may be explained by the up-regulation of some genes and transcription factors important in lesion regression process, such as *CD163*, *Fizz-1*, *Hmox-1*, *Arg-1* and *Ym-1*, as observed in S1P1/LysCre macrophages. Macrophages from S1P1/LysCre mice showed an up-regulation of *Klf4*, *C/ebpα*, *Bcl-6, Lgmn* and the activation of two transcription hematopoietic factors, *PU.1* and *Irf8*. This activation was then confirmed by the detection of Dectin-1 and CD115, two surface markers regulated respectively by Irf8 and PU.1. Moreover, macrophages overexpressing S1P1 showed an increase of CD206 and MHCII signal, typical surface markers of M2 macrophages and conversely a downregulation of CD86 and CD93, both recognized as M1 markers.
The role of S1P1 expression in modulating the immune response was also defined by the increased production of anti-inflammatory cytokines (IL-1RA, IL-10, IL-4 and CCL22) both under basal conditions and after stimulation with FCS in peritoneal macrophages and plasma (S1P1/LysCre).
Conclusions: Our evidences suggest that S1P exerts beneficial atheroprotective effects in macrophages by modulating inflammatory response mainly interacting with the macrophage S1P1. Hence, S1P1 may be considered a promising target to be explored in atherosclerotic disease.

UNATTENDED AUTOMATED OFFICE BLOOD PRESSURE MEASUREMENT AND CARDIAC TARGET ORGAN DAMAGE, A PILOT STUDY

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Aim: ESC-2018 guidelines focus on the lack of accuracy of manual ambulatory blood pressure (AOBP), placing the accent on the Unattended Automatic Office Blood Pressure (UAOBP) importance in obtaining more reliable measurements. However a difference of 5-15mmHg between AOBP and UAOBP is mentioned. This interval value underlines uncertainty in evaluating the two methods due to the extremely heterogeneous clinical studies on this topic.
The study's objective is to assess if the pressure difference between UAOBP and AOBP adopted in American and European guidelines is reproducible and to evaluate the correlation of UAOBP with markers of hypertension mediated target organ damage in hypertensive patients.
Methods: UAOBP and AOBP were taken on a cohort of 48 outpatients in Italy. The pressure difference between the 2 methods and their correlation with general anthropometric and cardiac parameters were analyzed. Finally the correlation of the 2 methods with the echocardiographic organ damage indices was studied.
Resullts: Systolic UAOBP values were lower than which of systolic AOBP (135±7 mmHg *vs*. 139±21 mmHg). Differences between values measured using the two methods were ΔSBP= 4±10 mmHg and ΔDBP= 3±9 mmHg for systolic and diastolic pressure respectively. ΔDBP was significantly directly correlated with female sex (r=0.347, p=0.016) and it was lower in men compared to women (0.11±8.9 *vs.* 6.07±7.42, p=0.016).
The difference between UAOBP and AOBP was 8 mmHg for systolic values, so that an unattended systolic blood pressure of 140 mmHg corresponded to an attended systolic blood pressure of 148 mmHg.
Finally unattended systolic BP was better correlated with left ventricular mass indexed/$h^{2.7}$ (r=0.381, p=0.008) than attended systolic BP (r=0.286, p=0.049).
Conclusions: UAOBP provides values significantly lower than which obtained with AOBP and it can better identify cardiac target organ damage. The difference in BP values between the two methods is much lower than which obtained in most clinical studies.

ROLE OF LIPIDS AND LIPOPROTEINS IN IPSC DERIVED CARDIOMYOCYTE MITOCHONDRIAL FUNCTION AND METABOLISM

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Background and aim: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is secreted into the circulation by the liver and can interacts with several receptors of the LDL-R family but also with CD36 and favors their degradation. An increased expression of receptors of the LDL-R family was shown to favor lipid accumulation, accumulation that in the heart results in cardiomyocyte dysfunction.
This project is aimed at testing the hypothesis that PCSK9 might regulate lipoprotein and fatty acid metabolism in the heart, thus modulating mitochondria physiology and cardiomyocyte metabolism.
Methods: Cardiomyocyte have been differentiated from iPSC and after 30 days of culture have been treated with different source of lipids with or without PCSK9. Cells have been pretreated for 24h with PCSK9 (2 ng/ml) and then treated with LPDS (10%), VLDL (50 µg/ml) and LDL(100 µg/ml). After the incubation the cells have been used for FACS analysis, real time, western blot and mass spectrometry.
Results: Gene expression analysis was performed to assess the maturation of cardiomyocyte, differentiated for 30 and 60 days from iPSC. After 30 days of differentiation, cells presented increased expression of Troponin T and genes involved in fatty acid metabolism (CD36, CPT1b, PPARg). Incubation of iPSC-CM with VLDL reduced the expression of mitochondrial fusion protein (MFN) while mitochondrial fission (DRP1) protein were increased. This profile was associated with increased accumulation of neutral lipids and a decreased mitochondrial mass compared to control. Of note preincubation with PCSK9 reverted the phenotype.
Conclusions: Our data suggest that incubation with VLDL lead lipid accumulation and reduced mitochondrial mass in cardiomyocyte. Moreover, PCSK9 might limit lipotoxicity and increase mitochondrial mass by regulating lipid receptors expression.

MYOCARDIAL MECHANOENERGETIC EFFICIENCY (MEE) IN PATIENTS WITH PRIMARY ALDOSTERONISM OR ESSENTIAL HYPERTENSION

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Background: Available data indicate that patients with primary aldosteronism (PA) have an increased risk of cardiovascular (CV) events; furthermore, CV risk seems to be, at least in part, independent of blood pressure (BP) elevation. Previous studies have shown that patients with PA have a greater prevalence of left ventricular (LV) hypertrophy, which might contribute to the increase in CV risk. Recently, a non-invasive approach for the estimation of LV mechanical efficiency through the calculation of the ratio between stroke work (SW) and heart rate (HR)–pressure product has been proposed by de Simone and coworkers. This index, which expresses the amount of blood pumped in a single beat in 1 second by the heart, may be easily obtained by echocardiography.
Aim: The aim of our study was to evaluate the determinants of myocardial mechanoenergetic efficiency index (MEEi), calculated as SV/HR and indexed to LV mass (MEEi=MEE/LVM) in a large group of patients with primary aldosteronism and in a control group of essential hypertensives (EH). PA was diagnosed in the presence of a positive aldosterone to renin ratio (>30) and post saline aldosterone >100 ng/ml). Ninety-nine patients with PA were compared with 99 EH patients matched for age and BP values.
Results: No differences between groups were observed for age, gender, BMI, BP values, glucose, lipid profile and renal function. LV mass index was greater in PA vs EH (101±34 *vs.* 84±20, gr/m^2, p<0,05); also relative wall thickness was greater in PA *vs.* EH (0.36±0.1 *vs.* 0.32±0.4, p<0,05). Ejection fraction was not different between groups, while endocardial and midwall fractional shortening were lower in PA *vs.* EH (40±7 *vs.* 43±6, and 18±3 *vs.* 21±2 both p<0,05). MEEI was lower in PA vs EH (0.44±0.14 *vs.* 0.52±0.10, p<0,05). A negative correlation was observed between MEEI and aldosterone levels (r=-0.20, p<0.05).
Conclusions: In patients with primary aldosteronism MEEI is lower as compared to EH. These findings may contribute to explain the increased risk of CV events in patients with PA.

ROLE OF CRYPTOCHROME-1 AND CRYPTOCHROME-2 IN ALDOSTERONE-PRODUCING ADENOMAS AND ADRENOCORTICAL CELLS

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Aim: A study by Doi M. et al. reported a form of hyperaldosteronism sustained by the upregulation of type VI 3β-hydroxyl-steroid dehydrogenase (*Hsd3b6*) in knock-out mice for the genes *Cry1* and *Cry2*, encoding the circadian clock components Cryptochrome-1 and Cryptochrome-2. *Hsd3b6* gene is the murine counterpart of the human Type I 3β-hydroxyl-steroid dehydrogenase (*HSD3B1*). The objective of this study was to analyse the role of *CRY1* and *CRY2* and their potential interactions with *HSD3B* isoforms in human aldosterone biosynthesis.
Methods: The study included 46 sporadic aldosterone-producing adenomas (APA) and 20 paired adjacent adrenal cortex samples. As *in vitro* model, we used human adrenocortical cells HAC15.
Results: In the cohort of sporadic adenomas, *CRY1* was 1.7-fold [0.75-2.26] more expressed (p=0.016) within the main node compared to adjacent cortex, while *CRY2* showed a 20% [0.80, 0.52-1.08] (p=0.04) decrease in APA tissue. Type II *HSD3B* is the principal isoform in APAs, with an expression 317-fold [200-573] higher than *HSD3B1*. Both *HSD3B* isoforms are more expressed in APA compared with paired adjacent cortical tissue, 5.7-fold (p<0.001) and 3.5-fold (p=0.001) respectively. In addition, *HSD3B1* is significantly more expressed in APAs composed primarily of *zona glomerulosa*-like cells. In HAC15 cells, angiotensin II (AngII) stimulation determines a significant increase of *CRY1* expression (1.7±0.25-fold, p<0.001) at 6 hours and a significant downregulation of *CRY2* (1.6±0.1-fold, p<0.001) at 12 hours, through type 1 AngII receptor activation. Independent *CRY1* and *CRY2* silencing causes a mild upregulation of *HSD3B2*, whereas it does not condition *HSD3B1* expression.
Conclusions: In conclusion, our results support the hypothesis that *CRY1* and *CRY2*, being AngII-regulated genes, and showing a differential expression in APAs when compared with the adjacent adrenal cortex, might play a role in adrenal cell function, and in the regulation of aldosterone production.

BLOOD PRESSURE EFFECTS OF OBSTRUCTIVE SLEEP APNEA TREATMENT BY CONTINUOUS POSITIVE AIRWAY PRESSURE: SYSTEMATIC REVIEW, META-ANALYSIS AND EVALUATION OF PHENOTYPES PREDICTING RESPONSE

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Aim: Treatment of obstructive sleep apnoea (OSA) has been shown to reduce blood pressure (BP). However, the effect size is modest and treatment of OSA is not recommended as the only treatment target when treating hypertension. The aim of the present systematic review and meta-analysis was to identify potential predictors for BP response in patients with OSA undergoing CPAP treatment.
Method: A systematic search was conducted in three databases (MEDLINE, Embase and Web of Science) using terms exploring obstructive sleep apnoea, CPAP and clinical trial. Inclusion criteria were:
1) randomised controlled clinical trials published between January 1st 1960 to December 31st 2017 and with a reasonable control group;
2) OSA diagnosis using polysomnography;
3) age >18 years;
4) OSA severity of at least 5 AHI/h. The random effect model was fitted to estimate the pooled BP reductions (difference between end-treatment minus baseline BP) in the CPAP and control group. Moreover, the original estimates have been stratified according to selected patient characteristics.

Results: Out of 2445 articles, 59 RCTs were included (n=7.329 subjects) comparing CPAP with control groups. CPAP was associated with a net reduction in systolic BP of -2.12 (95% CI: -2.82/-1.42) mmHg and in diastolic BP of -1.97 (95% CI: -2.46/-1.48) mmHg, favoring treatment of OSA using CPAP (both p<0.001). The subgroup analysis showed a greater reduction of SBP in subjects younger than 60 years (-2.88 for age 40-50, -2.78 for age 50-60 and -0.61 for age more than 60 years, p=0.007) and in patients with controlled BP at baseline versus uncontrolled BP (-1.45 *vs*. -4.14, p=0.002).
Conclusions: Younger patients (< 60 years) with uncontrolled BP at baseline are more likely to experience significant BP reductions with CPAP therapy. Phenotypisation of specific cohorts of patients can guide clinicians to target OSA treatment and help to optimise patients' cardiovascular risk.

USE OF COLOR DOPPLER ULTRASOUND WITH CONTRAST ENHANCEMENT IN THE EVALUATION OF TAKAYASU ARTERITIS

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Background: Takayasu disease is a large vessel arteritis characterized by systemic inflammation involving the vessel wall. Clinical patterns include fever, weight loss, malaise and ischemical damage of organs. Diagnostic imaging includes CT/RM angiography, PET and color Doppler ultrasound (CDUS). The treatment requires immunosuppressive drugs and surgical approach if necessary. Few studies have been performed to evaluate carotid vessel wall vascolarization by contrast enhanced ultrasound (CEUS).
Aim: The aim of this study is to evaluate sovraortic branches lesions by standard and contrast enhanced CDUS in patients affected by Takayasu disease.
Methods: We unrolled 12 patients (9 w, 3 m, mean age 45,4) affected by Takayasu arteritis. We performed CDUS of sovraortic branches. We evaluated intima-media thickness (IMT); lesion thickness; Doppler velocities and presence of stenosis or occlusion. Six patients (50%) underwent CEUS to evaluate vascularization within the vessel wall to improve the visualization of the lumen-vessel wall border. Two patients underwent PET.
Results: Mean IMT was 2,13 mm (reference range <0,9) with characteristic circonferenzial arterial wall thickening in ten patients. According to the peak of systolic velocity, stenosis >70% of CC was present in 2 patients, occlusion of CC in 1 patient; stenosis >70% of SA was present in 4 patients, occlusion of SA in 3 patients. CEUS demonstrated intralesional enhancement in four patients (66%) and two of these (50%) showed correlation with vascular F18-FDG uptake at PET study.
Conclusions: CDUS allows the assessment of wall thickening and degree of stenosis of Takayasu arteritis, circonferenzial arterial wall thickening was present in almost all patient, regardless of the presence of stenosis and/or occlusion, thus suggesting CDUS as a useful screening approach. CEUS allows dynamic assessment of wall vascularization, which is a potential marker of disease activity in patients with Takayasu arteritis.

EVALUATION OF ATHEROSCLEROTIC CORONARY ARTERY DISEASE BURDEN BY CT SCAN IN PATIENTS WITH MOLECULARLY DEFINED HETEROZYGOUS FAMILIAL HYPERCOLESTEROLEMIA (HEFH) AS COMPARED TO THOSE WITH POLYGENIC FAMILIAL HYPERCHOLESTEROLEMIA (PFH)

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Aim: HeFH is the most common genetic disorder associated with an increased risk of coronary heart disease (CHD). In addition to the monogenic variant, it has been reported that the HeFH phenotype may also have polygenic molecular bases (PFH). To date few studies have compared HeFH and PFH in terms of atherosclerotic burden.
The aim of this study is the evaluation of atherosclerotic coronary artery disease by CT scan in subject with monogenic HeFH as compared to individuals with PFH.
Methods: One-hundred twenty subjects, aging 18-75 years were screened for mutation in the major FH-causing genes, by next generation sequencing. Ninety-five patients were found to carry monogenic HeFH and 27 were classified as PFH. All patients were asked to undergo coronary CTA imaging. Coronary atherosclerosis burden was evaluated based upon the presence and severity of coronary stenosis.
Results: In comparison with PFH patients, those with HeFH were younger (50,8±8,5 *vs.* 47,1±13,3 yrs, respectively, p=0.08), had higher levels of LDL-C (150,8±51 mg/dl *vs.* 132 ± 84 mg/dl, respectively; P<0.05), were smokers (14,8% *vs.* 25,3%; *P=0,19*) and reported higher use of statins (47.8% *vs.* 71.9%, respectively; p<0.05). The prevalence of other cardiovascular risk factors was comparable between groups. A coronary involvement was almost absent in patients under the age of 30 years. Among HeFH, 29 patients (27,5%) had non-significant coronary artery lesions (stenosis <50%) and 22 patients (23.2%) had coronary lesions of which at least one significant (stenosis ≥50%). Among patients with PFH, 11 patients (40%) had one or more non-significant lesions while 4 patients (14.8%) had one or more lesions of which at least one significant.
Overall, 53.7% of HeFH and 55.6% of PHF showed any grade of coronary atherosclerosis and this difference was not statistically significance.
In a subset of 40 subject (22 HeFH and 19 PH) we evaluated the LDL-C burden before enrollment. While HeFH patients had LDL-C values significantly higher than PH at enrollment (173,6 mg/dl vs. 155 mg/dl respectively; p<0,05), the LDL-C burden values were similar (burden LDL-C pre-V1: 210 mg/dl *vs.* 191 mg/dl respectively, p=0,07). In this subset the coronary atherosclerotic burden was similar between the groups.
Conclusions: Coronary atherosclerotic burden is similar between HeFH and PH individuals.
In a subset of subjects we observed that the HeFH subjects had higher values of LDL-C at enrollment than the PH subjects, but mean LDL-C exposure values over time were similar in the two groups suggesting that HeFH individuals are well treated.
Larger studies are needed in order to better clarify this issue.

ACHILLES TENDON XANTHOMAS AND THICKNESS ARE ASSOCIATED WITH CLINICAL AND SUBCLINICAL ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA: AN ULTRASONOGRAPHIC STUDY

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Aims: Achilles tendon xanthomas (ATX) and thickness (ATT), an early sign of ATX, reflect the cumulative burden of LDL-cholesterol and are markers of atherosclerotic cardiovascular disease (ASCVD) risk. However, the ability to detect ATX and ATT by clinical examination is limited and may be improved by ultrasonography. We aim to clarify whether ultrasonographic (US) ATX and ATT are associated with clinical and subclinical ASCVD in familial hypercholesterolemia (FH).
Methods: 89 adult patients (men/women 45/44; age 48[18-82] years) with a clinical and/or molecular diagnosis of definite FH were submitted to bilateral Achilles tendon ultrasonography with measurements of ATT and evaluation of ATX, defined as focal hypoechoic lesions. Clinical ASCVD was retrieved by patient history and chart review; subclinical ASCVD was evaluated by carotid ultrasound with assessment of plaques and intima-media thickness (c-IMT).
Results: Ultrasonography increased the detection of ATX from 23 (25.8%) to 59 (66.3%) FH patients. FH patients with US-ATX had lower HDL-cholesterol and higher LDL-cholesterol and US-ATT. In its turn, US-ATT was significantly negatively associated with HDL-cholesterol (rho:-0.261; p=0.015) and positively associated with age (rho:0.235; p=0.026) and LDL-cholesterol (rho:0.476; p<0.001). 13 (14.6%) FH patients had a history of ASCVD, mainly coronary heart disease (CHD). FH patients with US-ATX showed a significantly higher prevalence of ASCVD than their counterpart without US-ATX (20.3% *vs.* 3.3%; p=0.032); this association was largely conveyed by CHD (18.6% *vs.* 3.3%; p=0.046). They also had a significantly higher prevalence of carotid plaques (52.9% *vs.* 25%; p=0.023) and presented significantly higher c-IMT (p=0.011). Consistently, US-ATT was significantly higher in FH patients with a history of ASCVD (p=0.023) or CHD (p=0.038) and in FH patients with carotid plaques (p=0.001); moreover, US-ATT was significantly and positively associated with c-IMT (rho:0.517; p=0.001).
Conclusions: US-ATX and US-ATT are associated with clinical and subclinical ASCVD in FH patients. Achilles tendon ultrasonography may have not only diagnostic but also prognostic implications in FH patients.

BISPHOSPHONATE EXPOSURE AND INCIDENCE OF CARDIOVASCULAR AND CEREBROVASCULAR EVENTS: A POPULATION-BASED RETROSPECTIVE COHORT STUDY

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Background and aim: Although bisphosphonates have been suggested to protect against atherosclerotic cardiovascular (CV) events, due to their ability to reduce calcification in arterial walls, evidence is still conflicting. We aimed to investigate the effect of bisphosphonates on CV events.
Methods: We carried out a retrospective cohort study selecting from administrative databases of Lombardy (Italy) patients aged ≥40 years, with a first prescription of bisphosphonates between 2003/01/01 and 2008/12/31. Subjects were followed until the first CV event, death or migration, or 2012/12/31. Exposure to bisphosphonates was characterized based on cumulative doses (proportion of days covered). A proportional hazards model was fitted to estimate the association between cumulative time-dependent exposure with bisphosphonates and CV events (hazard ratios [HRs] and 95% confidence intervals). The model was adjusted for age, sex, comorbidities (such as atrial fibrillation, fractures, and Charlson Comorbidity Index) at baseline, and concomitant treatments (as time-dependent variables).
Results: Among 82,704 new bisphosphonates users (women 87.0%, mean age 70.7±10.6 years), 16.1% had a CV event during the follow-up. Alendronic acid was the most commonly prescribed bisphosphonate (67.1%), followed by risedronic acid (22.1%). Compared with individuals with an exposure <25% of follow-up, those exposed for a period ranged between 25%-50%, 50%-75%, or longer than 75% showed HRs [CI 95%] of 0.92 [0.87-0.98], 0.89 [0.84-0.94], and 0.72 [0.68-0.77], respectively. Analogous trends were found separately for cardiovascular (HRs of 0.87 [0.79-0.95], 0.86 [0.79-0.95], and 0.70 [0.64-0.77], respectively) or cerebrovascular (HRs of 0.96 [0.89-1.04], 0.91 [0.84-0.99], and 0.74 [0.68-0.80], respectively) events.
Conclusion: These results suggested a significant CV risk reduction (ranged from 8% to 28%) with increasing number of days covered by bisphosphonate treatment. Bisphosphonate therapy seems to be more effective in reducing cardiovascular rather than cerebrovascular events. Further studies to confirm this protective effect of bisphosphonate therapy and to investigate possible mechanisms are warranted.

DETERMINANTS OF LONGITUDINAL CHANGES IN EXERCISE BLOOD PRESSURE IN A POPULATION OF YOUNG ATHLETES

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Aim: In adults, a higher exercise blood pressure predicts the development of overt hypertension and future cardiovascular events. The aim of this study is to investigate correlates and determinants of longitudinal variations in exercise blood pressure in young athletes.
Methods: Longitudinal retrospective study conducted on adolescent athletes who underwent at least two pre-participation screening visits that included maximal exercise test on treadmill by using standardized ramp incremental protocol. Blood pressure (auscultatory method) was assessed at rest (SBP_{rest}), at 3rd minute of exercise (SBP_{3min}) and at exercise peak (SBP_{peak}). Predictors of changes vs baseline (Δ) of SBP_{rest}, SBP_{3min} and SBP_{peak} were determined by multivariate regression models after adjustment for age, sex, follow-up duration, related baseline SBP values, characteristics of sport, baseline and ΔBMI.
Results: 351 young athletes (mean age at baseline 13±2 years, 54% boys, average follow-up duration 3.4±2.2 years) were enrolled. At baseline, mean SBP_{rest} was 103±14 mmHg, mean SBP_{3min} 124±18 mmHg, mean SBP_{peak} 154±23 mmHg. We observed significant between-visit increase in SBP_{rest} (ΔSBP_{rest} 7.0±17.4 mmHg; p vs baseline<0.001), ΔSBP_{3min} (4.8±11 mmHg, p<0.001), and ΔSBP_{peak} (11.7±24 mmHg, p<0.001). BMI increased by 1.5±1.8 kg/m2 (p<0.001). ΔSBP_{3min} was significantly predicted by male gender (p<0.01), baseline BMI (p<0.01), ΔBMI (p<0.01) and number of sports (p<0.05), whereas ΔSBP_{peak} was positively predicted by male gender (p<0.01), baseline BMI (R 0.13; p<0.05), ΔBMI (p<0.01) and negatively by baseline resting heart rate (p<0.01). In a logistic regression model, ΔBMI was the only independent determinant of switching from a lower to an upper quartile of SBP_{3min} (p<0.001) while ΔBMI and male gender were independent determinants of moving to a higher quartile of SBP_{peak} (p<0.001).
Conclusions: Increase in BMI and male gender are independent determinants of the increase of exercise blood pressure, recorded both at maximal and at light intensity, during time in a population of of teenage athletes.

MARKERS OF VASCULAR DAMAGE IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES MELLITUS

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Aim. Type 1 diabetes mellitus (T1DM) is associated with early development of atherosclerosis. The aim of this study was to measure some indices of vascular damage in a group of children and adolescents with T1DM and their relationship with haemodynamic and metabolic parameters.
Methods. In a cross-sectional study, peripheral (pSBP/pDBP) and central systolic and diastolic blood pressure (cSBP/cDPB), carotid intima media thickness (cIMT) and carotid distensibility (cDC), pulse wave velocity (PWV) were measured using ultrasound and the SphygmoCor XCel device. Metabolic parameters, including triglycerides, LDL and HDL cholesterol, were measured and glycated haemoglobin (HbA1c; average of the last two years) was calculated.
Results. 126 subjects with T1DM (61 females and 65 males, mean age 15.9±2.6 years) followed at the Paediatric Diabetology Unit of Verona were evaluated at the Vascular Laboratory of the General Medicine & Hypertension Unit. Eighteen per cent of the sample had cDC under the 5th percentiles and 60% had cIMT higher than the 95th percentiles per sex and height. Both cSBP and pSBP were positively correlated with BMI (respectively r=0.404; p<0.0001 and r=0.279; p<0.01). A significant correlation between cSBP and all the measures of vascular damage was found: cDC (r=-0.410; p<0.0001), PWV (r=0.447; p<0.0001), cIMT (r=0.227; p<0.01). The duration of diabetes, LDL-cholesterol and the
average HbA1c were not correlated to any vascular phenotype. In linear regression analysis, after adjustment for all metabolic and anthropometric parameters, central SBP remained independently associated with subclinical carotid damage (for cIMT β=0.002; p=0.023; forcDC β=-0.343; p=0.003) and PWV (β=0.025; p<0.001) whereas LDL-cholesterol with cIMT(β=0.001; p<0.05) and PWV (β=0.025; p<0.01).
Conclusions. Subclinical vascular damages are present in a high proportion of children and adolescents with T1DM. cSBP, more than pBP, is independently associated with indexes of vascular subclinical atherosclerosis, suggesting a pivotal role of blood pressure homeostasis, along with LDL-cholesterol, in determining vascular damage even during childhood and adolescence in patients with T1DM.

POTENTIAL ROLE OF MENAQUINONE-4 IN OSTEOGENIC TRANS-DIFFERENTIATION OF VASCULAR SMOOTH MUSCLE CELLS ISOLATED FROM SPONTANEOUSLY HYPERTENSIVE RATS

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Aim: Vascular calcification (VC) is a biomolecular process which shares common features with osteogenesis. Several studies demonstrate that in presence of risk factors, such as hypertension, vascular smooth muscle cells (vSMCs) lose their contractile phenotype and trans-differentiate into a synthetic osteoblastic-like phenotype contributing to VC development. Recently, menaquinones (MKs) family, also known as Vitamin K2, has been revealed to play an important role in cardiovascular health by decreasing VC. However, the MKs effects and mechanisms potentially involved on vSMCs osteoblastic trans-differentiation are still unknown.
Thus, the aim of this study was to investigate the possible role of menaquinone-4 (MK-4), an isoform of MKs family, in the modulation of vSMCs phenotype.
Experimental protocol: vSMCs isolated from the thoracic aorta of Spontaneously Hypertensive Rats (SHR) were used as an *in vitro* model of cell vascular dysfunction while vSMCs from normotensive Wistar Kyoto rats (WKY) were used as control. Both vascular cellular models were induced to a calcification process and daily treated with MK-4.
Methods and Results: In both WKY- and SHR-vSMCs the exposure to calcification medium induced a trans-differentiation into osteoblastic-like cells associated with the loss of their contractile phenotype (*real time* RT-PCR, flow cytometry, scratch wound assay). Notably, this phenomenon was reverted by MK-4 daily treatment. Moreover, the gamma-Glutamyl-Carboxylase (GGCX, MK-4 activated enzyme) down-regulation (RNA interference) abolished the MK-4 protective effects on rans-differentiation process, demonstrating that MK-4 inhibits the WKY- and SHR-vSMCs osteogenic trans-differentiation through the GGCX-dependent pathway.
Conclusions: Taken together these results describe, for the first time, the mechanisms potentially involved in the capability of MK-4 to preserve vSMCs contractile phenotype by inhibiting the VC process. Thus, our findings highlight the important role of MK-4 in the prevention of vascular dysfunction and atherosclerosis, thus encouraging further in-depth studies to confirm its use as natural food supplement.

FROM LABORATORY TO CLINICAL PRACTICE: THE FASCINATING RELATIONSHIP BETWEEN MICROPARTICLES AND STATINS

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Aim: Microvesicles (MV) are extracellular vesicles detectable in the plasma of healthy subjects and in different clinical conditions and are considered CV risk factors influencing atherogenesis and thrombosis. The main progenitor cells of MV are erythrocytes, leukocytes, platelets and endothelial cells. The objectives of our study were to identify circulating MV levels in patients at CV risk treated or not with statins.
Methods: We enrolled 100 subjects at CV risk, on low-dose aspirin for CV prevention, 43 of whom on statin treatment. We analyzed circulating levels of MV in patients treated *vs.* not treated with statins, subdividing them according to the type of molecule. Total MV, platelet MV (PMV) (CD41a+), endothelial MV (EMV) (CD31+) and leukocyte MV (LMV) (CD45+) were quantified and characterized by flow-cytometry. Blood sampling was collected at 24 hours from a witnessed administration of ASA (T24).
Results: Total MV, PMV, EMV were significantly lower in patients with dyslipidemia as compared to non dyslipidemic subjects (P=0.012, P=0.22, P=0.021).
These findings prompted us to hypothesize that statins may affect MV levels. As expected, Total MV, PMV, EMV were significantly lower in statin-treated patients as compared to untreated patients (P=0.010, P=0.020, respectively). No statistically significant between-group differences were observed as to LMV.
Conclusions: Circulating MV levels were lower in patients on statin treatment than in patients not on statin treatment. Although the underlying mechanism is unknown, we can speculate that statins, by acting on the mevalonate pathway, may inhibit the G protein-coupled signaling, implicated in cellular signal transduction, possibly regulating MV release. Due to the emerging role of circulating MV as biomarkers of CV risk, their reduction mediated by statins may contribute to the beneficial effects of these drugs in terms of atherothrombosis prevention. Future studies will better clarify this effect, differences among molecules and interactions with other drugs.

ADD ON LIPID LOWERING THERAPY WITH PCSK9 INHIBITORS RAPIDLY IMPROVES ARTERIAL STIFFNESS IN OFF-TARGET PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA: TWO CENTRE EXPERIENCE

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Background: FH is characterized by elevated LDL-C levels and high CV risk. Prognosis correlates with lifelong LDL-C levels, and therapy aims to achieve and maintain LDL-C target over years. Vascular dysfunction occurs years before atherosclerosis is detectable by instrumental tests. PWV is considered an early, reliable marker and independent prognostic predictor for CV mortality.
Aim: To evaluate the effectiveness of an add-on therapy on vascular arterial function, in a setting of LDL-C off-target HeFH patients (primary/secondary prevention).
Materials and Methods: we enrolled 78 patients (M/F 38/40, aged 18-70 years) with HeFH in primary (50) or in secondary (28) prevention. At the first visit, 62 patients were already on statin regimen, but off-LDL-C target. Thirty-nine patients were not receiving therapy with ezetimibe and it was added on. Thirty-nine patients already at best conventional therapy received a prescription with iPCSK9 (evolocumab/alirocumab=21/18). At baseline and 6 months after add-on treatment we tested for blood chemistry, arterial stiffness (SphygmoCor) and anthropometric parameters.
Results: CVD was more prevalent in iPCSK9 group (58% *vs*. 11%). At T1 we found a significant reduction of LDL-C: added-on ezetimibe patients experienced a 23.34% (mean LDL-C 98.2±19.7, 41.7% on-target) reduction while iPCSK9 patients a 62.41% (mean LDL-C 62.33±17.1, 77.5% on-target). PWV decreased in both groups (9.4% *vs*. 16.39%; p=0.025). Due to the small sample size we cannot adjust this result for primary/secondary prevention prevalence.
Conclusions: A 6-months add-on treatment with iPCSK9 mAbs appears effective in improving significantly PWV, with respect to ezetimibe, in high and very high risk patients.

PCSK9: A NEW PLAYER IN VASCULAR CALCIFICATION?

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Aim: Vascular calcification is a pathological process frequently occurring in patients with chronic kidney disease (CKD). It is mediated by osteochondrocytic-like cells arose by the transdifferentiation of vascular smooth muscle cells (VSMCs). Several mechanism underlying the onset and progression of the pathology are already known, but some pieces are still missing. Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) levels correlates with the presence of calcific aortic valve stenosis and carriers of the PCSK9 R46L loss-of-function variant have a low calcific aortic valve stenosis. Moreover, PCSK9 is secreted by SMCs. The aim of this project is to unveil the role of PCSK9 secreted by SMCs in the vascular calcification process.
Methods: Twenty-two male Sprague-Dawley rats were equally divided in two groups and fed for six weeks with a standard diet or with a low-protein diet supplemented with 0.5% adenine to establish a uremic status that led to medial vascular calcification, whose levels in aortas have been assessed both by von Kossa staining or HCl incubation followed by a colorimetric assay after sacrifices. PCSK9 expression level in kidneys and livers has been evaluated by western blotting analysis, and plasma PCSK9 levels by ELISA assay. *In vitro*, rat SMCs control and overexpressing PCSK9 (rSMC/rSMCs^{PCSK9}) have been incubated with 2.0 mM and 2.4 mM of NaH_2PO_4 in DMEM/2.5%FCS for 7 days. Calcium deposition has been measured with a colorimetric assay.
Results: The uremic condition in rats was documented by increased urine volume (1.1±0.5 ml/h *vs* 0.8±0.2 ml/h), plasma creatinine (25.7 µM *vs.* 208 µM) and phosphate levels (2.64 µM *vs* 6.11 µM). High phosphate concentration lead to higher deposition of Ca^{2+} into the media of aortas (0.34 mg/g tissue *vs.* 2.48 mg/g tissue). This pathological condition was associated to a significant increase of total cholesterol (from 75.3 mg/dL to 107.6 mg/dL) and PCSK9 levels (from 40.1 ng/mL to 109.7 ng/mL). Higher expression of PCSK9 was also observed in kidney (+4.8-fold) and liver (+1.5-fold). rSMCs^{PCSK9} showed a significant increase of extracellular Ca^{2+} deposition in response to 2.4 mM PO^{4-} treatment (+3.1-fold *vs* rSMCs).
Conclusions: The present study highlight a potential role of PCSK9 on vascular calcification associated to a uremic. Further analysis will attempt to identify the molecular mechanism of this action and to study the effect of monoclonal antibodies anti PCSK9.

SEX STEROIDS MODULATE ADIPOGENESIS MARKERS AND PRO-INFLAMMATORY MEDIATORS EXPRESSION IN HUMAN ADIPOCYTES: LOOKING FOR A LINK BETWEEN HORMONES, METABOLISM AND CARDIOVASCULAR DISEASE

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Aim and background: Sex hormones abnormalities have been associated with an increased risk of cardiovascular disease, but mechanisms responsible for this relationship are still largely unknown. Low testosterone plasma levels are commonly observed in metabolic syndrome and aging in men, while high testosterone levels are the classical feature of polycystic ovary syndrome (PCOS). All these conditions are characterized by a pathological ectopic distribution of fat mass (muscle, liver, abdomen) with an impaired insulin sensitivity leading, together with other factors, to a chronic systemic inflammatory state involved in the development and the progression of atherosclerotic process. In this study we evaluated the possible direct effects of sex steroids on adipocyte biology and inflammatory response, hypothesizing a role of sexual hormones in the inflammatory imbalance as an additional cardiovascular risk factor.
Methods: Preadipocytes isolated from subcutaneous adipose tissue of obese patients have been differentiated in vitro to mature adipocytes and incubated with testosterone and 17-β-estradiol and in the presence and absence of their respective receptor antagonist flutamide and tamoxifene. We evaluated the gene expression (mRNA) of markers of adipogenesis (PPARγ, FABP-4), adipokines such as leptin and adiponectin, and inflammatory mediators such as MCP-1, IL-6 and TNF-α.
Results: incubation of human adipocytes with testosterone reduced mRNA expression of FABP-4 and PPARγ and both leptin and adiponectin, while the pre-incubation with flutamide inhibited the negative effects of testosterone on the expression of these adipogenic markers. On the contrary, the incubation with 17-β-estradiol showed an increase in mRNA expression of these markers with a dose-dependent effect. Testosterone exposure was also associated with a clear increase of MCP-1 expression, while only a mild increase on IL-6 was observed. These effects were reversed by pre-incubation of adipocytes with flutamide. On the contrary, incubation of human adipocytes with estradiol reduced the basal expression of MCP-1 and IL-6 in a dose dependent manner. These effects were reversed by pre-incubation of adipocytes with tamoxifene.
Conclusions: sex hormones can influence the expression of adipogenesis markers, adipokines and pro-inflammatory mediators in human adipocytes; however, sex steroids seem to have a very complex role, ranging from apparent beneficial effects on one hand (estrogen) and deleterious effects on the other hand (testosterone). Further studies are needed to better explain their possible role in cardiovascular homeostasis and the gender differences in the phenotypes of cardiovascular diseases.

FATTY ACID PROFILE IN 7-11 YEARS OLD CHILDREN ATTENDING THE PRIMARY SCHOOL IN VERONA SOUTH DISTRICT

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Objective: Dietary and circulating fatty acids (FA) and desaturases activity, involved in FA metabolism, are associated with metabolic and cardiovascular disorders. In particular, a lower delta-5 desaturase activity (D5D) in contrast to a higher delta-6 desaturase (D6D) and stearoyl-CoA desaturase (SCD-16; the enzyme that metabolizes palmitic (PA) to palmitoleic acid (PtA) activities were related to obesity and metabolic disorders.
Method and aim: FA profile was determined on whole blood drop in 244 children (50.2% females; mean age 8.6±0.72 years) participating in a school-based cross-sectional study. The possible associations between individual FA and desaturases activity (evaluated as product: precursor ratios) with indices of adiposity and blood pressure (BP) was investigated.
Results: Omega-3 Index, marker of long-term eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) dietary intake, was low (3.9±0.88%). DHA inversely correlated with BMI and waist/height ratio, whereas oleic acid, PA and individual omega-6 PUFAs were not associated with either obesity or BP, except for a direct correlation of dihomo-gamma-linoleic acid (DGLA) with some indices of adiposity. PtA, an omega-7 FA, was directly associated with adiposity (BMI: $r=0.408$, $p<0.01$; waist circumference: $r=0.368$, $p<0.01$; fat mass: $r=0.402$, $p<0.01$) and BP (Systolic: $r=0.203$, $p<0.01$; Diastolic: $r=0.167$, $p<0.01$). D5D inversely correlated with waist circumference, D6D directly with waist/height ratio and SCD-16 directly with most adiposity indices and BP. Children with excess weight (>85th percentile) showed higher concentrations of PA, PtA and a higher SCD-16 activity compared to normal-weight children. In the excess-weight group PA directly correlated with some anthropometric measures and PtA confirmed the direct association with obesity and BP. Even SCD-16 directly correlated with anthropometric features and BP. Caucasian children (66.8%) had higher D6D activity than non-Caucasians (33.2%), and in Caucasians D6D directly correlated with obesity. In this subgroup PA, PtA and SCD-16 directly correlated with anthropometric characteristics and BP.
Conclusions: PtA and SCD-16 directly correlated with indices of adiposity and BP, especially in obese children. D6D activity is likely influenced by ethnicity, showing an apparent harmful effect especially in Caucasians.

A SYSTEMATIC REVIEW AND META-ANALYSIS OF RENAL TARGET ORGAN DAMAGE IN PRIMARY ALDOSTERONISM

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Aim: Aldosterone excess has been linked to the progression of renal disease in experimental animal models. Several studies evaluated the risk of renal damage in patients with primary aldosteronism (PA) with conflicting results. The aim of this study is to determine the relationship between PA, renal target organ damage and its reversibility, by integrating evidences from previous clinical studies.
Methods: We performed a meta-analysis of prospective and retrospective observational studies. We sought MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials for articles published from January 1960 up to August 2017. 44 studies were included in the analysis, accounting for a total of 4,438 patients with PA and 8,234 patients with EH.
Results: After 8.5 years from the diagnosis of hypertension, patients with PA diagnosis had an augmented glomerular filtration rate (GFR) compared to hypertensive patients without PA (by 3.93 ml/min IQR [0.60; 7.26]) and increased albuminuria (Std. mean difference 0.57 [0.11-1.03]), resulting into a significant association with microalbuminuria (OR 2.15 [1.21; 3.84]). After a median follow-up of 12 months after specific PA treatment, GFR was significantly reduced (by -10.57 ml/min [-13.60; -7.55]). The difference was significant in both patients treated with unilateral adrenalectomy and patients treated with mineralcorticoid receptor (MR) antagonists. Accordingly, a reduction in albumin excretion and augmented serum creatinine were observed after specific PA treatment.
Conclusion: Hypertensive patients with PA, compared to non-PA hypertensive patients, display a more severe renal target organ damage, which can be reduced by the specific PA treatment, highlighting the importance of an early diagnosis of PA.

SPECIFIC HDL FUNCTION MODIFICATIONS IN PATIENTS WITH ABDOMINAL AORTIC ANEURYSM

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Aim: Abdominal aortic aneurysm (AAA) is a bulging, weakened area in the wall of the aorta, correlated to genetic factors and smoke. The relationship between AAA pathogenesis/progression and serum lipid levels or function is not well understood. We analysed serum lipoproteins functions, as cell cholesterol efflux capacity (CEC) and cholesterol loading capacity (CLC), in patients with AAA to evaluate not their atherogenic potential but a possible relationship with AAA development.
Methods: AAA (n=30) and control patients (n=21), age- and sex-matched with no aneurysm but with the same pro-atherogenic comorbidities, were enrolled. HDL CEC was assessed by radioisotopic technique specific for aqueous diffusion (AD) and ABCG1 or ABCA1 transporters. Serum CLC was measured by a fluorimetric assay.
Results: We did not find any significant difference comparing serum lipid profile in patients with AAA and control patients. ABCG1-CEC was significantly reduced while ABCA1-CEC was higher in AAA patients, suggesting a block in HDL maturation. This is supported also by the finding that ABCG1-CEC inversely correlates to ABCA1-CEC and directly correlates to AD-CEC only in AAA patients. Again only within the AAA group, smokers had significantly lower ABCG1-CEC and higher ABCA1-CEC compared to non-smokers, indicating a role for smoke in altered HDL maturation in AAA patients. CLC did not differ between AAA patients and controls, but only in the AAA group CLC values correlated directly to HDL levels, ABCG1-CEC and AD-CEC, suggesting a role for HDL in serum-induced cell cholesterol accumulation only in AAA patients.
Conclusions: Our data indicate that lipoprotein function modifications specific for AAA patients occur, likely due to a block in HDL maturation and possibly favoured by smoke. Such lipoprotein function abnormalities are not necessarily involved in the atherosclerotic process, but could contribute to local aortic wall pathologic processes involved in AAA development.

ENDOCAN LEVELS AS MARKER OF CARDIOVASCULAR HEALTH IN IBD PATIENTS

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Background: Inflammatory Bowel Diseases (IBDs) are systemic disorders characterized by increased CV risk; however, CV risk assessment is not always *easy*, and it is often underestimated mainly in earlier phases of the ill, when CV involvement is generally subclinical. Endocan, a novel biomarker of endothelial dysfunction, plays a key role in endothelial and systemic inflammatory reaction; recently, Endocan was found significantly increased in IBD patients.
Aim: To evaluate novel potential markers of early cardiovascular involvement in patients with refractory (as considered after six months of conventional therapy with CCS plus mesalazine) IBD before and after treatment with biological drugs (infliximab, adalimumab, vedolizumab). Consistently, circulating CD34+ cell count and Endocan levels were assessed, along with Carotid-femoral Pulse Wave Velocity (cf-PWV), global longitudinal strain (GLS) assessment.
Methods: Clinical and instrumental examination was performed in 27 IBD patients; PWV, GLS, endocan levels and circulating CD34+ cells were evaluated before (T0) and after (T1) a six-months treatment with biological drugs. GLS was measured by speckle tracking echocardiography. Circulating CD34+ were counted by flow cytometry. In addition, inflammatory indices and EF% were also evaluated.
Results: At T1, we found a significant reduction of Endocan levels (-22.16%, p=0.045), and CRP (-67%, p<0.005) as well CD34+ cell count (+6.5%, p=0.047) and GLS (+26%, p<0.001) were increased. No statistically significant difference as regards ESR, PWV, EF with respect to T0. The interdependence analysis performed on the mean percent changes showed a significant correlation between ΔEndocan and ΔGLS (r=0.80), and a trend with ΔCD34+ (r=-0.31).
Conclusions: In our study we have shown that biological drugs may improve inflammatory status, clinical compensation and CV risk as suggested by favorable change of CRP, Endocan and CD34+ plasma levels and GLS values. This study is limited by short patients cohort to confirm this preliminary data. Endocan is involved in a variety of biological processes including cell proliferation, migration, and neovascularization. Its levels are closely related to the development and progression of CVD. Patients with IBD have a greater risk of developing CV disease, especially when IBD is clinically *uncontrolled*.

WHAT ABOUT THE CHILDREN? NON ALCOHOLIC FATTY LIVER DISEASE IS HIGHLY PREVALENT AND LINKED TO HYPERTENSION IN A GROUP OF OBESE CHILDREN

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Background and Aims: Obesity leads to the clustering of metabolic syndrome (MetS) and cardiovascular risk factors (CVRF), also in children. Non-alcoholic fatty liver disease (NAFLD) often accompanies MetS and is described like a new independent CVRF. The aim was to investigate the prevalence of NAFLD and its associations with single CVRF in a group of 70 obese children.
Method: Anthropometric parameters (weight, height, waist circumference) were taken together with routine blood test including lipid panel, HOMA IR, glucose, hepatic function indices and calculation of APRI, FIB-4 and PNFI (pediatric nafld fibrosis index) scores. Ultrasound (US) measurement of cIMT (carotid intima-media thickness) and carotid distensibility were taken as well as office and ambulatory blood pressure (BP) values. NAFLD was defined as ALT >25 and >22 IU/mL (males and females respectively) and/or evidence of liver fatty infiltration at abdominal US. Data are presented as median ± standard deviation.
Results: 70 obese children (male 57%;mean age of 11,5±2,5 years) were enrolled. Mean BMI-Z score was 2,17±0,35. NAFLD was identified in 59,6% of participants. ALT was elevated in 31,9%. HOMA IR was ≥2,5 in 71,4% (63,6% in those with NAFLD and 75% in those without NAFLD). Liver US showed the presence of steatosis in 59,6%. APRI score was significantly higher in the NAFLD group (0,12±0,09 *vs.* 0,24±0,12, p=0,003), instead of PNFI and FIB-4, compared to non-NAFLD subjects. Total Cholesterol, TG, HDL, LDL were similar in both group. BMI-Z score, HOMA IR, cIMT, waist/height ratio were not associated with NAFLD, while hypertension was associated with NAFLD (83,3% in NAFLD participants *vs.* 50,0% in non-NAFLD subjects, p=0,0017, at Fisher's exact test).
Conclusion: NAFLD is highly prevalent in obese children. Hypertension and APRI score are associated with NAFLD. These findings may serve to develop a non-invasive screening tool to help clinicians identify children with NAFLD avoiding liver biopsy.

DECIPHERING THE ROLE OF V200A AND N291S MUTATIONS LEADING TO LPL DEFICIENCY

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Aims: Type 1 hyperlipoproteinemia is a rare autosomal recessive disorder most often due to loss of function mutations in the lipoprotein lipase gene (LPL), a key enzyme in triglycerides (TGs) catabolism, catalyzes the hydrolysis of TGs in TG-rich lipoproteins resulting in severe hypertriglyceridemia and recurrent pancreatitis episodes. This study was aimed at evaluating the molecular mechanism of two known mutations in LPL gene by *ex vivo* and *in vitro* experiments and the effect of two splice site mutations by *ex vivo* experiments.
Methods: Two patients with hypertriglyceridemia were selected from the Lipid Clinic in Vienna. The first patient was compound heterozygous for c.680T>C (p.V200A) and c.1139+1G>A. The second patient compound heterozygous for c.953A>G (p.N291S) and c.1019-3C>A. LPL gene was sequenced and post-heparin plasma samples (ex vivo) were used to test LPL activity.
In vitro experiments were performed in HEK293T cells transiently transfected with wild type or mutant LPL plasmids. Cell lysate and media were used to evaluate LPL production, secretion and activity by western blot analysis and LPL enzymatic assay.
Results and conclusions: Immunoblot analysis and LPL activity measurement in plasma samples showed a faint LPL band and a strong reduction of LPL activity in both patients. *In vitro* experiments, showed a 10% reduction in LPL synthesis in both mutant cells whereas LPL secretion was significantly reduced (80%), only in V200A mutant. Concentrated media of cells transfected with wild type or mutant *LPL* plasmids were used to examine LPL activity. V200A is a mutation altering LPL production, secretion and activity; conversely, cells transfected with N291S showed normal levels of LPL protein but absent enzyme activity. In conclusion, we (i) described a novel pathogenic *LPL* splice site mutation leading to virtual absence of LPL activity and (ii) characterized at the molecular level these LPL mutations showing that are pathogenic.

ULTRASOUND-BASED ASSESSMENT OF HEPATIC STEATOSIS: A DEEP LEARNING APPROACH

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Aim: Non-Alcoholic Fatty Liver Disease (NAFLD) is becoming a global epidemic and represents a risk factor for cardiovascular disease (CVD) independently of the features in common with the metabolic syndrome. Ultrasound (US) is a non-invasive and highly available imaging technique but suffers from operator-dependency and low reproducibility; furthermore, it is used only in a qualitative or semi-quantitative way for the assessment of hepatic fat content. Deep learning (DL) techniques, employing convolutional neural networks (CNN), can overcome these limitations. The aim of this study was to develop a US-based DL approach for the evaluation of hepatic steatosis.
Methods: The study population included 150 subjects and was divided on the base of Magnetic Resonance Spectroscopy (MRS) measurements: a previously identified cut-off of 3.12% was employed to discriminate between presence and absence of steatosis. For each subject, US images were acquired in oblique subcostal view and properly pre-processed in order to be suitable for the DL approach. The well-known CNN InceptionV3, already pre-trained on natural images, was chosen and a transfer learning process with the customization of the final layers was performed. A fine tuning with a very low learning rate (0.0001) was implemented in order to achieve a CNN able to deal with US images. The whole dataset was divided as follows: 80% of the images were employed for the training phase while the remaining 20% for the test phase. The training dataset was further divided: 70% of the images were employed for the real training phase and the remaining 30% for the validation one.
Results: The final CNN applied on the test dataset provided 88.3% sensibility, 99.2% of specificity and 96.7% of accuracy.
Conclusions: These preliminary results suggest that this system could represent a valid approach for overcoming the US-related limitations in the assessment of hepatic fat content.

INCREASED GUT PERMEABILITY IS ASSOCIATED WITH OXIDATIVE STRESS IN PATIENTS WITH NAFLD

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Aim: Non-alcoholic fatty liver disease (NAFLD) represents the most common liver disease worldwide. Based on multiple hits hypothesis, oxidative stress plays a pivotal role in NAFLD onset and progression from simple steatosis to steatohepatitis (NASH). However, factors inducing oxidative stress in NAFLD are still unknown. Clinical e experimental data demonstrated that intestinal bacterial overgrowth and augmented gut permeability were associated with NAFLD. Aim of the study was to investigate the relationship between oxidative stress and LPS in patients with NAFLD.
Methods: We enrolled 44 were patients affected by non-alcoholic steatohepatitis (NASH); 50 subjects with simple steatosis (NAFL) and 50 subjects without hepatic steatosis balanced for age, gender and BMI were used as controls. Serum bacterial lipopolysaccharide (LPS) and serum zonulin, a marker of gut permeability, were measured with ELISA technique. Serum NOX2-derived peptide (sNOX2dp), a marker of NADPH oxidase activation, was detected in serum by enzyme-linked immuno- sorbent assay method.
Results: Patients had no difference in diabetes and arterial hypertension prevalence and in statin and aspirin use. NASH patients, as compared to NAFL and controls, had increased median sNOX2dp ($p<0.001$) and increased median LPS ($p<0.001$). At bivariate analysis sNOX2dp was correlated with LPS ($rs=0.608$, $p<0.001$), γGT ($rs=0.576$, $p=0.000$), AST ($rs=0.560$, $p=0.000$) and ALT ($rs=0.620$, $p=0.000$). LPS was correlated with zonulin ($rs=0.375$; $p<0.001$). At linear regression analysis, after correction for age, sex, BMI and diabetes and aspirin use, LPS above median (Beta=0.059; $p<0.05$) and liver diagnosis (Beta=0.913; $p<0.001$) were independently associated with sNOXdp.
Conclusions: Patients with NAFLD have higher sNOXdp indicating more oxidative stress in this setting. LPS increase may result from impaired gut permeability and induce oxidative stress.

COULD NAFLD AFFECT VASCULAR FUNCTION AND MORPHOLOGY BEYOND HYPERTENSION?

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Background: Non-alcoholic fatty liver disease (NAFLD) is associated with an increased risk of cardiovascular disease (CVD) that can be amplified by the presence of hypertension.
Aim: to assess the difference in markers of subclinical atherosclerosis, arterial stiffness, and in the degree of liver fibrosis in NAFLD patients with or without hypertension.
Methods: Forty-four participants (52,9±6,9yr; males=86,3%) were divided according to the presence of NAFLD and essential hypertension in three groups: a group carrying NAFLD but not hypertension (only-NAFLD; n=15), a Hypertension group (only-HT; n=10) and a NAFLD+Hypertension group (NAFLD+HT; n=19). Patients with class II/III obesity and diabetes were excluded. Central and peripheral blood pressure (BP), carotid ultrasonography, and fibroscan were performed by validated devices. Carotid intima–media thickness (cIMT) as marker of subclinical atherosclerosis and Carotid Distensibility (CD), as marker of local stiffness were measured using the "Carotid Studio" hardware (Quipu). Carotid-femoral pulse wave velocity (cf-PWV) was measured by the SphygmoCor XCEL.
Results: The prevalence of atherosclerotic plaques was significantly higher in the NAFLD+HT group as compared with the only-NAFLD group (47% *vs.* 6,7%; $p<0,05$). Only 3 patients in the whole population showed a pathological value of cIMT (>0,9mm) whereas cf-PWV was below 10m/s in all participants. The prevalence of significant hepatic fibrosis (liver stiffness≥7kPa) was 11%. No significant differences in central BP, cIMT, CD, cf-PWV and liver stiffness were found among the three groups.
Conclusion: Overt atherosclerosis, as witnessed by a higher prevalence of carotid plaques, rather than markers of subclinical atherosclerosis and arterial stiffness were more evident in patients with NAFLD+HT. Only when at least HT is present, NAFLD seems associated with an increased atherosclerotic risk.

POSTER

NON-ALCOHOLIC FATTY LIVER DISEASE, INSULIN-RESISTANCE AND HYPOCHOLESTEROLAEMIA: WHAT'S THE COMMON THREAD?

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Background: NAFLD is defined as the presence of steatosis over 5% into the hepatocytes and Its pathophysiological cause is the insulin-resistance (IR). Many subjects with NAFLD had high LDL cholesterol and triglycerides (TG) plasma levels; however, NAFLD may be found also in subjects with low LDL cholesterol such as hypobetalipoproteinemia.
Case report: a 37-year-old man with hypertransaminasemia was evaluated at the Day Service of Internal Medicine of Garibaldi Hospital, Catania. He had a family history of type 2 diabetes and he denied alcohol consumption. Blood samples showed normal fasting plasma glucose and HbA1c, TC 107 mg/dl, HDL 40 mg/dl, LDL 52 mg/dl, TG 54 mg/dl, ApoB 48 mg/dL, ApoAI 95 mg/dL, ApoB/apoAI 0.49, AST 60 UI/L, ALT 154 UI/L. Abdominal ultrasonography showed a severe liver steatosis. Blood tests excluded viral and autoimmune causes, Wilson's disease and haemocromatosis. To better evaluate glucose homeostasis, we performed a 75-gram oral glucose tolerance test; the plasma glucose and insulin levels were respectively 80 mg/dL and 113 µUI/ml at 120'. These findings confirmed IR. In consideration of low LDL-cholesterol, we hypothesized the hypobetalipoproteinaemia; so, to confirm this diagnosis, the patient performed a genetic test. This revealed the presence of three heterozygous variants: one in the ApoB gene (exon 14: c.1837A>C, p.Lys613Gln), and two in the MTTP gene (exon 4: c.294G>C, p.Glu98Asp; exon 5: c.497A>G, p.Asn166Ser). These variants were not present on the database of pathogenetic mutation database of FHB but the bioinformatic software indicated a "possibly damaging effect" of MTTP genetic variant c.294G>C.
Conclusion: the presence of a possible pathogenic MTTP variant may link the presence of low LDL cholesterol, IR and NAFLD in this patient. In these subjects, it should be planned a follow-up with liver ultrasonography and elastography; also, it should be considered a possible deficiency of liposoluble vitamins.

LOW PHYSICAL ACTIVITY INCREASING ATHEROSCLEROTIC LIPIDIC MARKERS IN NAFLD PATIENTS: PERSPECTIVES FROM THE 'H2020 FOIE GRAS' PROJECT

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Background and aim: Non-alcoholic fatty liver disease (NAFLD) affects ≈25% of adults. The prevalence is rising worldwide, alongside obesity, metabolic syndrome and markers of atherosclerosis, such as intima-media thickness (IMT) and epicardial fat (EF). Healthy lifestyles, including appropriate levels of physical activity, slow the progression of disease. We studied the cardiovascular profile of adult patients and young healthy subjects living in Apulia, Italy.
Methods: Fifty patients from a gastrointestinal clinic (M:F=32:18; mean age 45±1.6 yrs; range 21-66; 54% obese) were gender-matched with a younger healthy group (M:F=29:21; mean age 28±1.3 yrs; range 20-62). Liver steatosis, IMT and EF were assessed ultrasonographically (Noblus® Hitachi, 3.5, 7.5 MHz Probe, Italy; score 0-3 and ARFI=Acoustic Radiation Force Impulse by GE Healthcare, USA; score F0-F4). The International Physical Activity Questionnaire (Minetto et al. 2018) described levels of physical activity by Metabolic Equivalent Tasks (METs; 1 MET=3.5 mL/kg/min of oxygen consumption or 1.5 Kcal/kg/hr).
Results: NAFLD patients (mean degree of steatosis: 1.6) were heavier than healthy subjects (95.5±2.7 *vs.* 64.1±1.8 kg, respectively; P<0.0001 for both). Both IMT and EF were thicker in patients than controls (1.0±0.1 *vs.* 0.6±0.0 mm and 8.1±0.4 *vs.* 4.5±0.2 mm, respectively; P<0.0001 for both). Patients with abnormal IMT (≥1.0 mm) and abnormal EF (≥5.0 mm) were 34% (n=17), whereas 92% (n=46) presented abnormal EF. None of the healthy subjects had abnormal IMT, but 44% (n=22) had slightly increased EF. Patients were more sedentary (<675 METs/week) than controls (58% *vs.* 18%, respectively; P=0.001). Both groups preferred light physical activity (≈3.0 METs; 83% overall), with healthy subjects exercising longer at this intensity than patients (1310±147 *vs.* 579±127 METs, respectively; P<0.0001).
Conclusions: From southern Italy, we detect a worrisome trend towards increased risk for atherosclerosis in NAFLD patients. Physical activity remains a crucial cornerstone for ameliorating the fatty liver condition of Mediterranean adults.

CARDIOPULMONARY CONSEQUENCES IN HEPATITIS C (HCV) PATIENTS WITH LOW-MILD LIVER FIBROSIS

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Aim: It is currently evident that hepatitis C virus (HCV) infection is associated with a wide range of hepatic and extrahepatic systemic effects thanks to direct and indirect mechanisms. Our purpose is to evaluate the morpho-functional profile of heart and lung in patients with hepatitis C.
Methods: Sixteen patients with mild to moderate hepatic fibrosis (F0, F1, and F2) were subjected to transthoracic echocardiography, cardiopulmonary exercise test (CPET) and spirometry and were compared to sixteen healthy subjects.
Results: We documented an increase in the right atrium volume (Rav/BSA=19,88±1,83 mm^3 in HCV+ *vs.* 13,99±0,29 mm^3; p<0.001) and right ventricle diameter (Basal RVD=35,3±7.1 mm in HCV+ *vs.* 32,1±5.0 mm; p<0.01). In addition, the systolic excursion values of the tricuspid valvular plane (TAPSE) were altered (25.1±0.6 mm in HCV+ *vs.* 18.8±0.6 mm; p<0.001) and pulmonary artery pressure (PAPs) indicated higher values (29.6±7.7 mmHg in HCV+ *vs.* 24.5±7.9; p=0.005). The study of spirometric parameters showed no significant modifications while the evaluation of respiratory gases pointed out a newsworthy range of correlations with echocardiographic data. The VE/VCO2 slope values were inverse to the right atrial volume (p=0,009; r_s=-0.665) and the PetCO2 values at the anaerobic threshold were directly proportional to the right atrial values (p=0.001; r_s=0.798). The exhaled volume of CO2 was positively linked to right atrial volume (p=0,016; r_s=0.675) and right ventricular diameter (p=0,012; r_s=0.696).
Conclusions: The purport of such correlations could be an initial increased alveolar perfusion linked to an hyperdynamic right ventricle altered directly or indirectly by HCV. The physio-pathological nexus appears to be a primitive myocardial alteration with non-pathological consequences in the lungs.

OSA AND OXIDATIVE STRESS BEFORE AND AFTER TREATMENT WITH C-PAP THERAPY: COULD IT BE PART OF CARDIOVASCULAR PREVENTION?

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Introduction: Obstructive sleep apnea (OSA), a highly prevalent sleep disorder characterized by repeated disruptions of breathing during sleep, arouses a great interest today due to its features of systemic disease and to the growing evidence of a link between Intermittent Hypoxia (IH), sleep fragmentation on one side, and increased insulin-resistance, sympathetic outflow and hyperlipidemia on the other one. OSA induces inflammation and oxidative stress (OS) which cause endothelial dysfunction: this could explain the higher risk of hypertension, CAD, stroke, arrythmias and LV dysfunction and the increased cardiovascular mortality in OSA patients. It still remains unknown whether CPAP can modify this inflammatory state. Our goal was to verify if constant therapy with CPAP (at least 240 min/night) might improve OS and IH.
Methods: We enrolled 13 patients affected by obesity (mean BMI 35) and severe OSA. Arterial gas analysis and were also analyzed. Then we measured, at beginning and after 90 days of ventilator therapy: 8-iso-PFG2a, 8-isoprostane, marker of lipid peroxidation and NOX2-derived peptide(sNOX2-dp), an index of NADPH oxydase activation. Flow-mediated brachial artery dilatation was measured to assess endothelial function. Patients were assessed monthly for adherence.
Results: Polysomnographic parameters were normalized after CPAP (AHI:43.4 to 7 steps/h; ODI: 35.8 to 2.8 steps/h). Urinary 8-isoprostanes, sNOX2-dp levels were decreased significantly. After 3 months a significant decrease of sNOX2 (p<0.005) and urinary 8-iso-PGF2a (p<0.01) was observed.
Conclusion: CPAP therapy has a relevant impact on levels of sNOX2-dp, which correlates with severity of OSA and plays an important role in ROS production and endothelial dysfunction. Results suggest that CPAP decreases inflammation and OS. These findings may be relevant for a better understanding of the pathogenesis of cardiovascular disease in OSA and for validation of CPAP as a fundamental part of the therapy.

A RARE CASE OF OF PAROXYSMAL HYPERTENSION IN URINARY BLADDER PARAGANGLIOMA

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Aim: Primary bladder paragangliomas (PGLs) are rare ectopic chromaffin-cells derived tumors. Clinical manifestations include micturition hypertension and/or palpitations, syncopes, hematuria, and frequent urinary tract infections. However, these symptoms, very often, are paroxysmal and misdiagnosed. The aim of our case report is to focus the attention and better understanding this rare disease to prevent late diagnosis.
Case description: A young woman referred to our Unit for the first time when she was 21 years old, complaining, for about 4 years, for recurrent micturition episodes of throbbing frontal headache, associated to increased blood pressure (BP) values, palpitation, nausea, and flushing of legs. She had no family history for PGL. At the 24-h ambulatory blood pressure monitoring (ABPM) mean systolic and diastolic BP values were normal, but showed only paroxysms of blood pressure (BP) during micturition. We performed a screening for secondary hypertension without finding any pathological evidences. In particular, several measurements of 24-h urinary metanephrines were into the normal range. However, symptoms were very suggestive of PHEO, and the patient underwent to abdominal magnetic resonance (MR) that showed a thick-walled left adnexal mass (4,5x2cm) abutting the urinary bladder, and confirmed by ^{123}I-MIBG scintigraphy as strongly uptaking mass. Laparotomic surgery was performed and the mass was removed without complications; the histologic exam confirmed diagnosis of bladder PGL. The genetic testing for SDH genes was negative for mutations. At 12-months of follow-up our patient is completely asymptomatic and her BP are well-controlled.
Conclusions: Our case showed that extra-adrenal PHEOs, often, are not associated with increased value of 24-h urinary metanephrines, especially when the over secretion is stimulated in particular situations, as in our case by bladder contraction. Thus, it is mandatory, whether clinical manifestations are suggestive, to perform further investigations in order to avoid late diagnosis.

ASSOCIATION BETWEEN PLASMA CERAMIDES AND SEVERITY OF CORONARY-ARTERY STENOSIS IN PATIENTS WITH CLINICAL INDICATIONS FOR CORONARY ANGIOGRAPHY

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Objective: Recent prospective studies have identified specific plasma ceramides as strong predictors of major adverse cardiovascular outcomes in patients with established or suspected coronary artery disease (CAD). Presently, it is unknown whether higher circulating levels of specific ceramides are also associated with a greater angiographic severity of coronary-artery stenoses in this high-risk patient population.
Approach and Results: We measured six previously identified high-risk plasma ceramide molecules [Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/20:0), Cer(d18:1/22:0), Cer(d18:1/24:0) and Cer(d18:1/24:1)] in 167 consecutive patients with established or suspected CAD, who underwent urgent or elective coronary angiography. Approximately 60% of these patients had significant coronary stenosis (≥50%) at the level of left anterior descending (LAD) artery. Of the six measured plasma ceramides, higher circulating levels of Cer(d18:1/20:0) (adjusted-odds ratio 1.39, 95% CI 1.0-1.99), Cer(d18:1/22:0) (adjusted-odds ratio 1.57, 95% CI 1.08-2.29) and Cer(d18:1/24:0) (adjusted-odds ratio 1.59, 95% CI 1.08-2.32) were significantly associated with the presence of LAD stenosis ≥50% after adjustment for age, sex, smoking, pre-existing CAD, hypertension, diabetes, dyslipidemia, estimated glomerular filtration rate and plasma C-reactive protein levels. Similar results were found after excluding patients (n=30) undergoing urgent coronary angiography.
Conclusion: This cross-sectional study shows for the first time that higher circulating levels of specific ceramides are independently associated with a greater angiographic severity of coronary-artery stenoses in patients with suspected or established CAD referred for clinically indicated coronary angiography.

ANALYSIS OF SHORT-TERM BLOOD PRESSURE VARIABILITY IN PHEOCHROMOCYTOMA/PARAGANGLIOMA PATIENTS

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Aim: Data about short-term blood pressure variability (BPV), whose changes represent a well-established cardiovascular risk factor, in pheochromocytoma and paraganglioma (PPGL) patients is lack and still conflicting. The aim of this study is to analyse 24h ambulatory blood pressure monitoring (ABPM) derived short-term BPV markers in PPGL, before and after surgical removal of catecholamine-secreting tumour.
Methods: We retrospectively evaluated PPGL patients ≥18 years old, referred to our Hypertension Unit from 2010 to 2018. PPGL diagnosis, according to the guidelines, was assessed by 24h urinary metanephrines, adrenal gland imaging, such as computed tomography scan or magnetic resonance and/or I^{123}-meta-iodobenzylguanidine scintigraphy to localise extra-adrenal masses, and confirmed by histological exam. For each PPGL, at baseline and after follow-up (FU), markers of short-term BPV were assessed by 24h ABPM, including:
1) systolic (SBP) and diastolic (DBP) dipping percentage,
2) 24h, daily, and night-time SBP and DBP standard deviation (SD),
3) 24h, daily, and night-time SBP and DBP average real variability (ARV). We also collected a complete clinical assessment, including medical history, current treatment, physical examination, and biochemical evaluation.

Results: 24 patients (7M,17F) with PPGL (52±19 yrs), were evaluated at baseline and after FU (27±25 months). The mean 24h urinary metanephrines was 457.6±61.9 µg/24h at baseline *vs.* 62.9±29.0 µg/24h at FU (p=0.04).
37.5% of patients showed a physiological circadian pressure rhythm (dipping-pattern) at baseline and 41.7% after treatment. Comparing with baseline results, after treatment, we found a significant decrease of 24h SBP ARV (8.8±1.9 *vs.* 7.7±1.6, p=0.05), and a decreasing trend in 24h DBP ARV (7.6±1.9 *vs.* 6.9±1.7, p=0.3), and 24h SBP and DBP SD values. On a multivariate analysis 24h urinary metanephrines values resulted predictors of 24h SBP SD (r^2=0.59, p=0.009) and of night-time DBP SD (r^2=0.49, p=0.024).
Conclusions: Our study shows as in patients affected by catecholamine-secreting tumours, after successfully treatment, there is a reduction of short-term BPV indexes.

COMPARISON OF AUTOMATED OFFICE BLOOD PRESSURE WITH OTHER BLOOD RESSURE MEASUREMENT TECHNIQUES: A META-ANALYSIS

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Aim: Automated office blood pressure (AOBP) is an emerging fully automated unattended BP measurement technique. However, the availability of only three validated devices and the lack of established BP thresholds for hypertension diagnosis limits its use in clinical practice. The aim of this meta-analysis was to compare AOBP with other BP measurement methods and to analyse the differences between AOBP and physician's office BP, non-physician's office BP, daytime ABPM, and HBPM.
Methods: We conducted a systematic research through PubMed database for articles published up to April 2018; we included studies comparing unattended AOBP with office and out-of-office measurement techniques, using BP validated devices and reporting the BP differences or BP values obtained.
Results: 26 studies with 7116 patients were included in the analysis. AOBP mean values were lower than physician office measurements (SBP −10.48 mm Hg, 95% CI: −13.15 to −7.81; DBP −4.44 mm Hg, 95% CI: −6.07 to −2.80) and non-physician office ones (SBP −6.89 mm Hg, 95% CI: −8.75 to −5.04; DBP −3.82 mm Hg, 95% CI: −4.86 to −2.78). No significant differences were found between AOBP and daytime ABPM measurements (SBP −1.85, 95% CI: −4.50 to 0.79; DBP 0.12, 95% CI: −1.42 to 1.66) and between AOBP and HBPM values (SBP −2.65, 95% CI: −8.42 to 3.12; DBP −1.67, 95% CI: −4.20 to 0.87).
Conclusions: The AOBP measurement, although performed in an outpatient clinic, is not significantly different from the out-of-office techniques, probably because it allows to overcome some of the OBP limits, first of all the white-coat effect.

PROGNOSTIC VALUE OF VISIT TO VISIT VARIABILITY IN SYSTOLIC BLOOD PRESSURE

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Aim: High blood pressure levels in hypertensive patients are associated with increasing of cardiovascular events.
We assessed if visit-to-visit systolic blood pressure variability contribute to the risk of cardiovascular events independently of mean systolic blood pressure during follow-up.
Method: We selected 1586 hypertensive patients without prevalent CV disease, with mean age of 53±11 years . The mean duration of follow-up was 11± 4 years with mean number of visits of 16±6. We calculated Standard Deviation (SD) of mean systolic blood pressure from different visits and the patients were classified in quartiles of SD.
Results: Patients in the highest quartiles of SD (visit-to-visit blood pressure variability >16,7 mmHg) were frequently older men, women and diabetics. We found that they had increased risk of cardiovascular events [184 combined CV events; hazard ratio (HR) 1,49; confidence interval (CI 95%) 1,07-2,08; p=0,02] independently of significant association with older age.
Conclusion: High visit-to-visit systolic blood pressure variability should be considered a negative prognostic marker for incidence of cardiovascular events in hypertensive patients.

STATIN THERAPY AND AMBULATORY BLOOD PRESSURE PARAMETERS: A PROPENSITY SCORE ANALYSIS

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Aim: Hypertension and dyslipidemia often coexist and statins are cornerstones of the cardiovascular therapy. In a previous study, statin therapy was found to be associated with lower office blood pressure (BP). Our aim is to evaluate the effect of statin therapy on ambulatory BP parameters in a wide hypertensive population.
Methods: Retrospective observational study on 1827 consecutive outpatients referred to our Hypertension Centre for BP evaluation. All patients performed an ambulatory BP monitoring (ABPM). Anti-hypertensive treatment intensity score (TIS) was calculated to compare the different drug associations. We used a propensity score matching to compare two equally sized cohorts of patients with similar characteristics according to statin therapy. The variables included in the score were the following: age, sex, smoke, body mass index (BMI), estimated glomerular filtration rate (eGFR), diabetes mellitus, TIS, mean 24h pulse pressure.
Results: Mean age was 58.1±13.8 years, with male prevalence (55%). Four-hundred-and-two patients (22%) took statins. Statin therapy was associated with lower 24h (-2.8/-7.0 mmHg), daytime (-3.3/-7.5 mmHg) and nighttime BP (-2.5/-6.0 mmHg, all $p<0.001$). Accordingly, patients on statin therapy had better BP control, even after adjusting for age, sex, BMI, eGFR, diabetes mellitus and TIS (OR: 0.47 to be uncontrolled during 24h; OR: 0.52 to be uncontrolled during daytime; OR: 0.53 to be uncontrolled during nighttime, all $p<0.001$). The analyses on the propensity score matched groups (218 patients for group) confirmed these results.
Conclusions: Statin therapy is associated with significant lower ambulatory BP. These findings are independent of anti-hypertensive treatment intensity and comorbidities. The cardiovascular benefits of statins are mainly due to the lowering of cholesterol. Further studies are needed to establish their possible role on BP values.

RELATIONSHIP BETWEEN COMMON CAROTID DISTENSIBILITY/AORTIC STIFFNESS AND CARDIAC LEFT VENTRICULAR MORPHOLOGY AND FUNCTION

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Introduction: Increased arterial stiffness is associated with atherosclerosis, cardiac remodelling and cardiovascular adverse events. Common carotid artery rigidity could accurately predict cardiac abnormalities in morphology and function as well as ascending aortic parameters. The aim of the present study is to determine the relation between carotid and aortic markers of stiffness and the main echocardiographic parameters in patients affected by different types of chronic arthritis.
Methods: 208 patients participated (57,4±11,4yr; males=36,1%): 65,9% were previously diagnosed with rheumatoid arthritis, 20,2% with psoriatic arthritis and 13,9% with ankylosing spondylitis. For each subject medical history, use of drugs and gluco-metabolic parameters were assessed. Echocardiography, blood pressure (BP) measurement and carotid ultrasonography were performed. Carotid Distensibility (CD) and Aortic Stiffness index (AoS) were measured and taken as indices of arterial stiffness.
Results: Mean Left Ventricular Mass indexed by body surface area (LVM/BSA) and Relative Wall Thickness (RWT) were 98,8±20,7 g/m^2 and 0,46±0,06, respectively. In multiple regression analysis, between traditional risk factors for cardiovascular disease, CD correlated with age (β=0,325, p<0,0001), mean arterial pressure (MAP) (β=0,502, p<0,0001), gender and dyslipidaemia while AoS was not associated with any anthropometric, gluco-metabolic and hemodynamic covariates. About cardiac measurements, CD was inversely correlated with LVM/BSA (r=-0,20, p=0,005) whereas AoS directly correlated with left E/e' (a diastolic function index) (r=0,191, p=0,007).
Conclusion: Our data show an association between CD and left cardiac hypertrophy and remodelling and between AoS and left ventricular diastolic function. Carotid ultrasonography could be a valid monitoring tool for an early detection of vascular damage that can even predict sub-clinic cardiac remodelling in patients affected by chronic arthropathies.

PREVALENCE OF STATIN-ASSOCIATED MUSCLE SYMPTOMS IN ITALY: THE PROSISA STUDY

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Aim: Statin-associated muscle symptoms (SAMS) is one of the main reasons for statin non-adherence and/or discontinuation. The PROSISA is an observational, retrospective and multicenter study aimed at assessing the prevalence of statin intolerance, due to muscular symptoms, in dyslipidemic patients on statins followed by Italian lipid clinics.
Methods: Anamnestic data, biochemical values, and potential occurence of muscular symptoms were collected. Adjusted logistic regression was fitted to estimate odds ratio (OR) and 95% confidence intervals for the association between SAMS onset and several factors.
Results: In the first six months, the PROSISA database accounted for 6429 patients (mean age 66.7±12 years; 53.9% males) on statins. During statin therapy, 787 patients (12.2%) reported muscular symptoms (63.9% within the first year of treatment), mainly myalgia (74.2%) and cramps (25.8%). Among them, 375 underwent dechallenge, with disappearance of muscular symptoms in 87.2% of cases, while overall 503 patients underwent rechallenge (237 with change of statin/dose reduction without stopping therapy) mainly with low-intensity statins, with reappearance of muscular symptoms (myalgia 76.2%) in only 151 patients. Risk of SAMS onset was significantly higher for patients with diabetes (OR 1.58 [1.26-1.98]), using high potency statins (OR 1.32 [1.09-1.59]) and with an interacting drugs therapy (OR 1.65 [1.14-2.39]). Women had a lower risk of SAMS than men (OR 0.89 [0.76-1.03]). Although this result was not significant, it was possibly due to a higher prescription of low-intensity statins among women.
Conclusions: The PROSISA study offers a real life outlook of SAMS. The percentage of patients in whom intolerance has been confirmed by dechallenge/rechallenge is between 26-30%, emphasizing the need for a better management of muscle symptoms to provide a definitive diagnosis of SAMS and treatment re-evaluation.

LIPID LEVELS IN SUBJECTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA (HEFH) ACCORDING TO GENDER

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Purpose: Women affected by Heterozygous Familial Hypercholesterolemia (HeFH) are identified later, receive less lipid-lowering therapy and show higher lipid values than men. However, literature data are conflicting and no specific studies have been conducted in HeFH women. The aim of our study is to evaluate the clinical and phenotypic differences of adult subjects with molecular defined HeFH according to gender.
Methods: At the Department of Internal Medicine and Translational and Precision Medicine of Policlinico Umberto I Rome, after obtaining the informed consent, we consecutively enrolled 174 adults (91 women and 117 men age at enrollment between 18 -73 years old) with a DLCN score of ≥3. Mutation analysis in LDLR, APOB, PCSK9 and LDLRAP1 causative FH genes has been performed by next generation sequencing (LIPIGEN study). Clinical and anamnestic evaluation was performed and blood samples were collected for lipid profile analysis in all subjects . A p value of <0.05 was considered significant.
Results: Women were older (46.0±16.1 *vs.* 40.9±13.7 yo respectively; p=0.02) and were taking less potent statin therapy or combination therapy as compared to men (43% *vs.* 55% p=0.08). They showed higher TC (273.8±85, 2 mg/dl *vs.* 249.3±77.5, P=0.04), LDL-C (189.6±78.2 mg/dl *vs.* 174.5±72.5 mg/dl, P=0.18) and NON HDL-C (210.0±82.2 mg/dl *vs.* 196.2±75.6 mg/dl; p=0.25) values as compared to men, nevertheless these data became non significant when considering statin therapy or combination therapy [CT (adjusted p=0.26), LDL -C (adjusted p=0.811) NON HDL-C (adjusted p=0.90)]. The prevalence of the other cardiovascular risk factors was comparable between groups.
Conclusions: In the present study women showed a tendency towards higher cholesterol lipid values as compared to men: this may be partly due to the fact that they are undretreated. Further and larger studies are needed in order to clarify this important and alarming matter.

IDENTIFICATION OF RARE VARIANTS IN LPL AND APOA5 GENE IN PATIENT WITH SEVERE HYPERTRIGLYCERIDEMIA ASSOCIATED TO RENAL MANIFESTATIONS AND COMPLICATIONS

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Aim: Severe Hypertriglyceridemia (HTG) is a rare disease characterized by levels of triglycerides (TG) higher than 10mmol/L associated with eruptive xanthomas and pancreatitis. Transmission is autosomal recessive and several genes are involved i.e. those encoding 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*). Lipoprotein glomerulopathy is a pathological condition characterized by lipid accumulation in the glomerular capillaries that has been associated with the presence of rare mutations in the apolipoprotein E gene (*APOE*). We are reporting the case of a patient suffering from HTG and lipoprotein glomerulopathy.
Patient and methods: A 47-years-old woman affected by hypertriglyceridemia and type II diabetes reported recurrent pancreatitis attacks. The biochemical profile showed severe hyperlipidemia (triglycerides 2478 mg/dl, HDL-c 23 mg/dl, total cholesterol 420 mg/dl). The patient also suffered from hyperglycemia (glucose 224 mg/dl) and severe proteinuria (600 mg/dl and >10g/24h). The genetic screening was performed by direct sequencing of the coding region and the exon-intron junctions of *LPL*, *APOA5*, *APOC2*, *GPIHBP1*, *LMF1* and *APOE* genes.
Results: Two rare variants were found in two different genes at double heterozygous status: *LPL* p.Val442Gly (c.1325T>G) and *APOA5* p.Ala315Val (c.944 C>T). Based on the ACMG criteria, both variants could be classified as uncertain significance variants (USV). No rare variants were found in the *APOE* gene, while the genotype was E2/E3. A renal biopsy reported a condition of lipoprotein deposition in the glomeruli.
Conclusions: The severe HTG is plausibly caused by the two rare variants found in *LPL* and *APOA5*. Although the *APOE* rare variants causative of the reported cases of lipoprotein glomerulopathy were not found, in presence of genetic variants causative of HTG, the *APOE* genotype could further contribute to the lipoprotein glomerulopathy.

PREVALENCE OF FATTY LIVER AND ITS ASSOCIATION WITH ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Aims: Atherosclerotic cardiovascular disease (ASCVD) risk stratification in familial hypercholesterolemia (FH) is challenging due to high heterogeneity. Fatty liver (FL) is associated with an increased ASCVD risk. We aim at determining the prevalence and the predictors of FL, and at evaluating whether FL is associated with increased prevalence of ASCVD in FH patients.
Methods: 110 adult patients (men/women 54/56, age 53[18-92] years) with a clinical and/or molecular diagnosis of definite FH were retrospectively enrolled. Main study measures were detection of FL (by liver ultrasound) and ASCVD (by patient history and chart review).
Results: The prevalence of FL was 40% (n=44). Patients with FL were significantly more likely to be men and to have overweight/obesity, type 2 diabetes, high blood pressure and metabolic syndrome (MetS) than those without FL. They also had significantly higher BMI, waist circumference (WC), triglycerides, glucose, HbA1c, uric acid, liver enzymes and lower HDL-cholesterol. 21 (19.1%) FH patients had a history of ASCVD, mainly coronary heart disease (CHD). FH patients with FL showed a significantly higher prevalence of ASCVD than their counterpart without FL (29.5% *vs.* 12.1%; p=0.023); this association was largely conveyed by CHD (29.5% *vs.* 10.6%; p=0.012). In univariate logistic regression analysis, FL was significantly associated with ASCVD (OR:3.0, 95% CI:1.1-8.1; p=0.027) and CHD (OR:3.5, 95% CI:1.3-9.8; p=0.015). Together with FL, male sex, age, smoking status, WC, high blood pressure, glucose, HbA1c, HDL-cholesterol, LDL-cholesterol and MetS were significantly associated with ASCVD and CHD. The association between FL and CHD remained significant even after adjustment for sex, age, smoking status, LDL-cholesterol and MetS (adjusted OR:4.8, 95% CI:1.1-21.7; p=0.040).
Conclusions: FL, mainly attributable to nonalcoholic fatty liver disease (NAFLD), is common in FH patients and is associated with a higher prevalence of ASCVD, particularly CHD. Larger and prospective studies are needed to confirm whether NAFLD may help in ASCVD risk stratification of FH patients.

GENETIC AND METABOLIC PREDICTORS OF HEPATIC FAT CONTENT IN A COHORT OF ITALIAN CHILDREN WITH OBESITY

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Aim: To comprehensively explore metabolic and genetic contributors to liver fat accumulation in overweight/obese children.
Methods: Two hundred thirty Italian children with obesity were investigated for metabolic parameters and genotyped for *PNPLA3*, *TM6SF2*, *GCKR* and *MBOAT7* gene variants. Percentage hepatic fat content (HFF%) was measured by nuclear magnetic resonance.
Results: HFF% was positively related with BMI, $HOMA_{IR}$, metabolic syndrome, ALT, AST, γGT and albumin. Carriers of [G] allele in *PNPLA3*, [T] allele in *GCKR* and [T] allele in *TM6SF2* genes had significantly higher hepatic fat content than wild-type carriers. HFF% was explained for 8.7% by metabolic and for 16.1% by genetic factors and, a model including age, gender, BMI, $HOMA_{IR}$, *PNPLA3*, *GCKR* and *TM6SF2* variants was the best predictor of HFF%, explaining 24.8% of its variation (P<0.001). A weighted-genetic risk score combining *PNPLA3*, *GCKR* and *TM6SF2* risk alleles was associated with almost eightfold higher risk of NAFLD.
Conclusions: Our data highlighted the predominant role of genetic factors in determining the amount of liver fat content in children with obesity.

CEREBROTENDINOUS XANTHOMATOSIS, A METABOLIC DISEASE WITH DIFFERENT NEUROLOGICAL SIGNS: THREE CASE REPORTS

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Aim: Cerebrotendinous xanthomatosis (CTX) is an autosomal recessive disease caused by mutations in the *CYP27A1* gene encoding the mitochondrial sterol 27-hydroxylase enzyme. A defect of this enzyme leads to increased levels of cholestanol that accumulate in the body and cause juvenile cataracts, intellectual disability, neurological features, tendon xanthomas, and chronic diarrhoea. Our aim is to demonstrate that the sterol profile and genetic analyses are essential to make the diagnosis of CTX and to exclude other dyslipidemias.
Patients: Case 1 is a patient with neurological dysfunction, including moderate intellectual disability, cataract of right eye, and xanthomas; Case 2 is a patient with tendon xanthomas without neurological symptoms. Case 3 is a patient with a history of cognitive regression, bilateral juvenile cataracts, chronic diarrhea, but no evidence of xanthomas. Based on the suspect of CTX the plasma sterol profiles was performed. The sterols were extracted from plasma by a liquid-liquid procedure and trimethylsilyl derivatives were analyzed by gas chromatography coupled to flame ionization detector (GC-FID) and by mass spectrometry (GC-MS). Genetic screening was performed by amplification and direct sequencing of the promoter and the 9 exons with intronic junctions of the *CYP27A1* gene.
Results: Plasma sterols profile obtained in all three cases, showed higher levels of cholestanol and cholesterol biosynthetic precursors compared to unaffected subjects. Genetic screening of *CYP27A1* gene revealed that Case 1 and Case 2 were homozygous for the c.1263+5G>T (p.Leu396Profs29X) and c.1435C>G (p.Arg-479Gly) pathogenic variants , respectively , while the Case 3 present a new variant c.850_854delinsCTC at homozygous status.
Conclusions: We identify 3 patients suffering from a very rare disease that could be treated with chenodeoxycholic acid to prevent development of more severe clinical signs.
The role of biochemical together with the genetic analysis is crucial to make an early diagnosis.

DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA: PERFORMANCE OF DUTCH LIPID CLINIC NETWORK SCORE AND DISTRIBUTION OF VARIANT PATHOGENICITY

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Aim: Familial hypercholesterolemia (FH) is an inherited disorder characterized by high levels of blood cholesterol from birth and premature coronary heart disease. Thus, the identification of FH patients is crucial to prevent the onset of cardiovascular events. However, a significant proportion of patients remains without a definitive diagnosis of FH due to lack of information for Dutch Lipid Clinic Network (DLCN) score calculation.
Methods: After evaluating DLCN performance in FH patients enrolled in the LIPIGEN study, we focused on a subgroup who underwent genetic testing in a centralized laboratory to evaluate the impact of variant pathogenicity.
Results: First, DLCN score performance was evaluated on 1377 adults with genetically-confirmed FH to define the impact of each criteria and missing data on the final score, resulting in 28.5% of the sample classified as probable FH (score 6-8) and 37.9% as definite (score >8). About 43.4% of the sample had at least one missing data out of 8 and 10.0% had ≥4 missing data. Applying the DLCN score to the subgroup with additional information about variant pathogenicity (N=209, 15.2%), pathogenic (Type I) or likely pathogenic variants (Type II) were more frequent in patients with DLCN score≥6 (56.0% and 28.4, respectively *vs*. 15.6%) while the presence of only variants with uncertain clinical significance (type III) increased in patients with DCLN score<6 (33.3%). Among patients with Type I, Type II, or Type III variants, 72.1%, 64.4%, and 45.7% had DLCN score ≥6, respectively.
Conclusions: The DLCN score failed to identify a third of subjects with genetically-confirmed FH, requiring updates and validation. In-depth analyses for variant pathogenicity would be useful to better understand conditions in which genotype is not explained by phenotype.

IMIDS AND STEROIDS INDUCE A DIASTOLIC DYSFUNCTION IN MULTIPLE MYELOMA: PRELIMINARY RESULTS

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Introduction and aim: Multiple Myeloma (MM) is a plasma cell dyscrasia. New treatments include proteasome inhibitors (PIs) and immunomodulatory drugs (IMIDs). Few data are available on cardiotoxicity of these drugs. Aim of this research was to investigate the diastolic dysfunction and heart modifications in different MM treatment.
Materials and methods: We analyzed 10 F and 12 M aged 67.63±8.98 yrs treated in our department between January 2016 and December 2017. All patients underwent a complete echocardiography at the end of treatment. PIs based treatment was experienced in 6 pts, IMIDs in 7 pts while 9 pts had no PIs or IMIDs (NNt) treatment last year. Three patients were also treated with prednisone and 10 with dexamethasone.
Results: IMIDs showed statistical significant tendency to an increased E/A ratio compared to PIs (0,98±0,11 *vs.* 0,72±0,06; p=0.057) but not to NNt (*vs.* 0,80±0,09; p=0.23). In parallel, E/e' ratio was found increased with a statistical significant tendency in IMIDs compared to PIs (13,44±1,95 *vs.* 9,13±0,55; p=0.074) and to NNt (*vs.* 9,68±1,15; p=0.11). No differences were found in PIs compared to other regimens. Patient who experienced steroids had both a statistical significant tendency to an increased E/A (steroids 0,905±0,099 *vs.* no steroids 0,693±0,045; p=0.059) and increased E/e' (steroids 12,15±1,75 *vs.* no steroids 8,91±0,61; P=0.087). Left atrium volume (LAV) was not significantly changed in IMIDs compared to PIs or NNt, while it decreased in patients given steroids (steroids 61,82±4,10 ml *vs.* 82,27±9,88 ml; p=0,070). No differences were found both between different therapies in Right atrium area values and when LAV was normalized for body surface area. No differences were found in parameters evaluated comparing prednisone to dexamethasone.
Conclusion: Here we noticed worsened diastolic dysfunction after IMIDs treatment which was more frequent than in other regimens. The same results appear to be related to the use of steroids. The limited number of patients analyzed has to be considered as the main limitation of this study.

ENDOCAN LEVELS ARE ASSOCIATED TO CD34+ CIRCULATING CELL NUMBER IN SYSTEMIC SCLEROSIS

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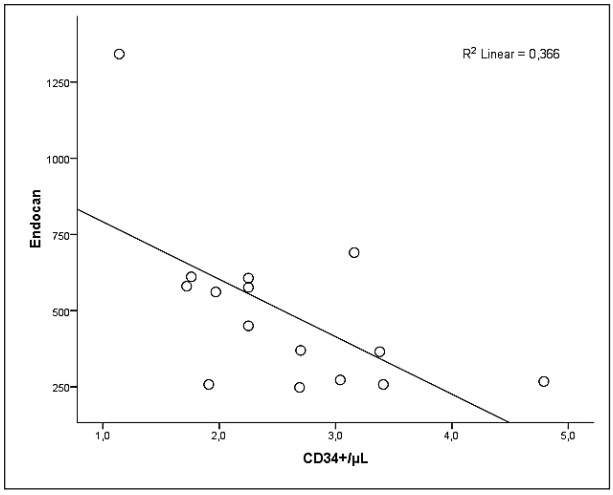
Background: Systemic sclerosis (SSc) is an autoimmune chronic disease characterized by vascular alterations of small arteries and microvessels. Circulating CD34+ cell number is acknowledged to be associated to cardiovascular health status in several chronic conditions, including chronic immune-inflammatory disease. CD34+ cell number was found inconstantly reduced in SSc. Endocan is a proteoglycan expressed by endothelial cells likely interacting with white blood cells, recently suggested as a marker of vascular stress.
Methods: We selected 27 (26 female, 61±13.5 years) patients out of 36 SSc patients (35 female) we have previously enrolled; CD34+ cell number (2.6±0.83), CRP (0.56±0.96), ESR (22.66±17.3), serum uric acid (SUA, 3.86±1.06), creatinine (0.77±0.38), Vitamin D3 (27.26±10.9), Rodnan skin score (28.89±10.10) and two frozen plasma samples should be available for all of these patients. Then, we randomly selected 15 patients to determine plasma Endocan levels.



Results: We found no correlation between Endocan and Rodnan skin score, ESR, fibrinogen, SUA or pulmonary artery pressure (as estimated by echocardiography); we found a trend of correlation between Endocan and Vitamin D levels (r=-0.315), red blood cells distribution width (r=0.310), CRP (r=0.299), but statistical significance was not reached due to the small sample size. The only significant (inverse) correlation we found was between Endocan and CD34+ cell number (r=-0.605, p=0.017).
Conclusion: In our study population, we found a significant correlation between CD34+ cell number and Endocan plasma levels; Endocan and CD34+ progenitor cells can be suggested as potential marker of disease status.

ACCELERATED ATHEROSCLEROSIS IN RHEUMATOID ARTHRITIS IS ASSOCIATED WITH CARDIOVASCULAR RISK FACTORS AND RESIDUAL DISEASE ACTIVITY

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Background: Patients with Rheumatoid Arthritis (RA) have an increased incidence of cardiovascular (CV) events. Ultrasound examination (US) of the carotid arteries can detect and measure the atherosclerotic subclinical process valuating intima-media thickness (cIMT) and the carotid segmental distensibility (cCD) measurement. The aim of the study was to demonstrate the association between traditional CV risk factors and the activity of arthritic disease.

Materials and methods: We enrolled 137 patients from March 2014 to March 2016, affected by RA without previous CV events, valuating immediately and after one year, carotid arteries with US to detect atheromatous plaques and to measure cIMT and cCD.

Results: After one year, all the indexes of subclinical atherosclerosis were worse than baseline (Δ-cIMT= 0.030 ± 0.10mm, p=0.005; Δ-cCD= -1.64 ± 5.83, 10^{-3}/ KPa, p=0.005; Δ-plaques= 8.6%, p=0.035). Traditional CV risk factors and corticosteroid therapy were independently associated with the acceleration of atherosclerosis. Interestingly, when considering RA patients divided according to the degree of disease activity (DAS28 [CRP]≥2.6), the worsening of subclinical atherosclerosis indices was detectable exclusively in the group of patients with active disease.

Dividing the population in quartiles, considering the 75° percentiles in which the consumption of DMARDs was high, we documented a progression of cIMT in patients with RA, showing an inflammation cCD significantly lower in patients with active disease than in those with remission of inflammatory state. In fact, in the multiple logistic regression analysis, considering the presence of cIMT above 75° percentile as dependant variable, while age, sex, BMI, CRP, pressure values and rheumatologic drugs as independent variables, only DMARDS and ACE-inhibitor assumption were predictive of higher and lower cIMT, respectively.

Conclusion: Our longitudinal study supports the hypothesis of an interaction between traditional CV risk factors and activity of arthritic disease and confirm the association between worsening indices of subclinical atherosclerosis and DAS28.

POST-PRANDIAL LIPEMIA AND CD36

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Aim: CD36 is a ubiquitous receptor which binds oxidized-lipoproteins and free fatty acids (FFA). Its expression at relatively high levels also in circulating cells (PBMCs) and its role in these are poorly understood. We therefore asked a) whether CD36 expression in these cells type associates with increased FFA flux during post-prandial lipemia (PP) and b) if this is related to altered inflammatory pattern, modulating cellular responses.

Methods: Subjects underwent a post-prandial study and the concentration of plasma lipoproteins, FFA in plasma and PBMCs and changes in the expression of gene and protein were evaluated. To study whether modulating CD36 expression *in vivo* associates with different post-prandial responses, carriers of a variant of *CD36* gene, the rs1761667, associated with reduced transcript and production of the protein, underwent a PP test.

Results: During PP phase we observed an increased expression of pro-inflammatory genes (IL-1β, IL-6, TNF-α, MCP-1). An increase of FFA in plasma and PBMCs was also observed, and was related to an elevation of the CD36 gene expression (+14±11 folds of induction) in the same cells. Moreover cells of carriers with mutant allele, showed a reduction in the transcript (0.44±0.29 folds of induction) of CD36 and in protein levels (-21%), and reduced concentration of FFA in PBMCs during the PP phase (-13%, p=0,3).

Conclusions: The post-prandial response is associated with changes in CD36 in PBMCs which appears to be related with changes in the inflammatory response. The physiopathological relevance of this observation and the molecular mechanisms involved remain to be explored.

IMPAIRED FATTY ACID SYNTHESIS AFFECTS THE ADAPTIVE IMMUNE RESPONSE: ROLE OF THE STEROL REGULATORY ELEMENT BINDING FACTOR-1C IN T CELLS

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Aim: Cellular metabolism defines T cell polarization and activation; indeed, whereas activated T cells rely on glycolysis, naïve, memory and regulatory Tcells use fatty acids oxidation (FAO) for their metabolic demand. We aimed at studying how key proteins regulating intracellular fatty acid (FA) metabolism (SREBP1c) affected the adaptive immune response with a focus on Tcells.
Material and Methods: A detailed immunophenotyping through flow cytometry and metabolic profiling of isolated Tregulatory (CD4+CD25+) and Tconventional (CD4+CD25-) cells were performed together with an in vivo immune challenge (skin allograft transplantation) in SREBP1c KO and WT littermates.
Results: SREBP1c KO mice presented a significantly delayed rejection after skin allograft transplantation compared to WT ($p<0.01$). This phenotype was associated with a significant reduction of T cells fueled by FAO: CD4+CD44+ ($p<0.01$), CD8+CD44+ ($p<0.05$), and Tregulatory CD4+CD25hiFoxP3+ cells ($p<0.01$). To unravel the role of FA in Tcells, metabolomic analysis was performed on isolated cells. SREBP1cKO Treg showed a reduction of FA synthesis with accumulation of acetilCoA ($p<0,01$) which led to an accumulation of medium-chain acetylcarnitines ($p<0,01$), suggesting an incomplete FAO; glycolysis was also affected with accumulation of lactate ($p<0,01$) in Treg of SREBP1cKO. This phenotype was peculiar of Treg as metabolites of Tconv were similar between SREBP1cKO and WT, thus addressing a key role of FA metabolism in Treg but no other CD4 Tcells.
Conclusion: Data collected suggest that SREBP1c represents a key player of FA metabolism in T cells. SREBP1c deficiency couples impairment of Treg metabolism with a delayed rejection after an in vivo model of transplantation. Therefore, reprogramming Tcell FA metabolism may represent a therapeutic target for diseases characterized by dysregulations of immune activation.

IMPACT OF LDL RECEPTOR ON CD8+ T LYMPHOCYTES ACTIVATION

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Aim: A finely tuned crosstalk exist between systemic lipid metabolism and cellular metabolism which also affects immune cells function and activation. Here we investigate whether LDL-receptor (LDL-R), a key player linking systemic and cellular lipid metabolism, affects the differentiation and functionality of Tcells.
Methods: Immunophenotypic analysis and cytokines production evaluation in T lymphocytes from WT and LDLR KO mice, after in vitro (anti-CD3/anti-CD28 stimulation, mixed lymphocytes reaction) and in vivo (vaccination) activation through flow cytometry and gene expression analysis.
Results: LDLR increases after in vitro activation of CD8+ T cells. By using LDLR KO mice, we found that LDLR deficiency impairs CD8+ proliferation in vitro both after anti-CD3/anti-CD28 stimulation and after allogenic stimulation (proliferated cells: 1764±38 WT, 1144±64 LDLR KO $p<0.01$). Furthermore, INFγ production was reduced in LDLR KO CD8+ Tcells (13.9±1% WT, 8.4±0.6% LDLR KO $p<0.01$). Similarly, in vivo antigen-specific activation (vaccination with ovalbumin) resulted in a reduced proliferation and cytokines production in CD8+ of LDLR KO mice (IFNγ $p<0.001$, IL13 $p<0.01$, perforin $p<0.05$). We investigated whether these effects were caused by an impaired T cells activation and we found a reduced expression of CD69 (WT 61.6±6.1%, LDLR KO 41.8±8.5%, $p<0.01$), a marker of early activation, and decreased phosphorylation of Akt, a molecule rapidly phosphorylated after T cell activation. Defects in signalling can be the consequence of altered lipid rafts formation, membrane portions enriched in cholesterol. Indeed, we observed a significant reduction of Ctxb expression, a marker of lipid rafts, in CD8+ T cells of LDLR KO mice after activation in vitro.
Conclusions: Our results show that LDL receptor plays a critical role for immunometabolic responses in CD8+ T lymphocytes. LDL receptor in lymphocytes could therefore represent a checkpoint linking cellular cholesterol metabolism to adaptive immune response.

THE SMALL INTESTINE TRANSCRIPTOME HINTS AT A POSSIBLE APOA-I AND DUOX2 MUTUAL RELATIONSHIP: A LESSON FROM KNOCKOUT MICE

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Aim: apoA-I, the main protein component of HDL, is mainly synthesized in the liver and the small intestine. Recently, it has been shown that APOA1 gene expression is down-regulated in the ilea of patients affected by Crohn's disease, whereas DUOX2 is up-regulated. DUOX2 is responsible for the production of hydrogen peroxide in the intestine, and known to be induced by mucosal homeostasis perturbations caused by dysbiosis.
The present study was aimed at studying apoA-I/HDL modulation of intestinal homeostasis.
Methods: Extremely low-HDL mice, knockout for both apoA-I and apoE (DKO) and high-HDL mice, on the same background but overexpressing human apoA-I (DKO/hA1) were fed chow diet from weaning until 30 weeks of age. Small intestine gene expression profiling was performed by small RNAseq.
Results: Among the 27 transcripts up-regulated in DKO mice, 5 could be related to perturbed lipoprotein metabolism and mucosal homeostasis: Slc10a2, Slc5a8, Saa1, Duox2 and its maturation factor Duoxa2. Duox2 and Duoxa2 gene expression, confirmed by qPCR, were 2.3-fold and 2.5-fold increased, respectively, in DKO mice compared to DKO/hA1.
Conclusions: We hypothesize that the lack of apoA-I/HDL can, beyond its classically described effects, also affect atherosclerosis development by modulating the expression of genes involved in the intestinal host-microbiota interaction. This can potentially mark the start of a new apoA-I/HDL biology scenario previously never investigated.

THE ANTI ATHEROGENIC ROLE OF SPHINGOSINE 1-PHOSPHATE (S1P) AND ITS RECEPTOR S1P3 IN THE REVERSE CHOLESTEROL TRANSPORT

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Aim: Sphingosine 1-phosphate plays a crucial role in atherosclerosis, even though the molecular mechanisms underlying its anti-atherogenic effects are still partially known. S1P exerts its biological activity by binding to its G protein-coupled receptors, such as S1P3. To date, we have no direct evidence connecting S1P3 role in cellular and systemic cholesterol handling. This study aims to investigate whether the anti-atherogenic activity of S1P is related to the modulation of lipid metabolism.
Methods: We evaluated the role of S1P3 receptor in a mouse model overexpressing S1P3 in myeloid lineage (S1P3-Lyz). ABCA1 and ABCG1 gene and protein expression in MPM were quantified through RT-qPCR and Western Blot analysis. ABCA1- and ABCG1-mediated cholesterol efflux was evaluated in control (C57BL/6) and S1P3-Lyz MPM through a radioisotope technique, using different mouse plasma concentration (0,1% and 2% v/v) and HDL (12,5 mg/ml) as cholesterol acceptors. *In vivo* RCT was measured through a radioisotope technique by injecting ^{3}[H] cholesterol-enriched MPM isolated from both C57BL/6 and S1P3-Lyz mice in C57BL/6 recipient.
Results: S1P3-Lyz MPM displayed an increased ABCG1 protein and gene expression compared to C57BL/6, while no differences were observed in ABCA1. Accordingly, ABCG1-mediated cholesterol efflux to mouse plasma was higher in S1P3-Lyz MPM compared to C57BL/6 MPM; similarly, acetylated LDL-loaded S1P3-Lyz MPM displayed an higher cholesterol efflux to plasma acceptors compared to C57BL/6 MPM. Finally, S1P3-Lyz MPM incubated with plasma together with a selective receptor antagonist (TY52156, 10μM) displayed a reduced cholesterol efflux compared to non-treated S1P3-Lyz MPM. *In vivo* total RCT resulted higher in S1P3-Lyz group, as ^{3}[H]-Cholesterol found in plasma, liver and faeces was higher compared to C57BL/6 group.
Conclusion: These results showed that endogenous S1P, through the interaction with its receptor S1P3 on myeloid cells, positively modulates cholesterol metabolism by improving reverse cholesterol transport, a well-known anti-atherogenic process.

RELATIONSHIP BETWEEN HDL CHOLESTEROL EFFLUX CAPACITY, CALCIUM CORONARY ARTERY CONTENT AND ANTIBODIES AGAINST APOLIPOPROTEINA-1 IN OBESE AND HEALTHY SUBJECTS

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Aims: to explore the associations between cholesterol efflux capacity (CEC), Coronary artery calcium (CAC) score, Framingham risk score (FRS), and antibodies against apolipoproteinA-1 (antiapoA-1 IgG) in healthy and obese subjects (OS).
Methods: ABCA1-, ABCG1-, passive diffusion (PD)-CEC, and anti-apoA-1 IgG were measured in sera from 34 controls and 35 OS who underwent CAC score determination by chest computed tomography. Anti-apoA-1 IgG ability to modulate CEC and macrophage cholesterol content (MCC) was tested *in vitro*.
Results: Controls and OS displayed similar ABCG1, ABCA1, and PD CEC, CAC and FRS score. Logistic regression analyses indicated that FRS was the only significant predictor of CAC lesion. In OS, significant correlations were observed between anti-apoA-1 IgG and ABCA1-CEC (r=0.48, p<0.0001), PD-CEC (r=-0.33, p=0.004), as well as CAC score (r=0.37,p=0.03). ABCA1-CEC directly was associated with CAC score (r=0.47, p=0.004) and FRS (r=-0.46,p=0.006), while PDCEC was inversely associated with the same parameters (CAC r=-0.46, p=0.006; FRS score r=0.40,p=0.01). None of these associations were replicated in healthy controls or after excluding antiapoA-1 IgG seropositive subjects. *In vitro*, anti-apoA-1 IgG inhibited PD-CEC (p=0.008), increased ABCA1-CEC (p=0.009) and increased MCC (p=0.002).
Conclusions: we report a paradoxical positive association between ABCA1-CEC and the CAC score, the latter being inversely associated with PD in OS. Corroborating our clinical observations, antiapoA-1 IgG enhanced ABCA1 while repressing PD-CEC, leading to MCC increase *in vitro*. These results indicate that anti-apoA-1 IgG have the potential to interfere with CEC and macrophage lipid metabolism, and to lead to paradoxical associations between ABCA1-CEC and cardiovascular risk

DECREASE OF EXTRACELLULAR VESICLES THEORETICAL SIZE CORRELATES WITH AN ENRICHMENT IN SATURATED FATTY ACIDS

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Aim: Extracellular vesicles (EVs) are a group of membrane-derived structures released by cells which, according to 2018 MISEV guidelines can be divided by their size in <100, <200 (small) or >200 large and/or medium or by their biochemical composition (CD63+ for smaller EVs) or by their biogenesis (intracellular for exosomes and from plasma membrane for other EVs). EVs circulate in biological fluids and transport and deliver lipids, proteins and nucleic acids to distant cells, and contribute to homeostasis, diseases development and progression. In this in vitro study, we utilized a lymph-node melanoma metastatic cell line (LM-16).
Methods: The most used EV isolation method is differential centrifugation (DC). By using an algorithm that takes in consideration g-force, time, viscosity of the medium and density of desired particles, to better define the properties of different EVs populations, we pelleted 4 different fractions (from 200 to 50nm). After DC we processed and analyzed the different pellets with Gaschromatography (lipid analysis) and Transmission Electron Microscopy.
Results: Our data show that the theoretical size decrease obtained by DC corresponds to an increase in relative percentage of saturated fatty acids (SFA) (from 53.77%±5.52 for 200nm to 68.80%±5.42 for 50nm, n=11, r2=0,90), counterbalanced by a decrease in unsaturated ones (from 39,29%±5.67 to 27.63%±6.23).
Conclusions: These data suggest that an enrichment in SFA can be considered as a new and still undiscovered feature of smaller EVs, due to the lack of available and reliable methods for their correct characterization. We plan to perform dot blot analysis to verify is the enrichment in SFA is correlated with an enrichment in CD63, a tetraspanin known to be marker of smaller EVs. We plan also to analyze the enrichment of a peculiar phospholipid, namely Bismonoacylglicerophosphate which is contained only in exosomes.

ALTERED ABCA1 EXPRESSION AFFECTS HDL ABILITY TO INHIBIT PROSTATE CANCER CELL PROLIFERATION THROUGH CHOLESTEROL EFFLUX

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Background and aim: Prostate cancer (PCa) is the most commonly diagnosed cancer and the second most common cause of cancer–related death in men. After an initial response to androgen deprivation therapy, PCa may evolve to a castration-resistant phenotype (CRPC), for which therapeutic options are poor and ineffective. The mechanisms responsible for this transition are not known and could be linked to metabolic alterations. In this context, it is known that cells need cholesterol to proliferate. It maintains the structural and functional integrity of cell membranes and it is the precursor of hormone synthesis. Since HDL are able to promote cell cholesterol efflux, aim of the study was to assess whether HDL are able to modulate cell cholesterol content in two PCa cell lines (LNCaP, androgen-dependent, and PC-3, androgen-resistant) and the consequent impact on cell proliferation.
Results: In LNCaP, cell exposure to LDL increased cholesterol content and the following treatment with HDL caused a significant efflux towards HDL. This modulation of cholesterol content was able to affect cell proliferation. Indeed, when LNCaP were incubated with LDL for 72h, cell proliferation increased by ~40%; however, when cells were co-incubated with LDL and HDL, the increase of cell proliferation was completely prevented. On the contrary, in PC-3 cells HDL were not able to promote cholesterol efflux after LDL loading, but caused a further increase of cell cholesterol content. As a consequence, HDL were not able to prevent LDL-induced increase of cell proliferation. The analysis of the expression of receptors and transporters involved in cholesterol efflux showed a significant reduction of ABCA1 protein in PC-3 despite high levels of mRNA, suggesting a role for post-translational modifications.
Conclusions: HDL-mediated reduction of cell cholesterol content inhibited cell proliferation in androgen-dependent PCa cell lines. On the contrary, cholesterol efflux is altered in castration-resistant ones. The identification of the mechanisms responsible for ABCA1 down-regulation could provide novel targets for the development of therapeutic stategies for CRPC.

MOLECULAR MECHANISMS OF TUBULAR DAMAGE IN LCAT DEFICIENCY

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Background and aim: LCAT deficiency is a rare genetic disorder caused by mutations in *LCAT* gene, which codifies for the only human enzyme involved in plasma cholesterol esterification and in HDL maturation. In carriers' plasma HDL-C is dramatically reduced, and the accumulation of small and discoidal HDL subclass, called preβ-HDL, and the presence of LpX were detected.
The principal cause of morbidity and mortality in LCAT deficient carriers is the kidney disease. The impact of the LpX in the onset of glomerular damage is already known, while the role of the accumulation of preβ-HDL in the pathogenesis of kidney disease is still under investigation; thus, the aim of this project is to assess the impact of preβ-HDL in the onset of tubular injury, focusing on the identification of the possible involved mechanisms.
Methods: For the *in vitro* study in tubular kidney cells, HDL were isolated from carriers and controls or synthesized (rHDL) resembling the shape and size of the endogenous particles. Oxidative stress, apoptosis and mitochondrial oxidative phosphorylation were evaluated.
Results: the incubation with carriers' HDL induces a higher ROS production in tubular cells compared to the HDL controls (28,57%, *p=0,0489 vs control);* the consistent result obtained with rHDL (30,10%, *p=0.028 vs control)* confirms that the shape and size of these particles are involved in ROS production, according to the increased oxidative phosphorylation observed in mitochondria. As the oxidative stress impacts on apoptosis, key genes of this process were evaluated, highlighting the majored expression of the pro-apoptotic genes *BAX* and *BAD* (169,44%, *p=0.034 vs control*; 83,6%, *p=0.033 vs control*, respectively) in presence of rHDL, which indicates a growth of cell death process.
Conclusion: The results demonstrate that preβ-HDL are involved in the onset of tubular damage, probably through the increase of ROS production and the alteration of apoptosis.

SERUM ENDOCAN LEVELS IN HIGH CARDIOVASCULAR RISK CLINICAL CONDITION

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Background: Endocan is a small soluble proteoglycan secreted by human endothelial cells, detectable in peripheral circulation, considered an indicator of angiogenesis and endothelial cell activation. It has been already established that Endocan levels increase in cancer and sepsis; its elevation during chronic inflammatory conditions is under evaluation so far, since its expression may be influenced by VEGF-α and TNF-α, cytokines involved in pathogenic pathways in chronic inflammatory disease.
Aim: To evaluate plasma Endocan levels in patients with different degree of vascular and/or systemic inflammation; here we present the results of an extensive evaluation of serum Endocan in different clinical conditions characterized by chronic inflammatory status and increased endothelial reaction.
Methods: We measured Endocan in healthy controls (10), statin-treated hypercholesterolemic (HC) patients (28), untreated scleroderma (SSc) patients (30), patients with refractory (as considered after six months of conventional therapy with CCS plus mesalazine) IBD (20), by a commercially available ELISA kit. CRP levels were also measured by routine methods.
Results: Endocan levels in controls were 337.73±64.01 pg/ml; in statin treated HC we found a mean value of 183.1±59.41; in SSc patients 497.15±281.43; in refractory IBD patients 348.9±196.63. HC patients presented with lower Endocan levels with respect to controls ($p<0.0001$). Endocan levels in SSc and IBD patients did not differ from controls. Endocan correlates to CRP levels in HC patients ($r=0.515$), while no significant relationship we found in controls, in IBD and in SSc patients.
Conclusions: Endocan is involved in a variety of biological processes including cell proliferation, migration, and neovascularization. Its levels are reported to be correlated with inflammatory cytokines, and to be closely related to the development and progression of CVD. However, while in statin-treated HC patients we found a significant correlation between CRP and Endocan plasma levels, we cannot confirm this relationship in controls, IBD and SSc patients.

ROLE OF SPLEEN IN DEVELOPMENT OF HYPERTENSION

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Objective: Immune system (in particular T lymphocytes) is involved in the pathogenesis of arterial hypertension and microvascular remodelling. It has also been recently suggested a crucial role for spleen in the development and onset of hypertension, through a neuroimmune mechanism mediated by a splenic factor, the Placental growth factor (PIGF), in different models of experimental hypertension (Carnevale D, et al. Immunity 2014, Carnevale D, et al. Nat Commun. 2016, Perrotta M, et al. Cardiovasc Res. 2018). However, to date no data are present in humans.
Design and methods: We investigated 29 patient previously splenectomized for idiopathic thrombocytopenic purpura or marginal lymphoma (clinical conditions associated with good survival) and 10 patients underwent an elective surgical intervention (cholecystectomy) in the same period, well matched for ages, sex and cardiovascular risk factors. In all patients clinical and 24-hours blood pressure (BP) were recorded. Wall to lumen ratio of retinal arterioles (WLR) was obtained by Adaptive Optics as index of microvascular damage. Functional (basal) and structural (total) capillary density were studied by capillaroscopy before and after venous congestion.
Results: The groups did not present statistically significant differences for clinical or 24-hours BP values (Clinical BP 126.94±8.20 / 72.38±13.7 mmHg in splenectomized patients *vs.* 125.56±12.9 / 76.67±5.59 mmHg in the cholecystectomized patients, p=NS; 24-hours BP: 119.7±13.8 / 66.4±6.60 *vs.* 119.20±8.76 / 69.10±7.53 mmHg respectively, p=NS). No differences in central BP and Augmentation index were observed between the groups. Retinal arteriole morphology and capillary density did not differ between the groups. However, WLR was slightly higher, albeit not significant, in cholecystectomized than in splenectomized patients (Table).

	Splenectomized patients (n=19)	Cholecystectomized patients (n=10)	p-value
WLR	0.27 ± 0.40	0.30 ± 0.38	0.10
Internal diameter (μm)	95.03 ± 13.06	89.57 ± 12.07	0.29
External diameter (μm)	121.41 ± 16.21	116.85 ± 15.55	0.48
Wall thickness (μm)	13.19 ± 2.38	13.64 ± 2.22	0.64
WCSA (μm²)	4553.5 ± 1254.70	4492 ± 1205.3	0.90

Conclusions: Our preliminary data do not confirm in humans the hypothesis of a difference in blood pressure values and indices of microvascular damage in splenectomized patients compared to cholecystectomized patients. However, a statistically not significant trend towards a greater WLR was observed in cholecystectomized patients. For a definitive conclusion about the involvement of the spleen in the genesis of hypertension also in humans it is necessary to extend the evaluation to a larger population.

EFFECT OF ANTIDIABETIC THERAPY ON BRACHIAL ARTERY FLOW-MEDIATED DILATION: A PILOT STUDY

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Introduction/Aims: Diabetes mellitus is a major risk factor for macrovascular complications in the long term with increased disability and the need for assistance. Considering the increasing number of diabetic patients is reasonable to expect an increasing economic and social welfare burden. Therefore the early detection of the vascular alterations is of great interest for the prevention of cardiovascular disease (1) (2). Development of atherosclerosis is preceded by alterations in endothelial and microcirculation function through different mechanisms (3). The evaluation of brachial artery flow-mediated dilation(FMD) allows early to assess endothelial dysfunction in atherosclerotic lesions and it is a independent predictor factor for major vascular complications like cardiovascular disease (4) (5). The aim of this study is to assess the effect of antidiabetic therapy on vascular function after 6 months of the treatment with SGLT-2 inhibitors.
Materials and Method: We studied a total of 21 patients, 9 without diabetes mellitus, 5 with diabetes mellitus in basal-bolus therapy and 7 with diabetes mellitus in antidiabetic oral whit SGLT-2. Diabetes mellitus was defined in agreement with American Diabetes Association Guidelines. FMD was performed at baseline and 6 months on a Philips Affiniti ultrasound scanner with a L12-3 linear transducer. All measurements were performed by the same physician. FMD was calculated in the right arm with the patient in the supine position. Differences in baseline characteristics were compared between groups using ANOVA Test for normally distributed continuous variables. Analysis in variables differences at 6 months between diabetes groups it was performed using T test for paired samples.
Result: The baseline characteristics are summarized below (see table). There was not differences between the groups for age (p 0.84), blood pressure (p 0.93), BMI (p 0.93), renal function (p 0.2) and disease duration (p 0.45). At baseline HbA1 in diabetes groups was similar (8.2±0.34 *vs.* 8.5±0.49, p0.21) as well as the average values of FMD (7.7±1.8 *vs.* 5.9±1.4, p0.89). At 6 month, FMD was significantly reduced compared at baseline in both diabetes groups (Basal bolus group 8.1±1.8, p0.01; SGLT-2 inhibitors 9.3±1.2, p<0.001). In SGLT2group FMD mean reduction was higher than the Basal-Bolus group (- 3.6±0.4 *vs.* – 0.3±0.2).
Discussion: In our pilot study we confirmed as optimized glycemic targets leading to an improvement of the endothelial function assessed through the increase of FMD values. We found a higher greater increase of FMD in SGLT-2inhibitors group with the same glycemic targets.
Conclusion: SGLT inhibitors could have a role in the remodeling of vascular function irrespective of hypoglycemic effect. However, further studies expanding the sample population are needed to confirm our data.

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RENAL ARTERY STENOSIS IN A YOUNG FEMALE PATIENT WITH SEVERE HYPERTENSION. A CASE REPORT

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Renal artery stenosis (RAS) is one of the most important causes of secondary hypertension, and affects 1 to 5% of all hypertensive patients. Atherosclerosis and fibromuscular dysplasia are the most common etiologies.
Strategies of treatment include medical therapy, percutaneous interventions with balloon angioplasty or stent implantation and surgery, but the identification of the best approach remains controversial. A real challenge for clinicians is to identify patients with renovascular disease who can potentially benefit from revascularization. Integration of information from ABPM, intrarenal doppler evaluation, plasma renin levels and GFR is of crucial importance to characterize patients and identify the best treatment option.
We report the case of a 27 years old woman with secondary hypertension due to RAS successfully treated with the implantation of a drug eluting stent. During the follow up, after 12 months, the patient exhibited excellent home blood pressure control with normal renal function and serum electrolytes and normal pattern at Doppler evaluation of intrarenal vasculature.

A RARE CASE OF PELVIC AND CAROTID PARAGANGLIOMA WITH PAROXYSMAL HYPERTENSION

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Aim: Pheochromocytomas (PHEOs) and paragangliomas (PGLs) (together as PPGLs) are neuroendocrine tumors arising from chromaffin cells of adrenal medulla and paraganglia of the sympathetic and parasympathetic nervous system, respectively. PPGLs produce and secrete excess of catecholamines, leading to various symptoms including hypertension, headache, palpitations, and diaphoresis. Rare PPGLs have shown to be multiple and recurring after surgical treatment. Herein, we report a case of a woman with pelvic and carotid PPGLs associated to paroxysmal hypertension.
Case description: A 76-year-old woman referred to Our Center complaining of paroxysmal hypertension. She had no family history of PPGL. Hormonal screening test showed normal levels of 24-hours urinary metanephrines. A computed tomography (CT) scan showed a mass of 52 mm diameter in the right pelvis. Strongest uptake of ^{123}I-Metaiodobenzylguanidine (^{123}I-MIBG) deposed for a pelvic PGL. The patient underwent successful laparatomic surgery to remove the pelvic mass, without complications. Histologic examination confirmed PGL diagnosis. At 6-months follow-up the patient showed normalised levels of blood pressure with no medications. Although, during a ultrasonography evaluation of carotid arteries, an incidental oval mass of 20 mm of diameter, closed to right carotid siphon, was found and later confirmed by CT scan, as a tumour compatible with a carotid-PGL. Unfortunately she refused surgical remove of this mass. The genetic testing of SDHx genes was negative for mutations.
Conclusions: It is important, in presence of PPGLs, to look for any other multiple lesions. Secondly, a genetic screening is mandatory to exclude hereditary syndromic PPGLs, in order to establish a proper treatment and follow-up.

SUBCLINICAL TARGET ORGAN DAMAGE IN A SAMPLE OF CHILDREN WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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Aim: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common monogenic hereditary kidney disease, characterized by the development of cysts along the renal tubule. Its prevalence is estimated at 1 per 1000 population. Previous studies suggest that hypertension and vascular damage can start during childhood. The aim of this study is the evaluation of markers of vascular damage and left ventricular geometry in a sample of children with ADPKD.
Methods: Several vascular measurements were obtained: Ambulatory Blood Pressure Monitoring (ABPM), carotid Intima Media Thickness (cIMT), Carotid Distensibility (CD), Pulse Wave Velocity (PWV) and Echocardiographic measurements: Relative Wall Thickness (RWT) and Left Ventricular Mass Index (LVMI).
Results: 11 children with ADPKD were recruited (4 females and 7 males, mean age 9.5±3.2 years): 3 children were overweight, 8 were normal weight. Five children resulted hypertensive at the ABPM, 4 were normotensive, 2 ABPM were not available. One child was already on therapy with an ACE-inhibitor. RWT was tendentially high (mean 0.47±0.39) while cIMT was above the 95° percentile for sex and height in 20% of children (0.5±0.005 mm). Average PWV and CD were between the normal range (5.5±4.6 m/sec and 89.6±16.1 x10-3/KPa respectively). We observed a positive correlation between the PWV and RWT (r=0.616; p=0.044) and a negative correlation between CD and RWT (r=-0.770; p=0.015). None of the subjects have altered Glomerular Filtration Rate (GFR).
Conclusion: Increased RWT and high cIMT, indicating subclinical organ damage, are present in ADPKD children. The level of RWT was significantly correlated to that of CD and PWV, implying that vascular stiffening is associated with cardiac remodelling. None of the children has an alteration of renal function, suggesting that subclinincal cardiovascular alterations may precede the overt decline in Glomerular Filtration Rate. These data underline the importance of a comprehensive cardiovascular screening in all the children with ADPKD.

ACHIEVEMENT OF LDL CHOLESTEROL THERAPEUTIC TARGETS IN A COHORT OF PATIENTS WITH A MOLECULAR DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA (FH): EXPERIENCE OF A SINGLE CENTER

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Introduction: Familial Hypercholesterolemia (FH) is an autosomal dominant disorder with high prevalence (1 / 350-500 in heterozygosy), characterized by high plasma values of total cholesterol (CT) and LDL cholesterol (LDL-C), leading to an accelerated atherosclerosis with high risk of cardiovascular morbidity and mortality in early age. Four genes are known causing FH: LDLR, APOB, PCSK9 and LDLRAP1. FH is clinically diagnosed with internationally validated scores, while confirmation is done by molecular analysis of candidate genes by direct sequencing.
Aim: The aim of this study is to evaluate the response to conventional therapy (statins and / or Ezetimibe) in patients with clinical diagnosis of FH with and without mutations in the causal genes. The primary purpose of this study is to calculate the overall percentage of patients achieving the therapeutic goals in terms of LDL-C. The secondary purpose is to identify the percentage of patients who, having failed the therapeutic goals with the therapy standard, are candidates for the prescription of the new anti-PCSK9 monoclonal antibodies according to the regulations of our country.
Methods: Within the LIPIGEN screening program for FH we enrolled 316 patients with clinical diagnosis of FH (DLCN score ≥ 3). By retrospective analysis of the medical records, 230 patients were identified for the follow-up study.
Results: The NGS method identified 175 mutation positive subjects (FH/M+) and 55 mutation negative FH/M-). 32.7% of FH/M+ subjects in primary prevention and 14% in secondary has reached the therapeutic targets. FH/M- patients reach targets with higher percentage (43%). 38% of FH/M+ subjects are candidates for PCSK9 inhibitors according to the AIFA guidelines.
Conclusions: In patients with FH, it is still difficult to reach therapeutic targets with standard therapy. Therefore, it's desirable, especially in patients with mutation that not reach the target, a greater diffusion of the new anti-PCSK9 antibodies therapy.

EFFICACY AND SAFETY OF PCSK9 INHIBITORS: THE REAL-LIFE EXPERIENCE OF THE LIPID CLINIC IN MODENA

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Aims: PCSK9 inhibitors (PCSK9i) have proven effective and safe for reducing LDL cholesterol (LDL-C) in clinical trials. We aimed at evaluating efficacy and safety of PCSK9i in the real-life setting of the lipid clinic in Modena.
Patients and Methods: 46 consecutive patients who started treatment with PCSK9i (evolocumab 24; alirocumab 22) between Feb.2016 and Jan.2019 were followed-up for a median period of 24[8-144] weeks. Baseline and on-PCSK9i therapy clinical data and lipid profile were registered.
Results: baseline patients characteristics were as follow: men/women 30/16; median age 58[26-76] years; 37 with familial hypercholesterolemia (FH); 33 on secondary cardiovascular prevention; 6 with total statin intolerance; 6 with type 2 diabetes (T2D); 8 on LDL-C apheresis. PCSK9i significantly reduced LDL-C by -61[-81 - +158]% ($p<0.001$); 70% of patients reached LDL-C therapeutic target. Lp(a) levels were also significantly reduced by -32[-77 - +56]% ($p<0.001$), but only 5 out of 15 patients with high baseline Lp(a) levels reached <50 mg/dl. PCSK9i LDL-C lowering efficacy was independent from baseline characteristics (FH vs. non-FH; primary vs. secondary cardiovascular prevention; presence vs. absence of T2D) and from the type of PCSK9i. LDL-C apheresis was discontinued in 88% of patients. Patients with total statin intolerance were less likely to reach LDL-C therapeutic target. No significant changes in glycemia, CPK and aminotransferases were seen during follow-up. Three patients experienced adverse events (1 local skin reaction, 1 diffuse skin rash, 1 rhinopharyngitis). Three patients experienced cardiovascular events (2 coronary and 1 carotid revascularization) during follow-up. Three patients discontinued PCSK9i therapy (2 low compliance, 1 non-responder).
Conclusions: PCSK9i are effective and safe in attaining LDL-C therapeutic target in our real-life cohort of high-risk patients. Open clinical issues are: management of high-risk patients with total statin intolerance or high Lp(a) levels; PCSK9i long-term safety; PCSK9i efficacy on cardiovascular outcomes.

CHARACTERIZATION AND CLINICAL MANAGEMENT OF STATIN INTOLERANCE IN A COHORT OF HYPERLIPIDEMIC SUBJECTS

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Aim: Statins are one of the most commonly prescribed drugs in clinical practice and effective treatment in prevention of cardiovascular diseases (CVDs). They are usually well tolerated, but rates of statin discontinuation after adverse events are high. We aimed to assess prevalence and risk factors of adverse events potentially attributable to statin therapy in lipid referral practice. Furthermore we explored clinical outcomes after statin re-challenge.
Methods: This is a retrospective study of 666 patients in therapy with statins in our referral lipid clinic between 01/01/2010 and 30/09/2018. Follow-up data of patients with statins intolerance were collected.
Results: Patients with adverse events were 150 (22.5%) including 48% with muscle symptoms, 38% with asymptomatic CPK elevation, 7% with hepatic transaminase increase and 7% with other events. Patients with muscle symptoms had higher levels of CPK, TSH, total and LDL-cholesterol compared to asymptomatic subjects ($p<0.01$), and both physical activity and history of CVDs were associated to greater risk of muscle symptoms. Patients who reported previous suspension had three times higher risk of further discontinuation (OR: 3.09, $p<0.0001$). Suspension of statin therapy was the main strategy adopted in patients with adverse events (62%) and muscle symptoms (77%), but led to elevation of total and LDL-cholesterol and no differences in rate of muscle symptoms compared to reduction or change of statin. After re-challenge only 3 patients described muscle symptoms again.
Conclusions: Real statin intolerance is a rare condition, but poor adherence to therapy due to adverse effects is widespread. If muscle symptoms occur, it is necessary to exclude subclinical hypothyroidism and effects of physical activity. Adequate counseling is essential to improve adherence in particular in high CV risk subjects. The suspension of statins in re-challenge might increases the risk of a low adherence to therapy, thus alternative strategies should be considered.

SEX DIFFERENCES IN ADHERENCE TO STATIN THERAPY IN THE CLINICAL PRACTICE

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Aim: Significant differences exist in the response to drugs and in their use between men and women. Our aim was to evaluate the effect of sex on adherence to statin therapy.
Methods: A retrospective study was carried out, including individuals aged≥40 years with a first prescription of statin between 2002/01/01 and 2009/12/31. Adherence was measured using the daily possession ratio (DPR). Persistence was evaluated as continuative drug use until a 30-days gap between two prescriptions occurred. Log-binomial models stratified by sex were fitted to estimate relative risk (RR) and 95% confidence intervals for the association between suboptimal adherence (DPR<0.8) and several factors.
Results: The cohort included 384,518 women and 379,824 men, with mean age 64.86±10.68 and 62.01±10.60 years, respectively. At 1-year follow-up, the mean DPR was higher for men (0.49±0.34 *vs.* 0.42±0.32, $p<0.0001$) and 19.16% of women and 27.43% of men had DPR>0.8 ($p<0.0001$). Regarding the analysis of persistence, the percentage of patients that showed a continued use of statin was lower for women than men (16.06% *vs.* 23.25%, $p<0.0001$). Compared with men, the risk of suboptimal adherence decreased more for women with diabetes (RR: 0.913 [0.909-0.917] *vs.* 0.971 [0.968-0.974]), with previous cardiovascular events (RR: 0.897 CI: [0.893-0.902] *vs.* RR: 0.918 CI: [0.915-0.921]) and with a Charlson Comorbidity Index ≥2 (RR: 0.905 [0.896-0.913] *vs.* 0.974 [0.969-0.979]). No sex-differences were identified for the effect of concomitant antihypertensive therapy and use of high potency statins on adherence. Among subjects with DPR >0.8 at 1-year, only 17.49% of women and 21.56% of men had still optimal adherence at 3 years.
Conclusions: Women were more likely to stop or be less adherent to statin treatment than men. There were significant sex-differences in factors known to affect adherence. Customize strategies to improve adherence and facilitate programmatic interventions are warranted.

HOME BLOOD PRESSURE ASSESSED BY SMS: ONE WEEK EXPERIENCE FROM CAMPANIA SALUTE NETWORK

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Aim: To compare a SMS-based remote monitoring system of reporting blood pressure to conventional office blood pressure measurement.
Methods: All patients were instructed to send by SMS blood pressure values collected from home monitoring devices once a week. All the SMS received in one week were examined and age, sex and BMI of the patients were evaluated, in addition to systolic and diastolic blood pressure values sent by SMS and systolic and diastolic blood pressure values found at the last office visit before the SMS were sent.
Results: We examined 24 blood pressure-related SMS. Most of the patients who sent an SMS were men (22 out of 24). The mean age of the patients was 47±15 years and the mean BMI was 26.11±4 kg/m^2. The mean systolic and diastolic blood pressures sent by SMS were respectively 127±12 mmHg and 77±9 mmHg whereas the mean office systolic and diastolic blood pressures were respectively 138±13 mmHg and 84±8 mmHg . A difference of 10±15 mmHg was observed between the mean systolic blood pressure found at the office visit and the one of the blood pressure readings sent by SMS. Regarding diastolic blood pressure this difference was estimated to be 7±8 mmHg.
Conclusions: A system of SMS-based remote monitoring of blood pressure may represent a useful tool to obtain more reliable values of blood pressure identifying white-coat and masked hypertension and may help to improve hypertension control and associated healthcare outcomes.

ATTENDED VS UNATTENDED BLOOD PRESSURE MEASUREMENT: AVERAGE VALUES AND DIFFERENCE DETERMINANTS

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Background: The results of the SPRINT study have called attention on the possible differences between blood pressure (BP) values obtained by health-care professionals in the office, during the visit ("attended BP") as compared to those obtained in the office leaving the patient alone ("automated office BP" or "unattended BP"). Only few studies have compared the two techniques and none of them implemented the approach for unattended BP measurement used in SPRINT by the use of completely automated device for both attended and unattended BP and by the measurement of 3 values after 5 minutes of rest,.
Methods: In 261 consecutive outpatients attending the outpatient clinic at an ESH Excellence Centre, BP values were measured by the physician with an automated oscillometric device (Omron HEM 9000Ai, mean of 3 measurements), after 5 minutes of rest. After the measurement of BP by the physician, the patient was left alone in the room, and the device was programmed to automatically perform 3 BP measurements after 5 minutes.
Results: Mean age was 61±16 yrs, 60% ♀, BMI 26.1±4.2, 88 % with a previous diagnosis of hypertension (64 % treated). Unattended systolic BP (SBP) and diastolic BP (DBP) were lower as compared to attended SBP (130.1±15.7 *vs.* 138.6±17.2 mmHg) and DBP (77.1±11.7 *vs.* 78.9±12.2 mmHg). The differences (Δ) between the values obtained using the two techniques were 8.5±7.9 mmHg for SBP and 1.8±5.6 mmHg for DBP. Δ SBP was directly correlated with age ($r=0.235$ $p<0001$) and with attended BP values ($r=0.407$ $p<0.0001$); Δ SBP was significantly lower in males than in females. At multivariate analysis Δ SBP remained independently correlated with age and attended SBP. Δ DBP was directly correlated with attended DBP ($r=0,322$ $p<0.001$) and was lower in males.
Conclusion: Our findings indicate that "unattended BP" measurement provides values significantly lower as compared to measurements obtained in the presence of the physician. Interestingly, the difference between the values obtained by the two approaches is not constant for all patients, being significantly affected by age, gender and BP values.