ABSTRACT

30° CONGRESSO NAZIONALE S.I.S.A.

ROMA
20/22 NOVEMBRE 2016

SOCIETA' ITALIANA
PER LO STUDIO
DELL'ARTERIOSCLEROSI

MODULAZIONE DELL'OMEOSTASI DEL COLESTEROLO DA PARTE DI PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) NEL MACROFAGO

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Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) è una proteina appartenente alla famiglia delle proproteine convertasi espressa principalmente nel fegato, nell'intestino tenue, nel rene e nel sistema nervoso centrale. La principale funzione di PCSK9 è quella di regolare i livelli circolanti di LDL-C attraverso la degradazione del recettore per le LDL (LDLR) a livello epatico. PCSK9 contribuisce anche alla regolazione di altre funzioni cellulari in tessuti extraepatici come il macrofago del lume vascolare. Lo scopo dello studio è stato quello di valutare un possibile ruolo di PCSK9 nella modulazione del processo di efflusso di colesterolo mediato dalla proteina ATP-binding cassette transporter A1 (ABCA1) nel macrofago. L'efflusso del colesterolo è stato valutato in macrofagi peritoneali murini ottenuti da topi wild type (WT) e knock-out per LDLR (LDLR-/-). Le cellule sono state stimolate per l'espressione del trasportatore ABCA1 mediante trattamento con agonisti dei recettori LXR/RXR ed esposte a PCSK9 ricombinante. L'efflusso ad apolipoproteina AI è stato misurato attraverso tecniche radioisotopiche. L'espressione proteica e genica sono state valutate rispettivamente tramite Western blotting e real time PCR. PCSK9 inibisce del 55% ($p<0.05$) l'efflusso cellulare del colesterolo ABCA1-mediato indotto dagli agonisti LXR/RXR nei macrofagi WT ma non nei macrofagi LDLR-/-. Parallelamente PCSK9 inibisce l'espressione della proteina ABCA1 solo nei macrofagi WT. L'induzione dell'espressione genica di Abca1 mediata dagli agonisti LXR/RXR, viene inibita da PCSK9 ricombinante del 64% ($p<0.001$) nei macrofagi WT e, in misura minore, nei macrofagi LDLR-/- (-35%, $p<0.001$). PCSK9 ha un effetto nullo o marginale sull'espressione genica rispettivamente di Abcg1 ed Sr-bi. PCSK9 ha un ruolo diretto sul processo di efflusso di colesterolo ABCA1-mediato nel macrofago attraverso l'inibizione dell'espressione del trasportatore ABCA1. Questo effetto necessita della presenza di LDLR probabilmente per mediare la sua internalizzazione. Questa azione di PCSK9 potrebbe essere rilevante nella patogenesi della malattia aterosclerotica promuovendo la formazione delle foam cells.

CLINICAL AND GENETIC FEATURES OF 2 PATIENTS WITH SEVERE HYPERTRIGLYCERIDEMIA DUE TO A MUTATION IN GPIHBP1 GENE

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Background. Familial chylomicronemia is a recessive disorder that may be due to mutations in ipoprotein lipase (LPL) and in other proteins such as apolipoprotein C-II and apolipoprotein A-V (activators of LPL), GPIHBP1 (the molecular platform required for LPL activity on endothelial surface), and LMF1 (a factor required for intracellular formation of active LPL).
Methods. We sequenced the familial chylomicronemia candidate genes in one adult patient presenting long-standing severe hypertriglyceridemia.
Results. The proband had plasma triglyceride >10 mmol/L but no mutations in the LPL gene. The sequence of the other candidate genes by Next Generation Sequencing (NGS) showed that the patient was homozygous for a frameshift mutation (c.413_429delTCCCACCCTGGCAAAGC p.Val138fs) in GPIHBP1 that is expected to result in a truncated protein devoid of function; this mutation affecs the Ly6 domain of GPIHBP1 that is required to maintain the structure of GPIHBP1 protein. The mutation has been previously described in a 5-week-old Hispanic girl with severe HTG in which whole exome sequencing revealed this mutation for the first time in compound heterozygosity. The proband's sister was carrier of the same mutation in homozygosity and she also showed severe hypertriglyceridemia.
Conclusions. We identified a frameshift mutation in GPIHBP1 gene in a family affected by severe hypertrigliceridemia by a NGS approach.

ESPERIENZA CLINICA PRELIMINARE DEGLI INIBITORI DI PCSK9 IN PAZIENTI CON IPERCOLESTEROLEMIA FAMILARE ETEROZIGOTE. CONSUDERAZIONI SUL LORO UTILIZZO IN ALTERNATIVA ALL'AFERESI LIPOPROTEICA E NEI PAZIENTI INTOLLERANTI ALLE STATINE

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L'introduzione degli anticorpi monoclonali anti-PCSk9 nello scenario di trattamento dei pazienti con ipercolesterolemia familiare (FH), rappresenta una importante ulteriore strategia terapeutica per il raggiungimento del target di LDL-colesterolo in soggetti a rischio Cardio-Vascolare (CV) molto elevato.
Metodi. Sono stati valutati complessivamente 12 HeFH, tutti con mutazione del gene LDLR (6M e 6F), età media 57.3 anni, range 36-74, con storia di malattia CV, avviati al trattamento con inibitori di PCSK9. In 9 pazienti, gli anticorpi monoclonali sono stati inseriti in aggiunta alla massima dose tollerata di statina+ezetimibe (gruppo A), mentre in 3 pazienti con totale intolleranza alle statine ed ezetimibe sono stati utilizzati in monoterapia (gruppo B). 6 pazienti (3M e 3F) sono stati trattati con evolocumab 140 mg sc ogni 2 settimane (4 del gruppo A e 2 del gruppo B) e 6 pazienti (3M e 3F) con alirocumab (75 mg ogni 2 settimane in 4 soggetti del groppo A, 150 mg ogni 2 settimane in un paziente del gruppo A e un paziente del gruppo B). 5 pazienti del gruppo B erano in trattamento con aferesi lipoproteica, sospesa con l'inizio della terapia con evolocumab (4) o alirocumab (1). Al basale, dopo 1-3 e 6 mesi è stato eseguito prelievo per colesterolo totale, LDL, HDL, trigliceridi, glicemia, transaminasi, GGT, CPK e creatinina.
Risultati. I pazienti del gruppo A presentavano valori basali di LDL-colesterolo di 140±126 mg/dl, dopo 1-3 e 6 mesi di trattamento con evolocumab (4pazienti) o alirocumab (5 pazienti) si è ottenuta

una riduzione del colesterolo-LDL rispettivamente del 57.4±12.9, del 64.6±20.7 e 61.1±9.3%. I pazienti del gruppo B presentavano valori basali di LDL-colesterolo di 375±81 mg/dl, dopo 1-3 e 6 mesi di terapia con evolocumab (2 pazienti) o alirocumab (1 paziente) si è ottenuta una riduzione del colesterolo-LDL rispettivamente del 21.2±3.3, del 29.2±2.9 e 35.1±4.2%. In tutti i pazienti i valori di transaminasi, glicemia, creatinina si sono mantenuti nel range di norma con risultati sovrapponibili per efficacia e tollerabilità con entrambi gli Ab monoclonali. I pazienti che provenivano dal trattamento con aferesi lipoproteica, hanno ottenuto valori di colesterolo-LDL <70 mg/dl come gli altri pazienti del gruppo A. Nei pazienti del gruppo B, pur con una riduzione assoluta del colesterolo-LDL a 3 mesi di 118 mg/dl, i valori di colesterolo-LDL rimanevano molto lontani dal target (a 6 mesi LDL-colesterolo >200 mg/dl).
Conclusioni. questi dati preliminari confermano l'efficacia e tollerabilità di Alirocumab e Evolocumab nel trattamento di soggetti HeFH. I pazienti che hanno sospeso l'aferesi hanno tutti raggiunto e mantenuto il target dei valori di colesterolo-LDL con un miglioramento della Qualità di vita. Tuttavia, la completa intolleranza alle statine ed ezetimibe in pazienti HeFH, condiziona una insufficiente riduzione del colesterolo-LDL con gli anti-PCSK9 in monoterapia (a 6 mesi LDL-colesterolo =244± 51 mg/dl). Pertanto, In questi pazienti, dovrà essere considerata l'opzione dell'aferesi lipoproteica.

VALUTAZIONE DEI LIVELLI DI LIPOPROTEINA(A) ED INSULINA DURANTE E DOPO LA GRAVIDANZA. DATI PRELIMINARI

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Background. La Lipoproteina(a) [Lp(a)] è una lipoproteina a bassa densità, simile alle LDL (low density lipoprotein), costituita dal legame tra una molecola di apolipoproteina B (apoB) e una di apolipoproteina(a) [apo(a)]. Elevati livelli di Lp(a) predicono in maniera lineare ed indipendente il rischio di malattia cardiovascolare (CVD). Tuttavia sembra che i livelli di Lp(a) possano essere inversamente correlati al rischio di insorgenza di diabete mellito tipo 2 (T2DM), condizione notoriamente associata per se allo sviluppo di CVD. Inoltre alcuni studi sperimentali hanno dimostrato la soppressione di apo(a) a livello post-trascrizionale in presenza di insulina. Scopo del nostro lavoro è stato analizzare, in una popolazione di donne in epoca gestazionale (età media 33.6±6.5) senza storia di T2DM e senza altre patologie concomitanti, che si sottoponevano a OGTT (oral glucose tollerance test, tra la 24ª e la 28ª settimana), anche i livelli di insulina, Lp(a) e delle altre frazioni lipidiche, per valutare se vi fossero correlazioni tra i livelli lipidici, inclusa Lp(a), e quelli di insulina.
Risultati. Abbiamo trovato che i livelli di Lp(a) correlavano inversamente con quelli di insulina (r -0.461, p 0.004), senza tuttavia trovare significative correlazioni con altri parametri (peso, frazioni lipidiche, età, HOMA). I livelli di insulina correlavano anche con peso pregravidico (r 0.535, p<0.001) e BMI attuale (r 0.585, p <0.001), oltre che con Lp(a). Infine l'analisi di regressione mostrava un'associazione significativa, ed inversa, soltanto tra insulinemia ed Lp(a) (B -0.461, p 0.012).
Conclusioni. I nostri dati mostrano come in una popolazione di donne in epoca gestazionale, periodo durante il quale si possono evidenziare alterazioni del profilo glico-lipidico, i livelli di Lp(a) sembrano essere influenzati solo dall'insulinemia, avvalorando l'ipotesi di una risposta di Lp(a) ai livelli di insulina. Analoga valutazione verrà eseguita dopo sei mesi dal parto per valutare se alla riduzione, prevedibile, dei livelli di insulina si associ anche la variazione in quelli di Lp(a).

ELEVATED LDL AND OXIDATIVE STRESS CONTRIBUTE TO ARRHYTHMOGENIC CARDIOMYOPATHY PHENOTYPIC MANIFESTATION

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Arrhythmogenic cardiomyopathy (AC) is characterized by progressive fibro-fatty replacement of the myocardium, arrhythmias and sudden death in young athletes. Due to a desmosomal gene mutation, AC patients present an autosomal-dominant trait of inheritance, with incomplete penetrance and variable expressivity, suggesting the involvement of possible co-factors determining the pathological phenotype.
Clinical studies conducted by Centro Cardiologico Monzino documented an increase in cholesterol (total, -LDL) and oxidized-LDL (ox-LDL) in AC patients' plasma vs controls, highlighting that altered lipid profile and oxidative stress may contribute to disease progression.
We conducted studies on cardiac mesenchymal stromal cells (MSC-C) derived from AC and NON-AC patients' biopsies. Already at baseline AC C-MSC show significant increases of oxidative stress (5.73±0.8 vs 3.73±0.4), PPAR-gamma- (5±1.1 vs 1.4±0.7) and CD36 expression (116±57.8 vs 1.8±0.5) compared to NON-AC. When grown in adipogenic medium, AC C-MSC showed a significant increase in free cholesterol- (9.37±1.65 vs 7.9±0.9) and triglycerides mass (35.80±5.8 vs 19.45±3.7) vs NON-AC C-MSC.
To understand the mechanism of action of the pathology, we are validating in C-MSC what shown in macrophages, i.e. PPAR-gamma hyperactivation and transcription of proadipogenic factors after interaction of the scavenger receptor CD36 with ox-LDL, through the oxidized fatty acid 13-HODE.
We therefore cultivated C-MSC in adipogenic medium plus 13-HODE, documenting significant neutral lipid accumulation (259.8±42.5 vs 135.5±24.4), increased PPAR-gamma- (4.1times) and CD36 expression (7,4 times) in AC vs NON-AC cells.
Based on these findings, we hypothesize that the gene mutation is necessary but not sufficient for the manifestation of the disease, while dyslipidemia and oxidative stress may be exacerbating co-factors.
We are investigating oxidative stress and dyslipidemia in a mouse model with desmosomal PKP2 mutation (involved in AC pathogenesis), fed a western diet plus 13-HODE and subjected to intense exercise, aiming at understanding the mechanism of AC's clinical manifestation and at identifying specific pharmacological treatments.

PROTEOMIC STUDIES TO ASSESS THE MECHANISM OF ACTION OF THE ANTIPROLIFERATIVE EFFECT OF FUROXANS IN SMOOTH MUSCLE CELLS

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Atherosclerosis is characterized by oxidation, altered NO bioavailability and increased smooth muscle cell (SMC) proliferation in vascular intima. To find innovative antiatherosclerotic approaches we synthesized NO-donor molecules (furoxans) able to inhibit SMC proliferation. We demonstrated that the antiproliferative effect of 4-phenyl-furoxans is related to the electron-attractor power of the substituent in position 3 of the aromatic ring.
Since our experimental evidence suggest that the antiproliferative properties are not mediated by NO, through the cGMP- or the polyamines pathway, to exclude a different NO-mediated mechanism, we evaluated whether a co-treatment with classical NO-donor scavengers (red globules, hemoglobin) would prevent this effect. Since these molecules indeed prevent growth inhibition, but their effect may be due to the presence of thiol groups which degrade furoxans, we used the non-thiol NO-scavenger PTIO, which, on the other hand demonstrated to be uneffective, thus concluding that NO is not directly reponsible of the antiproliferative effect.
We then tried different proteomic approaches aimed at assessing which is the portion of the furoxan able to inhibit SMC growth. Since we got positive feedback neither by 1D- and 2D-gel analysis coupled to mass spectrometry, nor by IodoTMT labelling (to evaluate S-nitrosylated proteins), we recently utilized SILAC analysis to evaluate the possible different expression of proteins which regulate G1/S cell-cycle progression, after furoxan treatment. Of more than 700 selected proteins, we found a significant variation in the expression of 12 proteins. Among these there are nuclear factors involved in nucleic acids' replication and proteins regulated by SUMO1, whose reduced expression determines a block in G1 phase, due to cyclin-dependent kinases inhibition, thus possibly explaining furoxans' antiproliferative effect on SMC.
We are now trying to confirm this finding that, if turns to be true, will allow to consider furoxans (either alone or conjugated with other pharmacophores) as interesting antiatherosclerotic molecules.

"DO YOU TAKE YOUR DRUG?": DEVELOPMENT OF AN UHPLC-MS/MS METHOD TO QUANTIFY ANTIHYPERTENSIVE DRUGS IN HUMAN PLASMA, FOR THE ASSESSMENT OF THERAPEUTIC ADHERENCE IN PATIENTS WITH "RESISTANT" HYPERTENSION

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Introduction. Nowadays hypertension is becoming increasingly regarded since its prevalence rises with the progressive aging of global population. In a little fraction of hypertensive patients (15-30%) the administration of one or two drugs is not enough to achieve adequate blood pressure values. These cases are defined as "resistant hypertension" (RH) and require at least three antihypertensive drugs. Not-pharmacological treatment, in these cases, consists in invasive surgery (e.g. renal denervation). Hypertension and, even more, RH are major risk factors for many other diseases, such as atherosclerosis, coronary artery disease, stroke, heart failure, peripheral vascular disease, vision loss and chronic kidney disease. For all these reasons management of resistant hypertension (RH) is a critical health problem and clinicians should face many issues, among which poor therapeutic adherence is relevant; in these cases patients are referred as "pseudo-resistant" hypertensives.
In this context Therapeutic Drug Monitoring (TDM) of antihypertensive drugs, which consists in the measurement of drug concentration in biological matrices, could be a helpful tool in order to discern problems in drug metabolism from cases of poor therapeutic adherence.
It has been hence validated an UHPLC-MS/MS method for simultaneous TDM on plasma samples of ten currently used antihypertensive drugs: amlodipine, atenolol, clonidine, chlortalidone, doxazosin, hydrochlorothiazide, nifedipine, olmesartan, ramipril and telmisartan.
Results have been then correlated and compared with the clinical and psychosocial profile of patients in order to globally evaluate the adherence to the prescribed therapy.
Materials and Methods. The proposed method has been validated according to FDA guidelines. Two hundred microliters of plasma sample, standard and quality control were added with internal standard (IS, 6,7-dimethyl-2,3-di(2-pyridyl)quinoxaline) and underwent a protein precipitation protocol with acetonitrile and centrifugation, in order to discard a large fraction of plasma proteins. Then, samples were subjected to a drying step and the resulting extracts were resuspended in water:acetonitrile 90:10 (v:v; +0.05% formic acid) and then analyzed through a Shimadzu Nexera X2® UHPLC system coupled with a LCMS-8050® tandem mass detector. The validated method was tested on real samples from patients with RH/pseudo-RH, enrolled in the SEAL study (protocol CS/504 03/09/2015), all giving informed consent.
Results. Accuracy, intra-day and inter-day precision, and others analytical parameters of the method fitted FDA guidelines for all analytes. 36 patients have been enrolled. On the basis of preliminary data, at first TDM, 56% of patients were found with detectable concentration of all the prescribed drugs, 25% of patients resulted partially non-adherent and 19% were totally non-adherent, with undetectable concentrations of all drugs. Continuing the TDM of those patients, after a period of tight control and repeated clinical consultation, two of them became adherent.
Moreover, through univariate and multivariate logistic regression testing, the diastolic pressure and the white-coat increase in heart rate resulted the best predictors of poor adherence. Through ROC curve analysis a diastolic pressure over 124 mmHg resulted strongly associated with total inhaderence, with a sensitivity of 85.7% and a specificity of 92.4%. The developed methodology could be applied to screen patients for adherence, preventing unnecessary surgery.

Conclusions. The simple and cheap extraction procedure makes this method eligible for a clinical routine use. From the clinical point of view we obtained encouraging results: we managed to discriminate some cases of poor adherence to the therapy preserving those patients from an invasive and expensive therapeutic approach, promoting adherence. Predictors of poor adherence could be a useful tool for the clinician in order to perform a prior screen of his patients.

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

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Background. PCSK9 (proprotein convertase subtilisin/kexin type 9), a 692-amino acid glycoprotein, came to the attention of the scientific community in 2003, when it was identified as the third gene involved in autosomal dominant hypercholesterolemia, beyond LDLR (low-density lipoprotein receptor) and ApoB (apolipoprotein B). Subsequently, several mutations in PCSK9 gene were described, both "gain-of-function" mutations associated to hypercholesterolemia and "loss of function" mutations linked to low levels of circulating LDL-C (LDL cholesterol), suggesting its important role in cholesterol metabolism. Briefly PCSK9, which is mainly synthesized and secreted by the liver, primarily regulates the levels of circulating low-density lipoprotein cholesterol (LDL-C) by enhancing the degradation of the hepatic LDL receptor (LDLR). PCSK9 targeting with monoclonal antibodies was approved as a novel therapeutic target for severe hypercholesterolemia. The rapid translational of PCSK9 from the bench to the clinic left some gaps open in relation to the potential role of PCSK9 in targeting the LDL-R in organs other than the liver. Indeed, the LDLR is abundantly expressed in pancreatic β cells in humans, mice and rats, where it plays a key role in the uptake of plasma LDL particles. Therefore, further investigations are needed to better clarify the physiological role of PCSK9, also in light of its pharmacological targeting.
Methods and Results. 2-months old WT and PCSK9 KO male mice were fed for 20 weeks with a HFD (High Fat Diet - 45% Kcal fat) or SFD (Standard Fat Diet -10% Kcal fat). As expected, PCSK9 KO mice exhibited significantly lower plasma cholesterol levels compared with WT littermates (51,8±12,3 mg/dl vs 79,8±11,0 mg/dl with SFD, 86,1±2,1 mg/dl vs 123,4±5,2 mg/dl with HFD, $p<0,05$), while no difference in plasma triglycerides concentrations and in weight gain arose between PCSK9 KO and WT mice. Interestingly the Glucose Tolerance Test (GTT) revealed that glycemia of PCSK9 KO mice, after glucose i.p. injection, remained significantly higher when compared with WT counterparts (GTT–AUC +25%±11% SFD; +40%±9% HFD, $p<0,05$), with no differences in the Insulin Tolerance Test (ITT). Since the response to exogenous insulin was comparable in both WT and KO mice, the impaired glucose tolerance observed in PCSK9 KO mice might be associated with? - cells dysfunction and not with insulin resistance. Of note, PCSK9 KO mice were characterized by a significant reduction in plasma insulin levels (4,3±0,6 ng/ml vs 3,2±0,2 ng/ml) and an increased insulin content in pancreas (104±7 ng/ml vs 138±12 ng/ml) compared to WT. Moreover, the histological analysis revealed a significant differences in dimensions of Langerhans islets between PCSK9 KO and WT mice (762±289 vs 425±117 Square Pixel) and insulin-positive areas significantly greater in the absence of PCSK9. Interestingly, there were no difference in GTT, ITT, plasma insulin levels and pancreatic insulin content between PCSK9 KO and WT mice on an LDLR KO background.
Conclusion. To conclude, our findings revealed that PCSK9 deficiency might affect not only liver, but also extra-hepatic tissue. We focused our attention especially on glucose metabolism observing an association between impaired glucose homeostasis and a defection in insulin secretion in PCSK9 KO mice, both in physiological state (SFD) and in a condition of obesity and metabolic dysfunction (HFD). The results obtained in the double KO mice suggest that the impaired glucose metabolism observed in PCSK9 KO mice could be due to the effect of the protein on pancreatic LDLR. These results reported compelling information considering the emerging importance of PCSK9 pharmacological inhibition for the treatment of hypercholesterolemia, but further investigations are still needed.

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

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Background. Experimental models demonstrate that adaptive T-cell response is promoted during atherogenesis and increased effector memory T subsets have been observed in patients with coronary artery disease. Aim of the study is to see whether expanded circulating T effector memory (TEM) (CD3+CD4+CD45RA–CD45RO+CCR7–) subtypes predict long term atherosclerosis progression and incidence of cardiovascular events in the general population.
Methods. We analyzed subsets of circulating CD4(+)T cells by flow cytometry (markers: CD3/CD4/CD45RO/CD45RA/CCR7/CCR5/CXCR3/HLA-DR/CD25/CD127) in peripheral blood of 163 (79 women and 84 men, 58 (43-65) years-old) subjects from the general population (PLIC Study), followed for up to 4 years. Anthropometric parameters, pharmacological history and incidence of cardiovascular events (CVE) during the observational period were collected; biochemistry and lipid parameters were measured. Common carotid artery Intima-Media Thickness (CCA-IMT) was determined by ultrasound at both visits to measure its progression over time.
Results. T-cell subsets were differently correlated with cardiometabolic risk factors; in particular T(EM) were increased in obese, in dyslipidemic subjects and in smokers while opposite trend were observed for T naïve (CD3+CD4+CD45RA+CD45RO-CCR7+) subtypes. No effect of pharmacological treatment was observed.
CCA-IMT progression was 0.008 (-0.003-0.021) mm/year and 14 incident CVE were reported during the observational period.

At basal visit HLA-DR(+)T(EM) count predicted faster CCA-IMT progression over follow-up (beta= 0.207, p= 0.012), adjusting for confounders (BMI, LDL lipoprotein cholesterol, triglycerides).
At the same time, HDLADR(+)T count was higher in subjects who developed incident CVE during observation (766.69 +/- 69.82 vs 607.97 +/- 25.35 cell count compared to those who did not developed events, p= 0.040).
Conclusions. Circulating T(EM) (HLADR+TEM in particular) cells are associated with cardio-metabolic parameters, with faster subclinical atherosclerosis and increased incidence of CVE in the general population. Further analyses unveiling cellular aspects will contribute to understand the inflammatory pathogenesis of atherosclerosis.

DISEASE TRENDS OVER TIME AND EFFECTOR MEMORY T-CELLS PREDICT ATHEROSCLEROSIS DEVELOPMENT IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Introuction. Patients with Systemic Lupus Erythematosus (SLE) present an increased cardiovascular mortality compared to general population. In this study we aimed at investigating the association of immunoinflammatory disease activity and of classical cardiovascular risk factors (CVRF) with five years atherosclerosis development.
Materials and Methods. Clinical, pharmacological history and information on principal CVRFs were collected at basal visit and after 5 years in 40 SLE patients (36 women, 42 +/- 9 years-old; 14 +/- 7 years as mean disease duration) and 50 age matched controls. SLEDAI score (disease activity index) was annually calculated, flare-ups and disease trends over time were reported according to guidelines. Ultrasound at both visits to detect carotid atherosclerosis was performed. Basal circulating T cell subsets were characterized by flow-cytometry analysis (FACS).
Results. At basal visit SLE showed only higher waist circumference compared to controls; however, 32% of SLE patients developed carotid atherosclerosis. Carotid atherosclerosis development was not predicted by lupus serology.
SLE patients showing elevated basal SLEDAI experienced further annual SLEDAI increase and showed the highest incidence of carotid atherosclerosis during follow up. FACS confirmed reduced lymphocyte and T helper (CD4+) counts in SLE compared to control (26832.24 +/- 1815.80 vs 40136.24 +/- 1111.60 and 8648.60 +/- 692.61 vs 12631.18 +/- 383.68, respectively, p less than 0.001); in addiction, expanded effector memory cells (CD4+/RO+/RA-/CCR7-/HLADR+) were observed in SLE who developed carotid atherosclerosis (750.38 + 189.33 vs 457.81 + 69.33 cell count, p= 0.006).
Conclusions. SLE patients present a more rapid carotid atherosclerosis development, which is associated more to disease activity and activated T cell subtypes rather than classical CVR factors, supporting a key role for the inflammatory response during vascular disease progression in patients with autoimmune diseases.

EFFETTI DEL TRATTAMENTO PER 8 SETTIMANE CON TERAPIA MONOCLONALE ANTI-PCSK9 SULLA FUNZIONALITÀ PIASTRINICA IN SOGGETTI AFFETTI DA IPERCOLESTEROLEMIA FAMILIARE

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Introduzione. L'ipercolesterolemia, in particolare la forma familiare, si configura come un importante fattore di rischio cardiovascolare e la riduzione del colesterolo LDL conduce ad una sostanziale riduzione del rischio di eventi e di mortalità cardiovascolari. Proprotein Convertase Subtilisin/Kexin 9 (PCSK9) svolge un ruolo centrale nella regolazione della funzione del recettore della lipoproteina LDL, favorendone l'internalizzazione e la degradazione nei lisosomi. La terapia ipolipemizzante con anticorpi monoclonali anti-PCSK9 ha mostrato grande efficacia nella riduzione dei livelli del colesterolo LDL ma non ne sono noti gli effetti sulla funzione delle piastrine che svolgono un ruolo importante nella patogenesi dell'aterotrombosi.
Scopo dello studio. Verificare, in soggetti affetti da ipercolesterolemia familiare, gli effetti del trattamento con anticorpi monoclonali anti-PCSK9 sulla funzione piastrinica.
Soggetti e metodi. Sono stati valutati 21 soggetti (14M/7F, età 56.2±11.5 anni, BMI 25.5±4.5 kg/m^2) affetti da ipercolesterolemia familiare, in gran parte (n=14) in terapia antiaggregante con aspirina. Prima e dopo trattamento per 8 settimane con Alirocumab (n=14) o Evolocumab (n=7), sono stati valutati il quadro metabolico e i seguenti parametri piastrinici:
1) test di aggregabilità in campioni di plasma ricco di piastrine (PRP) e sangue intero (WB) in risposta ad ADP (10-20 micromol/l), collagene (4 mg/L), acido arachidonico (1 mmol/l) ed epinefrina (5 micromol/l) (metodo di Born o ad impedenza);
2) test di sensibilità all'aspirina mediante adesione e aggregazione in condizioni di elevato shear stress (CEPI-PFA-100);
3) espressione sulla membrana del marcatore di attivazione CD62P (citofluorimetria).

Risultati. Il trattamento con Alirocumab o Evolocumab per 8 settimane ha determinato la riduzione dei seguenti parametri lipidici: colesterolo totale (da 283.6±81.1 a 166.7±63.6 mg/dl, p<0.001), e trigliceridi (da 137.2±54.9 a 117.4±49.7 mg/dl, p<0.016). A livello delle piastrine:
1) nel gruppo in terapia con aspirina, si è osservata: una riduzione della risposta aggregante (maximal aggregation) all'ADP (da 65.3±8.5 a 43.9±6.3, p<0.01), al collagene (da 41.3±4.7 a 30.5±6.7, p<0.001), e all'epinefrina (da14.3±3.4 a 4.2±1.3, p<0.01); un aumento dei tempi di chiusura al PFA-100 (secondi) (da 113±26 a 192±28, p<0.02); una riduzione dell'espressione di CD62P (fluorescenza media) (da 37.1±8.3 a 18.8±4.4, p<0.03);
2) nel gruppo non in terapia antiaggregante, si è osservata: una riduzione della risposta aggregante in PRP all'ADP (da 75.3±6.5 a 57.2±11.2, p<0.05), al collagene (da 77.5±6.2 a 53.8±13.4, p=0.08), e all'epinefrina (da 54.5±8.3 a 29.3±6.2, p<0.01); una riduzione della risposta aggregante in WB (ohm) all'ADP (da 9.7±2.8 a 4.8 ±2.0, p<0.04), al collagene (da 10.7±1.7 a 6.0±1.6, p<0.02), e all'acido arachidonico (da 19.7±8.3 a 29.3±6.2, p<0.01); una riduzione dell'espressione di CD62P (da 42.3±6.1

a 22.6±4.1, p<0.003). I pazienti trattati con Alirocumab o Evolocumab non presentevano differenze significative tra loro nei parametri metabolici e piastrinici al baseline e al termine del periodo di trattamento.
Conclusioni. In pazienti con ipercolesterolemia familiare, il trattamento per 8 settimane con terapia monoclonale anti-PCSK9 migliora significativamente il quadro lipidico, riduce la reattività delle piastrine agli agonisti e aumenta la sensibilità agli effetti inibitori dell'aspirina. I risultati dello studio suggeriscono che il trattamento con i nuovi farmaci ipolipemizzanti anti-PCSK9 potrebbe avere un impatto positivo sulla riduzione del rischio cardiovascolare anche per l'azione protettiva esercitata sulle piastrine.

ADHERENCE TO MEDITERRANEAN DIET AND NON-ALCOHOLIC FATTY LIVER DISEASE: IMPACT ON METABOLIC PROFILE

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Introduction. Many different mechanisms are supposed to play a role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). NAFLD is strongly associated with obesity and diabetes. Several nutritional approaches have been proposed for NAFLD patients. The Mediterranean Diet (MD) is a dietary pattern with beneficial properties in primary and secondary prevention of cardio-metabolic diseases.
Aims. To investigate the relationship between MD and NAFLD in a cohort of metabolic patients and to investigate how MD adherence affects metabolic phenotype in NAFLD patients.
Material and Methods. 584 consecutive patients underwent ultrasonography to assess the presence of fatty liver. Adherence to the MD was investigated by the administration of a short dietary questionnaire. For the analysis patients were divided in two groups according to the MD score: low adherence (0-5 points), and high adherence (6-9 points).
Results. Mean age was 56,1±12,8 years, 38,2% were women. According to the MD score, 227 patients (38,9%) had low, and 357 had a good adherence to MD (61,1%). Overall, the prevalence of NAFLD was 82,6%. NAFLD was significantly higher in the poor adherence group (88.5% vs 79,0%; p=0,003). MD score was significantly lower in patients with NAFLD compared to those without [6 (4/7) vs 5 (4/6); p<0,001].
Among 483 NAFLD patients, at univariate analysis, MD questionnaire score was positively correlated with age (Rs=0,135; p=0,003), while was inversely correlated with triglycerides (Rs=-0,106; p=0,023), fatty liver index (Rs=-0,163; p<0,001), lipid accumulation product (Rs=-0,135; p=-0,002), Homa-IR (Rs=-0,127; p=0,009) and BMI (Rs=-0,101; p=0,027).
Conclusions. Adherence to MD is associated with a lower prevalence of NAFLD. Moreover, NAFLD patients with a good adherence to MD disclose a better metabolic profile. Our findings suggest that MD could be considered the optimal diet to obtain the weight loss recommended by guidelines as first-line intervention in NAFLD treatment.

LDL-CHOLESTEROL REDUCTION WITH PCSK9 INHIBITORS: A META-ANALYSIS OF RANDOMISED CONTROLLED TRIALS

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Background. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease involved in lipid metabolism by mediating LDL receptor (LDLR) clearance through a post-transcriptional mechanism. The inhibition of PCSK9 results in increased expression of the hepatic LDL-R and in a consequent lipid-lowering effect. Since the role of PCSK9 in LDLR degradation and LDL-cholesterol (LDL-c) metabolism was discovered, different pharmacological approaches to inhibit this protein have been developed in a very short time. Among monoclonal antibodies (mAbs) developed against PCSK9, clinical trial results are available for alirocumab (SAR236553/REGN727), evolocumab (AMG145), bococizumab (RN316/PF-04950615), and LY3015014. The aim of this meta-analysis was to compare the efficiency of PCSK9 inhibitors in all published randomized controlled trials (RCTs).
Methods. Pubmed, MEDLINE, and EMBASE were searched from inception to 08 September 2016. The search strategy included keywords and MeSH terms relating to lipid-lowering effects of PCSK9 mAbs. The inclusion criteria were:
1) English language;
2) phase 2 or 3 RCTs;
3) comparing PCSK9 antibodies vs no PCSK9 antibody (active treatment or placebo);
4) with effects on LDL-c reported;
5) with treatment duration longer than 8 weeks.

We also manually searched bibliographies of included studies as well as existing systematic reviews for any other articles that may be potentially suitable. We excluded articles published as abstract. The average difference (MD) between change in LDL levels before and after treatment in the group of patients treated with anti-PCSK9 antibodies and in the control group was considered as primary endpoint. We pooled the estimates and the corresponding confidence intervals (95% IC) by using the random-effects model according to DerSimonian & Laird method.
Results. Twenty-nine RCTs were selected, comprising 15,838 patients; 9469 were treated with PCSK9 inhibitors and 6369 were in control groups. This meta-analysis included 11 phase II trials and 18 phase III trials. Regarding baseline patient's characteristics, 8 trials included HeFH patients, 8 trials included patients at high cardiovascular risk, 4 trials included patients intolerant to statins. Overall, treatment with PCSK9 antibodies led to marked reductions in LDL-c, with overall MD -52.82 (IC 95% -56.36 to -49.27), vs placebo MD -57.58 (-61.16 to -53.99), vs ezetimibe MD -38.49 (-42.64 to -34.32). MDs in LDL-c were -51.71 (-58.72 to -44.69) for alirocumab and -54.95 (-59.09 to -50.81) for evolocumab. Pooled estimates according to the different doses showed a dose-response relationship for alirocumab: 75 mg MD -41.55 (-54.74 to -28.37), 150 mg MD -59.81 (-66.35 to -53.27), and evolocumab: 140 mg MD -51.59 (-60.36 to -42.82), 420 mg MD -52.17 (-57.31 to -47.04).
Conclusions. This updated meta-analysis confirms the evidence emerged from previous meta-analysis and clinical trials about the

efficacy of different PCSK9 antibodies in reducing LDL-c, across different type of patients (at high cardiovascular risk, statin intolerants, or with familial hypercholesterolemia) and both vs active treatment and placebo.

THE INCIDENCE OF CARDIOVASCULAR EVENTS IS LARGELY REDUCED IN HYPER-LP(A) PATIENTS ON LIPOPROTEIN APHERESIS. THE G.I.L.A. (GRUPPO INTERDISCIPLINARE LDL AFERESI) PILOT STUDY

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Aim. Lipoprotein apheresis (LA) is the elective therapy for homozygous and other forms of Familial Hypercholesterolemia resistant/intolerant to lipid lowering drugs and hyperlipoproteinemia(a).
The aim of our study was to analyse, in a multicentre Italian study performed in subjects on LA treatment for hyper-Lp(a) (>60 mg/dl) and chronic ischemic heart disease, the incidence of adverse cardiac or vascular events (ACVE) before apheresis and during the LA treatment.
Methods. We collected data of 22 patients (mean age 63±9 years, male 77%; from hospital of Pisa, Pistoia, Verona and Padova), with hyper-Lp(a), pre aphaeretic LDL-cholesterol <130 mg/dl, cardiovascular disease on maximally tolerated lipid lowering therapy and LA treatment (median 7 years, interquartile range 3-10 years). The LA treatment was performed by heparin-induced LDL precipitation apheresis (15/22), dextran-sulphate (4/22), cascade filtration (2/22) and immunoadsorption (1/22). Time between first cardiovascular event and beginning of apheresis was 6 years (interquartile range 1-12 years).
The ACVE incidence, before and after treatment, was evaluated by statistical analyses (paired t test, Wilcoxon, Chi-square test).
Results. The recorded ACVE, before and after the LA treatment inception, were 37 and 9 respectively ($p<0.001$); notably, the AVCE rates/year were 0.27 and 0.05 respectively ($p<0.001$) with a 81% reduction of events occurrence.
Conclusions. Our data confirm long-term efficacy and positive impact of LA on morbidity, by dramatically reducing ACVE in patients with hyper-Lp(a) and atherosclerotic disease at maximally tolerated lipid lowering therapy.

MAPPATURA GENETICA DELL'IPERCOLESTEROLEMIA FAMILIARE: DATI PRELIMINARI DELL'ESPERIENZA PADOVANA

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Introduzione. L'Ipercolesterolemia Familiare (FH) è un disordine del metabolismo lipidico su base genetica, rara in omozigosi (1/1000000), ed al contrario interessante 1 soggetto ogni 250 abitanti nella forma eterozigote (HeFH). Il fenotipo lipidico è caratterizzato da livelli molto elevati di LDL-C dalla nascita, e da un rischio elevato di aterosclerosi coronarica che predispone ad eventi clinici cardiovascolari precoci. Scopo del nostro studio è stato quello di indagare la potenziale associazione tra genotipo, fenotipo clinico e severità del profilo di rischio cardiovascolare, e valutare il potenziale impatto della caratterizzazione genotipica sulla risposta alla terapia farmacologica in pazienti con diagnosi genetica di FH.
Materiali e Metodi. Sono stati studiati 69 pazienti con FH, di cui 36 maschi e 33 femmine, con diagnosi clinica e probabile o definita di FH secondo i criteri del Dutch Lipid Clinic Network Score (DLCNS ≥6). I pazienti sono stati sottoposti ad un prelievo ematico per la ricerca di mutazioni genetiche associate ad FH (geni di LDLR, PCSK9, LDLRAP1 e APO B) come da protocollo dello studio LIPIGEN, ed alla raccolta di parametri clinici e bioumorali, assetto lipidico, parametri di sicurezza epatica e muscolare, metabolismo glucidico, determinati secondo metodi laboratoristici standardizzati.
Risultati. Tra i pazienti con HeFH, il 49.2% assumeva una terapia con statine ad elevata efficacia (± ezetimibe), ma solo il 4.3% dei pazienti raggiungeva livelli target di LDL-C nonostante una riduzione media del LDL-C del 50% (LDL-C alla diagnosi 332.7±91.5 mg/dl vs in terapia attuale 167.2±53.1 mg/dl). Dei pazienti HeFH affetti da malattia cardiovascolare precoce, il 90% era in terapia ad elevata efficacia, ma nessun paziente raggiungeva il target per LDL-C (LDL-C<70 mg/dl). L'analisi del genotipo ha messo in luce che 55 pazienti (79.7%) presentavano mutazioni nel gene LDLR, 1 paziente nel gene LDLRAP1 in forma omozigote, mentre in 13 pazienti (18.8%) non veniva riscontrata alcuna mutazione nota nei geni testati. Dei pazienti con mutazioni del gene LDLR, 20 (pari al 36.3%) appartenevano alla classe funzionale allele nullo e i restanti 35 pazienti (pari al 63.7%) appartenevano alle altre quattro classi funzionali. Di questi ultimi, 11 presentavano la mutazione FH Padova-1, 2 la mutazione FH Napoli-1, 2 la mutazione FH Napoli-4 e 6 la mutazione FH Palermo-1. Tra i pazienti FH senza mutazione nota, il 70% presentava una diagnosi clinica probabile ed il 30% una diagnosi clinica definita di FH secondo il DLCNS. I pazienti portatori di allele nullo in eterozigosi presentavano un fenotipo clinico più severo rispetto alle altre classi funzionali od ai pazienti privi di mutazioni note. In particolare era evidente un DLCNS più elevato, una presenza significativamente maggiore di xantomi e gerontoxon, livelli di colesterolo totale e LDL-C alla diagnosi significativamente maggiori rispetto agli altri sottogruppi. Per quanto riguarda la risposta alla terapia ipolipemizzante, i pazienti di tutti e tre i sottogruppi sopra considerati presentavano una risposta simile alla terapia farmacologica (LDL-C -50% vs basale), senza differenze statisticamente significative tra i gruppi.
Conclusioni. L'ottanta percento dei pazienti con diagnosi clinica di FH formulata mediante DLCNS con cut-off ≥6 è risultato portatore di mutazione genetica nota; la caratterizzazione funzionale del genotipo si associa a caratteristiche cliniche fenotipiche diverse,

mentre non sembra, nella nostra casistica, predire la risposta alla terapia ipolipemizzante. Viene infine confermata l'assoluta inadeguatezza della terapia ipolipemizzante attuale anche se utilizzata in modo massimale.

IMPAIRED FATTY ACID SYNTHESIS AFFECTS IMMUNE CELLS ACTIVATION: FOCUS ON STEROL REGULATORY ELEMENT BINDING FACTOR-1C ON T LYMPHOCYTES

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Background. Intracellular metabolism has recently been recognized as a key determinant of immune cells differentiation and activation and animal models lacking crucial genes of lipid metabolism are currently used to unraveil their impact on immune responses. Therapeutic targeting of metabolic checkpoint is a new challenge for the treatment of immunometabolic diseases. SREBP1c is one of the three isoforms of the sterol regulatory element-binding proteins, involved in the regulation of fatty acid synthesis. Indeed, ACC1 (acetyl-CoA carboxylase 1), a target gene of SREBP1c, has recently been shown to have a crucial role in modulating the polarization toward the Tregulatory cells.
Aim. To study how key proteins regulating intracellular metabolism, as SREBP1c, affect the activation of T lymphocytes
Matherial and Methods. A detailed immunophenotyping through flow cytometry, gene expression and metabolic profiling of secondary lymphoid organs were performed in SREBP 1c KO and WT littermates.
Results: SREBP1c deficiency resulted in a different distribution of T lymphocyte subsets in secondary lymphoid organs and splenomegaly (0.31±0.015 vs 0.25±0.014 g spleen/g body weight, p<0,001). Proportions of CD4+CD44+ (18.48±1.03% vs 22.85±0.54%, p<0,01), CD8+CD44+ (11.63±0.82% vs 14.86±1.24%, p<0,05), and Tregulatory CD4+CD25hiFoxP3+ cells (2.61 ± 0.25% vs 3.38 ± 0.10%, p<0,01) were significantly decreased in SREBP1c KO mice. Gene expression and metabolomic analysis of secondary lymphoid organs confirmed that fatty acid synthesis was switched off in SREBP1c KO animals and revealed an impairment in the glycolisis and Kreb cycle which led to a reduced production of ATP.
Conclusion. SREBP1c is a key player of intracellular metabolism also in immune cells and its deficiency affected the distribution of T lymphocytes in secondary lymphoid organs, thus suggesting that reprogramming T cell fatty acid syntheisis may represent a therapeutic target for the treatment of diseases characterized by a dysregulated immune activation.

ASSOCIAZIONE TRA ALTERAZIONE DELLA RIFLESSIONE DELL'ONDA SFIGMICA E DURATA DEL SONNO IN INDIVIDUI ESPOSTI A ELEVATI LIVELLI DI RUMORE AEROPORTUALE: LO STUDIO SERA-CV

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Background. Exposure to environmental noise might exert negative effects on cardiovascular function. Aim of the study is to explore whether chronic stress and/or sleep fragmentation and loss, associated with exposure to aircraft noise, have a detrimental effect on vascular function.
Methods. 22 individuals heavily exposed (E) to aircraft noise pollution (>50 DbA) were recruited and matched with a group of non-exposed individuals (NE). Pulse wave velocity (PWV), augmented pressure (AP) and augmentation index (Aix) were performed. Furthermore, central, brachial and 24 blood pressure (BP) were measured. The following standardized questionnaires were administered: Perceived stress score (PSS), state anxiety (SAS), Pittsburgh sleep quality index (PSQI), Epworth Sleepiness Score (ESS). 7-day actigraphy was performed for the assessment of total sleep time (TST), wake after sleep onset (WASO), and sleep efficiency (SE).
Results. E and NE were superimposable for age (51±15 vs 45±17 years, p=0.15), sex, BMI (26±3 vs 25±5 kg/mq) and mean BP (91±14 vs 92±14, p=0.84). Furthermore, E showed similar TST (7.2±1.8 vs 7.1±1.3h, p=0.77), WASO (50±46 vs 47±30 min, p=0.49) and SE (89±11 vs 90±7%, p=0.76) compared to NE. E showed higher Aix (26±12 vs 14±16, p=0.006) and AP (11±7 vs 7±8, p=0.03) than NE, in the presence of similar PWV (7,6±2.1 vs 7.5±2.3 m/s, p=0.87) and HR (70±12 vs 68±14 bpm, p=0.70).
In E group, Aix was related to height (r=-0.56, p=0,009) and TST (r=-0.65, p=0.002), while was not related with mean BP, PWV and HR and showed only a trend with age (r= 0.41, p=0,055) and PSS (r=-0.40, p=0.06). The association between Aix and TST remained significant in a multiple regression model adjusted for these variables (beta =-2.92, p=0.01), with TST accounting for 12.9% of Aix variance (r2 full model 0.84).
In NE Aix was related with age (r=-0.82, p<0.001), HR (r=0.76, p<0.001), TST (r=-0.49, p=0.01), mean BP (r=0.61, p=0.01), PWV (r=0.57, p=0.004). The only independent determinants of Aix in NE were age (beta =0.64, p=0.02) and HR (beta =-0.37, p=0.03).
Conclusions. Central pressure augmentation is independently affected by sleep duration in individuals exposed to high levels of environmental aircraft noise.

EVALUATION OF EFFICACY AND SAFETY OF EVOLOCUMAB 140 MG IN GENETICALLY CHARACTERIZED HE-FH PATIENTS. FIRST EXPERIENCE OF GENOA LIPID CLINIC

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Introduction. Primary objective was to establish how lipid profile is modified in patients affected by Heterozygous Familial Hypercholesterolemia (He-FH) treated with high intensity statin therapy

in association to Ezetimibe 10 mg after eight weeks of therapy with Evolocumab, the new antiPCSK9 monoclonal antibody; secondary objective was to verify the tolerability of the product.
Methods. We enrolled 5 genetically characterized He-FH patients treated with high intensity statin therapy and ezetimibe and not reaching low density lipoprotein cholesterol (LDL-C) recommended goal (LDL-C<70 mg/dl). 4 patients were in secondary prevention, 1 patient was in primary prevention with carotid stenosis near 20%. At baseline the mean of LDL-C levels was 155.2±19.8 mg/dl.
Results. After eight weeks of treatment with Evolocumab 140 mg every 14 days, LDL-C was reduced to 115±23.2 mg/dl (p<0.0001). In all patients, the reccomended LDL-C target <70 mg/dl was reached. No differences of triglycerides and high density lipoprotein cholesterol (HDL-C) levels were observed. Determination of Lipoprotein(a) levels is in progress. In two patients we stopped the administration of ezetimibe and reduced the statin dose because of LDL-C<20 mg/dl. No adverse effects were reported by patients.
Conclusions. Evolocumab 140 mg every two weeks is a very effective and safe therapy for obtain the reduction of LDL-C levels in He-FH.

IL RUOLO DEL MICRORNA-155 NELLA PATOGENESI E NEL TRATTAMENTO DELL'ARTRITE PSORIASICA

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Premessa. I microRNA (miRNAs) sono piccole sequenze di RNA non codificante capaci di legarsi a specifici mRNA regolandone i processi di crescita, differenziazione e apoptosi. Una disregolazione della loro funzione è causa di alterati processi di trascrizione genica e quindi anche causa scatenante di molti processi patologici. L'artrite psoriasica (AP) è un'artropatia infiammatoria cronica la cui insorgenza è legata a livelli di miRNAs "disregolati" in più sedi e in varie forme. Il miRNA-155 (miR-155) è un classico esempio di miRNA multifunzionale responsabile del controllo di processi patologici che contribuiscono allo sviluppo dell'AP. La comprensione del ruolo e delle influenze del miR-155 mostra ampie prospettive di comprensione dei meccanismi molecolari che comportano lo sviluppo della patologia e può aprire importanti prospettive terapeutiche.
L'obiettivo dello studio era di valutare i livelli sierici del miR-155 all'inizio della terapia biologica e a distanza di 6 mesi di trattamento.
Metodi. Lo studio è stato condotto presso l'Ambulatorio di Reumatologia della Clinica Geriatrica della ASL Lanciano-Vasto-Chieti. Abbiamo prelevato campioni di plasma da 9 pazienti con diagnosi di AP (sec. criteri CASPAR). Tali pazienti, già trattati con farmaci di prima linea (DMARDs) senza arrivare al controllo clinico della malattia, necessitavano dell'introduzione di un farmaco biologico (molecole appartenenti alla famiglia degli anti-TNFα: Infliximab, Etanenercept, Adalimumab). Il primo prelievo è stato eseguito prima di iniziare la terapia con farmaco biologico; un secondo prelievo è stato fatto dopo 6 mesi per studiare le possibili modificazioni indotte dall'evoluzione della malattia e dalla terapia biologica in atto.
Risultati. L'analisi ha mirato a individuare una variazione dei livelli di miR-155 su plasma per valutare se, e in che misura, la terapia biologica potesse influire sulla sua espressione.
I dati completi includono solo i 3 pazienti che avevano completato la finestra terapeutica di 6 mesi. Per gli altri 6 pazienti, ancora in fase di trattamento, in attesa dei dati laboratoristici riguardanti il miR-155, abbiamo ottenuto dei dati preliminari con un approccio valutativo esclusivamente clinico. La rivalutazione si è basata sull'utilizzo di uno score validato (CDAI SCORE) per stabilire il grado di malattia in atto e ottenere un parametro predittivo clinico capace di evidenziare un eventuale miglioramento parziale della malattia. Tutti i pazienti mostravano un significativo miglioramento clinico con riduzione dei valori iniziali dello score e questo dato correla con la riduzione significativa dei livelli di miR-155 - sia quantitativa (rispettivamente: 252,3 → 59,2; 64,1 → 11,6; 77,3 → 4,2) sia relativa al valore iniziale (= fold-difference: 4,3; 5,5; 18,2) - osservata in risposta alla terapia nei pazienti che avevano completato il ciclo semestrale.
Conclusioni. Questi risultati, sia pur parziali e preliminari, suggeriscono che i miRNAs rappresentino non solo dei protagonisti essenziali nella patogenesi della malattia, ma anche dei potenziali bersagli terapeutici da modulare con farmaci specifici per ripristinare e correggere la disregolata espressione genica di quelle proteine coinvolte nei processi fisiopatologici dell'AP. Correggere i miRNAs espressi in maniera aberrante equivale a bloccare il processo patologico e in alcuni casi addirittura invertirlo, agendo, a vari livelli, sull'espressione genica di più elementi coinvolti nello sviluppo della patologia e migliorando il quadro clinico del paziente trattato.

MICROBIOTA COMPOSITION AFFECTS LIPID METABOLISM AND INTESTINAL HOMEOSTASIS

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Undernutrition, defined as inadequate intake or assimilation of nutrients, still determines worldwide half of all deaths in children younger than 5 years of age.
Protein-energy malnutrition is currently the most important nutritional problem in most low-income countries and leads to a wide range of continuing health and developmental problems. Protein-energy malnutrition is characterized by stunted growth, hepatic steatosis and a damaged gut mucosal architecture, associated with malabsorption, impaired mucosal permeability and inflammation.
Nowadays, it is widely recognized that the intestinal content, such as dietary constituents and commensal bacteria, influences physiological and pathological processes throughout the body. The mammalian gastrointestinal tract harbors trillions of microbes that belong to all three domains of life (Bacteria, Archaea, and Eukarya) and their viruses, the so-called gut microbiota. An increasing number of clinical and preclinical studies indicate that food is a critical factor that influences the composition of the gut microbial community and that the microbiota, in turn, can influence the efficiency of energy harvesting from ingested food. The microbiota modulates the bioavailability and metabolism of macro- and micronutrients as well as metabolites, including bile acids, lipids, amino acids, vitamins and short-chain fatty acids.

This study was aimed at determining whether different microbiota can limit the devastating effects of a protein-deficient diet by modulating energy extraction and availability from food. To this aim, germ-free mice were colonized with different microbiota communities obtained from mice eating different diets. Germ-free mice are those born and reared without exposure to any live microbes.
To obtain microbiota donors, Specific-Pathogen-Free male 8-week-old C57BL/6 mice were fed for 5 weeks a chow diet (CD), a high-fat diet (HFD), or a low-protein diet (LPD). Gut microbiota transfer in germ-free mice was performed by harvesting cecal contents from donor mice and introducing them, by gavage, into 5-week-old germ-free male C57Bl/6 recipient mice, maintained in separate, sterile isolators.
Even though body weight at the end of the experimental period was unaffected by different microbiota consortia, subsequent analyses indicated that:

1) germ-free mice receiving the CD microbiota were more prone to develop hepatic steatosis;
2) germ-free mice receiving the HFD microbiota had an increased concentration of plasma phospholipids and a different caecal composition in terms of short chain fatty acids, with an increased percentage of Propionate and a reduced percentage of Butyrate.In addition, the intestine showed shortened villi and cypts, both in the small intestine and in the colon. These histological modifications were accompanied by a robustly upregulated expression of Duox2 and Duoxa2, indicative of a perturbed intestinal mucosal homeostasis;
3) germ-free mice receiving the LPD microbiota had an increased plasma concentration of HDL-cholesterol. To clarify this finding, the expression of a wide panel of genes related to lipid/lipoprotein metabolism is currently under investigation.

In conclusion, the results obtained clearly indicate that different microbiota are able to modulate plasma lipid levels and the accumulation of lipids in the liver parenchyma leading to steatosis. Additionally, the microbiota shaped by a high-fat diet severely modifies the histological architecture of the gut and the intestinal expression of genes indicative of an increased microbiota/mucosa interaction, reflecting an altered microbiota composition or a defective host defense mechanism.

SEVERE CAROTID ATHEROMATOUS STENOSIS REGRESSION AFTER SUBCUTANEOUS ADMINISTRATION OF TOCILIZUMAB

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Takayasu arteritis (TA) is a chronic inflammatory disease of unknown etiology that involves large and medium-sized arteries, primarily the aorta and its major branches. TA is a therapeutic challenge because corticosteroids and conventional immunosuppressive agents are not always safe and/or efficacious. Interleukin 6 (IL-6) has emerged as a key cytokine in the pathogenesis of TA and its serum levels have been shown to well correlate with disease activity.
We report the case of a normotensive, normolipemic 19 years old female patient with TA refractory to conventional immunosuppressive agents successfully treated by subcutaneous administration of tocilizumab. The follow-up period was 3.6 years. At the first evaluation, despite the current use of prednisone at dosage of (50 mg/kg/day), the patients presented with high clinical indices of disease activity, and high inflammatory markers; moreover, at the age of 22-years old, ultrasonography doppler scan diagnosed a severe atheromatous carotid involvement, with estimated stenosis of 75%, bilaterally; after tocilizumab was started, the patient was clinically and instrumentally evaluated every 16 weeks, and a progressive normalization of clinical and bio-humoral indices of disease was observed (stably normal since the 5th month of treatment); prednisone dosage could be consistently tapered and finally stopped since the 4th month of treatment. At the last US evaluation (6th month of treatment), carotid stenosis were estimated as 45%, bilaterally. At 12th month since the treatment has been started the carotid stenosis has been estimated lower than 25%, bilaterally. No significant side effects have been reported, and the patient is continuing to take the drug.
Subcutaneous administration of tocilizumab appears a good option in refractory TA with an effective steroid-sparing effect. In addition, it seems to have very favorable effects on endothelial function improving cIMT, and significantly reducing artery hypertrophy.

ALIROCUMAB IN HIGH CARDIOVASCULAR RISK PATIENTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: EFFICACY AND SAFETY IN LIPID LOWERING LEVELS

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Background. Many patients with Heterozygous Familial Hypercholesterolemia (FH), at high cardiovascular risk are not at target for Low Density Lipoprotein Cholesterol (LDL-C) levels despite maximal tolerated traditional treatment. Alirocumab is an anti-protein convertase subtilisin/kexin type 9 (PCSK9) monoclonal antibody, approved for the treatment of hypercholesterolemia.
Aim. We investigated the efficacy in lipid lowering and safety of Alirocumab in patients with FH.
Material & Methods. In this, Multicenter, Single Arm, Open-Label Phase IIIb clinical trial (APPRISE) we enrolled thirteen patients (70% Male; average age: 54 years) with FH, where not at target with a maximal-tolerated stable daily dose of statin for ≥4 weeks prior to the screening visit, with or without other lipid-lowering drug. In addition to usual therapy patients were treated with Alirocumab 150 mg by subcutaneous auto-injection every 2 weeks. We evaluated efficacy and safety parameters at week 0 and at week 12 by blood sample collection.
Results. After data analysis we found a significant reduction of Total cholesterol (p<0,001), LDL-C (p<0,001), Triglycerides (p=0,005), HDL-C (p<0,001). There were not significant differences for Serum Creatinine, Glicemia, HbA1C, AST, ALT, GGT, CK, Leukocytes. Risk-based LDL-C goals (<70 mg/dL or <100 mg/dL) were achieved by 45% of patients. Average LDL-C reduction was 109.3 mg/dL (55.3%).
Conclusion. Alirocumab combined with maximal-tolerated statin therapy, significantly reduces LDL-C levels and CVrisk in 12 weeks. This treatment is well tolerated, safe and effective to achieve LDL-C target level in a large percentage of patients at high cardiovascular risk.

MICROPARTICELLE CIRCOLANTI E PATOLOGIA CORONARICA: RUOLO PREDITTIVO NELLA RI-OCCLUSIONE DEL BYPASS AORTOCORONARICO

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Introduzione. La pervietà del graft, dopo bypass aortocoronarico (CABG), è uno dei principali determinanti di successo a lungo termine della procedura di rivascolarizzazione. L'identificazione di biomarker in grado di predire la ri-occlusione del graft risulta quindi di fondamentale importanza. Numerosi studi hanno valutato l'effetto della procedura chirurgica e delle variabili genetiche, ma solo pochi hanno indagato il ruolo di marker biologici (infiammatori ed emostatici). Le microparticelle (MPs) sono vescicole rilasciate da diversi tipi cellulari dopo attivazione o apoptosi. Sebbene la formazione di MPs rappresenti un fenomeno fisiologico, molte patologie, tra cui quelle a carattere infiammatorio e aterosclerotico, sono associate ad aumento delle MPs, suggerendo il loro potenziale ruolo come biomarker di patologia. Sebbene dati in letteratura indichino che le MPs vengono prodotte durante CABG, non ci sono tuttavia studi volti a indagare l'associazione tra i livelli di MPs ed esito del CABG. Pertanto gli scopi di questo lavoro sono stati:
1) individuare un profilo di MPs potenzialmente associato con la ri-occlusione del bypass;
2) valutare se le MPs possano essere predittori indipendenti della ri-occlusione del bypass.

Metodi. Il profilo delle MPs è stato analizzato nel plasma di pazienti sottoposti a CABG, a cui è stato effettuato un prelievo venoso il giorno prima (T0) e 1 anno dopo (T1) il bypass, in concomitanza con una TAC per valutare la pervietà del graft: sono stati confrontati 30 pazienti con graft occluso con 30 pazienti con graft pervio dopo 1 anno, comparabili in termini di caratteristiche cliniche e trattamento farmacologico. Il numero di MPs, l'origine cellulare e l'espressione di marker di attivazione piastrinica [P-selettina, CD40L e Tissue Factor (TF)] sono stati analizzati mediante citofluorimetria. E' stato applicato un modello multivariato (curve ROC) per determinare se i livelli di MPs possano incrementare il valore predittivo di occlusione del bypass rispetto ai classici fattori di rischio cardiovascolare.

Risultati. Al T1, i pazienti con bypass occluso avevano un profilo di MPs significativamente diverso rispetto a quello dei pazienti con bypass pervio. In particolare, il numero di MPs di derivazione piastrinica e leucocitaria che esprimevano TF era significativamente superiore nei soggetti con bypass occluso. Tali pazienti presentavano inoltre un numero maggiore di MPs piastriniche che esprimevano P-selettina (6 volte superiore) e CD40L (10 volte superiore) rispetto a quelli con bypass pervio (p<0.0001 per entrambi i marker).

L'analisi delle MPs al T0, effettuata per valutare se il profilo preoperatorio di MPs fosse indicativo della prognosi a 1 anno dal bypass, ha evidenziato che i pazienti che avrebbero avuto il bypass occluso avevano un numero significativamente maggiore di MPs piastriniche, totali e positive per i marker di attivazione P-selettina e CD40L (6 e 11 volte, rispettivamente), rispetto ai pazienti con bypass pervio. Inoltre le MPs piastriniche TF+ erano 3 volte maggiori nei pazienti che dopo 1 anno avrebbero avuto il bypass occluso (64±40 vs 17±7 MPs/µl nei pazienti con bypass pervio, p=0.0002). Le curve ROC hanno evidenziato che i livelli preoperatori di tali MPs risultavano avere un valore predittivo indipendente di ri-occlusione del graft entro 1 anno dall'intervento. Inoltre, i livelli di MPs di origine piastrinica TF+, P-selettina+ e CD40L+ incrementavano significativamente il valore prognostico rispetto ai classici fattori di rischio cardiovascolare (AUC=0.82, 0.79, 0.83, rispettivamente vs 0.67, p<0001).

Conclusioni. Questo studio mostra che i pazienti che presentano ri-occlusione del bypass dopo 1 anno dall'intervento sono caratterizzati da un significativo aumento nel numero di MPs derivanti da piastrine attivate e che esprimono TF suggerendo che uno specifico profilo preoperatorio di MPs è predittore indipendente dell'occlusione del graft e candidando le MPs a potenziali biomarker per l'identificazione dei soggetti ad alto rischio di complicanze.

SINDROME DELLE APNEE OSTRUTTIVE NEI PAZIENTI OBESI: EFFETTI SUL PROFILO METABOLICO E CARDIOVASCOLARE E RISPOSTA A 6 MESI DI TERAPIA COMPORTAMENTALE

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Obesità e Sindrome delle Apnee Ostruttive Notturne (OSAS), sono noti fattori di rischio cardiovascolare, la cui prevalenza è in costante aumento. L'effetto cumulativo di queste condizioni sul profilo metabolico e cardiovascolare non è ancora del tutto compreso.

Scopo dello studio.
1) caratterizzare metabolicamente i soggetti obesi con OSAS (OSAS+) rispetto a quelli senza OSAS (OSAS-);
2) valutare prospetticamente l'impatto di 6 mesi di terapia comportamentale negli OSAS+ vs OSAS- e l'influenza della ventilo-terapia sui parametri metabolici.

Materiali e Metodi. La presenza di OSAS nei pazienti obesi afferenti al nostro ambulatorio è stata indagata mediante score clinici, quindi confermata tramite polisonnografia. Tutti i soggetti sono stati valutati al basale, mentre 15 OSAS+ e 15 OSAS- (di pari età, sesso e BMI) sono stati rivalutati dopo 6 mesi di trattamento con dieta ipocalorica personalizzata e attività fisica aerobica.

Risultati. Abbiamo studiato 111 pazienti (76 OSAS- e 35 OSAS+, di cui 21 in ventilo-terapia), di età media 53±13 anni (M/F 61/50). Il gruppo OSAS+ era caratterizzato da una maggiore età (P=0,02), prevalenza del sesso maschile (P=<0,0001), superiori valori di circonferenza vita (P=0,007) e collo (P=0,002) con un trend per valori inferiori di colesterolo-HDL (P=0,053) e maggiore prevalenza di Sindrome Metabolica (P=0,09).

Al follow-up entrambi i gruppi presentavano riduzione di BMI e circonferenza vita. Dal punto di vista metabolico, gli OSAS+ mostravano incremento nei soli valori di colesterolo HDL (p=0,01), mentre negli OSAS- si osservava un miglioramento significativo dell'intero profilo biochimico. Tra gli OSAS+ in ventilo-terapia era rilevabile un miglioramento significativo dei parametri metabolici (BMI, circonferenza vita, massa grassa, colesterolo-HDL e nonHDL).

Conclusioni. In questo studio pilota abbiamo osservato che i soggetti obesi OSAS+ sembrano essere caratterizzati da un peggior profilo metabolico al basale. Le OSAS si associavano a una ridotta risposta alla terapia comportamentale dell'obesità, seppure parzialmente compensata e migliorata dalla ventilo-terapia notturna.

REVISED DUTCH LIPID CLINIC NETWORK SCORE CRITERIA ADAPTED FOR PEDIATRIC AGE: EVALUATION OF THIS TOOL IN PEDIATRIC PATIENTS WITH HYPERCHOLESTEROLEMIA

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Introduction. Revised Dutch Clinic Network Score criteria (rDLCNSc) are worldwide used to detect patients with familial hypercholesterolemia (FH). Various Pediatric Lipid Centers have experimentally adapted rDLCN criteria to pediatric age. The criteria included are: LDL-cholesterol levels, presence of tendon xanthoma or corneal arcus, LDL-cholesterol higher than 190 mg/dl in a parent/first degree relative (160 if aged less than 18 years), premature coronary artery disease (pCAD) in a parent /first degree relative. "Premature" is meant before 55 years if male and before 65 years if female. Score between 6 and 8 qualify for probable FH, higher than 8 for definite FH.
Matherials and Methods. The aim of this study is to evaluate the efficacy of rDLCNc adapted for pediatric age to select pediatric patients for DNA mutational analysis for FH in patients referring to our Lipid Center. In a 12 months-period, 50 patients were referred to our Lipid Center for hypercholesterolemia. rDLCNc adapted for pediatric age was calculated for each patient. 14/50 (28%) showed a score of 6 or higher and underwent genetic analysis for LDL-R mutation. 2 tests are still ongoing.
Results. Among our 12 tested patients, 4/12 (33%) had a mutation of LDL-R gene. 7/12 (58%) had positive family history for hypercholesterolemia. 0/12 had positive pCAD in parents or first degree relatives, 6/12 (50%) had pCAD in second degree relatives. Out of the 4 patients with positive genetic test, 3/4 (75%) had pCAD in second degree relatives.
Conclusions. rDLCNc adapted for pediatric is a useful tool to detect patients eligible for FH molecular diagnosis, especially in healthcare systems with limited genetic testing resources. As children's parents age is often lower than the threshold proposed for pCAD, they may not have had a pCAD yet. Considering pCAD also in second degree relatives (such as grandparents) might even improve this tool for FH detection in pediatric population.

EXTREME LIPOPROTEIN(A) LEVELS IN HYPERCHOLESTEROLEMIC PATIENTS: RELEVANCE AND MANAGEMENT IN PEDIATRIC POPULATION

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Introduction. Elevated lipoprotein(a) levels have been considered a causal risk factor for coronary artery disease (CAD) since many years. Due to its biochemical structure, lipoprotein(a) may contribute to both atherosclerosis and thrombosis. Few data are available in hypercholesterolemic children.
Matherials and Methods. The aim of this study is to evaluate the prevalence of extreme lipoprotein(a) levels in hypercholesterolemic children referring to our Lipid Center and to develop a clinical management strategy. In a 12 months-period, 50 patients were referred to our Lipid Center for hypercholesterolemia. 14/50 (28%) matched clinical criteria for suspected familial hypercholesterolemia and underwent further analysis, included dosage of lipoprotein(a). Extreme lipoprotein(a) levels were defined as higher than 90th centile in two different blood samples, considering adult centile as no pediatric centile are available so far. Patients with extreme lipoprotein(a) levels underwent a thrombophilic panel screening (thromboplastin partial time, prothrombin time, mutation of V factor and II factor, dosage of protein c , protein S and antithrombin III). Haematologic evaluation and detailed CAD-oriented family history collection were also performed.
Results. 1/14 patients showed lipoprotein(a) levels higher than 90th centile. 1/14 patient showed lipoprotein(a) level higher than 99th centile. Among these two patients, 2/2 had positive family history for hypercholesterolemia, 1/2 had positive family history for CAD and for thrombosis. Thrombophilic screening showed no abnormalities. Specific dietetic and lifestyle indications for hypercholesterolemia were given.
Conclusions. Despite no specific pharmacological treatment is recommended yet in pediatric patients with extreme lipoprotein(a) levels, lipoprotein(a) levels determination is advisable in selected hypercholesterolemic patients. Detecting pediatric patients with extreme lipoprotein(a) levels is important in order to detect possible other pro-thrombosis risk factors. Moreover, patients and their families are educated to avoid the acquisition of other risk factors, such as smoking and weight excess, and to lead a healthy lifestyle, in order to preserve their cardiovascular health.

CIGARETTE SMOKE AQUEOUS EXTRACT: EFFECTS ON ENDOTHELIUM, MONOCYTES AND THEIR INTERACTION

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Cigarette smoke is a risk factor for cardiovascular disease that causes vascular endothelial cells (EC) dysfunction, a key event in atherogenesis. We recently demonstrated that cigarette smoke condensate, which contains the hydrophobic components of cigarette smoke, induces human monocytes (HM) to release chemotactic factors and to stimulate the EC expression of adhesion molecules, contributing to plaque progression. Moreover, the incubation with medium conditioned by HM treated with cigarette smoke condensate caused a change in cellular morphology and the shrinking of the EC cytoplasm. In the present study, we investigated the effects of the Aqueous Extract (AE) of cigarette smoke, which contains water-soluble components of both the condensate and the gas/vapor phase, on HM and EC behavior. Cells were incubated with 10% AE or with medium conditioned by HM alone (CHM) or exposed to AE (CMAE). AE shows a dual behavior: on one hand, it causes atherogenic effects by inducing morphological changes in EC and the shrinking of their cytoplasm (-55% vs control). Moreover, the incubation of EC with CMAE increases the expression of VCAM-1 and ICAM-1 (+2 fold vs control, measured by qPCR) and the NF-kB activity (+2 fold vs control for EC treated with AE or CMAE, measured by luciferase assay). Furthermore, in HM exposed to AE the expression of pro-inflammatory cytokines IL-8, IL-1β, MCP1 and TNFα is in-

creased (+8, +25, +3, +6 fold vs control, respectively, measured by qPCR). On the other hand, AE shows anti-atherogenic properties by increasing in EC the expression of KLF2, an atheroprotective transcription factor (+2 fold vs control, measured by qPCR) and by enhancing in HM the expression of anti-inflammatory cytokines such as IL-10 (+6 fold vs control, measured by qPCR). Our results suggest that AE determines multiple effects on HM and EC that may differently affect the atherogenic process.

RIDUZIONE DEL RISCHIO CARDIOVASCOLARE IN SOGGETTI OBESI DIABETICI DOPO CHIRURGIA BARIATRICA

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Introduzione. La chirurgia bariatrica (CB) rappresenta un valido strumento nel determinare un persistente calo ponderale, un miglioramento del compenso glucidico e una riduzione del rischio cardiovascolare (RCV) in soggetti obesi affetti da diabete mellito tipo 2 (DM2).
Scopo. Valutare l'effetto della CB sul RCV di pazienti obesi diabetici utilizzando due diversi modelli di rischio, lo score UKPDS e lo score Progetto Cuore (PC).
Metodi. Sono stati studiati 98 pazienti obesi affetti da DM2 (durata media diabete 5.4±5.2 anni), M/F 37/61, età 49.9±8.6 anni, BMI 46.2±7.8 kg/m^2, sottoposti a bendaggio gastrico (LAGB, N=8), bypass gastrico (RYBP, N=71) o sleeve gastrectomy (SG, N=19) nel periodo 2005-2014. In tutti i soggetti sono stati analizzati i fattori di RCV al basale e 12 mesi dopo l'intervento e abbiamo applicato gli score di rischio UKPDS e PC per predire gli eventi CV a 10 anni.
Risultati. 12 mesi dopo l'intervento si è osservato un calo ponderale del 26,4±8,8% con remissione di DM2 nel 69,4% dei casi (4/8 dei LAGB, 49/71 di RYBP, 15/19 di SG). Ad 1 anno dall'intervento si è assistito a una riduzione del RCV misurato sia con lo score UKPDS (deltaUKPDS -27,9%, p=0,0001) che con lo score PC (deltaPC - 40,8%, p=0,000<0.001. La riduzione del RCV era maggiore nei soggetti che andavano incontro a remissione di DM2 (deltaPC p=0,0001) e in chi apparteneva alle categorie di rischio moderato-elevato (deltaUKPDS p=0,022, deltaPC p=0,259), mentre era minore nei pazienti sottoposti a LAGB rispetto a RYGB e SG (UKPDS p=0,146, PC p=0,016).
Conclusioni. La CB determina una riduzione significativa del RCV misurato con gli score UKPDS e PC. La riduzione del rischio varia in base alle caratteristiche dei soggetti, alla tecnica chirurgica e al modello di rischio utilizzato.

BIOACTIVE COMPONENTS FROM ANTARTIC KRILL EXERT ANTI-ATHEROSCLEROTIC ACTIVITY IN APOE-DEFICIENT MICE

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Krill oil from Antarctic krill has emerged as good source of EPA and DHA for their increased bioavailability compared to fish oil, being esterified in phospholipids rather than in triglycerides. Additionally, Antarctic krill is characterized by a high-quality protein content, whose biological effects have not been explored. Aim of the present study was to evaluate the effect of Antarctic krill components on plasma lipids and atherosclerosis development.
Sixty apoEKO mice were randomly divided into four groups and fed, for 12 weeks, with Western diet (CONTROL) or diets identical to the Western diet except for the protein or fat content. Specifically, the amount of casein or fat in CONTROL was partially replaced by krill proteins (PRO), krill oil (KRILL OIL), or both (KRILL OIL+PRO). Blood was collected at different time points for lipid analysis. At sacrifice, aorta, heart and liver were harvested for subsequent analyses.
In KRILL OIL+PRO and KRILL OIL mice, cholesterol levels measured at 6 and 12 weeks of dietary treatment were significantly lower than those of CONTROL and PRO animals. Glycogen accumulation and steatosis, commonly present in CONTROL and PRO animals, were sporadic in KRILL OIL+PRO and KRILL OIL mice ($p<0.005$). The presence of krill oil or krill proteins in the diets significantly inhibited atherosclerosis development in the aorta, whereas, at the aortic sinus, a significant reduction in lesion area was only observed in KRILL OIL mice compared with CONTROL (-22.6%, $p<0.05$). Krill oil containing diets strongly modified the fatty acid composition of aortic plaques and affected the expression of several genes involved in lipid metabolism. Moreover, krill proteins seem to exert a moderate anti-inflammatory effect.
Altogether, the results indicate krill oil as the most active component of krill, being able to reduce cholesterol levels, inhibit plaque development and prevent liver damage. Krill proteins appear to exert a moderate effect on atherosclerosis development.

CHARACTERIZATION OF METABOLIC SYNDROME IN PLIC COHORT

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Background. Metabolic Syndrome (MetS) is considered a "cluster" of risk factors (hypertension, visceral obesity, impaired glu-

cose metabolism, atherogenic dyslipidemia) that occur simultaneously in the individuals and results in increased cardiovascular risk. The aim of this study was to determine the prevalence of MetS and its determinants in a cohort of healthy Italian adults, evaluating their temporal trends.
Methods. We selected the study sample from PLIC cohort. PLIC (studio sulla Progressione delle Lesioni Intimali Carotidee) is a single-centre, observational, cross-sectional and prospective study of subjects enrolled on a voluntary basis in 1998-2000 and followed for 11 years on average. These analyses were performed on subjects with all visits completed. The prevalence of MetS was defined by the harmonized definition of previous criteria (IDF, NHLBI, AHA, ATP-III). According to this definition, a diagnosis of the MetS is made when any 3 of the 5 following risk factors are present: waist circumference >102 cm in men and 88 cm in women; elevated triglycerides (TGs), defined as ≥150 mg/dL or fibrate therapy; decreased HDL cholesterol (HDL-c), defined as 100 mg/dL, or hypoglycemic therapy. Prevalence was determined for each of the four planned visits.
Results. The sample included 1445 patients, of which 21.6% (24.3% M; F 19.7%) suffered from MetS at baseline. The prevalence increased during the study, reaching 25.2% in visit 4. The prevalence of MetS was higher in men than in women in all visits. Stratifying by age, we observed a higher prevalence of MetS in the age group ≥65 years (V1 29.2%), followed by those aged 40-64 years (V1 22.6%). In each visit, the most prevalent determinant was BP higher than the cut-off (95.5% in MetS patients at baseline; 69.3% in the total sample). MetS patients with glucose values higher than the cut-off increased during the study, from 52.6% at baseline (18.8% in the total sample), reaching 77,2% at visit 4 (30.6% in the total sample); the same trend was evident for waist circumference, while the prevalence of determinants related to HDL-c and TGs decreased during the visits.
Conclusions. The high prevalence of subject with MetS and its increasing trend, should call the attention of health care professionals on the management and prevention of cardiometabolic risk factors which, individually or in combination, can lead to an increased incidence of cardiovascular events, regardless of age.

RUOLO DELL'HDL E DELL'ACIDO URICO NEL DANNO VASCOLARE IN SOGGETTI AFFETTI DA IPERTENSIONE BEN CONTROLLATA FARMACOLOGICAMENTE

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Introduzione. È noto che negli ipertesi le alterazioni metaboliche concorrono nell'aumentare il rischio CV. Lo scopo di questo studio è stato di valutare il rapporto tra valori di HDL, acido urico e danno vascolare.
Materiali e Metodi. Abbiamo valutato 436 ipertesi ben controllati farmacologicamente (198 M; età media 59,8±9,9 anni); il 50.4% presentava obesità addominale; il 23,2% è risultato normopeso, il 39,9% sovrappeso, il 36.9% obeso. Tutti i pazienti assumevano farmaci antiipertensivi, il 37,2% statine.
In corso di terapia con ACEi, sartani, tiazidi rispetto ai betabloccanti o calcio-antagonisti (ns) i valori di HDL e uricemia sono risultati sovrapponibili.
Risultati. L'HDL è risultato correlato negativamente con la PAD (p=0.015), le dimensioni della radice aortica (p<0,0001).
Nel sottogruppo di pazienti in terapia con statine è confermata la correlazione tra HDL e le dimensioni della radice aortica (p=0,0009), correlazione inversa con l'uricemia (p=0,0219); assente correlazione con l'ASI. I valori di urato, in corso di terapia con statine, risultano non correlati ai valori di PA, il grado di stenosi carotidea e l'ASI. L'uricemia mantiene una correlazione diretta con il BMI (p=0,05), la CA (p=0.0125), le dimensioni aortiche (p=0.0002), l'IMT (0,0141) e l'ASCVD-10-yr (p=0,0007). È altresì degno di nota come i valori di uricemia ma non quelli di HDL sono risultati più elevati nei pazienti in terapia con statine.
Conclusione. Valori elevati di HDL sembrano costituire un fattore protettivo per l'insorgenza di ectasia della radice aortica, sebbene il loro ruolo nella prevenzione della perdita di elasticità della parete vascolare risulti ancora poco chiaro. L'acido urico sembra indurre una alterazione endoteliale confermato dalla riduzione dell'elasticità vasale e dall'ispessimento medio-intimale. Il rischio di sviluppare un danno vascolare mediato dall'urato permane anche in corso di terapia con statine.
Questi dati sembrano confermare il rapporto tra HDL, acido urico e disfunzione endoteliale.

NON ALCOLIC FATTY LIVER DISEASE: A MARKER OF INSULIN RESISTANCE AND VASCULAR DYSFUNCTION IN HYPERANDROGENIC PATIENTS

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Introduction. Overweight and insulin-resistence often associated with Non alcoholic fatty liver disease (NAFLD) are reported in women with polycystic ovary syndrome (PCOS) and their coexistence can amplify the individual risk for cardiovascular (CV) disease.
Aim of the Study: To evalutate by ultrasound the early atherosclerotic morphological and functional damage at common carotid and femoral arteries and NAFLD occurrence in hyperandrogenic PCOS patients.
Material and Methods. Study population consisted of 39 hyperandrogenic patients and 17 control age-matchetd subjects. The occurrence of traditional CV risk factors was made accordingly with current guidelines; in all principal anthropometric parameters, hormonal pattern, glycidic metabolism (HOMA-IR, insulin levels), lipid profile, ultrasound assessment for NAFLD evaluation, and intima-media-thickness and pulse wave velocity at common carotids and femoral arteries were investigated.
Results. PCOS patients showed a worse CV risk profile than control population. In particular, patients showed higher values of body mass index (BMI) (25.54 ± 4.89 vs 21.3 ± 2.50, p=0.002), waist to hip ratio (WHR) (0.82 ± 0.07 vs 0.76 ± 0.04, p=0.017) and BIA for fat mass (24.54 ± 7.49% vs 16.58 ± 5.47%), and lower values of BIA for lean mass (73.94 ± 8.35% vs 83.57 ± 5.49%, p <0.0001) in comparison to controls. Moreover, PCOS patients showed a worse glycidid and lipid profile, because of higher LDL-c (97.87 ± 25.18 vs 81.88 ± 18.40, p<0.05) and triglycerides values (106.81 ± 74.58 vs 62.85 ± 29.49, p<0.05), and lower HDL-c values (49.83 ± 9.51 vs 62.08 ± 12.28, p=0.002). Twenty-5 out of 39 PCOS patients and 3 control subjects had ultrasound detectable NAFLD.

PCOS patients showed higher carotid and femoral IMT (0.85 ± 1.15 mm vs 0.47 ± 0.08 mm, p=0.001; 0.76 ± 0.16 mm vs 0.54 ± 0.09 mm, p <0.0001), a worst carotid and femoral compliance (7,83 ± 2,78 m/s vs 4,71 ± 0,58 m/s, p<0.0001; 8,03 ± 2,31 m/s vs 5,75 ± 0,95 m/s, p<0.0001), and (3,25 ± 1,57 vs 0,49 ± 0,51, p<0.0001), in comparison to controls.
Conclusions. As confirmed at multivariate analysis, the presence of insulin resistance and overweight associated with liver steatosis were independent factor for early vascular atherosclerosis in PCOS patients.

PROFILO LIPIDICO E SOPRAVVIVENZA IN PAZIENTI RICOVERATI PER SEPSI

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Background. I livelli sierici delle lipoproteine si riducono drammaticamente in corso di una risposta di fase acuta e la magnitudine di tale riduzione potrebbe essere associata, in pazienti settici, alla severità e mortalità dell'evento. Da studi precedenti è noto che le lipoproteine giocano un ruolo importante nella sepsi, favorendo il legame e la neutralizzazione dell'LPS, endotossina presente sulla membrana esterna dei Gram-, inibendo l'espressione di molecole di adesione endoteliale e stimolazione l'espressione di NOS endoteliale.
Obiettivi. L'obiettivo di questo studio retrospettivo è analizzare il profilo lipidico in un gruppo di soggetti ricoverati per sepsi in un reparto di medicina e valutarne eventuali correlazioni con la sopravvivenza.
Metodi. Dei 253 soggetti ricoverati con diagnosi di sepsi da marzo ad ottobre 2013, 151 sono stati esclusi per concomitante terapia con farmaci immunosoppressori o patologie ematologiche, 59 per mancanza di dati sul profilo lipidico e 7 per terapia ipolipemizzante in atto. Dei 36 soggetti rimanenti (33.3% femmine, età media 80.1±9.5) abbiamo valutato colesterolo totale, trigliceridi e colesterolo HDL, misurati dal laboratorio centrale al giorno 1, e colesterolo LDL, calcolato tramite la formula di Friedewald.
Risultati. 16 (44.4%) pazienti sono deceduti nel corso della degenza. Confrontati con i sopravvissuti, i non sopravvissuti presentavano valori più bassi di colesterolo totale (114.1±16.5 vs. 129.0±18.8, p=0.02), LDL (65.5±12.5 vs 76.7±14.2, p=0.02) e, seppur non significativi, HDL (26.2±3.4 vs 27.5±4.3, p=0.36) e trigliceridi (104.0±20.0 vs 116.6±24.4, p=0.12) Abbiamo documentato una correlazione significativa tra sopravvivenza e livelli maggiori di colesterolo totale (r 0.38; p 0.03) ed LDL (r 0.37, p 0.03), mentre non è stata evidenziata alcuna correlazione significativa con i livelli di colesterolo HDL (r 0.08; p 0.64) e trigliceridi (r 0.26, p 0.13).
Conclusioni. I risultati preliminari di questo studio mostrano che bassi livelli di colesterolo totale e LDL sono associati ad una maggiore mortalità in corso di sepsi. Solo studi con numerosità maggiore permetteranno di confrontare tali risultati con quelli di soggetti ricoverati per eventi non infettivi.

EFFETTO DI CARBOIDRATI RICCHI IN PEPTIDE LTP2 SU PRESSIONE ARTERIOSA, REATTIVITÀ ENDOTELIALE ED ALTRI FATTORI DI RISCHIO CARDIOVASCOLARI: UNO STUDIO CLINICO RANDOMIZZATO IN DOPPIO CIECO CON DISEGNO CROSS-OVER

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Razionale. Farine derivate da diversi tipi di frumento contengono diverse quantità di peptidi bioattivi con diversi effetti farmacologici. Alcuni peptidi del frumento come LTP2 hanno note azioni ACE-inibitorie in vitro.
Scopo. Testare gli effetti emodinamici e metabolici di diete basate su cibi a base di frumenti naturalmente poveri o ricchi (Khamut®) in LTP2.
Metodi. Nel contesto del progetto europeo 7FP EU "Beneficial Effects of Bioactive Compounds in Humans (BACCHUS)", abbiamo arruolato 60 soggetti non-diabetici (età 40-70 anni), con PAS 130-139 mmHg e/o PAD 85-90 mmHg, in prevenzione primaria, e li abbiamo randomizzati in due bracci sperimentali dopo un periodo di dieta di stabilizzazione. Il trial era disegnato con randomizzazione in doppio cieco, con cross-over. Ogni fase di trattamento aveva durata di 4 settimane.
Risultati. Parametri antropometrici, pressione in ospedale, colesterolemia, parametri renali ed epatici non si sono modificati durante entrambe le fasi di trattamento, così come PWV ed AI.
PAS diurna e notturna sono migliorate significativamente solo dopo il periodo di assunzione dei prodotti a base di Khamut, sia vs. baseline che vs. controllo (da 136,3±4,2 a 132,4±4,5 mmHg, p <0.05, e da 120,8±5,4 a 116,4±4,1 mmHg, p<0,05, rispettivamente).
La variazione del volume di polso (marcatore di reattività endoteliale) è migliorato solo dopo il periodo di assunzione dei prodotti a base di Khamut, sia vs. baseline che vs. controllo (da 64,3±6,6 a 68,1±4,2, p<0.05).
Trigliceridi e glicemia a digniuno sono migliorati significativamente solo dopo il periodo di assunzione dei prodotti a base di Khamut, sia vs. baseline che vs. controllo (da 123,5±32,9 a 107,2±21,5 mg/dL, p <0.05, e da 86,3±8,8 a 84,2±6,3mg/dL, p<0,05, rispettivamente).
Conclusione. Sostituire nella dieta prodotti preparati con frumenti standard rispetto a frumenti ricchi in peptide LTP2 sembra migliorare leggermente la PAS delle 24 ore, reattività endoteliale, glicemia e trigliceridemia in soggetti sani con livelli pressori subottimali.

GENE EXPRESSION PROFILING OF GLUTEAL ADIPOSE TISSUE AFTER PROLONGED BEDREST

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Adipose tissue is a complex and highly active metabolic and endocrine organ, with an important role in immune and inflammatory

processes. Its dysfunction has been linked to development of insulin resistance, type 2 diabetes and cardiovascular disease.
A sedentary lifestyle is associated to a quantitative and qualitative alteration of adipose tissue mass and function. Bed rest has been utilized as an experimental model to simulate physical inactivity.
The aim of this study was to investigate the impact of physical inactivity on adipose tissue gene expression in healthy subjects.
A total of 7 healthy subjects underwent gluteal adipose tissue biopsies before and after 14 days of bed rest; RNA was isolated and hybridized on RNA microarray chips in order to detect gene expression differences.
A total of 308 genes were differently expressed after bed rest intervention; these genes were analyzed with bioinformatics database support (DAVID gene functional classification tool), to find potential functional-related gene groups and gene-disease associations.
In silico analysis showed that 14 genes in our list have been previously involved in cardiovascular diseases, 8 genes in metabolic disorders and 12 genes in both. Among these, UCP3 (Uncoupling Protein 3) and BMP7 (bone morphogenetic protein 7) have been shown to be involved in energy expenditure and adipose tissue "browning"; TTR (transthyretin) is a known transport protein that carries the thyroid hormones (T4) and retinol-binding protein bound to retinol. Moreover several genes involved in inflammatory response such as CRP (C-Reactive Protein), IL1-beta and IL18 are differentially expressed after bedrest.
In conclusion, our data suggest that adipose tissue may play an important role in the detrimental effect of physical inactivity and bed confinement on cardiovascular system and metabolism.

EVALUATION OF LYSOSOMAL ACID LIPASE LEVELS IN PATIENTS WITH LIVER DISEASE OF UNKNOWN ORIGIN

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Introduction. Lysosomal acid lipase (LAL) deficiency is a rare autosomal recessive disease characterized by progressive accumulation of cholesterol and triglycerides in the liver, spleen, lymph nodes and other organs. It is caused by deleterious mutations of the LIPA gene.
Infants with LAL rarely survive beyond 6 months of age, while children and adults typically show a combination of dyslipidemia, elevated transaminases and liver damage, with progression from hepatosteatosis to cirrhosis. LAL deficiency should be particularly suspected in non-obese patients with steatosis or cryptogenic cirrhosis, especially in those presenting abnormalities in lipids metabolism.
Materials and Methods. We evaluated LAL levels in a clinical series of 29 patients with hypertransaminasemia of unknown cause since 12 months and/or evidence of ultrasound fatty liver. We excluded secondary causes of liver damage, history of alcoholic intake, use of drugs, viruses B and C, autoimmune disorders and alterations of iron and copper metabolism. Only one patient had compensated liver cirrhosis.
Results. Twenty-nine non-obese patients with cryptogenic liver disease, 16 women (55.2%), age 48.9 ± 13.3 yrs (mean ± SD), were recruited;21/29 patients (72.4%) had low levels of LAL with a functional reduction of 30.4 ± 14.2 % (mean ± SD) (range: 7.4 – 52.5%) and 47.8% had hypertriglyceridemia.
A positive correlation between degree of ultrasound steatosis and LAL deficiency was found (Spearman's Rho: 0.563; p=0.008). Levels of LDL were significantly higher in patients with advanced LAL deficiency (>50° percentile LAL deficit than in patient with deficit of LAL <50° percentile (131.0 mg/dL ± 36.3 vs 98.0 ± 26.3; p=0.047).
Conclusion. In this group of patient LAL deficiency is significantly correlated both with ultrasound steatosis and LDL levels; therefore could be useful to evaluate LAL levels in non-obese patients with cryptogenic liver disease. Further studies are needed in larger population samples in order to confirm our findings.

CHARACTERIZATION OF CHOLESTEROL BIOSYNTHESIS DEFECTS: A NEW CASE OF STEROL-C4-METHYL OXIDASE DEFICIENCY IN ITALY

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Inborn defects of cholesterol biosynthesis are metabolic disorders presenting with multi-organ and tissues anomalies. Recently, a new autosomal recessive defect in 4 patients involving the demethylating enzyme C4-methylsterols (SC4MOL) has been described. In infancy, all showed congenital cataracts, growth delay, microcephaly, psoriasiform dermatitis, immune dysfunction, and intellectual disability (1).
Here, we describe a new case of SC4MOL deficiency, showing bilateral congenital cataracts, psychomotor and development delay and learning disabilities in the early life.
At 15 years, he showed small stature and behavioral disorder. His skin never demonstrated a marked psoriasiform rash, but only abundant dandruff of scalp. Despite numerous biochemical and genetic examinations, the diagnosis was missed until 19 years. Based on clinical evidences, such as congenital cataracts and developmental delay, a cholesterol biosynthesis defect was suspected. Blood C4-monomethyl- and C4-dimethylsterols levels, analyzed by gas-chromatography and mass spectrometry, were significantly higher than controls, suggesting a deficiency of SC4MOL. Sequencing analysis of SC4MOL gene showed mutations in both alleles (1st variant: c.731A>G, p.Y244C, already known; 2nd one: c.605G>A, p.G202E, new variant). Both mutations were absent in both EXAC database and healthy controls. His parents were found heterozygous.
Finally, integrating clinical, metabolic, and genetic tests, we diagnosed the SC4MOL deficiency definitively. Notably, the interactions of multi-field skills are fruitful to diagnose a new defect of cholesterol biosynthesis. Therefore, we suggest that plasma sterol profile should be taken early into account for all undiagnosed patients showing clinical signs overlapping that of patient presented here. (Reference: 1. He et al 2014, BBA 1841:331).

CONTROLLO DEI FATTORI DI RISCHIO IN PAZIENTI CON RECIDIVA DI SINDROME CORONARICA ACUTA

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Il mancato controllo dei fattori di rischio cardiovascolari implicati nella genesi e nella progressione della malattia aterosclerotica, ivi compresi i valori di colesterolemia totale, LDL, HDL, comporta un rischio aumentato di nuovi eventi. Non è chiaramente riconosciuto il ruolo svolto in questo campo dalle apolipoproteina A, apolipoproteina B, rapporto apolipoproteina B/A1 ed Lp(a).
Abbiamo arruolato in modo consecutivo 539 pazienti con diagnosi di Sindrome Coronarica Acuta (STEMI, NSTEMI, Angina Instabile) documentata tramite evidenza coronarografica di lesioni ostruttive (>75%), afferenti presso la nostra UTIC successivamente divisi in due gruppi: 1 (primo evento); 2 (secondo evento).
Abbiamo osservato che i pazienti del secondo gruppo hanno più spesso NSTEMI (59% vs 28%) o angina instabile (14% vs 7%), sono più anziani (69 vs 63 anni), in sovrappeso, ipertesi (88% vs 57%) e diabetici (38% vs 27%). Questi pazienti presentano, inoltre, scarso controllo delle dislipidemie con valori non a target di colesterolemia totale (159 mg/dl vs 185 mg/dl), LDL (99 mg/dl vs 122 mg/dl), apoB (93 mg/dl vs 108 mg/dl), Lp(a) (41 mg/dl vs 30 mg/dl) e rapporto apoB/A1 (0,86 vs 0,97), sebbene migliore rispetto ai valori riscontrati nel primo gruppo, in quanto questi ultimi risultavano spesso naif dal punto di vista terapeutico. Sorprendentemente abbiamo notato che molti pazienti (40%) del secondo gruppo non assumevano terapia ipolipidemizzante e, di conseguenza, la maggior parte (77%) non raggiungeva valori di colesterolemia target (LDL <70 mg/dl). I pazienti del secondo gruppo avevano più frequentemente più di un vaso coronarico affetto (75% vs 55%) e un grado più elevato di insufficienza renale (valore medio di filtrato glomerulare di 73 vs 84 ml/minuto).
I nostri dati evidenziano che dopo una SCA il controllo dei fattori di rischio, in particolare di quelli legati alle dislipidemie, è subottimale e ciò si correla ad un aumentato rischio di recidive.

ROLE OF TGFBR1 AND TGFBR2 GENETIC VARIANTS IN DETERMINING OR MODULATING MARFAN SYNDROME

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Background. Genetic variants in TGF-β receptors type 2 (TGFBR2) and type 1 (TGFBR1) genes may be involved in cardiovascular (CV) manifestations as they have been associated with different connective tissue disorders sharing thoracic aortic aneurysm and dissection (TAA/D). Mutations in both TGFBR2/1 genes have been described in patients with TAA/D and Marfan syndrome (MFS), and they are associated with Loeys-Dietz syndrome. Existing literature shows no concordant data resulting from mutational screening of TGFBR2/1 genes in MFS patients with no fibrillin-1 (FBN1) gene mutations; therefore, their role in classical MFS remains unclear. Aim of the study was to investigate the role of TGFBR2/1 genetic variants in determining and/or modulating CV manifestations of MFS .
Methods. 75 unrelated patients who underwent FBN1 mutational screening (47 patients with pathogenetic and 28 without pathogenetic FBN1 mutation) were subjected to direct sequencing for TGFBR2/1genes.
Results. Patients with or without FBN1 pathogenetic mutations were comparable for traditional CV risk factors. No TGFBR2/1 pathogenetic mutations were detected in both groups. Ten polymorphisms in TGFBR2 and 6 polymorphisms in TGFBR1 were identified which were evaluated for their association with CV features. Carriers of rs11466512 A allele showed significantly reduced Z-score with respect to homozygous wild-type MFS patients (p=0.032). Carriers of delA allele of c.383delA polymorphism showed significantly reduced Z-score and number of subjects undergoing aortic surgery with respect to homozygous wild-type MFS patients (p=0.083 and p=0.088, respectively), and rs-2276767carriers of A allele showed significantly increased Z-score with respect to homozygous wild-type MFS patients (p=0.252). Carriers of delT allele of c.1256-15del1T polymorphism showed significantly reduced Z-score and subjects undergoing aortic surgery with respect to homozygous wild-type MFS patients (p=0.211 and p=0.049, respectively).
The effect of a genetic TGFBR1/2 score including the 4 polymorphisms was also evaluated.
Patients with severe CV manifestations (aortic Z-score ≥2 or aortic surgery) showed a significantly lower prevalence of subjects with 2 or more protective alleles (29.7%) than patients with no or milder CV involvement (63.6%, p=0.029). At the logistic regression analysis the patients with 2 or more protective alleles showed an OR=0.241 (95%CI 0.063-0.922, p=0.038). MFS patients with 2 or more protective alleles had statistical significant reduced aortic Z-score levels [2.20 (IQR 1.48-3.37)] with respect to patients with 1 or no protective alleles [4.20 (IQR 2.48-7.12)] (p=0.007). The logistic regression analysis remains statistically significant when adjusted for the presence of pathogenetic FBN1 mutations [OR=0.213 (95%CI 0.054-0.843, p=0.028)].
Conclusions. TGFBR2/1 genetic score plays a role in determining CV manifestation severity in MFS patients. That leads to the hypothesis of a potential similar role in other pathologies.

MODIFICATION OF PERIPHERAL MICRO- VASCULAR REACTIVITY AFTER EXERTION IN PATIENTS WITH SYSTEMIC SCLEROSIS: BLUNTING THE HYPERTONE

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Background. Vascular alterations are important features in pathogenesis of Systemic sclerosis (Ssc) being consequence of vascular damage induced by inflammation and promoting tissue damage through an ischemic/ipossic recurrence that enhances fibrotic modifications.
The decrease of tissue perfusion due to a progressive capillary reduction must be accompanied and anticipated by significative and progressive alterations of perfusion "quality". Several authors have demonstrated a progressive decrease in endothelial dependent dilation and reduction in hyperemic function. We hypothesize that also vasodilation due to exercise and sympathetic stimulation may

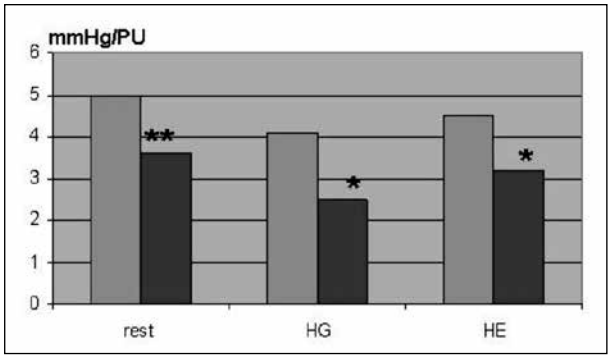

Figure 1 - Microcirculatory resistances.
**p<0,05 vs correspondent rest; **p<0,05 vs Ssc patients*

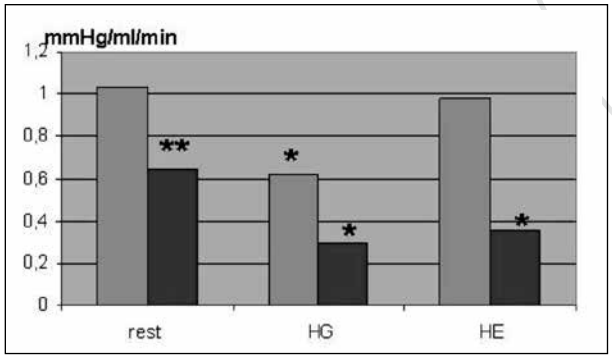

Figure 2 - Forearm resistances.
**p<0,05 vs correspondent rest; **p<0,05 vs Ssc patients*

be blunted in these patients with a progressive failure in adaptation to metabolic requests from tissues.

Aim. To evaluate the effects on hand cutaneous microcirculation of ischemia, handgrip and exercise in Ssc patients.

Methods. We enrolled 12 adult, aged 61±6 y, non-obese, non-smoker, non-diabetic, and non-hypertensive women, who fulfilled the American College of Rheumatology criteria for the diagnosis of SSc. Mean time from diagnosis was 11,5±4,2 y. The were all without ulcers; 2/12 had previous digital ulcers healed . Latest iloprost infusion was at least 28 days before the exams (28-32 days); no pulmonary hypertension signs were detected by means of echocardiography; no vasoactive drugs were assume the day of exams. No obstructive pathology at upper limb was detected using color-Doppler ultrasound, while all had thickening of radial and ulnar vessels with rare dotted calcifications. 6 healthy, age matched subjects were enrolled. We analyzed microcirculatory flux by means of Laser Doppler flowmetry (LD) with probe placed on volar face of the right hand ; flow at humeral artery was measured with ultrasound.

Blood pressure was measured with oscillometric device on the some arm at the end of each stimulation. Resistances were calculated as mean pressure/flow . Post ischemic hyperemia was evaluated after 3 minutes ischemia obtained with cuff placed on brachial artery and inflated 20 mmHg over systolic value; hand grip (HG) was determined with fist clunching on cuff with exerted pressure 70% of maximum for 3 minutes on the other hard; hand exertion (HE) was done with repeated near-maximal fist clunching for 3 minutes (at least 1 /sec). Data were analyzed with Student's t test for paired data.

Results. LD flow at rest was slightly lower in Ssc (19,7±9,2 vs 25,4±11,7 PU ns); post ischemic hyperemia showed a lower microcirculatory peak flow with LD in Ssc patients (41,5±8,6 vs 65±11,3% increase p<0,05); HG determined an increase in LD flow only in healthy (p<0,05), while a slight decrease in Ssc; HE showed an increase in LD flow only in healthy (p<0,05).. Microcirculatory resistances were increased in Ssc patients and slightly reduced only by HG; in healthy they were reduced both by HG and HE. Forearm resistances were significantly lower at rest in healthy (0,64±0,02 vs 1,03±0,06 mmHg/ml/min p<0,05 - figure 2), they were reduced by HG in both groups (p<0,05), while HE reduced them only in healthy (p<0,05);

Conclusions. Our study demonstrates that Ssc patient have impairment in microcirculatory flux and increased micro/marcocirculatory resistances at rest. Post ischemic hyperemia and the answer to exertion is impaired in Ssc as well. HG can reduce resistances at the forearm also in Ssc patients with a slight increase in microcirculatory flux. These data show a reduced adaptation Ssc flow regulation when increase in requests occur. HG evokes an noradrenergic stimulation with a better response in Ssc that probably is due to abrupt blunting after sustained stimulation over a chronic hyperactivity, we can also hypothesize positive effect of noradrenaline in reactive vasodilation post-exertion, still working in Ssc. Furthermore HG hyperemia is sustained also by NO production by flow stimulation of endothelial cells. As a conclusion we can hypothesize that HG exertion may be useful in Ssc patients for improving forearm perfusion.

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RESPONSE TO TREATMENT AND OCCURRENCE OF CARDIOVASCULAR (CV) COMPLICATIONS IN PATIENTS WITH AUTOSOMAL RECESSIVE HYPERCHOLESTEROLEMIA (ARH): A RETROSPECTIVE ANALYSIS

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Objectives. ARH is a rare, severe form of recessive hypercholesterolemia due to mutations in the LDLRAP1 gene. As a few data on clinical management and outcome are available, we have carried out a retrospective analysis of reported ARH cases.
Methods. All published ARH cases were identified by electronic search through PubMed and Medline. Corresponding authors were invited to provide published and unpublished information of patients according to a pre-specified database. The major endpoints of retrospective analysis were: 1) proportion of ARH patients under pharmacological and/or LDL apheresis (LA) treatment, 2) LDL-C lowering effect of treatments, and 3) incidence of CV outcomes such as death, coronary and peripheral artery disease, aortic stenosis.
Results. We were able to collect complete data for 39 ARH patients (22 females/17 males ; mean age 40.4, range 9-80 yrs). The mean observational period from diagnosis was 10.8 ± 6.4 yrs. At follow up, 61.5% of patients were treated with LA combined with lipid lowering drugs, while 38.5% were on drug therapy alone. Average maximum LDL-C reduction was 67 ±17.2% (from 535 mg/dl to 169.2 mg/dl) and only 14.3% of ARH patients reached the LDL-C goal of <100 mg/dl. The cumulative incidence of major CV outcomes were: CV death: 9.7%, coronary heart disease: 27.7%, severe aortic stenosis: 40%, peripheral artery disease: 20%.
Conclusions. Despite standard care including LA, LDL-C levels remain high and residual CV risk significant. These data confirm that an effective treatment for ARH is still lacking.

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

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Background. Genome Wide Association studies have identified many loci associated with NAFLD. However, the definitive confirmation of these genetic associations may derive from the demonstration of the enrichment of genetic variants in the gene(s) of interest.
Aim. Characterize the enrichment of non-synonymous (NS) variants in APOC3, GCKR, LIPA, PPP1R3b, NCAN, LYPLAL1 and TM6SF2 genes in NAFLD patients compared to controls. These genes have been selected among those reported as being strongly associated with the NAFLD trait (threshold > P<10-4).
Methods. A total of 362 subjects, 183 with ultrasound-defined NAFLD and 179 controls, were re-sequenced by next generation sequencing (NGS) techniques employing the Ion Torrent PGM platform. Statistical analyses were performed using SPSS v.20.0 (IBM, Armonk, NY, USA).
Results. Overall, we identified 194 sequence variants within the selected genes, 82 of which were NS. Compared to controls, NAFLD patients showed higher frequencies of rs1260326 (L446P) in GCKR, rs58542926 (E167K) in TM6SF2 and rs2070666 in APOC3 variants. Conversely, rs1051338 (T16P) in LIPA showed lower frequency in NAFLD (all P<0.05). After adjustment for traditional NAFLD risk factors, carriers of TM6SF2 EK+KK and GCKR PP genotypes had a significantly increased risk of NAFLD (OR, 4.5; 95%CI, 1.6-12.6, P=0.004; OR, 1.9; 95%CI, 1.1-3.4; P=0.029; respectively). Interestingly, carriers of LIPA PP genotype showed a negative correlation with NAFLD, suggesting a possible protective effect of this variant (OR=0.3, 95%CI, 0.1-1.1, P=0.054). When the cumulative frequencies of all NS variants within each gene were considered, only the TM6SF2 emerged as gene conferring a significantly increased risk of NAFLD (OR, 2.9; 95%CI, 1.3-6.6; P=0.011 in the adjusted model).
Conclusions. Our data confirm the major role of TM6SF2 as genetic predictor of NAFLD risk and suggest the usefulness of NGS technology to further characterize the genetic burden in complex inherited disorders.

NON-INVASIVE, NON-IONIZING FATTY LIVER ASSESSMENT BY MEANS OF A NEW ULTRASOUND-BASED IMAGING SYSTEM: THE STEATOMETER

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Fatty-liver is becoming a global epidemic and is associated with an increased cardiovascular risk. For this reason, reliable, non-invasive, cheap and non-ionizing imaging measurement systems are needed. Aim of this study was to develop a system (Steatometer) for assessing liver fat content based on ultrasound (US) images analysis.
Fifty-seven subjects (53.3±10.3-years, 37 males) undergoing abdominal US-scan because of metabolic syndrome were examined. Liver fat content was assessed with the Steatometer software, by Magnetic Resonance Spectroscopy (MRS) and using the CAP-index (Fibroscan, Echosense) which measures the US-attenuation-rate (decreased amplitude of US-waves propagating through the liver). Steatometer values were obtained in all the participants acquiring US images with a standard equipment in right inter/subcostal views and processing them to calculate 5 parameters

(hepatic-renal ratio, hepatic-portal-vein ratio, attenuation-rate, diaphragm and portal-vein-wall visualization); the overall score was obtained as their linear combination. MRS measures were obtained in 15 subjects with a clinical 3T scanner (Philips Medical Systems). CAP-index values were achieved in 42 individuals using a dedicated equipment.
Steatometer-score values (3.03±2.75) were significantly correlated with both MRS measures (8.6±11.8%) and CAP assessments (280±17dB/m) (R=0.95, p<0.001; R=0.56, p<0.001, respectively). Regression analysis of the relationship between Steatometer and MRS/CAP fat content evaluations defined the Steatometer score as 0.54+(0.26*MRS-score) and -4.21+(0.25*CAP-index), respectively.
The stronger association between Steatometer-MRS than Steatometer-CAP could be explained by the fact that MRS provides a direct measure of fat content and Steatometer-score is based on a combination of parameters associated with it, while CAP-index relies on just one of these parameters, the attenuation rate. The Steatometer system represents a valid approach for non invasive, non-ionizing quantification of fatty liver applicable to any standard US scanner and can be useful for screening and monitoring fatty liver in both clinical trials and clinical practice.

ETEROGENEITÀ GENETICA NELLE IPOBETALIPOPROTEINEMIE PRIMITIVE RIVELATA DAL NEXT GENERATION SEQUECING (NGS)

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Introduzione. L'ipobetalipoproteinemia familiare (FHBL-1) è un disordine monogenico a trasmissione co-dominante caratterizzato da ridotti livelli plasmatici di colesterolo totale e lipoproteine contenenti ApoB. Nella maggior parte dei casi l'FHBL è dovuta a mutazioni del gene APOB che determinano la formazione di proteine ApoB troncate di dimensioni variabili (APOB-linked FHBL); meno frequentemente FHBL-1 è dovuta a mutazioni con perdita di funzione (LOF) nel gene PCSK9. Recentemente, una condizione di ipobetalipoproteinemia associata a ipoalfalipoproteinemia dovuta a mutazioni LOF del gene ANGPTL3 è stata denominata ipolipidemia familiare combinata (FHBL-2).
Materiali e Metodi. Mediante la tecnologia Next Generation Sequencing (NGS) abbiamo investigato tre soggetti con severa ipocolesterolemia compatibile con una diagnosi di FHBL1 o FHBL-2 .
Risultati. Il primo probando è un ragazzo di 16 anni giunto all'osservazione clinica per la presenza di manifestazioni neurologiche associate ad ipobetalipoproteinemia. Il sequenziamento mediante NGS ha rilevato la presenza di due varianti di sequenza allo stato eterozigote del gene PCSK9: la presenza di una variante rara nell'esone 2 (p.Arg96Cys) indicata come potenzialmente dannosa dall'analisi in silico mediante algoritmi specifici e la presenza di una nuova mutazione nell'esone 12 che determina una alterazione della trama di lettura dell'RNA messaggero con abolizione del codone di stop canonico. Il prodotto della traduzione di questo mRNA anomalo è una proteina PCSK9, più lunga di quella normale, contenente 708 aminoacidi rispetto ai 692 della proteina wildtype (p.Trp630LeufsX81).
Il secondo probando è un bimbo di tre anni con ipobetalipoproteinemia associata ad una lieve steatosi epatica. Il sequenziamento mediante NGS ha evidenziato la presenza allo stato eterozigote di una sostituzione nucleotidica nell'esone 1 del gene APOB (c.2 T>A) che determina la sostituzione del codone di inizio della traduzione dell'mRNA, codificante metionina, con il codone codificante lisina (p.Met1Lys). Questa variante non è presente nei database disponibili e non è riportata in letteratura.
Il terzo paziente è un donna di 38 anni che presentava ipocolesterolemia associata ad una moderata steatosi. Il sequenziamento mediante NGS ha evidenziato la presenza allo stato omozigote di una delezione in trama di 3 nucleotidi nell'esone 1 del gene ANGPTL3. La mutazione determina l'eliminazione in trama dell'acido glutammico in posizione 96 della proteina (p.Glu96del). Si tratta di una variante già riportata in letteratura e indicata come "dannosa" dal software predittivo PROVEAN.
Conclusioni. L'analisi mediante NGS di un pannello di 15 geni potenzialmente patogenetici di ipobetalipoproteinemia ci ha permesso di identificare la base molecolare di ipocolesterolemia severa in tre pazienti che sono risultati portatori di mutazioni in tre diversi geni candidati di FHBL-1 e FHBL-2.
La mutazione del gene PCSK9 (c.1883_1884insG; p.Trp630LeufsX81), identificata nel primo probando, è da considerarsi una nuova variante patogenetica come causa di FHBL.
La mutazione del gene APOB (c.2 T>A; p.Met1Lys), presente nel secondo probando, è una variante che coinvolge il primo codone dell'mRNA e rappresenta un evento estremamente raro che non è mai stato riportato nel caso dell'apoB. Infine, la mutazione identificata nel gene ANGPTL3 (c.286_288delGAA; p.Glu96del) è una variante nota già riportata in letteratura ed associata a ipolipidemia familiare combinata.

HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA IN CAMPANIA

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Introduction. Familial Hypercholesterolemia (FH) is an autosomal dominant disease caused by a defective metabolism of LDL cholesterol leading to its increased plasma levels and to premature atherosclerosis. Genes causing FH encode for the LDL receptor (LDLR), the Apolipoprotein B (APOB) and the Proprotein convertase subtilisin/kexin type 9 (PCSK9) regulating LDLR recycle.
Two forms of FH are known: 1. Heterozygous FH (HeFH) characterized by the presence of a single mutation in one of the causative genes; 2. Homozygous FH (HoFH) characterized by the presence of a mutation at homozygous status or of 2 mutations at compound heterozygous status. The HoFH form shows a dramatic increase of LDL cholesterol and can lead to cardiovascular accidents since the childhood.
Materials and Methods. Based on clinical diagnosis, 466 patients (342 unrelated) with a clinical suspect of FH have been enrolled. Genetic screening has been performed by amplification and direct sequencing of the exons with the flanking intronic regions of the causative genes (LDLR, PCSK9 and the exons 26 and 29 of APOB encoding the binding domain). Multiplex Ligation-dependent Probe Amplification (MLPA) was used to identify large rearrangements of LDLR.

Results. Among 466 patients, 19 are HoFH (4.1%) of which 4 are true homozygotes and 15 are compound heterozygotes for mutations in LDLR. Mutations in other genes have been found only at heterozygous status. Total and LDL cholesterol levels are statistically increased in HoFH vs HeFH patients (both p<0.0001).
Considering only the unrelated patients, the HoFH patients are 15 and since all originate from the Campania region that consists of about 6,000,000 of inhabitants, the prevalence of HoFH in our region is estimable as at least 1:400,000, since the presence of other HoFH patients cannot be excluded.
All HoFH patients undergo regular follow-up strictly followed and some case has been treated with anti-PCSK9 antibodies. The analysis of patient relatives allowed to perform a genetic diagnosis of several additional patients suffering from HeFH (cascade screening).
Conclusions. The phenotype of HoFH patients is severe already in the childhood and allows an early diagnosis that leads to perform a cascade screening to identify HeFH patients with a mild phenotype. High prevalence of HoFH in Campania is consistent with recent evaluations performed in Northern Europe. This data indicates that also the prevalence of HeFH should be very high and suggests the need of more accurate diagnostic procedures to identify undiagnosed patients allowing an adequate prevention of cardiovascular disease.

CONSUMPTION OF BUCKWHEAT PRODUCTS AND CARDIOVASCULAR RISK PROFILE: A RANDOMIZED, SINGLE-BLINDED CROSSOVER TRIAL

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Background. Lifestyle modifications, especially dietary interventions, assume an increasingly more important role in the population-based approach to cardiovascular diseases risk reduction. Buckwheat is a highly nutritional food component that has been shown to provide a wide range of beneficial effects.
Objective. The aim of the study was to examine whether a replacement diet with buckwheat products could provide additive protective effects in reducing cardiovascular risk factors, including blood glucose, insulin, lipids, oxidative damage and pro-inflammatory markers, in comparison to a similar replacement diet using products made from organic wheat.
Methods. Twenty-one participants at high risk for cardiovascular disease (11 F; 10 M; mean age 51.3±13.4) were randomized to receive products (bread, pasta, biscuits and crackers), made from either buckwheat-enriched semi-wholegrain wheat or control semi-wholegrain wheat for 8 weeks in a single-blinded crossover trial. A washout period of 8 weeks was implemented between the two intervention phases, in which participants were permitted to eat all foods according to their normal eating habits. Blood analyses were performed at the start and end of each intervention period, respectively.
Results. Consumption of buckwheat products resulted in a significant amelioration in total cholesterol (-4.7%), low-density lipoprotein cholesterol (-8.5%), triglycerides (-15%), glucose (-5.8%) and insulin (-17%) from baseline levels, independently of age, sex, body mass index and hypertension. Moreover, thiobarbituric acid reactive substances (TBARs) levels were significantly reduced by 29.5%. A concomitant significant increase in plasma ORAC levels (+9.7%) was observed. No significant differences from baseline in the same participants were observed after consumption of the control products.
Conclusion. Our results suggest that a replacement diet with buckwheat products exert a protective effect on the development of cardiovascular disease by reducing circulating cardiovascular risk factors and markers of oxidative stress.

MEDITERRANEAN DIET AND MULTIPLE HEALTH OUTCOMES: AN UMBRELLA REVIEW OF META-ANALYSES OF OBSERVATIONAL STUDIES AND RANDOMIZED TRIALS

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Background. Research has shown that following a Mediterranean diet is associated with a reduced risk of major chronic disease; however, the existing literature led to debates for different issues.
Aim. To summarize the evidence and evaluate the validity of the association between adherence to Mediterranean diet and multiple health outcomes
Design. Umbrella review of the evidence across meta-analyses of observational studies and randomized clinical trials (RCTs) reporting adherence to Mediterranean diet and health outcomes
Data sources. Medline, Embase, Scopus, Cochrane database of systematic reviews, Google Scholar, and screening of citations and references
Eligibility criteria. Meta-analyses of observational studies (cohort, cross-sectional, and case-control design) and RCTs that examined the association between adherence to Mediterranean diet and health outcomes
Results. 12 meta-analyses of observational studies and 14 meta-analyses of RCTs investigating the association between adherence to Mediterranean diet and 32 different health outcomes, for a total population of over than 12 700 000 subjects, were identified. A robust evidence, identified by a p value < 0.001, a large simple size, and not a considerable heterogeneity between studies, for a greater adherence to Mediterranean diet and a reduced the risk of overall mortality, cardiovascular diseases, coronary heart disease, myocardial infarction, overall cancer, neurodegenerative disease, dementia and diabetes was found. On the basis of the available literature, for most of the site-specific cancers, as well as for inflammatory and metabolic parameters, the evidence was only suggestive or weak and further studies are needed to draw firmer conclusions. No significant evidence, on the other hand, was reported for bladder, endometrial and ovarian cancers, as well as for LDL-cholesterol levels.
Conclusions. The present umbrella review of meta-analyses investigating the possible association between adherence to Mediterranean diet and clinical outcomes reported a robust evidence for the beneficial role of Mediterranean diet versus some relevant clinical outcomes such as overall mortality, cardiovascular disease, cancer, neurodegenerative disease and diabetes.

VALIDATION OF A LITERATURE-BASED ADHERENCE SCORE TO MEDITERRANEAN DIET: THE MEDI-LITE SCORE

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Background and Aim. Numerous studies have demonstrated a relationship between adherence to Mediterranean diet and prevention of chronic degenerative diseases. The aim of this study was to validate a novel instrument to measure adherence to Mediterranean diet based on the literature (the MEDI-LITE score).
Methods. The study included 204 clinically healthy subjects (114 M, 90 F; age range: 23-78 years). All completed both the MEDI-LITE score and the validated MedDietScore (MDS) for estimating the adherence to Mediterranean diet.
Results. Significant positive correlation between the MEDI-LITE and the MDS scores was found in the study population (R=0.70; p<0.05) were found for all the nine different food groups, as follows: cereals (R=0.64), fruit (R=0.70), vegetables (R=0.74), legumes (R=0.61), meat and meat products (R=0.74), milk and dairy products (R=0.73), olive oil (R=0.90). Lower, but still significant correlations were seen for fish (R=0.56), and alcohol (R=0.27). The discriminative power or correct-subject classification capacity of the MEDI-LITE was analysed with the Receiver Operating Characteristic (ROC) curve, using the MDS as reference method. According to the ROC curve analysis, MEDI-LITE evidenced a significant discriminative capacity between adherents and non-adherents to the MD pattern (optimal cut-off point=8.50; sensitivity=96%; specificity=38%).
Conclusion. Our findings show that the MEDI-LITE score well correlate with MDS in both global score and in most of the items related to the specific food categories. Whether confirmed, the present tool can be utilized as a valid and quick measure to capture adherence to Mediterranean diet, with good accuracy and minimal cost.

LIVELLI SIERICI DI LIPOPROTEIN(A) E MICRO/MACRONUTRIENTI ALIMENTARI: DATI DAL BRISIGHELLA HEART STUDY

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Introduzione. La concentrazione sierica della lipoprotein(a) è per lo più geneticamente determinata e, da letteratura, sembra risentire solo marginalmente dell'alimentazione.
Metodi. Escludendo soggetti con patologie dell'assorbimento (gastro-enteriche o endocrine n=81), donne in gestazione o allattamento (n=2) o in stato neo-menopausale (n=83), abbiamo valutato i dati clinici e laboratoristici di 305 soggetti (M:163 ; F:142) appartenenti alla corte storica del Brisighella Heart Study e visitati consecutivamente nel survey epidemiologico del 1988 ed in quello del 1992. Con la somministrazione del "Seven-days questionnaire" (questionario validato) abbiamo potuto stimare approssimativamente, per ciascun soggetto, l'intake medio giornaliero di macro e micronutrienti alla baseline e, quindi, a quattro anni di distanza. È stata quindi eseguita un'analisi di regressione lineare stepwise, considerando la variazione di Lp(a) sierico quale variabile dipendente e, quali variabili indipendenti, le variazioni di intake medio giornaliero dei principali macro e micronutrienti (glucidi, proteine, lipidi, grassi saturi e monoinsaturi, vitamina A, vitamina B1, vitamina B2, vitamina B6, vitamina C, vitamina D, vitamina E, vitamina K, minerali, fibre, calcio, ferro, rame, magnesio, potassio, sodio, zinco e fosforo). La correlazione è stata corretta per sesso, età e BMI alla baseline. Le variabili con distribuzione non normale in popolazione sono state trasformate in scala logaritmica.
Risultati. All'analisi di regressione (R2=0.224, P=0.000016) è risultato come, nella nostra corte di studio:
- un aumentato introito alimentare di vitamina D correli positivamente con un incremento dei livelli sierici di Lp(a) (B=0.423, P<0.0001);
- l'intake alimentare di grassi monoinsaturi correli negativamente coi livelli sierici di lipoprotein(a) (B=-1.052, P<0.001).
Conclusioni. Il nostro modello di regressione spiega come, nella nostra coorte di studio, considerando l'introito medio giornaliero dei principali macro e micronutrienti, il 22.4% della variazione dei livelli sierici di lipoprotein(a) possa essere, con buona probabilità statistica, attribuibile all'intake di grassi monoinsaturi e vitamina D

SCREENING STRATEGIES FOR CARDIOVASCULAR DISEASE IN PEDIATRIC PATIENTS

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Introduction. Hypercholesterolemia is one of the main modifiable risk factors for atherosclerosis and cardiovascular diseases. Cholesterol screening in childhood is necessary to prevent cardiovascular morbidity.
Aim. To identify families at higher risk for cardiovascular disease.
Methods. We distributed a questionnaire to the parents (mean age 20-40 y) of the newborns at Neonatology U.O., San Paolo Hospital, Milan. The questionnaire was meant to assess: family history for premature cardiovascular diseases, family history for lipid disorders, parents' awareness of their own lipid profile, parents' awareness of the normal values of the lipid profile.
Results. We have collected 310 questionnaires. 114 couple of parents (36.7%) have first or second degree relatives with premature cardiovascular events but, nevertheless, 82 out of 114 of them (71.9%) have never determined their own lipid profile. 118 couple of parents (38%) have a positive family history for lipid disorders, but 52.5% of them is unaware of their own lipid profile. 88 couple of parents (28,3%) know their own lipid profile. 162 couple of parents (52.2%) know the correct normal values of lipid profile.
Conclusions. Collecting an accurate and targeted family history seems to be a very good strategy to select subjects at higher risk for cardiovascular disease, but this strategy has anyway some limits, such as the parents' poor awareness of the problem. General Practitioners seem to be poorly aware of preventive strategies for cardiovascular diseases: 36.7% of the couple of parents refer a positive family history for premature cardiovascular diseases, but 71.9% of them have never had a determination of their own lipid profile. These data suggest that the percentage of adults with a

positive family history for cardiovascular diseases and undetected lipid disorders is higher than expected. This implies that the number of undetected pediatric patients at risk for cardiovascular diseases might be higher than expected: our results are worth further investigations in order to identify the best screening strategy for cardiovascular disease in childhood.

ESPRESSIONE E REGOLAZIONE DEI RECETTORI PER I PEPTIDI NATRIURETICI NEL TESSUTO ADIPOSO: MECCANISMI MOLECOLARI ALLA BASE DEL "NATRIURETIC HANDICAP" IN PAZIENTI CON OBESITA' D'ALTO GRADO

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Introduzione. Il BNP (Brain Natriuretic Peptide) è un ormone con importanti funzioni nel controllo dell'omeostasi cardiovascolare e del bilancio energetico. L'obesità è caratterizzata da bassi livelli sistemici di BNP ("natriuretic handicap") e alcune evidenze suggeriscono che un'alterata espressione dei suoi recettori a livello del tessuto adiposo possa giocare un ruolo cruciale nel favorire tale deficit. Obiettivo dello studio è stato quello di valutare i fattori che regolano l'espressione del recettore attivo NPR (Natriuretic Peptide Receptor)-A e del recettore di clearance NPR-C nel tessuto adiposo umano, e di confrontare l'espressione degli stessi tra pazienti obesi e controlli.
Metodi. Nello studio sono stati arruolati 34 pazienti obesi con BMI medio di 46,5 ± 8 kg/m^2 e 20 pazienti di controllo, con BMI medio di 26 ± 1 kg/m^2. Per ciascun paziente sono stati determinati i livelli circolanti di BNP, i principali parametri clinici e biochimici e, in campioni di tessuto adiposo viscerale (VAT) e sottocutaneo (SAT) prelevati in corso di chirurgia bariatrica o addominale, i livelli di espressione dei recettori NPR (real-time PCR) e la secrezione di IL-6 e IL-10 (ELISA).
Risultati. Il VAT dei pazienti obesi è caratterizzato da una maggiore espressione di NPR-C, da una ridotta espressione di NPR-A e da una maggiore secrezione di IL-6 rispetto al VAT dei pazienti di controllo. All'analisi univariata, l'espressione del recettore NPR-C presenta una correlazione positiva con BMI, insulinemia e IL-6 e negativa con il BNP; inoltre, l'IL-6 correla negativamente con l'espressione del recettore NPR-A ($p<0,05$).
Conclusioni. Il tessuto adiposo di soggetti obesi è caratterizzato da un sistema recettoriale NPR disfunzionale. La riduzione del rapporto NPR-A/NPR-C, favorita da IL-6 ed insulina, potrebbe rappresentare uno dei meccanismi molecolari che contribuiscono al "natriuretic handicap" tipico del soggetto obeso e che sottostanno dunque alla relazione tra obesità, insulino-resistenza, infiammazione e disordini cardiovascolari.

LA VALUTAZIONE ECOGRAFICA DEL TENDINE DI ACHILLE NELLA DIAGNOSI DI IPERCOLESTEROLEMIA FAMILIARE: UNO STUDIO CASO-CONTROLLO.

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Introduzione. Abbiamo inteso valutare il ruolo di un semplice test ecografico standardizzato del tendine di Achille, nella diagnosi di Ipercolesterolemia Familiare (FH), definendone il ruolo in rapporto a scale diagnostiche già validate e la correlazione con i livelli di colesterolemia e la presenza di manifestazioni cardiovascolari precoci (CHD).
Pazienti e Metodi. In uno studio Caso-Controllo sono stati arruolati 30 soggetti maschi e 30 soggetti femmine di età 30-65 anni, con diagnosi di Ipercolesterolemia Familiare eterozigote, confermata all'analisi genetica, che si sono presentati consecutivamente presso 2 Centri per le malattie metaboliche. Per ognuno di tali pazienti è stato individuato un soggetto affetto da ipercolesterolemia poligenica o altra iperlipemia non monogenica ed un soggetto di controllo normolipemico, confrontabili per sesso ed età. Ciascun individuo è stato sottoposto a studio ecografico bilaterale del tendine di Achille, valutando lo spessore tendineo (a 2 cm dall'inserzione calcaneare) e la presenza di caratteri eco-strutturali propri della xantomatosi (XT).
Risultati. Sono stati determinati cut-off sesso/età specifici di spessore tendineo quale criterio di validità della procedura nel classificare correttamente i soggetti (M, <50 aa: 5.1 mm; M, >50 aa: 5.9 mm; F, <50 aa: 4.5 mm; F, >50 aa: 5.3 mm). Il riscontro ecografico di ispessimento tendineo (IT) ha mostrato una buona sensibilità (70%) e specificità (72%) per la diagnosi di FH, superiori a quelle calcolate per altri cut-off presenti in letteratura. Tuttavia il confronto (ROC analysis) con il punteggio DLS-clinico (Dutch Lipid Score, omettendo lo score relativo alla diagnosi genetica) ha evidenziato una miglior predittività di quest'ultimo per valori >5. Lo spessore del tendine di Achille è apparso correlato positivamente con i livelli di colesterolemia-LDL all'analisi uni- e multi-variata, anche dopo aggiustamento per terapia ipolipemizzante e storia di coronaropatia. Solo nel gruppo di soggetti FH è stata evidenziata la presenza di ATX (sensibilità 32% e specificità 100%), risultata patognomonica per tale condizione ed inoltre associata ad una anamnesi positivita per CHD precoce.
Conclusioni. L'ecografia del tendine Achilleo appare un esame utile e di semplice esecuzione per un adeguato inquadramento diagnostico e la stratificazione del rischio nel soggetto ipercolesterolemico.

GENETIC HETEROGENEITY OF PATIENTS WITH SEVERE HYPERTRIGLYCERIDEMIA

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Introduction. Severe hypertriglyceridemia is characterized by very high levels of plasma triglyceride (TG) levels, usually more than 10 mmol/L. Clinical signs include eruptive xanthomas, lipaemia retinalis and recurrent pancreatitis. The disease is caused by mutations in the Lipoprotein lipase (LPL), Apolipoprotein A-V (APOA5), Apolipoprotein C-II (APOC2), Glycosyl-phosphatidylinositol-anchored HDL-binding protein (GPIHBP1), and Lipase maturation factor-1 (LMF1) genes. A large percentage of patients do not carry mutations in these genes.
Methods. Sixteen unrelated patients with severe hypertriglyceridemia were recruited based on fasting plasma TG>10 mmol/L. Exclusion criteria were the presence of diabetes mellitus (when started before pancreatitis), alcoholism, renal disease, untreated hypothyroidism, estrogen use and autoimmune diseases. The coding regions with the flanking intronic regions of LPL, APOA5, APOC2, GPIHBP1 and LMF1 genes were amplified by PCR and directly sequenced. Multiplex ligation-dependent probe amplification (MLPA) was used to search for large rearrangements in the LPL gene. The APOE gene was also sequenced restricting the analysis to the portion of exon 4 encoding the region ranging from the aminoacid Cys130 to the Arg176, that are substituted in the E4 and E2 allele respectively.
Results. Likely pathogenic rare variants in the LPL, APOA5, APOC2 and LMF1 genes have been identified in 8 patients (50%). Among the 8 patients with rare variants, 4 are compound heterozygotes (3 for variants in LPL gene and 1 for LMF1 gene), 1 is double heterozygote (variants in LPL and APOC2 genes), 3 are heterozygotes (2 for variants in the LPL gene and 1 for a variant in the APOA5 gene). For all compound heterozygotes, the parents were analysed to verify that the two variants are present on different chromosomes, inherited from the two parents. Overall, we identified 7 different rare variants in the LPL gene (comprising 3 new variants), one rare variant in APOA5, one rare variant in the APOC2 gene and 2 rare variants in LMF1. No statistical differences of lipid levels were observed between patients carrying 2, 1 or none mutation with the exception of total cholesterol levels that are higher in heterozygote patients than in patients with 2 mutations (p=0.034). No statistical differences of E2 or E4 carriers frequencies have been observed between patients with and without rare variants and no difference in lipid levels were observed between patients carrying different APOE alleles.
Conclusions. Results indicate the high heterogeneity of genetic background of severe hypertriglyceridemia, including half of patients without mutations. This indicates that other genetic or non-genetic factors could be responsible of the disease development and that genetic diagnosis could be used for diagnosis confirmation but cannot be used to exclude the disease if no mutations are found.
The presence of increased levels of total cholesterol in patients with a single mutation suggest that other factors could modify the lipid profile influencing also cholesterol levels. This also supports the hypothesis that several cases of severe hypertriglyceridemia could have a polygenic basis.

CORRELAZIONE TRA LIVELLI DI ACIDO URICO, SINDROME METABOLICA E ATEROSCLEROSI CAROTIDEA IN UNA POPOLAZIONE AMBULATORIALE ANZIANA

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Introduzione. Le malattie cardiovascolari sono una delle principali cause di morbidità e mortalità nei paesi occidentali. Il ruolo dell'acido urico come potenziale fattore di rischio di tali patologie, rimane controverso e i meccanismi fisiopatologici che legano l'aterosclerosi ai livelli sierici di acido urico non sono ancora noti. Scopo del nostro studio è stato quello di valutare la presenza di correlazioni tra uricemia, aterosclerosi carotidea e parametri metabolici in un gruppo di pazienti diabetici, confrontato con pazienti non diabetici.
Materiali e Metodi. Abbiamo studiato 209 soggetti, 87 diabetici e 112 non diabetici, di età compresa fra 65 e 85 anni afferiti agli ambulatori dell' UOS di Fisiopatologia Clinica delle Malattie Metaboliche (UOC Medicina 1) dell'Azienda Ospedaliera Universitaria Senese. Ogni soggetto è stato sottoposto ad una valutazione delle caratteristiche antropometriche, ad un prelievo ematochimico per il dosaggio di glicemia, Hb glicata, assetto lipidico completo e uricemia. Tutti i pazienti sono stati sottoposti ad un esame ecocolordoppler dei tronchi sovra-aortici.
Risultati. La Sindrome Metabolica è risultata prevalente nei soggetti diabetici rispetto ai non diabetici ($p<0.05$) così come la prevalenza di placche aterosclerotiche ($p<0.05$). In entrambi i gruppi i soggetti con Sindrome Metabolica presentavano livelli di uricemia significativamente ($p<0.01$) più elevati rispetto a quelli senza Sindrome metabolica; sia nei diabetici che nei non diabetici è stata riscontrata una correlazione significativa ($p<0.01$) tra uricemia e numero dei criteri della sindrome metabolica. Nei pazienti diabetici abbiamo riscontrato una associazione statisticamente significativa ($p<0.05$) fra valori sierici di acido urico e percentuale di stenosi carotidea. Trend simile è stato riscontrato nei non diabetici.
Conclusione. I risultati di questo studio evidenziano una correlazione positiva fra livelli di acido urico e numero di criteri della sindrome Metabolica in entrambi i gruppi di pazienti. Nei diabetici la percentuale di stenosi carotidea è risultata significativamente correlata all'uricemia. Sono necessari ulteriori studi per valutare la rilevanza causale dell'acido urico nella sindrome metabolica e nella progressione dell'aterosclerosi carotidea.

GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF PATIENTS WITH AUTOSOMAL DOMINANT HYPERCHOLESTEROLEMIA IDENTIFIED IN THE LIPIGEN CENTRE OF PALERMO

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Background. Autosomal dominant hypercholesterolemia (ADH) is an autosomal dominant disorder characterized by high serum

low density lipoprotein-cholesterol (LDL-C) levels. The clinical manifestations of ADH might vary among affected subjects and the phenotype correlates with the severity of mutation and the specific gene involved.
Objective and Methods. The aim of this study was to evaluate the clinical expression and clinical outcomes in a cohort of ADH subjects. 223 ADH probands with a DUTCH score > 6 were enrolled in this study and the analysis was extended to the family members of these index cases. Anthropometric measures, clinical and biochemical parameters, life style (smoker and/or alcohol habits) and cardiovascular outcomes were evaluated. A total of 296 relatives were identified and characterized as well.
Results. Molecular diagnosis was defined in 78.8% of probands; 97% were carriers of pathogenic mutations in LDLR gene (55 different mutations).
The phenotypic characterization of LDLR mutation carriers (ADH-1) and revealed that in both sexes independent predictors of the presence of tendon xanthomas were LDL cholesterol, the presence of coronary heart disease (CHD) and of receptor negative mutations. Independent predictors of CHD were male gender, tendon xanthomas.
Conclusions. This study confirms the genetic heterogeneity of ADH and underscores that the variability in phenotypic expression of ADH-1 is greatly affected by the type of LDLR mutation.

UN CASO CLINICO DI DISLIPIDEMIA SECONDARIA

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Introduzione. La dislipidemia associata al Diabete ha prevalenza altissima; essendo per sua natura secondaria, è consuetudine trattarla senza intraprendere ulteriori accertamenti.
Descrizione del caso. A.V. (01/02/1974) giungeva alla nostra osservazione a Novembre 2014, per Diabete esordivo: gli accertamenti documentavano Hb glicata 7,6 %, Colesterolo LDL calcolato 231, Creatinina 1,40 (0,70-1,20). Il paziente, asintomatico, con BMI 31.1, P.A. 166/117 mmHg, in anamnesi autismo nel primo anno di vita, dimostrava Performance Status del tutto normale. Era stato valutato il mese precedente presso il Centro di riferimento regionale per le Dislipidemie, presentando i seguenti esami ematochimici di ottobre 2014: CT 294, HDL 46, TG 314, G 149, creatinina 1.28 mg/dl, CPK 208 UI/L, HbA1c 7.9%. Su base anamnestica era improbabile la diagnosi di dislipidemia familiare primitiva e già nel 2012 si era presentata una dislipidemia associata a iperglicemia, in pochi mesi corretta da dieta e supplementazione nutraceutica, ma poi aumentata, da noi giudicata troppo severa per essere secondaria esclusivamente al Diabete. Veniva richiesto assetto ormonale tiroideo che risultava: TSH 454,296 (V.N. 0,350-5,50); fT3 1,29 (V.N. 2,30-4,20); fT4 0,34 (V.N. 0,89-1,76). Veniva sospesa l'Atorvastatina, e prescritta terapia sostitutiva scalare con Levotiroxina, che portava a Maggio 2015 a normalizzazione del TSH e degli ormoni tiroidei liberi. In assenza di terapia ipolipemizzante, il colesterolo LDL calcolato risultava 115 mg/dl, identificando così nel severo ipotiroidismo, la causa patogeneticamente prevalente di questa singolare dislipidemia "doppiamente" secondaria. Solo dopo aver ottenuto assetto ormonale fisiologico, veniva reintrodotta in terapia l'Atorvastatina.
Discussione e Conclusioni. Nella nostra casistica, il paziente con ipotiroidismo primitivo con valori di TSH > 180 µUI/ml, dimostravano bradicinesia e sindrome psichica di grado discreto. In questo caso il Performance Status era del tutto conservato. L'aumento di incidenza della miopatia da statine in soggetti con ipotiroidismo non trattato, giustifica uno screening del TSH a tutti i pazienti con ipercolesterolemia? La coesistenza di ipotiroidismo primitivo, col Diabete 2, è rara, per cui non deve essere ricercata sistematicamente, ma in casi selezionati, suggestivi anche se il quadro clinico può essere estremamente sfumato.

ADERENZA DI ITALIANI E IMMIGRATI ALLA DIETA MEDITERRANEA IN UN PROGRAMMA DI SALUTE CARDIOVASCOLARE

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Introduzione. L'aderenza alla dieta mediterranea (DM) è stata associata a minore mortalità cardiovascolare (CV) in studi osservazionali e alla riduzione dell'incidenza di eventi CV nello studio di intervento "PREDIMED". Quindi, la DM è uno degli stili alimentari raccomandati in prevenzione CV. Tuttavia, l'adesione alla DM potrebbe essere influenzata dalle tradizioni culturali della nazione di origine o dalle condizioni socioeconomiche dei singoli soggetti.
Obiettivo. Valutare e confrontare l'aderenza alla DM dei residenti nativi e immigrati partecipanti a ProSALUTE, un programma di prevenzione cardiovascolare svolto presso un quartiere multietnico periferico di Milano.
Materiali e Metodi. ProSALUTE è rivolto a residenti di entrambi i sessi tra i 40 e 65 anni. Questo studio si riferisce ai primi 260 partecipanti (34% immigrati) dei 1300 candidati contattati. L'adesione alla DM è stata stimata dalle risposte alle 14 domande del questionario PREDIMED (score <5 significa scarsa adesione).
Risultati. Lo score PREDIMED in tutto il campione è 6.8±1.8 (media±DS). In un'analisi multivariata, correggendo per i principali fattori confondenti, lo score è leggermente superiore in immigrati vs italiani (β=0.649; p=0.049). In termini di singoli alimenti, gli immigrati hanno un consumo di carne bianca (β=0.183; p=0.03) e legumi (β=0.547; p=0.03) maggiore ed un consumo di carne rossa (β=0.949; p=0.003) minore degli italiani. Inoltre, limitatamente all'analisi univariata, lo status di immigrato correla in modo negativo anche con il consumo di vino (p=0.0001; r=-0.26) e di olio d'oliva (p=0.015; r=-0.15).
Conclusioni. L'adesione media alla DM nel campione osservato è in linea con dati riportati in un precedente studio in italiani ed è minore di quella descritta per la popolazione spagnola (8.9±2). Italiani e immigrati residenti in Italia hanno abitudini alimentari differenti, soprattutto nella scelta degli alimenti proteici. Il bilanciamento tra gli item che compongono lo score PREDIMED, tuttavia, non mette in evidenza pienamente queste differenze.

EFFECTS OF A SINGLE INFUSION OF SYNTHETIC HDL CONTAINING APOA-IMILANO (MDCO-216) IN HEALTHY VOLUNTEERS AND STABLE CORONARY ARTERY DISEASE PATIENTS

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Synthetic HDL are currently under clinical development as anti-atherosclerotic agents. Among these, MDCO-216 is composed by phosphatidylcholine and apoA-IMilano and proved to be safe in a multiple dose toxicity study in cynomolgus monkeys. In the present study, the effects of single infusion of MDCO-216 on plasma lipid/lipoprotein levels, cholesterol efflux capacity and esterification system were assessed in human healthy volunteers (HV) and in patients with stable coronary artery disease (CAD).
After a single infusion of 5 to 40 mg/kg MDCO-216, serial blood samples were collected up to 30 days. MDCO-216 caused a rapid and dose-dependent increase of unesterified cholesterol in plasma (UC) peaking at 8h and remaining above baseline until 48 h, whereas levels of esterified cholesterol (CE) were unchanged or even decreased at the highest dose. The increase of UC correlated with the improved ability of sera to promote cell cholesterol efflux through the SR-BI receptor after the infusion of MDCO-216. NMR and 2D electrophoretic analyses showed a rapid and dose-dependent increase in HDL particle size, due to the fusion of discoidal MDCO-216 particles with endogenous spherical small-sized HDL generating large HDL, thus explaining the increased SR-BI-mediated efflux. The massive increase of UC and the reduced ability of apoA-IMilano to activate LCAT caused a drop of plasma cholesterol esterification rate (CER) between 4 and 8h; indeed, an inverse correlation was found between CER and UC plasma levels. Upon FPLC analysis UC was found to increase first in HDL and VLDL and later in LDL. The observed changes were most pronounced in CAD patients compared to HV and parameters returned to baseline values within 7 days.
In conclusion, MDCO-216 proved to be safe in humans and interacted with endogenous HDL causing a rapid increase of plasma cholesterol efflux capacity. This increase transiently impaired plasma esterification system but lipid/lipoprotein profile normalized within 7 days.

PSYCHOSOCIAL FACTORS IN PROSALUTE, AN ONGOING CARDIOVASCULAR HEALTH PROGRAM IN A MULTIETHNIC COMMUNITY IN NORTH ITALY

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Introduction. Psychosocial factors have a major role in coronary heart disease (CHD). In particular, individuals with anxiety, depression and low social support are at increased risk of first occurrence, recurrence and mortality of CHD events. Yet, psychosocial factors are seldom assessed systematically in cardiovascular primary prevention programs.
Objective. To evaluate prevalence and features associated with psychosocial heart-threatening factors among participants to ProSALUTE, an experimental Cardiovascular Health Program carried out in a low-income and multiethnic community from a peripheral neighborhood of Milan, Italy
Methods. ProSALUTE involves residents of both genders aged 40 to 65. This study refers to the first 260 subjects (57% females) that adhered to the Program (among 1300 contacts). Prevalence of immigrants was similar in the sample (34%) and in the population (38%), suggesting fair inclusion. Psychosocial variables were assessed using the following questionnaires:PHQ4 for anxiety and depression and FSSQ for social support. Considering multi-ethnicity, tools were administered in 5 different languages. Univariate and multivariate analyses were performed to assess associations between psychosocial variables and demographic and lifestyle characteristics.
Results. Scores for anxiety, depression and social support were above the standard cutoff in 27%, 16% and 20% of the sample, respectively. In univariate analyses, anxiety negatively correlated with education ($r=-0.5$; $p<0.05$), depression negatively correlated with education ($r=-0.4$; $p<0.005$) and positively with smoking habit ($r=0.6$; $p<0.05$) and immigrant status ($r=0.4$; $p=0.06$) whereas low social support positively correlated with immigrant status ($r=-0.3$; $p<0.005$, respectively)
Conclusions. At least 1 heart-threatening psychosocial condition affects 63% of participants in ProSALUTE. Education level and immigrant status seem to be the most influent determinants of adverse psychosocial factors, suggesting the need of personalized preventative interventions for these conditions.

IMPROVEMENT OF CELL CHOLESTEROL TRAFFICKING-RELATED LIPOPROTEIN FUNCTIONS IN RHEUMATOID ARTHRITIS PATIENTS TREATED WITH TOCILIZUMAB

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Introduction. Rheumatoid Arthritis (RA) is associated with accelerated atherosclerosis, partly attributable to disturbances in serum lipoprotein functions. Tocilizumab treatment, targeting IL-6, is increasingly being used in RA patients but its effects on circulating lipoproteins, including a decrease in total high density lipoproteins (HDL), are generating concern about its possible pro-atherogenic effect. We studied serum HDL capacity to promote cell cholesterol efflux (CEC) and serum cholesterol loading capacity (CLC) in RA patients before and after tocilizumab treatment.
Methods. Serum was drawn from 8 patients with RA before (t0) and after 4 (t1) and 12 (t2) weeks of intravenous treatment (8 mg/kg/4 weeks). CEC was measured with radioisotopic technique and CLC with a fluorimetric technique.
Results. SR-BI-mediated CEC increased significantly after treat-

ment (mean±SEM 2.43±0.33, 2.88±0.30, 3.41±0.35 at t0, t1 and t2 respectively; p=0.025 t0 vs t1, p=0.008 t0 vs t2) while total HDL serum levels were unmodified, so the ratio SR-BI-mediated CEC/HDL levels increased significantly (p<0.05 t0 vs t2). The same trend, very close to statistical significance (3.97±0.33, 4.44±0.57, 4.97±0.26 at t0, t1 and t2 respectively; p=0.064 t0 vs t2) was observed for ABCG1-mediated CEC, while no modification was detected in ABCA1-mediated CEC. CLC was significantly reduced at t2 versus t1, but not t0 apparently for t0 values high dispersion (mean±SEM 5.59±1.10, 5.16±0.80, 4.71±0.62 at t0, t1 and t2 respectively; p=0.035 t1 vs t2). At t2 total LDL serum levels were significantly increased so CLC/LDL ratio was reduced after treatment.
Conclusions. The results of this pilot study point to an anti-atherogenic effect of tocilizumab, despite apparently unfavourable changes in lipid profile. In particular tocilizumab treatment may improve and reduce anti-atherogenic HDL and pro-atherogenic LDL function respectively, known to be dysregulated in RA. These effects, overall opposing to foam cell formation, may be especially relevant in RA patients particularly prone to accelerated atherosclerosis.

HIGH ON-TREATMENT PLATELET REACTIVITY AND ADVERSE CARDIOVASCULAR EVENTS: ANY ROLE FOR GENDER? RESULTS FROM THE RECLOSE2-ACS STUDY

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Backgroup. High residual platelet reactivity (HRPR) in patients receiving clopidogrel and aspirin has been associated with high risk of ischemic events after percutaneous coronary intervention (PCI). Evidence existson difference in pathophysiological mechanisms of acute coronary syndrome in relation to gender. Methods: The RECLOSE 2-ACS study was a prospective, observational, single-center cohort study of 1789 consecutive patients with ACS undergoing an invasive procedure in whom platelet reactivity after PCI was prospectively assessed. The study enrolled consecutive patients undergoing coronary stent implantation for ACS from April 2005 to April 2009 at the Division of Cardiology of Careggi Hospital, Florence, Italy. Platelet reactivity was assessed by light transmittance aggregometry (LTA), using adenosine diphosphate (ADP) and arachidonic acid (AA) as agonists, on blood samples obtained 12-18 hours from PCI. High residual platelet reactivity by ADP was defined as platelet aggregation of 70% or greater. HaPR by AA was defined as platelet aggregation of 20% or greater. Results: Platelet reactivity by ADP or by AA were significantly higher in women than in men [ADP: 49(31-64) % vs 45 (26-30)%, p<0.01; AA: 13 (8-19)% vs 12 (7-17)%, p<0.01]. According to gender, in the high residual platelet reactivity by ADP group the primary end point event rate in women was 36.2 % and 18.9 % in men; stent thrombosis rate was 8.8% in women and 6.2% in men. In the high residual platelet reactivity by AA group the primary end point event rate in women was 21.4% and 17.1% in men; stent thrombosis rate was 8.3 % in women and 7.5 % in men. By multivariable Cox regression analysis, high residual platelet reactivity by ADP was independently associated with the primary end point in men but not in women [hazard ratio [HR], Men:1.6; 95% CI, 1.1-2.5; P =0.027; Women HR:1.1 (0.5-2.5), p=0.520]. High residual platelet reactivity by AA was independently associated with the primary end point in men but not in women [HR, Men:1.4; 95% CI, 1.1-2.4; p=0.049; Women HR:1.6 (0.9-3.2), p=0.142]. and with cardiac mortality (HR, 1.81; 95% CI, 1.18- 2.76; p=.006). Similarly, high residual platelet reactivity by ADP was independently associated with stent thrombosis in men but not in women [HR, Men:1.9; 95% CI, 1.1-3.8; p=0.049; Women HR:2.2 (0.8-7.5), p=0.111]. High residual platelet reactivity by AA was independently associated with stent thrombosi in men but not in women [HR, Men:2.0; 95% CI, 1.1-3.6; P=0.031; Women HR:2.8 (0.9-9.2), p=0.850]. Conclusions: Despite the higher prevalence of adverse cardiovascular events in women than in men, high on treatment platelet reactivity by ADP or by AA represents an indepent predictor of major adverse cardiovascular events or stent thrombosis only in male patients.

CORRELAZIONI GENOTIPO-FENOTIPO IN PAZIENTI PEDIATRICI CON IPERCOLESTEROLEMIA FAMILIARE ETEROZIGOTE

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Introduzione. L'ipercolesterolemia familiare eterozigote (HeFH) è una patologia monogenica a trasmissione autosomica dominante sostenuta da mutazioni dei geni LDLR, APOB e PCSK9, ad espressione fenotipica assai variabile. Poiché gli effetti dannosi a carico dell'endotelio associati all'eccesso di LDL-C si manifestano sin dall'infanzia, una diagnosi precoce risulta necessaria per prevenire eventi cardiovascolari.
Obiettivi. Sulla base di queste premesse, il nostro studio si prefigge di valutare il profilo biochimico e molecolare, e l'affidabilità diagnostica di due score basati su parametri clinici e biochimici, in pazienti con HeFH.
Metodi. 124 bambini con valori di LDL-C incrementati, e storia familiare positiva per ipercolesterolemia ed eventi cardiovascolari precoci sono stati valutati per la presenza di HeFH. Tutti i pazienti sono stati sottoposti a: caratterizzazione del profilo delle lipoproteine, mediante kit commerciali; genotipizzazione di ApoE, tramite software Pyrosequencing PSQ99MA-SNP; analisi molecolare di LDLR, APOB e PCSK9, tramite amplificazione con PCR e sequenziamento del promotore e degli esoni di LDLR e PCSK9, e degli esoni 26 e 29 di APOB; costruzione dell'albero genealogico; applicazione degli score Lipigen e Simon Broome per la valutazione clinica.
Risultati. Mutazioni in eterozigosi sono state identificate 107 dei 124 pazienti (86.2%), di cui 105 a carico di LDLR, una in PCSK-9 ed una in APOB. Nei pazienti con mutazioni LDLR-defective è stata identificata una correlazione tra genotipo APOE4 e valori di colesterolo totale ed LDL. Quando confrontata con i risultati dell'analisi molecolare, l'applicazione degli score Lipigen e Simon Broome ha permesso l'identificazione del 93 e 98% dei casi, rispettivamente.
Conclusioni. L'applicazione degli score risulta estremamente utile, quindi indicata nella pratica clinica, per la diagnosi di HeFH, estremamente difficoltosa per l'assenza di sintomi suggestivi nella maggior parte dei casi. Al contrario, il reale beneficio clinico associato all'indagine molecolare (gravata da alti costi), quindi le sue indicazioni, rimangono dibattuti.

FAMILIAL HYPOBETALIPOPROTEINEMIA: ANALYSIS BY NEXT GENERATION SEQUENCING AND IDENTIFICATION OF A NOVEL FRAMESHIFT MUTATION IN THE APOB GENE.

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Background. Hypobetalipoproteinemia (HBL) is an autosomal codominant disorder of lipoprotein metabolism characterized by low plasma levels of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and apolipoprotein B (apoB) below the 5th percentile of the distribution in the population. Familial hypobetalipoproteinemia (FHBL) (OMIM 107730) is the most frequent monogenic form of HBL. It may be due to loss-of-function mutations in APOB or, less frequently, in PCSK9 genes. Most APOB gene mutations lead to the formation of truncated apoB protein of various size. Missense nontruncating mutations of the APOB gene can also cause FHBL. FHBL heterozygotes are generally asymptomatic but often develop fatty liver. Here we report the medical history and the molecular characterization of one patient and his father affected by FHBL.
Objective and Methods. We designed a custom panel for next generation sequencing in order to analyze known genes involved in FHBL by Ion Torrent PGM. The laboratory workflow consists of the following steps: library construction, preparation of template, run of sequence, data analysis. We used a 316 chip with a range of sequence reads from 3–4 millions bases. Bioinformatic analysis was conducted by the Ion Reporter System which is a combined hardware and software solution for analyzing human sequencing data.
We sequenced the FHBL candidate genes in 51 years old subject presenting LDL-C and ApoB levels < 5th percentile (21 mg/dl and 29 mg/dl respectively).
Results. The proband was found to be carrier of a novel herozygous mutation in the exon 22 of the APOB gene (c.3422delGTCinsTGTGG - p.Trp1141fs) that is expected to result in a truncated ApoB form shorter than apoB48.
The proband's 18 years old son was carrier of the same mutation in herozygosity and he also showed low very levels of LDL-c and ApoB (2,5 mg/dl and 15 mg/dl respectively).
Conclusions. In this work we describe a novel frameshift mutation of the APOB gene responsible for FHBL identified by a Next generation sequencing approach.

WHOLE GENE EXPRESSION PROFILING OF THE CARDIOPROTECTIVE EFFECT OF INTERMITTENT HYPOXIA

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Background. Hypoxia is a potentially fatal condition that results from an inadequate intake of oxygen (O2) than required. Although hypoxia has long been considered cardioprotective to humans, literature data are controversial. Studies in humans and animal models indicated the coexistence of several mechanisms underlying cardioprotection induced by intermittent hypoxia (IH). Aims of the study were 1) to develop and apply a cardioprotective mouse model of IH against the myocardial injury induced by ischemia/reperfusion (I/R); and to investigate genes and biological processes associated with IH induced cardioprotection by using whole transcriptome profiling of left ventricle (LV).
Method. Twenty mice were studied: n=5 normoxic (N); n=5 normoxic undergoing ligation of the descending coronary artery (LAD); n=5 undergoing IH; n=5 undergoing IH and LAD. IH protocol: mice were exposed to 4 daily IH cycles (3-min at 6-8% O2 followed by 2-min reoxygenation for 5 times) for 14 days. I/R protocol: 30-min occlusion by LAD followed by reperfusion and measurement of the area at risk and infarct size. Gene expression was evaluated by Affymetrix GeneChip technology (Mouse Genome 430 2.0).
Results. The infarct size, expressed as a percentage of the risk area was greater in controls than in animals subjected to IH [41.0% (±3.9%) vs 22.0% (±2.7%), p=0.02], indicating the significant cardioprotective effect of IH. The SAM (Significant Analysis of Microarray) analysis among IH animals, sacrificed after 24 hours of the last IH session, and N animals not undergoing LAD identified 72 genes differentially expressed. Among the 72 differentially expressed genes were Ikbkb and Rps3, both related to the regulation of the transcription factor NF-kB. The analysis of genes differentially expressed in IH animals subjected to LAD compared to IH animals without LAD 8421 genes. The analysis in N animals with or without LAD showed 5481 differentially expressed genes. The genes differentially expressed in a similar manner, in both comparisons are 3986: these genes could be representative of the biological processes underlying the I/R injury response. On the other hand, the genes differentially expressed in only a comparison or expressed in both comparisons but in an opposite manner may be considered to represent the mechanisms that characterize the different response to I/R damage in IH and N animals. The post-pre LAD discordant genes are 4435 in IH and 1495 in N mice. The Gene Ontology classification analysis of discordant genes showed the alteration of several biological processes including platelet activation, inflammatory processes and response to stress.
Conclusion. In conclusion, these data confirm that IH has a cardioprotective effect on the I/R damage. LV gene expression profiles of mice undergoing IH and N with or without LAD indicate that IH conditioning determines a different response to I/R insult resulting in cardioprotection.

ASSOCIATION BETWEEN TRIGLYCERIDE-RICH-LIPOPROTEIN SUBFRACTIONS AND CAROTID ATHEROSCLEROSIS IN POST-MENOPAUSAL WOMEN

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Objective. The aim of this study was to evaluate the relationship between cholesterol contained in VLDLs, IDLs, LDLs, HDLs, calculated by the Lipoprint® method, and carotid intima-media thickness (IMT) in women aged >60 years.
Methods. A sample of 134 post-menopausal women older than 60 years was included in the analysis. They participated to the Atena Project and underwent clinical, biochemical, and carotid IMT ultrasound tests. separated. The high-resolution B-mode carotid ultrasound examination was performed by an expert sonographer using a Esaote AU4. Measurements were taken on the anterior and posterior wall of the distal segment (equal to 1 cm) of the common carotid artery (CC) on both sides following a standardized protocol. The separation of the lipoproteins was assessed by the Lipoprint System®, a method which provides information on the distribution of lipoproteins and measures the concentration of esterified cholesterol in each fraction. The inter- and intra- coefficients of variation for the 12 subfractions were <10%.
Results. The women sample had mean age of 67.7 years (SD=5.1, range 61-81) and BMI of 28.3 Kg/m^2 (SD=4.6). Fifty-two per cent of the participants had carotid atherosclerosis. The main results showed that tertiles II and III of VLDL-C + IDL-C were associated with a higher risk of carotid atherosclerosis compared to tertile I (OR=2.41, 95% CI 1.01-5.79, p=0.048 and OR=2.51, 95% CI 1.02-6.17, p=0.04, respectively). In addition, the tertile III retained this association also after adjustment for age, smoking, systolic blood pressure, glucose, BMI and HDL-C (p=0.03).
Conclusions. This study demonstrates that high concentrations of Triglyceride-Rich-Lipoproteins (i.e. the sum of VLDL-C plus IDL-C) are independently associated with the risk of carotid atherosclerosis in post-menopausal women aged >60 years. The measurement of the level of Triglyceride-Rich lipoproteins represents a useful tool for improving the assessment of the risk of cardiovascular disease in post-menopausal women.

EARLY LEFT VENTRICULAR DYSFUNCTION, ARTERIAL STIFFNESS, PROANGIOGENIC HAEMATOPOIETIC CELLS AND VITAMIN D LEVELS IN PATIENTS WITH PSORIASIC ARTHRITIS

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Background and Aims. Patients with psoriatic arthritis (PsA) have an increased prevalence of cardiovascular risk factors such as hypertension, myocardial dysfunction, and type 2 diabetes mellitus, and cardiovascular diseases (CVD) are the leading cause of death in these patients compared with controls.
Recent studies have shown that PsA patients have a high prevalence of vitamin D (vit-D) deficiency; this condition that is considered an independent predictor of cardiovascular diseases and all-cause mortality in several clinical settings.
We aimed to evaluate left ventricular (LV) mechanics in patients diagnosed with PsA and no clinical evidence for cardiovascular disease (CVD) using a novel, more sensitive technique, which evaluates myocardial deformation in multidimensional planes for the detection of impaired LV function. Furthermore we evaluated carotid intima media thickness (cIMT) and pulse wave velocity (PWV), circulating proangiogenic haematopoietic cells (PHCs),as markers of endothelial dysfunction.
We investigated the association between vitamin D levels, inflammatory mediators, markers of endothelial and myocardial dysfunction in patients with PsA.
Methods and Results. The study enrolled 19 PsA patients and 16 sex- -age matched healthy controls. All participants underwent conventional echocardiography and 2-dimensional speckle tracking echocardiography (STE). Global longitudinal, circumferential, and radial strain were measured. PHCs,Vitamin D levels, C-reactive protein (CRP), fibrinogen, (PWV), (cIMT) were also evaluated. PHCs count and vitamin D levels were lower in PsA patients as compared to controls, while fibrinogen, CRP, PWV and cIMT were higher in PsA patients. STE analysis showed that PsA patients had significantly lower global longitudinal strain (-16.11±2.89% and -19.15±1.9%, respectively, p=0.05) and global circumferential strain (-14.21±2.7% and -20.22±4.13%, respectively, p<0.01) versus control group.
No correlation was found between longitudinal and circumferential strains and disease-related risk factors.
Vitamin D levels was found to correlate with longitudinal strain, ejection fraction, PHCs, diseases activity markers, and fibrinogen levels.
Conclusion. Subclinical impaired myocardial deformation and endothelial dysfunction were common in patients with PsA even when there is no clinical evidence for CVD. Furthermore, vitamin D seems to may have a role in the endothelial homeostasis and myocardial function.
Further studies on larger sample sizes could clarify whether a supplementation of Vitamin D could modify PHCs levels inflammatory indices, myocardial function and arterial stiffness in patients affected by PsA, therefore contributing to reduce cardiovascular risk in this patients.

ASSOCIAZIONE TRA STEATOSI EPATICA NON ALCOLICA (NAFLD) ED INCIDENZA DI MALATTIA CARDIOVASCOLARE IN PAZIENTI CON DIABETE TIPO 1

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Scopo. Recenti studi hanno documentato un'associazione significativa tra la steatosi epatica non alcolica (NAFLD) ed il rischio di malattia cardiovascolare (CVD) nei pazienti con diabete tipo 1 (T1DM). Tuttavia, tale associazione necessita di essere ulterior-

mente verificata in studi longitudinali. Abbiamo, pertanto, valutato se esiste una relazione tra la NAFLD e l'incidenza di CVD nei pazienti con T1DM.
Metodi. Sono stati studiati 286 pazienti con T1DM (età media 43±14 anni; maschi 42.3%) esenti da epatopatia cronica da causa nota e seguiti per un periodo medio di 5.3±2.1 anni per lo sviluppo di CVD (definita come riscontro di cardiopatia ischemica non fatale, ictus ischemico non fatale o rivascolarizzazione coronarica/periferica). La diagnosi di NAFLD è stata formulata mediante ecografia epatica.
Risultati. Complessivamente, al baseline, 150 (52.4%) pazienti avevano la NAFLD. Durante il follow-up sono stati osservati 28 casi di CVD. L'incidenza cumulativa di CVD era maggiore nei pazienti con NAFLD rispetto a quelli senza (17.3% vs. 1.5%, P<0.001, rispettivamente). Nella regressione di Cox la NAFLD si associava ad un aumentato rischio di incidenza di CVD (Hazard Ratio [HR] 8.16, 95% CI 1.9-35.1, P=0.005). Dopo aggiustamento per età, sesso, BMI, fumo, durata di diabete, HbA1c, ipertensione, dislipidemia, nefropatia, storia di cardiopatia ischemica e valori di GGT, l'associazione rimaneva significativa e non si attenuava (adjusted-HR 6.73, 95% CI 1.2-38.1, P=0.031).
Conclusioni. Questo è il primo studio longitudinale che dimostra l'esistenza di un'associazione significativa tra la NAFLD e l'incidenza di CVD nei pazienti con T1DM, indipendentemente dalla coesistenza di molteplici fattori di rischio.

SYMPATHETIC NEUROTRANSMISSION DURING ATHEROSCLEROSIS DEVELOPMENT: AN UNRECOGNIZED TARGET OF DYSLIPIDEMIA?

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Aim. With the aim of discovering new genes/pathways involved in dyslipidemia-driven atherosclerosis, transcriptomic analysis was performed on aortas of several transgenic mouse lines, with different lipid/lipoprotein profiles and different susceptibility to atherosclerosis.
Methods. C57Bl/6, apoE-deficient (EKO), apoE/apoA-I-deficient (EKO/A-IKO) and apoE/apoA-I-deficient mice overexpressing human apoA-I (EKO/A-IKO/hA-I) were studied. Mice were fed chow or Western diet, starting from 8 weeks of age. After 22 weeks of diet, plasma lipoprotein distribution was profiled by FPLC and aortic atherosclerosis evaluated by en-face analysis. The entire gene expression profile of murine aortas was investigated by a high-throughput sequencing approach (Illumina).
Results. On chow diet, plaques could only be detected in the aortic arch of EKO (high VLDL-LDL, low HDL) and EKO/A-IKO mice (high VLDL-LDL, absent HDL). Western diet worsened hyperlipidemia and plaque formation in the aortic arch of EKO and EKO/A-IKO mice, but only led to modest atherosclerosis development in EKO/A-IKO/hA-I mice, characterized by elevated VLDL-LDL cholesterol levels and displaying a large HDL cholesterol peak.
Out of a total of 23,000 genes, about 2,300 genes were identified as differentially expressed in at least one condition (dietary or genetically determined). In the athero-prone genotypes, Western diet, with respect to chow diet, dramatically lowered the expression of genes coding for key enzymes of catecholamine synthesis and synaptic vesicular structure. Interestingly, a similar down-regulation was found in EKO/A-IKO mice (low HDL) compared to EKO/A-IKO/hA-I mice (high HDL), when fed the same diet (chow or Western).
Conclusions. Our data suggest that dyslipidemic conditions, predisposing to atherosclerosis development (i.e. hyperlipidemia; low HDL levels), may interfere with the arterial sympathetic innervation by down-regulating the expression of genes involved in catecholamine biosynthesis, as well as in synaptic plasticity and transmission.

VITAMIN D STATUS AND HDL FUNCTIONALITY IN HEALTHY PRE-MENOPAUSAL WOMEN

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Background. Low vitamin D (vitD) status has been linked to increased cardiovascular (CV) risk. Serum HDL cholesterol efflux capacity (CEC) is a metric of HDL functionality that is inversely correlated to CV risk, independently of HDL-cholesterol (HDL-C) plasma levels. At present, there is no data on the possible correlation between HDL CEC and vitD levels. We evaluated whether impaired HDL functionality occurs in otherwise healthy vitD deficient pre-menopausal women. In addition, we evaluated in macrophages the serum capacity to promote cholesterol loading (serum cholesterol loading capacity, CLC), index of its pro-atherogenic potential. We finally evaluated the effect of a vitD integration.
Material and Methods. Flow-mediated dilatation (FMD) and pulse wave velocity (PWV) were measured by standard techniques as markers of subclinical atherosclerosis. HDL CEC was assessed by radioisotopic technique while serum Serum CLC was measured by a fluorimetric assay.
Results. Healthy pre-menopausal women (n=43) were stratified in two groups according to their vitD levels: ≤10 ng/mL (very low group, VL), status defined as severe hypovitaminosis and over 10 ng/mL (low/normal group, LN). No differences were found between the two groups in total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride levels. FMD was significantly lower in VL group in comparison with LN group (9.90% ± 0.26 compared to 10.81 ± 0.31; p=0.03); PWV was higher in VL vitD group (6.094 m/s ± 0.26 compared to 5.25 m/s ± 0.09; p=0.01). HDL CEC through aqueous diffusion and SR-BI was similar between groups. ABCA1-mediated CEC was increased in VL group compared to LN group (3.079% ± 0.31 compared to 2.042% ± 0.19; p=0.005). ABCG1-mediated CEC was lower in the VL group compared to LN group (2.48% ± 0.18 compared to 3.30% ± 0.22; p=0.01). Finally, VL group serum CLC was significantly increased (+1.21 fold p=0.049). After integration ABCG1-mediated CEC in the VL increased reaching the values of control group at baseline (4,09% ± 0,12 compared to 4,08% ± 0,25; p = 0,97), while the LN group after supplementation did not improve compared to baseline.
Conclusions. VitD status influences circulating lipoprotein functions relevant for atherosclerosis in healthy pre-menopausal women which may be, involved in the association between severe vitD deficiency and increased cardiovascular risk.

PCSK9 MODULATES PHENOTYPE, PROLIFERATION AND MIGRATION OF SMOOTH MUSCLE CELLS IN RESPONSE TO PDGF-BB.

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Introduction. Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) represents the most recent pharmacological target for the treatment of severe hypercholesterolemia, due to its action on hepatic low-density lipoprotein receptor (LDLR) (1, 2). Secreted PCSK9 derives mainly from the liver, but other tissues, such as intestine, pancreas, kidney and brain express PCSK9 at significant levels (3). PCSK9 is also found in Smooth Muscle Cells (SMC) present in human atherosclerotic plaques (4). In addition, the absence of PCSK9 determines a partial protection to neointimal formation in response to perivascular manipulation in mice (5). Based on these premises we hypothesized that PCSK9 has a positive effect on neointima formation by influencing the phenotype, the proliferation and the migration of SMCs in response to Platelet-Derived Growth Factor (PDGF-BB).
Materials and Methods. SMCs were isolated from the aorta of PCSK9-/- mice and their cellular behaviour was compared to the same cell strain with the expression of PCSK9 reconstituted with a retroviral plasmid encoding human PCSK9 (PCSK9-/- SMCs vs PCSK9rec SMCs). qRT-PCR was used to establish the expression levels of phenotypic markers. iCelligence system, cell counting and the evaluation of cell-cycle proteins levels (p21Cip1, p27Kin1, Cyclin E and Cyclin D1) were used to monitor the cell proliferation rate. Finally, cell migration was analysed with the Boyden's chamber assay. All these experiments were performed in response to PDGF-BB (20ng/mL).
Results. PCSK9rec SMCs express lower levels of contractile markers α-SMA (-56±2%; p<0.001).
Conclusion. The presence of PCSK9 induced a switch towards the synthetic phenotype in SMCs. In addition, PCSK9 ameliorates the proliferative and migratory response to PDGF-BB. Those results suggest that there is a dual modulation between PCSK9 and PDGF, even if the mechanism is still under investigation. This study may provide a basic molecular mechanism for the lower neointimal formation observed in PCSK9-/- mice.
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CORRELAZIONE TRA ATEROSCLEROSI CAROTIDEA E MALATTIA CORONARICA: UNO STUDIO RETROSPETTIVO SU 1067 PAZIENTI

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Background. Numerosi studi in letteratura hanno dimostrato che il rischio di eventi cardiovascolari è maggiore nei pazienti con evidenza ultrasonografica di aterosclerosi carotidea. Obiettivo del nostro studio è stato dimostrare la correlazione tra aterosclerosi carotidea e severità della malattia coronarica in pazienti ricoverati per dolore toracico tipico e sottoposti a eco-color Doppler dei tronchi sovra-aortici (eco-TSA) e coronarografia (CVG).
Materiali e Metodi. Abbiamo arruolato in maniera retrospettiva 1067 pazienti ricoverati per dolore toracico presso la nostra U.O. di Cardiologia e sottoposti, durante il ricovero, a una CVG ed un eco-TSA. Secondo le linee guida ESC, abbiamo definito come aterosclerosi carotidea la presenza di un IMT > 1,5 mm. Durante la CVG abbiamo considerato la presenza di malattia coronarica quando abbiamo riscontrato stenosi > 50%. Criterio di esclusione è stata la positività dei biomarkers di infarto miocardico.
Risultati. I risultati dell'esame ultrasonografico hanno evidenziato una prevalenza di placca carotidea asintomatica nel 81% dei soggetti in studio. All'esame angiografico solamente il 12% della popolazione presentava coronarie indenni, i restanti erano affetti da malattia coronarica. La presenza di placca carotidea è risultata prevalentemente associata alla presenza di aterosclerosi coronarica alla CVG nel 72,8% dei pazienti (p=0.0015). In particolar modo la presenza di una placca carotidea di diametro maggiore a 2,5 mm (p<0.0001) o di aterosclerosi carotidea bilaterale (p<0.0001) si associava ad una maggior prevalenza di malattia coronarica. Le dimensioni delle placche carotidee erano significativamente correlate con la severità della malattia coronarica, calcolata con il Syntax Score (p<0,0001). Inoltre il riscontro di aterosclerosi carotidea è risultato essere correlato alla severità della malattia coronarica stessa (overall p=0,006).
Conclusioni. In considerazione dei dati, riteniamo che la valutazione eco-color Doppler dei tronchi sovraortici potrebbe fornire al cardiologo clinico informazioni aggiuntive sull'effettivo rischio cardiovascolare globale del paziente con dolore toracico e markers di miocardionecrosi negativi.

SLEEVE-GASTRECTOMY EFFICACY ON METABOLIC AND CARDIOVASCULAR DYSFUNCTION WITH A FOCUS ON THE ROLE OF COMORBIDITIES

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Background and Aims. Little is known about pathogenesis of sleeve-gastrectomy (SG) benefits. The endpoints of our study were to evaluate the effect of SG on metabolic and cardiovascular parameters on the basis of weight loss, visceral fat area (VFA) and HOMA decrease, and to study the influence of comorbidities (diabetes/hypertension).

Methods and Results. At baseline and 10-12 months after sleevegastrectomy we assessed, in 110 obese patients (131 patients in total, 21 lost at follow-up), anthropometric and biochemical parameters, bioimpedentiometry, ultrasonographic VFA, liver steatosis, flow mediated dilation (FMD), echocardiography. Patients who normalized glycosilated haemoglobin (HbA1c) experienced a larger weight loss (p=.028). The probability of normalizing HbA1c was directly correlated to TWL (rho=.461; p=.027). Diabetics experienced a greater improvement of systolic blood pressure (p=.008), VFA (p=.036), HDL (p=.046), HOMA (p=.001) and HbA1c (p<.001) than non hypertensive.
Conclusion. According to our data, in diabetics the most important determinant of glucose control after surgery is weight loss. Diabetics and hypertensive patients experienced a greater improvement of vascular and metabolic status than non diabetics and non hypertensive; hence these data strengthen indications to bariatric surgery in presence of comorbidities. No parameter seems to be directly responsible for surgery-associated cardiovascular improvement, suggesting the presence of others mechanisms involved.

EFFECTS OF DIABETES MELLITUS ON MYELIN LIPID PROFILE IN THE RAT CEREBRAL CORTEX

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Introduction. Due to the emerging association of diabetes with several psychiatric and neurodegenerative events, the evaluation of the effects of this pathology on the brain function has now a high priority in biomedical research. In particular, the effects of diabetes on myelin compartment have been poorly taken into consideration.
Methods. We here set up a method using both flow-injection analysis (FIA-MS/MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS) to perform a deep myelin lipidomic analysis in cerebral cortex of rat-raised diabetic by streptozotocin.
Results. Data obtained have shown that three months of diabetes induced an extensive impact on the levels of phosphatidylcholines (Ctrl: 376±55 pg/µg of protein vs. Diab: 205±41 pg/µg of protein, p<0.05) and phosphatidylethanolamines (Ctrl: 602±21 pg/µg of protein vs. Diab: 292±46 pg/µg of protein, p<0.001), plasmalogens (Ctrl: 153±11 pg/µg of protein vs. Diab: 66±12 pg/µg of protein, p<0.001) as well as phosphatidylserines (Ctrl: 119±23 pg/µg of protein vs. Diab: 45±11 pg/µg of protein, p<0.001) and phosphatidylinositols (Ctrl: 9.7±0.2 pg/µg of protein vs. Diab: 4.03±0.9 pg/µg of protein, p<0.01). In addition, the levels of cholesterol (Ctrl: 6.1±1.5 ng/µg of protein vs. Diab: 2.9±0.55 ng/µg of protein, p<0.01) and myelin basic protein were also decreased in the myelin of the same brain area. Interestingly, one-month treatment with a neuroprotective molecule such as dihydroprogesterone, a metabolite of progesterone, restored the lipid and protein myelin profiles to the levels observed in non-diabetic animals.
Conclusion. Given the key functional and structural roles of lipid and protein in myelin, our data indicate, for the first time, that cerebral cortex myelin is severely compromised in diabetic status.

IL METODO EPIDEMIOLOGICO IN DIREZIONE SANITARIA: STRATEGIE DI APPROPRIATEZZA PRESCRITTIVA E PREVENZIONE NEI PERCORSI DI DIAGNOSI E CURE DELLE MALATTIE CARDIOVASCOLARI IN OSPEDALE

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Il ricorso all'autonomia prescrittiva (art 3, c 2 l 94/98) non può costituire riconoscimento del diritto del paziente all'erogazione di medicinali a carico del SSN se non specificatamente previsto. Obiettivo primario dei sistemi sanitari nazionali europei è fornire assistenza farmaceutica di buona qualità a costi accettabili per la spesa pubblica. Negli ultimi 20-30 anni i tassi di incremento della spesa farmaceutica sono risultati superiori ai tassi di crescita del prodotto interno lordo. Le strategie di prevenzione delle malattie cardiovascolari in ospedale prevedono inteventi di setting; processo ed esiti.
Gli interventi di setting includono
- Struttura, tecnologia
- Servizi di diagnosi e terapia
- organizzazione, carichi di lavoro
- Educazione continua

Quelli tipici del percorso prevedono Aderenza a linee-guida evidence-based , percorsi diagnostici di terapia; infine gli indicatori di esito riguardano end-point robusti quali i Miglioramenti: di mortalità, di morbidità, di qualità di vita.
Una soluzione è efficiente se nessuna delle distribuzioni alternative delle risorse consente un minor fabbisogno .Una prescrizione può essere considerata appropriata se è effettuata all'interno delle indicazioni cliniche di Linee Guida o all'interno delle indicazioni d'uso per le quali è dimostrata l'efficacia. Un indicatore di qualità della prescrizione è un elemento misurabile della prescrizione, per il quale ci sia evidenza o consenso che esso possa essere utilizzato per valutare e, di conseguenza, modificare la qualità dell'assistenza fornita. Pertanto dal punto di vista organizzativo dare un formato agli interventi, cioè strutturarli in Protocolli, Linee guida o Percorsi permette di lavorare su casistiche concrete ed indicatori indicatori forti, da trasferire alla pratica quotidiana, in modo routinario e non discrezionale. Dal punto di vista epidemiologico-valutativo la creazione di un database di dati da analizzare mettendo in relazione pazienti, terapie ed esiti può dimensionare anche il valore aggiunto di marcatori ed end point surrogati, dove siano predittivi e più facilmente valutabili nei percorsi ospedalieri.

STATINS AND RISK OF NEW-ONSET DIABETES: A META-ANALYSIS OF OBSERVATIONAL STUDIES

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Background. Several previous meta-analysis of randomized control trials (RCTs) investigating the association between incidence of diabetes and statin use showed an increased risk of new-onset diabetes (NOD) from 9% to 13% associated with statins. However, short follow-up period, unpowered sample size, and lack of prespecified diagnostic criteria for diabetes detection could be responsible of an underestimation of this risk.
Aim. To evaluate the association between statins use and risk of NOD performing a meta-analysis of published observational studies.
Methods. In according with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, a systematic review of literature was conducted by searching on PubMed, EMBASE and MEDLINE from inception to 30 June 2016. We searched for cohort and case-control studies with risk of NOD in users vs nonusers (any or single statin), on ≥1000 subjects followed-up for ≥1 year. Studies published as abstract and in languages other than english were excluded. Pooled estimates were calculated by a random-effects model and between-study heterogeneity was tested and measured by I2 index. Furthermore, stratified analyses by length of follow-up, geographic area and propensity score matching, influence analysis, and the evaluation of publication bias were performed.
Results. The meta-analysis included 20 studies, 18 cohort and 2 case-control studies. Duration of follow-up ranged from 2 to 20 years. Overall, NOD risk were higher in statin users than nonusers (RR 1.44, 95% CI 1.31-1.58), with no substantial impact from a single study, as from the influence analysis. High between-study heterogeneity (I2=97%) was found. Estimates for all single statins showed a statistical significant NOD risk, with rosuvastatin (RR 1.61, 1.30-1.98) and atorvastatin (RR 1.49, 1.31-1.70) showing the higher risk. Stratified analysis did not point out any significant differences. Little evidence of publication bias was found only for atorvastatin.
Conclusions. The present meta-analysis confirmed and reinforced the evidence of a diabetogenic effect by statins utilization, showing a higher increase risk of NOD than previous RCTs meta-analysis, suggesting an underestimates of this risk in clinical trial. Furthmore, all single statins showed an increased risk of NOD, supporting a class effect, independently of their chemico-physical characteristics. These observations confirm the need of a rigorous monitoring of patients taking statins, in particular pre-diabetic patients or patients presenting with established risk factors for diabetes.

INTERACTION OF LCAT AND APOA-I ON LIPID METABOLISM IN HUMAN LCAT X HUMAN APOA-I DOUBLE TRANSGENIC MICE

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Aim. Lecithin: cholesterol acyltransferase (LCAT) is a key enzyme that catalyzes the esterification of free cholesterol in plasma high-density lipoproteins (HDL) but its role in atherosclerosis has not been clearly established.
Methods. We recently developed a novel mouse model of human LCAT x human apoA-I double transgenic mice in C57Bl/6N and in apoE-KO backgrounds. We compared lipid metabolism and atherosclerosis development in our models with normal C57Bl/6N, hLCAT transgenic and apoE-KO mice.
Results. Double transgenic mice on normal chow diet had highest level of plasma total cholesterol, which was significantly higher than in apoE-KO animals and reached more than 1000 mg/dL. FPLC analysis showed that in contrast with apoE-KO mice, in double transgenic mice most of lipids were located in HDL fraction. Despite their higher total cholesterol, LCAT x apoA-I transgenic mice were protected against development of atherosclerosis. Expression analysis of the different tissues of the mice showed that in double transgenic mice had induction of genes involved in the reverse cholesterol transport (RCT) pathway. Genes related to inflammation were suppressed in the aorta of double transgenic mice compared to control mice and apoE-KO mice on HFHC diet.
Conclusion. The obtained results marked significance of HDL and efficient RCT for atheroprotection even in situation of very high total plasma cholesterol. The interaction between LCAT and ApoA-I is able radically enhance RCT and HDL. These data can be helpful in development of new prospective treatments of atherosclerosis and acute coronary syndrome with combination of LCAT and ApoA-I or ApoA-I mimetic peptides.

TREATMENT WITH LOMITAPIDE IN HOMOZYGOUS HYPERCHOLESTEROLEMIA: A CASE REPORT

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Background. Homozygous familial hypercholesterolaemia (HoFH), an inherited lipid disorder due to genes mutations involved in cholesterol metabolism, is characterized by accelerated and premature atherosclerosis.
In HoFH statins have often limited effectiveness in targeting lipid levels and patients require lipoprotein apheresis, which is some-

times insufficient to manage LDL cholesterol levels and not well tolerated by patients.
Case report. A 40year old man with diagnosis of HoFH (LDLR c.1135T>C p.Cys379Arg homozygous and an estimated LDR-R residual activity of 10-15%) and aortic valve replacement surgery at the age of 23. At baseline total cholesterol, LDL-C, HDL-C, triglyceride levels were respectively 720, 659, 41, 100 (mg/dl).
Despite apheresis combined with subcutaneous administration of Evolocumab twice a month, rosuvastatin 40 mg/d and ezetimibe 10 mg/d, time-averaged LDLC levels were above 250 mg/dl.
Therefore, the patient stopped Evolocumab administration and started treatment with lomitapide in February 2016.
Lomitapide dose was titrated up from a starting dose of 5 mg/d, to 10 mg/d (week 5), 20 mg/d (week 11) and 30 mg/d (week 16) with liver enzyme levels reaching a peak soon after starting dose of 30 mg/d (AST 192U/L, ALT 270U/L).
Consequently Lomitapide was discontinued for one month until AST and ALT normalized and the patient was re-titrated from a dose of 5 mg/d to 20 mg/d.
At this dose, the patient stopped apheresis and a decrease of 83% in LDLC and 76% in total cholesterol compared with baseline, was detected. Plasma levels of AST, ALT, and GGT remained stable within normal limit and even liver ultrasound did not reveal any hepatic fat accumulation.
Conclusion. To our experience Lomitapide could be an effective and safe drug in association with standard lipid-lowering therapy in HoFH patients, particularly in those who are receptor negative.

COMPARISON BETWEEN MEDITERRANEAN AND VEGETARIAN DIETS FOR CARDIOVASCULAR PREVENTION: THE CARDIVEG STUDY

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Background. Cardiovascular disease is the leading cause of death worldwide. Incidence and/or mortality from cardiovascular disease can be easily prevented with adequate changes in lifestyle. The optimal dietary strategy for the prevention of cardiovascular diseases remains a challenging and a highly relevant preventive health issue. Several models of diet have been imposed on public attention, but those that got the most interest are certainly Mediterranean and Vegetarian diets.
Aim. To conduct a randomized dietary intervention trial with the use of two different diets, Vegetarian and Mediterranean, in order to compare the effects of these diets on several anthropometric and circulating cardiovascular biomarkers.
Methods. Eighty-eight clinically healthy subjects (68 F; 20 M; mean age: 50.7±12.9) were randomly allocated to Mediterranean (Med) or Vegetarian (Veg) isocaloric diets lasting three months each, and then crossed over. Adherence to the specific dietary intervention was established through questionnaires and 24-h dietary recall. Anthropometric measurements, body composition and blood sampling were obtained from each participant at the beginning and at the end of each intervention phase.
Results. At the end of the 3-months intervention phase, Med and Veg both determined a significant ($p<0.05$) reduction of total body weight, fat mass and BMI, without any significant difference between the two diets [body weight: -2.0 kg (-2.5%) vs. -2.4 kg (-3.0%)], [fat mass: -1.8 kg (-6.1%) vs. -1.6 kg (-5.6%)] [BMI: -0.7 kg/m^2 (-2.4%) vs. -0.8 kg/m2 (-2.8%)], for Med and Veg, respectively. With regard to circulating biomarkers, Veg determined a significant ($p<0.05$) decrease for total cholesterol [-6.0 mg/dL (-2.9%)], LDL cholesterol [-6.5 mg/dl (-5.1%)] and insulin levels [-0.7 mU/L (-6.9%)], while Med determined a significant reduction of triglycerides [-11 mg/dL (-8.9%)].
Conclusions. Both Mediterranean and Vegetarian dietary patterns appear to be equally effective in reducing anthropometric parameters among clinically healthy subjects, but Vegetarian diet determined a significant reduction of total cholesterol, LDL cholesterol and insulin levels, while Mediterranean diet determined a significant reduction of triglycerides.

RELATIONSHIP BETWEEN SLEEP PATTERN AND EFFICACY OF CALORIE-RESTRICTED MEDITERRANEAN DIET IN OVERWEIGHT/OBESE SUBJECTS

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Background. An adequate sleep pattern is essential to maintain a good health. Short sleep duration and/or poor sleep quality has been associated with type 2 diabetes, hypertension and cardiovascular disease, as well as weight gain and obesity.
Objective. The aim of the study was to evaluate the possible association existing between sleep quality and quantity and efficacy of calorie-restricted Mediterranean dietary intervention in a group of overweight/obese subjects.
Methods. 403 overweight or obese subjects (179 M; 224 F, mean age 52.6±14.1 years) were provided with a detailed Mediterranean hypocaloric 1-week menu plan and followed-up for 9 months. Personal information and lifestyle habits, including patients' sleep pattern assessed by a modified version of the Pittsburgh Sleep Quality Index, was obtained at the baseline. Body weight and body composition were measured every 3 months for a total of 9 months.
Results. An overall better sleep resulted significantly ($p=0.02$) more prevalent among men [n=134 (74.9%)] than women [n=146 (65.2%)], at baseline. Poor sleeper subjects reported to have significantly ($p<2$). Women who reported to sleep 6 - 8 hours or >8 hours were more likely to lose fat mass than women who sleep <6 hours (OR=4.47, 95%CI=1.42 - 14.04; $p=0.010$ and OR=5.10, 95%CI 1.15 - 22.70; $p = 0.032$, respectively).
Conclusion. Our results confirm that regular sleep pattern is necessary to maintain a good general health, including optimal body weight and composition, and raise the possibility that an insufficient sleep may represent a significant risk factor for a failure to a calorie-restricted dietary plan, especially among women.

DYSREGULATED EXPRESSION OF ANKYRIN REPEAT DOMAIN 1 IN THE DEVELOPING MYOCARDIUM CAUSES ANOMALOUS VENOUS RETURN AND MORPHOGENETIC DEFECTS BY IMPAIRING CARDIAC REMODELLING

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Introduction. Total anomalous pulmonary venous return (TAPVR) is a severe congenital heart disease characterized by failure of pulmonary veins to connect exclusively to the left atrium. Cardiac Ankyrin Repeat Protein (CARP), encoded by the Ankyrin repeat domain 1 (Ankrd1) gene, is a mechanosensor protein, involved in physiological and pathological remodelling of myocardium. Recently, we have identified Ankrd1 as a candidate gene for TAPVR in isolated patients. Our reported TAPVR patients presented either increased Ankrd1 transcript levels or a missense T116M mutation, resulting in greater protein stability. To date, the role of this gene in venous pole development, as well as the link between an Ankrd1 gain of function condition and TAPVR pathogenesis in humans remain unexplored.
Methods. Two transgenic mouse lines overexpressing wild-type (WT) CARP or T116M-CARP, under the control of the α–MHC promoter, have been previously generated. Embryos from control and transgenic mice have been harvested at 10.5 and 14.5 days post coitum and processed to perform morphological and expression analyses.
Results. Our results showed that Ankrd1 expression delineates discrete morphogenetic subdomains in the developing heart of controls. Myocardial overexpression of WT-CARP or T116M-CARP resulted in strong impairment of early cardiac remodelling steps, including rotation and cranial expansion of the sinoatrial region. Mid-fetal transgenic hearts presented complex morphogenetic defects and abnormal pulmonary venous connections, accompanied by strong alteration of structural properties, but not of the molecular patterning program. Additionally, cardiac chamber trabeculation was more extended.
Conclusions. Our data indicate that CARP is a critical sensor-signalling molecule that modulates cardiomyocyte cellular properties during development. Moreover, Ankrd1 regionalized expression is required to refine shape and relative position of cardiac compartments and increased WT-CARP or T116M-CARP levels lead to TAPVR by impairing remodelling of early venous pole myocardium. These findings uncover novel levels of complexity in genetic regulation of cardiac development.

CHOLESTEROL EFFLUX CAPACITY MODULATION BY THE NUTRACEUTICAL COMPOUND "OLEACTIVE®"

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Aim. In the last decades, the use of nutraceutical compounds to support or replace medical therapies is spreading, due to fewer side effects, and more tolerability by patients compared to traditional drugs. Nutraceutical compounds may contribute to the qualitative and quantitative modulation of lipid and lipoprotein profile. HDL are the responsible of cholesterol efflux from the atherosclerotic plaque, through the interaction with cholesterol transporters expressed in macrophages composing the atheroma. Recent studies have shown the inverse correlation between the cholesterol efflux capacity of the serum (CEC), mostly mediated by HDL, and cardiovascular risk. The aim of this study is to evaluate the effects of the Oleactive® compound on lipid and lipoprotein profile in golden Syrian hamsters. In particular, ABCA1 mediated efflux has been evaluated, as it is the main transporter involved in cholesterol efflux from macrophages. Oleactive® is a nutraceutical compound patented by Fytexia company (Vendres, France). Experimentations are part of an industrial project, which is not finalized yet and the Oleactive® composition is secreted.
Material and Methods. 40 Golden syrian hamsters were divided in 4 groups. Standard group (STD group), which followed normolipidic diet, received daily vehicle for 12 weeks. The remaining groups, fed with a hypercholesterolemic diet(2g/kg of cholesterol), received daily vehicle (CTRL group), Atorvastatin (1.23 mg/kg/day-Atorva group), or Oleactive® formulation (OLA group) for 12 weeks. After sacrifice, HDL, total cholesterol (TC), LDL, triglycerides (TG) of the plasma were analyzed. We analyzed the ABCA1 mediated efflux and passive diffusion using murine macrophages J774 treated with cpt-cAMP, which induces an overexpression of ABCA1 transporter. Afterwards cells were incubated with sera (1%, v/v).
Results. Lipid profile of OLA group showed differences compared to CTRL group. In detail, it has been observed a slightly decrease of total cholesterol(-1,04mmol/L), LDL-C(1,14 mmol/L) and a significant decrease of triglycerides(-1,21mmol/L). Conversely, HDL-C levels are similar in two groups. Atorva group showed an increased plasma total cholesterol (+4,02mmol/L,$p<0.05$) reduction of total body weight, fat mass and BMI, without any significant difference between the two diets [body weight: -2.0 kg (-2.5%) vs. -2.4 kg (-3.0%)], [fat mass: -1.8 kg (-6.1%) vs. -1.6 kg (-5.6%)] [BMI: -0.7 kg/m^2 (-2.4%) vs. -0.8 kg/m^2 (-2.8%)], for Med and Veg, respectively. With regard to circulating biomarkers, Veg determined a significant ($p<0.05$) decrease for total cholesterol [-6.0 mg/dL (-2.9%)], LDL cholesterol [-6.5 mg/dl (-5.1%)] and insulin levels [-0.7 mU/L (-6.9%)], while Med determined a significant reduction of triglycerides [-11 mg/dL (-8.9%)].
Conclusions. Our results show that the administration of Olective® influence the lipid profile in hamsters, which have a lipid and lipoprotein profile more akin to the human one, compared to other animal models. In addition, the increased CEC, due to an increased ABCA1 mediated efflux by Oleactive®, may be a potential mechanism against atherosclerotic diseases. The administration of this nutraceutical compound could be used as support of pharmaceutical therapy, or in case of light hypercholesterolemia.

PHENOTYPIC EXPRESSION AND GENOTYPE ANALYSIS OF ELEVEN PATIENTS WITH CHOLESTERYL ESTER STORAGE DISEASE AND IDENTIFICATION OF A NOVEL LIPA GENE VARIANT.

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Background. Cholesteryl ester storage disease (CESD) is a recessive disorder due to a defect of Lysosomal Acid Lipase (LAL) that hydrolyses cholesteryl esters and triglycerides derived from internalized apoB containing lipoproteins. The disease is characterized by a multi-organ involvement including liver, spleen, intestine and cardiovascular system inducing premature atherosclerosis.
Objective. Aim of the study was the characterization of clinical and molecular features of 11 (10 unrelated) previously unreported CESD patients subjected to a follow up schedule for several years.
Methods. Data collected included clinical and laboratory investigations, liver imaging, liver biopsy, the response to lipid-lowering medications or liver transplantation and LIPA gene analysis.
Resuslts. CESD was suspected in infancy (4.9-3.5 years) for the presence of:
1) hepatomegaly;
2) elevated serum transaminase;
3) recessive hypercholesterolemia and was confirmed by liver biopsy/imaging and LAL assay. Mean follow up period was 7.6 years. Patients treated with statins with or without ezetimibe showed 31% reduction of plasma LDL-cholesterol. This treatment was not associated with a tangible effect on the progression of the liver damage. Eight out of ten patients were carriers at least one c.894G>A common allele (4 homozygotes and 4 compound heterozygotes). We identified four previously reported missense variants: p.(Thr288Ile) in homozygosity and p.(His295Tyr), p.(Gly342Arg), and p.(Leu294Ser) in compound heterozygosity. Two patients were carriers of p.(Asp345Asn), a novel variant affecting the LAL catalytic triad. The p.(Thr288Ile), p.(His295Tyr) and p.(Asp345Asn) were found so far only in Italian patients from restricted geographical areas.
Conclusions. This study provides additional data on the clinical and molecular features of CESD patients and describes a novel variant affecting LAL catalytic site.

VISCERAL FAT EVALUATED BY ULTRASOUND IS ASSOCIATED WITH INSULIN RESISTANCE AND LIVER DAMAGE IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Background. Non-alcoholic fatty liver disease (NAFLD) has been regarded as the hepatic manifestation of metabolic syndrome (MetS). Insulin resistance is a hallmark of MetS and is considered to play a pivotal role in NAFLD progression. Visceral adiposity tissue (VAT) evaluated by TC has been reported to be independently associated with liver fibrosis in NAFLD patients. Ultrasonography (US) is an easy and safe technique able to detect steatosis and VAT. NAFLD fibrosis score (NFS) has been proposed as a non-invasive tool for the assessment of liver fibrosis in NAFLD.
Aim. Aim of the study was to investigate the relation between VAT, insulin-resistance and liver damage in adult patients with NAFLD.
Methods. We enrolled 176 consecutive patients referred for suspected metabolic disease and with a US scanning positive for NAFLD. Liver steatosis severity was defined according to Hamaguchi's criteria. VAT was determined during US exam. A NFS was defined low when <-1.455 and intermediate/high when ≥-1.455.
Results. Mean age was 55.8 (± 13.5) years and 36.9% of patients were female. Prevalence of MetS and diabetes were 61,6% and 29,9%, respectively. Median VAT was 6.1 (5.1/7.6) cm and median HOMA-IR was 3.4 (2.3/5.3). Median VAT was significantly higher in patients with intermediate/high NFS than in those with low NFS. Linear bivariate regression analysis showed a positive correlation between VAT and HOMA-IR ($r=0.42$; $p<0.001$), Hamaguchi score ($r=0.47$; $p<0.001$), ALT ($r=0.38$; $p<0.001$), AST ($r=0.27$; $p<0.001$) and NFS ($r=0.19$; $p=0.026$). In a multiple linear regression analysis, VAT ($p=0.03$) and age ($p<0.001$) were independently associated with NFS.
Conclusions. Our data show a positive correlation between VAT evaluated by US, insulin resistance and liver damage in a population of NAFLD patients. Evaluation of VAT during US exam may be useful to identify NAFLD patients with a more severe liver disease.

ANGPTL3 FACILITATES β-ADRENERGIC-DEPENDENT LIPOLYSIS IN ADIPOCYTES: EVIDENCE FROM IN VITRO STUDIES

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Background. ANGPTL3 deficiency is associated with decreased plasma concentration of free fatty acids (FFA). The molecular mechanism underlying this effects remains unclear. The interprandial lipolysis of TG contained in adipose tissue is considered to be the major source of FFA. Therefore, we hypothesized that ANGPTL3 may play a role in modulating the β-adrenergic-dependent lipolysis in adipocytes.
Methods. 3T3-L1 adipocytes were treated with recombinant human ANGPTL3 (100 nM) with and without the addition of β-agonist isoproterenol. The amount of FFA and glycerol released in the colture medium were measured at 30' and 60' by a colorimetric assay. Changes in expression of kinases HSL, ERK1/2 and AMPKα involved in the lipolytic intracellular pathway were determined by western blot before and after treatments.
Results. Compared to untreated, ANGPTL3-treated cells showed a lower release of FFA (at 30 minutes 126.6 uM vs. 21.6 uM, respectively; $p<0.05$) and a markedly skewed FFA to glycerol ratio (<1.5).

In contrast, when adipocytes were treated with the β-agonist isoproterenol (ISO), either before or after the addition of ANGPTL3, the release of FFA levels were markedly increased (ISO 499 uM; ANGPTL3 pre-treatment 773 uM, p <0,03; ANGPTL3 post-treatment 1676 uM; p <0.01). The combined treatment of ANGPTL3 with ISO determined an increased expression of HSL, AMPKα and ERK1/2 in adipocytes.
Conclusion. Our findings indicated that ANGPTL3 may act by facilitating the β-adrenergic-dependent lipolysis in adipocytes. This mechanism might explain the lower FFA levels in subjects with ANGPTL3 deficiency.

ANTHROPOMETRIC ASSESSMENT AND ULTRA-ELASTO-SONOGRAPHIC EVALUATION OF LIVER STEATO-FIBROSIS IN HYPERCHOLESTEROLEMIC PATIENTS ON TREATMENT

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Background. Hypercholesterolemia is a condition often associated with other metabolic disorders, including liver steatosis (LS). Treatment of hypercholesterolemia includes the HMG-CoA reductase inhibitors statins, while the nutraceutic monacolin K (equivalent to lovastatin low dose) and diet might confer additional benefits. LS might also improved upon treatment.
Methods. 3 groups of naïve hypercholesterolemic patients (tot chol ≥200mg/dl; LDL ≥150 mg/dl) were enrolled and studied at baseline (T0) and 12 weeks (T12): SIMVA (6F, 6M age 57.0±2SD yrs, simvastatin 20 mg/day); MK (5F, 3M, 48.0±5SD yrs, monacolin K 10/mg/day); DIET (2F, 2M, 47.0±8SD yrs, on initial hypolipidic controlled diet). LS (grade 0 to grade 3) and elasticity for fibrosis (L/F index <2.73 for normal liver parenchyma; 2.73 to 3.93 for increased fibrosis) were assessed with Noblus-E ecocolordoppler (Hitachi Medical Corporation, Tokyo, Japan). Anthropometric data (BMI, and waist circumference) were measured.
Results. Treatment with SIMVA led to a significant decrease (T0-T12) of LS (from 1.9±0.1 to 1.6±0.1 p<0.038), L/F (from 3.1±0.9 to 2.8±0.8 p<0.024) and BMI (from 32±2.4 to 30.4±2 Kg/m^2, p<0.007). Interestingly, females displayed the greatest and significant decrease of waist circumference (from 92.7±5.8 cm to 88.3±4.4 cm, equal to -4.4±1.3%, P=0.035) when compared with males (from 96.1±4.2 cm to 95.2±3.8 cm, equal to -0.9±1.0%, P=NS). Treatment with MK and DIET did not produced changes in LS or L/F but BMI slightly decreased in MK females (8.3±4.2% vs. T0, P=0.043).
Conclusions. In hypercolesterolemic patients, SIMVA during 3 mo. is associated with significant reduction of LF and LS compared to monakolin and diet alone. Females display additional improvement of anthropometric parameters (BMI, abdominal fat), suggesting better lifestyles compliance than men.

SFINGOSINA 1-FOSFATO ED ATEROSCLEROSI: NUOVE EVIDENZE DA MODELLI ANIMALI TRANSGENICI

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Obiettivi. Evidenze recenti suggeriscono che gli effetti ateroprotettivi delle HDL possano essere in parte attribuiti alla sfingosina 1-fosfato (S1P), un lisosfingolipide capace di legare ed attivare specifici recettori accoppiati a proteine G (GPCR). Dei cinque sottotipi recettoriali finora identificati, le isoforme S1PR1 e S1PR3 sembrano essere le più rilevanti a livello cardiovascolare. Pertanto, il nostro lavoro mira a chiarire l'impatto di S1P endogena sull'aterosclerosi in vivo, utilizzando peculiari modelli murini transgenici capaci di sovraesprimere in modo tessuto specifico le isoforme recettoriali S1PR1 e S1PR3.
Materiali e Metodi. La sovraespressione dei recettori S1PR1 e S1PR3 in cellule rilevanti per l'aterosclerosi, come macrofagi ed endotelio, è stata ottenuta generando topi transgenici che sfruttano la tecnologia Cre-LoxP. Gli animali, in background genetico suscettibile all'aterosclerosi (ApoE+/- o chimere LDLR-/-), sono stati sottoposti a dieta iperlipidica per 16-24 settimane e poi sacrificati. L'analisi dell'aterosclerosi è stata condotta su criosezioni di cuore a livello delle radici aortiche e su arteria brachiocefalica dopo colorazione con Oil-Red-O/ematossilina ed espressa come area totale delle lesioni o come rapporto tra l'area della placca e l'area totale del vaso (media ± SD).
Risultati. Gli animali che sovraesprimono S1PR1 nei macrofagi presentano una riduzione massiva delle lesioni aterosclerotiche rispetto ai controlli, sia a livello delle radici aortiche (719749,75 ± 76145,30 vs 378623,85 ± 98072,97) sia a livello dell'arteria brachiocefalica (47383,53 ± 20251,24 vs 11288,12 ± 11085,16). Analogamente, nell'endotelio, la sovraespressione di S1PR1 (0,35 ± 0,28 vs 1,38 ± 0,53) o di S1PR3 (0,30 ± 0,13 vs 1,38 ± 0,53) induce una riduzione significativa delle lesioni rispetto ai controlli.
Conclusioni. Il nostro studio dimostra, per la prima volta, che l'amplificazione del signalling mediato da S1P endogena è un fattore protettivo nei confronti dell'aterogenesi e tali azioni possono essere attribuite, almeno in parte, ad entrambi i recettori S1PR1 e S1PR3.

SMOKING STATUS AND TYPE D PERSONALITY AFFECT THE CLINICAL OUTCOME IN PATIENTS WITH RECENT MYOCARDIAL INFARCTION UNDERGONE TO PRIMARY PERCUTANEOUS CORONARY INTERVENTION

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Background. Smoking cessation is correlated to several psychological, social, biological, and pharmacological aspects. It's important to identify differences concerning personality traits in order to increase chances to discontinue cigarette smoking. The combined tendency to experience negative emotions and to inhibit the ex-

pression of these emotions is indicated as "Type D personality", already known as an independent risk marker for worse clinical outcome in cardiac diseases. Despite this effect of Type D personality on cardiovascular diseases, it is still unclear whether this trait of personality may influence smoking cessation after a myocardial infarction.

Methods and Results. 231 smokers with ST-elevation myocardial infarction (STEMI), treated with primary percutaneous coronary intervention (pPCI), were enrolled in this study. Type D Scale-16 (DS16) was administered during hospital stay. Patients have been followed-up for 4.5 years. After controlling for demographic and clinical confounders, non-Type D patients reported statistically significant higher frequencies of smoking cessation (84%) when compared with Type D group (31%). Stop smokers had a better lipid profile, mainly due to HDL-C plasma levels raising (+9.3% vs -1.1%, $p<0.001$), and better cardiovascular outcome. Moreover, type D patients had a significantly higher incidence of cardiovascular events during the long-term follow-up: type D former smokers showed a similar cumulative rate of new CV events with respect to current non-Type D smokers during the follow-up, but the occurrence reported in current type D smokers was almost 4-fold ($p<0.0001$ for Chi square).

Conclusions. Type D smokers quit smoking hardly, even after an acute cardiovascular event (less than one out of three patients), and had an higher recurrence rate also with respect to current non-Type D smokers. Type D personality is confirmed as an independent risk factors for cardiovascular disease, also in addition to smoke habit. In order to be effective, offering and providing a genuine cessation counseling and suggesting a correct treatment are fundamental in reducing smoking addiction, especially in subpopulations with previous cardiovascular events and specific personality traits (i.e. type D personality).

DIAGNOSI MOLECOLARE DELLE IPERTRIGLICERIDEMIE PRIMITIVE ATTRAVERSO LA PROCEDURA "NEXT GENERATION SEQUENCING" (NGS): RISULTATI E QUESTIONI APERTE.

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Obiettivo. Caratterizzazione molecolare di pazienti con ipertrigliceridemia primitiva severa (Trigliceridemia >10 mmol/L) con accumulo di chilomicroni (chilomicronemia familiare). Questo disordine può essere dovuto a mutazioni di geni coinvolti nella cascata lipolitica, il processo di idrolisi dei trigliceridi trasportati da chilomicroni e VLDL.

Materiali/Pazienti e Metodi. È stata utilizzata la tecnologia "Next Generation Sequencing" (NGS, tecnologia "Ion Torrent") che prevedeva l'analisi in parallelo di un pannello di 19 geni coinvolti nel metabolismo delle lipoproteine contenenti trigliceridi [geni: LPL, APOC2, APOA5, GPIHBP1, LMF1, APOE, LIPC, LIPG, APOC3, GPD1, ANGPTL3, ANGPTL4, ANGPTL8, CREB3L3, GALNT2, MLXIPL, TRIB1, GCKR, APOB (esoni 26 e 29)]. Sono stati investigati 12 pazienti con ipertrigliceridemia primitiva identificati in strutture Ospedaliere italiane o straniere. Le varianti genomiche riscontrate con NGS sono state confermate con il metodo di sequenziamento Sanger.

Risultati. L'analisi ha portato all'identificazione di tre condizioni definite come assetto genotipico:
1) "atteso" caratterizzato da mutazioni o varianti funzionali dei geni candidati maggiori;
2) "inatteso" caratterizzato da mutazioni o varianti funzionali di geni minori;
3) "complesso" caratterizzato dalla presenza di multiple varianti genomiche rare in vari geni.

Sei pazienti con assetto genotipico "atteso" sono risultati portatori di mutazioni patogenetiche o varianti funzionali nei geni candidati "maggiori" coinvolti nella cascata lipolitica: LPL, APOA5, GPIHBP1, LIPC. Questo gruppo comprendeva: i) un omozigote per una mutazione non senso p.Cys89* di GPIHBP1; ii) un eterocomposto per mutazioni (p.Arg214Ile/p.Glu282*) di LPL ed eterozigote semplice per la mutazione p.Gly248Val di LIPC; iii) un doppio eterozigote per una mutazione di LPL (p.Trp113Arg) ed una variante funzionale (p.Gly185Cys) di APOA5; iv) un doppio eterozigote per due varianti funzionali di LPL (p.Asn318Ser) e di APOA5 (p.Ser19Trp); v) due eterozigoti semplici per varianti funzionali di LPL (p.Asn318Ser) e di GPIHBP1 (p.Cys14Phe).

Un paziente risultava avere un assetto genotipico "inatteso" essendo eterozigote semplice per una nuova variante missenso (p.Ala4148Glu) del gene APOB non coinvolto direttamente nella cascata lipolitica.

Un paziente risultava avere un assetto genotipico "complesso" essendo eterozigote per 4 varianti:
1) un polimorfismo funzionale di LPL (p.Asn318Ser);
2) una nuova mutazione di LIPC (p.Gly247AlaFs*12);
3) una nuova mutazione di APOB (p.Gln4368Argfs*26);
4) una nuova mutazione di ANGPTL8 (p.Arg83Trp).

Quattro pazienti risultavano avere un assetto genotipico negativo per varianti nei geni esaminati.

Conclusioni. La procedura NGS consente di analizzare in parallelo un gruppo di geni ritenuti coinvolti nel metabolismo dei trigliceridi e quindi rivelare, in tempi brevi, le basi molecolari delle ipertrigliceridemie, che possono coinvolgere più geni, alcuni dei quali in precedenza trascurati perché considerati "minori". Nel contempo, questa tecnologia porta all'identificazione di varianti genomiche rare in geni multipli il cui impatto funzionale e ruolo patogenetico nell'indurre ipertriglicerimia rimane al momento indefinito.

PCSK9 AND RESISTIN: NOT ONLY A STRUCTURAL SHARING

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Chronic inflammation is directly linked to several metabolic diseases, such as atherosclerosis, and obesity, where inflammatory cells infiltrate adipose tissue and release pro-inflammatory mediators, contributing to generate a state of chronic low-grade subclinical inflammation. Resistin is an adipokine, secreted primarily by monocytes and macrophages in humans and it interacts with adenylyl cyclase-associated protein 1 (CAP1) receptor, with its carboxy-terminal region, leading to an increase of cAMP intracellular levels and, consequently, to the activation of NF-kB. In obese subjects, resistin levels are strongly correlated with the developing of atherosclerotic cardiovascular diseases and with the promotion of

foam cells formation. In recent study, it was observed a positive correlation between circulating resistin levels and fasting serum triglycerides. Moreover, this protein directly stimulates VLDL production in human hepatocites, by inducing VLDL synthesis through the induction of apoB100, triglycerides and cholesterol.
Resistin can also induce the degradation of LDLR with a mechanism that involves both a PCSK9-dependent and a PCSK9-independent way.
PCSK9 is mainly produced and secreted by liver and promotes LDLR degradation. Patients with gain-of-function mutation of PCSK9 exhibit consistently elevated VLDL triglycerides and apoB100 levels, attributed to an increased hepatic VLDL production. PCSK9 levels are also significantly correlated with fasting serum triglycerides.
In a recent work, published by Hampton et al., it is shown that PCSK9 and resistin share their cysteine-rich carboxy-terminal domain structure, the one with whom resistin interacts with its receptor, CAP1, but now we know that they also share some common biological activities. Very little, instead, is known about the possible role of PCSK9 in the inflammatory process. For this reason, in this work we wanted to understand if PCSK9 could have a pro-inflammatory action, maybe with a resistin-like mechanism.
In our laboratory, we previously demonstrated that PCSK9 is also secreted by smooth muscle cells in the atherosclerotic plaque and degrades macrophages LDLR. For this reason, we decided to better understand PCSK9 possible proinflammatory effect on macrophages, using two different cell lines: THP-1 derived macrophages, differentiated from THP-1 monocytes with PMA 10-5M, and human macrophages, obtained from Centro Cardiologico Monzino from different healthy donors. We seeded them in RMPI 10% FCS media and then we threated them for 24h with increasing concentrations of PCSK9 (0.25, 0.5, 1.0 and 2.5 µg/ml) and resistin (20 and 50ng/ml), in RPMI 0.4% FCS media.
Then, we evaluated different pro-inflammatory cytokines mRNA levels, in order to understand if PCSK9, like resistin, can improve their secretion. We observed a dose dependent increase both with resistin and PCSK9, but, more surprisingly, at the higher concentration of PCSK9 (2.5 µg/ml), cytokines mRNA levels shown a very huge increase in THP-1 derived macrophages, like ~160 fold for IL6, 67 for TNF-a, ~42 for MIP2α, ~17 for MCP-1, and ~14 for IL-1β. Moreover, through ELISA assay, we observed a significantly higher amount of TNF-α and IL-6 protein in the conditioned media, after treatment with 2.5 µg/ml of PCSK9. We observed the same, significant, trends also in human macrophages, even if the increase after the higher concentration with PCSK9 was not so huge as in THP-1 macrophages and even if they seem to be more sensitive to resistin. In order to verify the activation of NF-kB pathway in THP-1 macrophages, we evaluated cAMP intracellular levels after the tratments and, in response to the higher concentration of both resistin and PCSK9, we observed a significant increase.
These results support a possible pro-inflammatory activity of PCSK9, potentially with a resistin-like activity. In the future, it will be determined the involvement of LDL receptor and CAP1 receptor, both expressed in macrophages, on the pro-inflammatory response.

DETERMINANTS OF LOW LEVELS OF BRAIN NATRIURETIC PEPTIDE IN MORBID OBESITY

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Background & Aims. Morbid obesity is associated with cardiovascular comorbidity. A noteworthy feature of this relationship could regard low levels of brain natriuretic peptide (BNP). The study investigates the relationship between BNP and obesity-related markers in a morbid obese population, along with echocardiographic and vascular parameters.
Methods: In 154 morbid obese patients we evaluated anthropometric parameters, glycometabolic/lipid profile, bioimpedenziometry, echocardiography, visceral fat area and flow-mediated dilation (FMD) by ultrasonography.
Results. We divided population in two groups on the basis of median BMI levels; patients with higher BMI had significantly lower BNP (p=.008), FMD (p=.014) and HDL-C (p=.001) and showed a more impaired heart function. A similar trend emerged subdividing patients on the basis of median visceral fat area. BNP showed a significant inverse correlation with BMI (p<.001), left ventricular mass (p=.026) and inter-ventricular septum thickness (p=.007) and a significant positive correlation with FMD (p=.008), HDL-C (p=.022), and ejection fraction (p=.013). BMI and triglycerides were independent predictors of BNP levels.
Conclusions. Patients with higher BMI show lower BNP levels associated with greater total body fat amount. The correlation of BNP with endothelium-dependent vasodilation and cardiac impairment could represent another link between obesity and cardiovascular damage.

MALATTIA CORONARICA E PROPROTEINA CONVERTASI SUBTILISINA/KEXINA 9: UN NUOVO ATTIVATORE DELLA RISPOSTA PIASTRINICA

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Introduzione. La proproteina convertasi subtilisina/kexina 9 (PCSK9) svolge un ruolo nell'omeostasi del colesterolo LDL (LDLC), incrementando la degradazione epatica del recettore delle lipoproteine a bassa densità (LDLR), risultando così in un aumento del LDLC. Dati recenti della letteratura indicano come i livelli plasmatici di PCSK9 predicano eventi cardiovascolari ricorrenti in pazienti con angina stabile (SA), anche quando il livello di LDLC sia ben controllato. Studi sperimentali suggeriscono che il contributo di PCSK9 agli eventi cardiovascolari potrebbe essere mediato anche attraverso meccanismi diversi dal legame con

LDLR che ad oggi tuttavia non sono ancora noti. Le piastrine sono attori importanti nell'aterosclerosi e la loro attivazione svolge un ruolo fondamentale non solo nelle complicazioni trombotiche ma anche nella progressione della malattia. Ben nota è l'aumentata reattività piastrinica descritta nelle malattie cardiovascolari e nel diabete mellito di tipo II (DM), noto fattore di rischio cardiovascolare. Recentemente è stata valutata la relazione tra PCSK9 e gli indici piastrinici in pazienti con malattia cardiovascolare e si è osservata un'associazione positiva tra i livelli plasmatici di PCSK9 e la conta piastrinica. Non ci sono tuttavia ad oggi studi che abbiamo valutato l'effetto diretto di PCSK9 sui parametri di attivazione piastrinica; pertanto lo scopo di questo studio è stato quello di:
1) comprendere se PCSK9 moduli attivazione e aggregazione piastrinica;
2) valutare se sia espresso nelle piastrine di soggetti sani e se tale espressione venga alterata in pazienti SA+, DM+ e DM-, al fine di comprendere se PCSK9 sia coinvolto nei meccanismi che conducono all'iperreattività piastrinica caratteristica dei pazienti SA+/DM+.
Materiali e Metodi. L'effetto di PCSK9 (5ug/ml) sull'attivazione piastrinica è stato valutato sia mediante aggregazione indotta da epinefrina (in concentrazioni che non inducano risposta aggregante massimale), con metodo di Born, in plasma ricco in piastrine (PRP), sia attraverso la valutazione dell'espressione dei marcatori di attivazione piastrinica (P-selettina, glicoproteina IIb/IIIa attivata, PAC-1, Fattore Tessutale, TF) mediante citofluorimetria in sangue intero. Con quest'ultimo metodo è stata analizzata anche l'espressione di PCSK9 nelle piastrine in sangue intero. Inoltre i livelli di PCSK9 sono stati misurati in lisati di piastrine isolate dal sangue intero da 30 pazienti SA+, 15 DM+ e 15 DM-, 10 pazienti SA-/DM+ e 10 volontari sani (VS) mediante ELISA.
Risultati. PCSK9 potenzia significativamente l'aggregazione piastrinica indotta da concentrazioni di epinefrina che non stimolano in modo massimale (0.6 µM; +40% AUC; +78% slope; -15% lag time; +15% massima aggregazione; $p<0,05$). A sostegno di questa evidenza, abbiamo osservato un'aumentata espressione di PAC-1, P-selettina e TF (+50%, +40%, +25%, rispettivamente) in piastrine stimolate da epinefrina+PCSK9 rispetto a quelle stimolate solo con epinefrina. In aggiunta, mediante citofluorimetria abbiamo osservato che il 20% delle piastrine è PCSK9+ e 1/6 di esse è anche TF+. L'analisi quantitativa dei livelli di PCSK9 mediante ELISA ha mostrato che le piastrine isolate da pazienti SA+/DM+ contengono una quantità doppia di proteina rispetto agli altri gruppi (20±5 pg/µg proteina, $p<0,001$) e questo si associa ad una maggiore attivazione piastrinica in termini di livelli basali di espressione di PAC-1, P-selettina e TF.
Conclusione. Questi dati mostrano per la prima volta che PCSK9 è espressa nelle piastrine umane e che livelli significativamente maggiori di proteina sono presenti in pazienti SA+/DM+ rispetto ai pazienti SA+/DM-, ai pazienti SA-/DM+ e ai VS. In aggiunta abbiamo osservato che la proteina svolge un ruolo nell'attivazione e nell'aggregazione piastrinica. Considerando la rilevanza del contributo piastrinico alle malattie cardiovascolari, questi risultati forniscono nuove conoscenze utili per chiarire la basi molecolari dell'iperreattività piastrinica in pazienti SA+ e, in particolare, in pazienti SA+/DM+.

PRO-INFLAMMATORY CYTOKINES AND ADIPOKINES REGULATE PCSK9 EXPRESSION IN HEPG2 CELLS

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Background and Aim. Clinical, genetic and experimental evidence indicates that proprotein convertase subtilisin/kexin 9 (PCSK9) may be either a cause or an effect of metabolic syndrome (MetS). We have recently demonstrated that PCSK9 is regulated by pro-inflammatory cytokine TNF-α in a SOCS3-dependent manner (Ruscica et al., JBC, 2016). Thus, the present work aimed to further extend this observation and studied the possible molecular mechanisms linking the effects of cytokines (TNF-α) and adipokines (leptin and resistin) on de novo lipogenesis and PCSK9 expression.
Methods. Human hepatocellular liver carcinoma cell line (HepG2) and HepG2 overexpressing PCSK9 (HepG2_PCSK9) were used as in vitro tools. qPCR, Western blot, ELISA and luciferase reporter assays, together with siRNA directed to STAT3 and SOCS3, were used.
Results. HepG2 cells express leptin (ObRb) and resistin (Adenylyl cyclase-associated protein 1, CAP1) receptors; the overexpression of PCSK9 in HepG2 (HepG2_PCSK9) does not alter that of ObRl and CAP-1. Fourty-eight-h treatment of HepG2 with TNF-α (10 ng/mL), leptin (200 ng/mL) and resistin (50 ng/mL) induced the expression of both PCSK9 (2.3-, 2.0- and 3.5-fold, respectively) and JAK/STAT pathway (3-, 1.8- and 1.9-fold, respectively). TNF-α and leptin increased the amount of secreted PCSK9 by 15%. Only leptin stimulated PCSK9 promoter activity after 24- and 48-h treatment (+52 and +26%, respectively). TNF-α, leptin and resistin induced the gene expression of apoB, sterol regulatory element-binding protein 1 (SREBP1), stearoyl-CoA desaturase-1 (SCD-1), fatty acid synthase (FAS) and microsomal triglyceride transfer protein (MTP). These effects, along with the activation of PCSK9, were inhibited by transfection with siRNA anti-STAT3, suggesting the involvement of the JAK/STAT pathway.
Conclusions. Pro-inflammatory cytokines and adipokines up-regulate PCSK9 expression and the key genes involved in the de novo lipogenesis. Future analyses will investigate the potential role of JAK/STAT pathways in mediating these effects.

EFFECT OF SOY ON METABOLIC SYNDROME AND CARDIOVASCULAR RISK FACTORS: EVIDENCE FROM A RANDOMIZED CONTROLLED TRIAL

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Background. Cardiovascular diseases are currently the commonest cause of death worldwide. Different strategies for their primary prevention have been planned, taking into account the main known risk factors, which include an atherogenic lipid profile and visceral fat excess. Functional foods and nutraceuticals have been assessed in several clinical studies and meta-analytical have indicated these as effective approaches for the management of primary CVD risk in the MetS.
Aim. To assess the effects of a low lipid diet with whole soy foods, on abdominal adipose tissue and related adipokines, lipid/lipoprotein profiles and glucose metabolism, and to compare them with the effects of standard low lipid diet with animal protein.
Methods. Randomized, parallel, single-center study with a nutritional intervention duration of 12 weeks. Whole soy foods corresponding to 30 g/day soy protein were given in substitution of animal foods containing the same protein amount.
Results. The soy nutritional intervention resulted in a reduction of the number of MetS features in 13/26 subjects (p=0.094 vs control diet). In the soy group we observed a significant improvement of median percentage changes for body weight (-1.5%) and BMI (-1.5%), as well as for atherogenic lipid markers, namely TC (-4.85%), LDL-C (-5.25%), non-HDL-C (-7.14%) and apoB (-14.8%). These changes were not affected by those in BMI. Since the majority of the studied variables were strongly correlated, three factors were identified which explained the majority (52%) of the total variance in the whole data set. Among them, factor 1, which loaded lipid and adipose variables, explained the 22% of total variance, showing a statistically significant difference between treatment arms (p=0.002).
Conclusions. The inclusion of whole soy foods (corresponding to 30 g/day protein) in a lipid-lowering diet significantly improved a relevant set of biomarkers associated to cardiovascular risk.

RELAZIONE FRA DUCTH SCORE E CONFERMA GENETICA DI IPERCOLESTEROLEMIA FAMILIARE. ESPERIENZA VERONESE DELLO STUDIO LIPIGEN

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L'ipercolesterolemia familiare (FH) è una malattia genetica in cui gli elevati valori di LDL-colesterolo sono responsabili di malattia cardiovascolare prematura. La diagnosi molecolare dei difetti genetici alla base delle forme FH clinicamente diagnosticate permette di realizzare interventi più incisivi per la prevenzione e cura delle malattie cardiovascolari ad essa correlate. Scopo del presente lavoro è stato di valutare la relazione fra parametri clinici di diagnosi di FH (DUCTH score) e la conferma genetica di mutazioni patogenetiche per FH.
Metodi. Da giugno 2012 a maggio 2016 sono stati valutati 81 soggetti (31M, 50F) partecipanti al Progetto LIPIGEN. L'origine geografica era così suddivisa: Veneto 57 soggetti, Lombardia 8, Trentino 6, Puglia 2, Sardegna 2, Piemonte 1, Sicilia 1; 3 avevano origine sudamericana, e 1 africana. In tutti i pazienti sono stati valutati parametri clinici quali storia personale e familiare per dislipidemia, malattia cardiovascolare, esame obiettivo, profilo lipidico, glicemia, creatinina, transaminasi, TSH, terapia ipolipemizzante ed esami strumentali. In tutti è stato calcolato il DUCTH score. L'analisi genetica ha indagato sulla presenza o meno di mutazioni sui geni di LDLR, ApoB, PCSK9 e LDLRAP1.
Risultati. In 53 pazienti è stata documentata storia familiare di malattia cardiovascolare precoce, 11 con storia personale di cardiopatia ischemica precoce, 20 con ateromasia carotidea. In 48 soggetti sono state identificate mutazioni, in eterozigosi, a carico del gene LDLR e in due sorelle mutazione del gene ApoB. Fra i pazienti con mutazioni di LDLR, 43 riportavano un punteggio DUTCH score >8, in tre compreso fra 6 e 8, in 2 era di 5. Il DUCTH score delle sorelle con mutazione di ApoB era, rispettivamente di 5 e 6. Nei pazienti in cui non sono state riscontrate mutazioni, 8 presentavano DUCTH score >8, mentre in 11 compreso fra 6 e 8.
Considerazioni. Il DUCTH score è un utile strumento per la diagnosi FH; tuttavia la clinica rimane l'elemento discriminante soprattutto quando lo score è basso, non essendo possibile raccogliere dati della storia familiare, o quando gli interventi sullo stile di vita (corretta alimentazione, attività fisica) non si accompagnano ad una adeguata riduzione di LDL-colesterolo. Inoltre i pazienti con Dutch score >6 in cui non sono state documentate mutazioni dei geni di LDLR, ApoB, PCSK9, LDLRAP1, potrebbero essere rivalutati per lo studio del gene STAP1.

LA PCSK9 È PRODOTTA NEL TESSUTO ADIPOSO VISCERALE UMANO E INDOTTA DALL'INSULINA IN ADIPOCITI MATURI DIFFERENZIATI IN VITRO

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Introduzione. La proproteina convertasi subtilisina/kexina tipo 9 (PCSK9) ha un ruolo chiave nel metabolismo delle lipoproteine legandosi al recettore delle LDL e avviandolo alla degradazione lisosomiale. Ad oggi, non si hanno dati sulla PCSK9 nel tessuto adiposo umano anche se è fisiologicamente un importante tessuto per il metabolismo delle lipoproteine. Lo studio vuole verificare la presenza di PCSK9 nel tessuto adiposo viscerale (TAV) umano e, in vitro, la sua regolazione insulina-mediata utilizzando una linea cellulare di adipociti umani.
Materiali e Metodi. L'espressione genica ed i livelli proteici della PCSK9 sono stati valutati nel TAV di pazienti sottoposti a nefrectomia. Adipociti differenziati sono stati trattati con insulina e con peptide natriuretico atriale (ANP). L'analisi proteica è stata condotta sulle cellule e sul terreno di coltura per valutare la forma secreta matura della PCSK9.
Risultati. L'espressione della PCSK9 nel TAV, rispetto al tessuto epatico di controllo, risulta ben determinabile anche se molto variabile. La proteina PCSK9 nel tessuto adiposo viscerale è come nel fegato in maggior parte come pre-forma rispetto alla sua forma matura che viene secreta. I livelli di mRNA e proteina nella linea cellulare SGBS confermano:
1) l'abbondante espressione sia di pre-forma che di forma matura di PCSK9 anche nel mezzo di coltura;
2) una significativa induzione dell'espressione dopo stimolo con insulina già a 10 nM;
3) un parziale effetto inibitorio sull'induzione PCSK9 insulino-mediata da parte del sistema controregolatore ANP-mediato.

Conclusioni. Per la prima volta abbiamo dimostrato che PCSK9 è abbondantemente espressa nel TAV umano e che adipociti umani

esprimono sia la preforma che la forma matura secreta. Inoltre, PCSK9 in condizioni di glucosio fisiologiche è indotta dall'insulina e che l'ANP, antagonista dell'insulina nell'inibizione della lipolisi, è in grado di ridurne parzialmente l'effetto, suggerendo un nuovo livello di azione della PCSK9 a livello del tessuto adiposo umano.

A CASE OF "ANAPHYLACTIC LIKE" REACTION DURING LDL APHERESIS: A PATHOPHYSIOLOGICAL HYPOTHESIS ON WHITE WINE CONTAINING METABISULPHITE

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Case Presentation. This report regards a 60-year-old man affected by heterozygous familial hypercholesterolemia associated with high levels of lipoprotein (a), coronary and polidistrectual atherosclerotic disease. Patient has been treated with Atorvastatin 20 mg OD and LDL-apheresis (Heparin mediated LDL precipitation system every 2 weeks treating 4000 ml of plasma for session) for eight years. Patient was regularly treated without any relevant problem, but unexpectedly, he presented two consecutive episodes of systemic malaise characterized by sweating, paresthesias of the lips and flush. In the second episode, these symptoms were followed by bradycardia and presyncopal episode. Both events occurred after more than 2 hours treatment, and were cured with fluid administration and bolus of 500 mg methylprednisolone i.v. Blood pressure, normal before the LDL apheresis start of, was low but responsive to administration of fluids. The symptoms, however, were so grave to require the discontinuation of LDL apheresis procedures.
At the revaluation of the possible causes of these unexpected episodes, occurred in absence of technical problems and without drug therapy modifications (aspirin 100 mg OD, atenolol 50 mg OD and ramipril 5 mg OD), we observed changes in the lifestyle: at dinner, the patient drank 2 or 3 glasses of white wine craft produced. The following apheresis was performed without drinking wine and we did not report any inconvenient. Furthermore, no problems occurred in the subsequent 9 apheresis performed so far with this setting.
Discussion. The symptoms pointed out in our patient are likely imputable to the common use of metabisulphite who has a long history as a preservative in the food industry. Metabisulphite is highly appreciated in winemaking for its ability to control bacteria and apiculate yeast, without damaging S. cerevisiae cells, the main agents of the winemaking process.
Exposure to sulfite-containing compounds induced adverse clinical effects. In our patient, who had introduced white wine craft produced more than 12 hours before the symptoms onset, we can assume that sulphur dioxide compounds were stored linked to proteins, as hemoglobin. These compounds modify their molecular forms at different pH values with subsequent effects on biocidic activity against yeast and human cell cultures.
In LDL apheresis, performed with a selective elimination of low-density lipoproteins and fibrinogen by heparin precipitation at acid pH systems, plasma obtained by filtration of whole blood is continuously mixed with an equal volume of an acetate buffer (pH 4.85) containing heparin. After removal of the precipitated heparin complex by filtration, excessive heparin is adsorbed to a specially developed filter, and the clear plasma filtrate is subjected to bicarbonate dialysis/ultrafiltration to restore physiologic pH and remove excessive fluids. The "restored" plasma that joins the whole blood, however, in own patients maintains a pH of 6.85. This pH level could modify the sulphur dioxide compounds linked to protein. Furthermore, sulfite species enhance carbon monoxide release from CO-releasing molecules, as hemoglobin and have an impact on physiological buffering systems of the human organism useful to mitigate the slight pH variations secondary to LDL apheresis treatment. The pathophysiological pattern described can explain the symptoms presented by our patient.
During LDL apheresis, a case of reaction secondary to herbal remedy was described but "anaphylactic like" reaction, imputable to metabisulphite, have not been previously linked with LDL apheresis.
Nowadays, the use of handcraft production leads to an increased risk of interactions between drugs or therapeutic procedures (as LDL apheresis) with serious consequences, as spontaneous taking medications/herbal products and nutrition with semi-prepared foods containing food-additives.

RUOLO DEL TEST AL CICLOERGOMETRO NEL DOLORE TORACICO AD INTERMEDIO-BASSO RISCHIO

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Scopo dello studio. Il dolore toracico rappresenta uno dei principali motivi di accesso al Dipartimento di Emergenza. Scopo dello studio è analizzare il valore diagnostico e predittivo del test al cicloergometro per eventi cardiovascolari maggiori nel breve-medio termine in soggetti con dolore toracico e rischio medio-basso di SCA assistiti presso la Clinica Medica 1 dell'Università di Padova. Inoltre sono state esaminate le caratteristiche cliniche dei pazienti per individuare i fattori di rischio in grado di influenzare la sensibilità e specificità della prova.
Metodi. 302 pazienti (185 maschi, 117 femmine) sottoposti a test da sforzo per dolore toracico o sintomi equivalenti suddivisi in due gruppi: a) soggetti con test positivo o dubbio (105 pazienti di cui 47 'Positivi' e 58 'Dubbi'); b) 165 soggetti 'Negativi' e 32 'Non valutabili'. Sono stati rilevati: fattori di rischio cardiovascolare, caratteristiche del dolore toracico, ECG iniziale, TroponinaI, rischio di morte e complicanze per SCA mediante il TIMI score, l'HEART score e l'algoritmo ACC/AHA per la gestione della angina instabile/NSTEMI e la probabilità pre-test per CHD. E' stato analizzato: l'outcome a 12 mesi per verificare la prevalenza di eventi cardiovascolari maggiori.
Risultati. Il test ha mostrato una sensibilità dell'82,7% e una specificità dell'71,1%; il valore predittivo positivo e negativo è stato rispettivamente del 40% e 95,1%. Sulla base del reale stato di malattia coronarica, sono stati individuati 52 soggetti CHD e 239 controlli; i soggetti CHD risultano avere un'età più elevata (66,08 anni vs 58,87; p=0,002), maggiore prevalenza di maschi (78,8% vs 57,3%; p=0,005), di anamnesi positiva per malattia cardiovascolare (56,9%, p<0,001), di ipertensione (76,4%, p=0,005), di dislipidemia (58,8%, p=0,047) e di fumo (58,8%, p=0,013). Maggiore presenza tra i soggetti CHD di dolore tipico. All'analisi multivariata, i fattori maggiormente associati a CHD sono l'anamnesi positiva per pregressi eventi cardiovascolari (OR=3,996; p<0,001); e il tabagismo (OR=2,125; p=0,033).

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

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Background and Aims. Prediabetes is associated with an increased risk of developing diabetes and cardiovascular disease. Our objective was to examine the cardiovascular (CV) risk profile of nondiabetic patients with and without prediabetes according to HbA1c using macroangiopathic imaging biomarkers.
Methods. Our population consisted in 272 non diabetic patients aged between 40 and 70 years, with a normal fasting plasma glucose (FPG < 5.6 mmol/L) and at least 1 CV risk factor. Exclusion criteria were prior history of CV disease or clinical evidence of advanced renal disease. Prediabetes was defined based on American Diabetes Association (ADA) criteria. Coronary artery calcium (CAC) score as well as mean common carotid intima media thickness (IMT) and plaque presence were assessed using consensus criteria.
Result and Conclusion. CAC score and mean IMT were higher in the prediabetes group compared to non prediabetic subjects (p value for all <0.001). The proportion of prediabetic patients with CAC=0 was significantly lower compared to controls (35% vs 63%, p<0.01). In opposite, the proportion of patients with a CAC>400 was significantly higher in the prediabetes group (10% vs 3%, p<0.05). Moreover, carotid plaques were significantly more present in patients with prediabetes than in the normoglycemic subjects (p<0.01). Multivariate analysis showed that both CAC and mean IMT were independently associated to HbA1c (p value for all <0.001). Among patients with normal fasting glucose, HbA1c increase is associated with higher coronary and peripheral atherosclerotic burden in non-diabetic patients.

VARIANTE GENETICA C.1402G>A (EXON 10 LDLR)

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L'ipercolesterolemia familiare (familial hypercholesterolaemia, FH) è la più frequente tra le cause genetiche di malattia coronarica precoce (coronary heart disease, CHD), in quanto causa l'esposizione per tutta la durata della vita a elevati livelli di colesterolemia LDL. La FH eterozigote e causata da mutazioni (in eterozigosi) con perdita di funzione nel gene LDLR, da mutazioni (in eterozigosi) nel gene APOB che alterano il dominio di legame dell'apolipoproteina B con il recettore LDL o da mutazioni (in eterozigosi) con guadagno di funzione nel gene PCSK9 .Attualmente, in tutto il mondo, sono state documentate oltre 1.200 mutazioni nel LDLR; queste modificano tutti i domini funzionali della proteina recettoriale e comprendono singole mutazioni nucleotidiche, variazioni del numero di copie e mutazioni di splicing distribuite in tutto il gene LDLR.
Vi presento il caso di un uomo di 40 aa valutato in data 08/01/2016 presso il servizio di Day Service della U.O di Medicina Interna del Garibaldi Nesima di Catania per una ipercolesterolemia pura. Nell' anamnesi familiare si è evidenziata una familiarità per malattie cardiovascolari in età precoce (padre IMA a 55 aa, un fratello IMA a 42 aa). Il soggetto assumeva, su prescrizione del medico curante, atorvastatina 10 mg una cpr al giorno da Ottobre 2015. Non praticava attività sportiva, non fuma, non è iperteso (PA 120/80 mmHg), non è diabetico e ha un BMI di 25,6 kg/m^2. All'esame obiettivo non sono stati riscontrati arco corneale o xantomi tendinei. Agli esami di laboratorio l'assetto lipidico era il seguente: Colesterolo totale 194 mg/dl - HDL 46 mg/dl - TG 100 mg/dl - LDL 128 mg/dl (dopo correzione per la terapia statinica praticata: 204 mg/dl). Il punteggio al Dutch score era 4, rientrando tra i casi possibili di FH. E' stata cosi effettuata l'analisi genetica presso il Laboratorio di Genetica Molecolare e Genomica Cardiovascolare dell' Ospedale Bassini di Milano. All'analisi genetica è stata riscontrata una variante genetica riguardante una singola mutazione nucleotidica c.1402G>A a livello dell esone 10 del gene LDLR (proteina identificata p.Val468Ile). Tale mutazione è stata catalogata come possibile variante patogenica di FH.
Discussione. in letteratura un solo lavoro descrive tale mutazione e pare abbia un effetto neutrale sulla funzione del recettore LDL. Dato che non c'e una validazione in letteratura della patogenicità di tale mutazione, per stabilire se tale genotipo è associato ad elevati valori di col. LDL, abbiamo comunque eseguito uno screening genetico a tutti i familiari.

VARIANTE GENETICA C.1118G>A - ESONE 8 LDLR (POTENZA-2)

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L'ipercolesterolemia familiare (familial hypercholesterolaemia, FH) è la più frequente tra le cause genetiche di malattia coronarica precoce (coronary heart disease, CHD), in quanto causa l'esposizione per tutta la durata della vita a elevati livelli di colesterolemia LDL. La FH eterozigote e causata da mutazioni (in eterozigosi) con perdita di funzione nel gene LDLR, da mutazioni (in eterozigosi) nel gene APOB che alterano il dominio di legame dell'apolipoproteina B con il recettore LDL o da mutazioni (in eterozigosi) con guadagno di funzione nel gene PCSK9 .Attualmente, in tutto il mondo, sono state documentate oltre 1.200 mutazioni nel LDLR; queste modificano tutti i domini funzionali della proteina recettoriale e comprendono singole mutazioni nucleotidiche, variazioni del numero di copie e mutazioni di splicing distribuite in tutto il gene LDLR.
Vi presento il caso di un uomo di 30 aa valutato in data 08/06/2016 presso il servizio di Day Service della U.O di Medicina Interna del Garibaldi Nesima di Catania per una ipercolesterolemia pura. In anamnesi familiare sia la madre che la sorella affette da ipercolesteroplemia pura (Col.totale >310 mg/dL e Col. LDL >200 mg/dL) ed all' esame obiettivo la madre presentava arco corneale bilaterale. Il soggetto non assumeva nessuna terapia ipocolesterolemizzante. Non praticava attività sportiva, non fuma, non è iperteso (PA 120/80 mmHg), non è diabetico e ha un BMI di 20,3 kg/m^2. All'esame obiettivo non sono stati riscontrati arco corneale o xantomi tendinei. Agli esami di laboratorio l'assetto lipidico era il seguente: Colesterolo totale 424 mg/dl - HDL 61 mg/dl - TG 110 mg/dl -

LDL 341 mg/dl. Il punteggio al Dutch score era 10, rientrando tra i casi definiti di FH. È stata cosi effettuata l'analisi genetica presso il Laboratorio di Genetica Molecolare e Genomica Cardiovascolare dell'Ospedale Bassini di Milano. All'analisi genetica è stata riscontrata una variante genetica riguardante una singola mutazione nucleotidica c.1118G>A a livello dell esone 8 del gene LDLR (proteina identificata p.Gly373Asp). Tale mutazione è stata catalogata come possibile variante patogenica di FH.
Discussione. In letteratura 4 lavori descrivono tale mutazione in diverse popolazioni ed è stata designata come Potenza-2 e fino ad ora pare non sia stata valutata l' attività recettoriale residua del recettore LDL. Dato che non c'e una validazione in letteratura della patogenicità di tale mutazione, per stabilire se tale genotipo è associato ad elevati valori di col. LDL, abbiamo intanto eseguito uno screening genetico a tutti i familiari.
Il passo successivo, se tale mutazione venisse confermata nei familiari, è valutare l' attività recettoriale residua del recettore LDL.

ROLE OF LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 IN ISCHEMIC STROKE PATIENTS TREATED WITH TPA THROMBOLYSIS: THE MAGIC STUDY

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Background. Lipoprotein-associated phospholipase A2 (Lp-PLA2), a member of phospholipase A2 proteins family that plays a key role in the metabolism of pro-inflammatory phospholipids, as oxidized low-density lipoproteins, and in the generation of pro-atherogenic metabolites, including lysophosphatidylcholine and oxidized free fatty acids which may affect vessel walls. Several studies have demonstrated that LpPlA2 is an independent risk marker for ischemic stroke. However, scarce data exist in IS patients treated with tPA. We evaluated the role of LpPlA2 on adverse clinical outcomes in tPA treated IS.
Methods. Blood was taken at baseline (B) and 24 hours after tPA from 327 patients (mean age 68, mean NIHSS 11). LpPlA2 activity levels were measured by immunoturbidimetry, and inflammatory markers by Bioplex assay. B, post-tPA and pre-/post-thrombolysis variations of LpPlA2 activity were analyzed according to SICH, death and 3-month mRs.
Results. LpPlA2 activity levels at B were significantly higher in males than in females [F 172 (140-199.5) nmoL/min/mL, M 200 (165-235) nmoL/min/mL, p<0.001]. At baseline, no significant correlations between age, glycemia, NIHSS, IL-6, CRP, white blood cell count, erythrocyte sedimentation rate, vWF levels and LpPlA2 activity were observed. A slight, but significant correlation was found between baseline total cholesterol levels and LpplA2 (r=0.194 p=0.002). B LpPlA2 activity levels did not differ between patients with and without SICH and between patients who died or who were alive at 3-month of follow-up [SICH: 219(177-254) vs 184(150.2-223) nmoL/min/mL; 3-month death: 176(140.8-220.5) vs 185 (153-220) nmoL/min/mL]. B LpPlA2 activity levels were significantly higher in patients with mRS 3-6 than patients with mRS 0-2 [194(161-232) vs 182(145-220) nmoL/min/mL; p<0.05], whereas neither post-tPA LpPlA2 activity nor pre-/post-thrombolysis variations of LpPlA2 activity differ in relation to mRS. At logistic regression analysis, after adjustment for major clinical determinants of outcomes, B LpPlA2 activity remain a significant and independent determinant of mRS 3-6 [OR (95% CI) 1.37 (1.07-1.76), P=0.012 for every 50 nmol/min/mL increase]. Conclusions: Our data suggest that LpPlA2 may contribute to the pathophysiological mechanism of IS and poor outcomes after tPA, suggesting that a vascular inflammation may have a detrimental role in this clinical setting.

LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 CIRCULATING LEVELS IN THE GENERAL POPULATION OF THE MONTIGNOSO STUDY

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Background. Lipoprotein-associated phospholipase A2 (Lp-PLA2), a member of phospholipase A2 proteins family that plays a key role in the metabolism of pro-inflammatory phospholipids, as oxidized low-density lipoproteins, and in the generation of pro-atherogenic metabolites, including glysophosphatidylcholine and oxidized free fatty acids which may affect vessel walls. Several studies have demonstrated that LpPlA2 is an independent risk marker for cardiovascular disease. However, few data about the relationship between LpPlA2, lipoprotein(a) [Lp(a)] and calcium score exist in the general population.
Methods. LpPlA2 activity levels were measured by immunoturbidimetry, lipoprotein(a) by using an immunoturbidimetric assay and calcium score as CACS Agaston score in 307 subjects of Montignoso Study.
Results. LpPlA2 activity levels were significantly (p 187 nmoL/min/mL), subjects with CACSAg >=10 had significantly (p<10 (31%). No association between CVS >1, hsCRP >3mg/L and Lp-PlA2 tertiles.
Discussion. Our data suggest that LpPlA2 may contribute to the pathophysiological mechanism in the early phase of atherosclerotic plaque formation, suggesting that a vascular inflammation may have a detrimental role.

DIFFERENZE DEI FATTORI DI RISCHIO CARDIOVASCOLARI TRA UOMINI E DONNE NELLA SINDROME CORONARICA ACUTA

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Ci siamo proposti di indagare il differente controllo dei fattori di rischio cardiovascolari quali colesterolemia totale, LDL, HDL, apolipoproteina A, apolipoproteina B, rapporto apolipoproteina B/A1 ed Lp(a) all'esordio della malattia aterosclerotica nei due diversi generi.
Abbiamo arruolato in modo consecutivo 539 pazienti con diagnosi di Sindrome Coronarica Acuta (STEMI, NSTEMI, Angina Instabi-

le) documentata tramite evidenza coronarografica di lesioni ostruttive (>75%), afferenti presso la nostra UTIC successivamente divisi in due gruppi: 1 (uomini) e 2 (donne).
Nel campione analizzato il 77% dei pazienti sono uomini e il 23% donne.
Le donne sono più anziane all'esordio della cardiopatia ischemica o in corso di recidiva. Probabilmente perché più anziane le donne risultano più spesso ipertese (83% vs 63%), diabetiche e con ridotta funzionalità renale.
Non sono emerse differenze significative riguardo ai valori di colesterolemia totale, LDL e apolipoproteina B.
Nelle donne i valori di trigliceridemia sono inferiori rispetto agli uomini (133 mg/dl vs 151 mg/dl), mentre quelli di HDL sono mediamente più elevati (46 mg/dl vs 39 mg/dl).
Nelle donne i livelli di apoA1 risultano più elevati (125 mg/dl vs 111 mg/dl) e di conseguenza il rapporto apolipoproteina B/apolipoproteina A1 (0,81 vs 0,97) appare inferiore.
I livelli plasmatici di Lp(a) sono risultati elevati nel 37% della popolazione totale e in particolare nelle donne in cui la concentrazione media è di circa 40 mg/dl: la differenza fra i generi non è risultata statisticamente significative.
Dai nostri dati appare che la distribuzione dei fattori di rischio per sindrome coronarica acuta sia sesso dipendente e che, alla luce della diversa prognosi post SCA in alcune sottopopolazioni di pazienti, possano essere necessarie diverse strategie terapeutiche.

BARRIERE E FACILITATORI PER L'ATTIVITA' FISICA IN PREVENZIONE CARDIOVASCOLARE PRIMARIA

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Introduzione. La sedentarietà in Italia è in crescita (Dati PASSI: 29% nel 2008 a 34% nel 2015) e prevale in soggetti immigrati, con bassi livelli di istruzione e socio-economico. Identificare barriere e facilitatori per l'attività fisica (AF) potrebbe contribuire ad adottare strategie personalizzate di intervento. Tali informazioni possono essere ricavate attraverso il Colloquio Motivazionale (CM), uno stile collaborativo di conversazione mirato a rafforzare la motivazione e l'impegno al cambiamento della persona.
Obiettivo. Individuare barriere e facilitatori per l'AF in un campione di soggetti sedentari partecipanti a un Programma di Salute Cardiovascolare (CV).
Materiali e Metodi. Il livello di AF dei primi 260 partecipanti a ProSALUTE, un programma di prevenzione primaria dedicato ai residenti adulti di un quartiere periferico di Milano (Ponte Lambro), è stato indagato utilizzando lo score PASSI. I soggetti sedentari sono stati invitati a svolgere incontri di CM, condotti da un ricercatore infermieristico formato in merito. Il contenuto degli incontri, audio registrato previo consenso, e trascritto ad verbatim, è stato sintetizzato in categorie di significato, sia di barriere che di facilitatori.
Risultati. La prevalenza di sedentarietà è stata del 24% (n=63). A 18 soggetti non è stato proposto il CM per problemi psicofisici o linguistici. 21 soggetti, in prevalenza immigrati e disoccupati, hanno rifiutato il CM (chi quadro: p=0.042 e p=0.006, rispettivamente). Sono state eseguiti 43 CM con 24 soggetti. Le categorie di facilitatori prevalentemente riferite sono state la buona percezione della proprie capacità di eseguire AF (Self efficacy) e l'adeguato supporto sociale. Carenze nelle medesime categorie sono state le barriere maggiormente riportate.
Conclusioni. Nell'ambito della prevenzione CV primaria, self efficacy e supporto sociale sono sia le barriere che i facilitatori prevalenti e rappresentano possibili target di intervento per favorire l'adozione di uno stile di vita fisicamente attivo.

CAUSATIVE MUTATIONS AND PREMATURE CARDIOVASCULAR DISEASE IN PATIENTS WITH SEVERE HYPERCHOLESTEROLEMIA

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Familial Hypercholesterolemia (FH) is a common lipid disorder, caused by mutations affecting the clearance of LDL cholesterol. FH patients have elevated LDL cholesterol and if untreated develop premature cardiovascular disease (CVD). Young adults, attending our Lipid Clinic, with LDL> 190mg/dL, after excluding Familial Combined Hyperlipidemia, were included in the study. Search for causative mutations, MedPed/WHO score and non-invasive ultrasound examination of carotid arteries were performed. Among the 225 patients there were 179 with LDLR mutations, 2 with apo B gene Mutations, 1 PCSK9 gene mutation, and 43 patients without mutations. Among 171 unrelated index cases there were 129 mutations (75 %). Among patients with LDLR mutations, we found 13 homozygotes or compound heterozygotes, 83 patients with radical mutations and 83 with missense mutations. An increase has been detected in LDL cholesterol, prevalence of xanthoma and of carotid plaques, among different categories of LDLR mutations or increasing MedPed/WHO score (all p<0.001). History of premature CVD was more prevalent in homozygotes/compound heterozygotes as compared to patients with other mutations (p=0.012) and in patients with MedPed/WHO score >8 as compared to those with score <8 (p<0.001), MedPed/WHO score (p<0.001), after controlling for age, gender, smoking habits , blood pressure. In all patients, with LDL>190 mg/dL, after excluding those with Familial Combined Hyperlipidemia, intensive lipid lowering treatment is indicated. Molecular analysis and MedPed/WHO score are helpful in identifying priorities for treatment with PCSK9 inhibitors. Prevalence of plaques is strongly associated with level of LDL cholesterol, even in a cohort of patients who had severe hypercholesterolemia.

IDENTIFICATION OF A NOVEL LMF1 NONSENSE MUTATION RESPONSIBLE FOR SEVERE HYPERTRIGLYCERIDEMIA BY TARGETED NEXT-GENERATION SEQUENCING

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Background. Severe hypertriglyceridemia (HTG) may result from mutations in genes affecting the intravascular lipolysis of triglyceride rich lipoproteins.
Objective and Methods. The aim of this study was to investigate monogenic causes of severe HTG by a targeted Next Generation Sequencing (NGS) approach to capture the coding exons and intron/exon boundaries of 18 genes affecting the main pathways of triglyceride synthesis and metabolism.
Results. The targeted resequencing of candidate genes of severe HTG led to the discovery of a novel homozygous nonsense mutation in one patient with severe hypertriglyceridemia. The mutation causes a C>G substitution in exon 9 (c.1380C>G), leading to a premature stop codon (W460X). The clinical and molecular familial cascade screening allowed the identification of two additional affected siblings and seven heterozygous carriers of the mutation. Homozygosity for the c.1380C>G mutation resulted in a severe HTG phenotype in the proband (II-2) while two other siblings (II-1 and II-4) showed mild to moderate HTG suggesting a different and variable pattern of penetrance among carriers of the same mutation of the LMF1 gene. None of them have suffered of acute pancreatitis or recurrent abdominal pain.
Conclusions. We describe the third novel nonsense mutation of LMF1 gene (c.1380C>G -p.Y460X) identified by a customized NGS panel for targeted gene sequencing of 18 genes involved in hypertriglyceridemia. More studies are needed to understand the reasons of the phenotypic variability of the same molecular defect in the same family.

BICUSPID AORTIC VALVE AND LIPOPROTEIN (A)

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Background. In bicuspid aortic valve (BAV), the most frequent congenital cardiopathy, the presence of calcification and stenosis (SAo) predicts a worse prognosis. In our study, we investigated the association between Lp(a) levels, kringle IV type 2 (KIV2) repeat number polymorphism, and the presence of calcification in BAV patients.
Methods. Sixty-nine patients [79.7% males; median age: 45(30-53) yrs],admitted at the Center for Cardiovascular Diagnosis or Referring Center for Marfan syndrome and related disorders, AOU Careggi-University of Florence, from June to November 2014, were investigated. Blood withdrawal was performed in order to determine Lp(a) levels (Randox Immunoturbidimetric Assay) and KIV2 repeat number. Patients were compared with 69 control subjects [78% males; median age: 52(50-54) yrs].
Results. BAV type 1 was present in 86.9% of patients (n=60), remaining patients showed BAV type 2. In 58.5% of patients, calcifications were not present; among remaining patients, mild calcifications in 27.7%, moderate in 9.2% and severe in 4.6% were detected. BAV patients did not show significantly different Lp(a) levels with respect to controls, with a trend towards lower values [108(58-190) mg/L vs 126(51-346), p=0.487]; a significantly higher KIV2 repeat number (9 vs 6, p=0.028) was documented. Lp(a) levels were significantly higher in patients with valvular calcifications [no-calcifications: 84(47-163) mg/L, mild or moderate: 138(75-192) mg/L; severe: 421(241-1782) mg/L, p=0.019], with a lower KIV2 repeat number. Higher Lp(a) levels in patients with SAo (105 mg/L vs 187, p=0.032) with lower KIV2 repeat number were documented.
Conclusions. In this study, we demonstrated for the first time an association between calcifications in BAV patients, KIV2 repeat number and Lp(a) levels measurement. Based on these information, needing a confirmation on larger populations, Lp(a) levels and KIV2 repeat number represent possible risk markers useful to stratify, among BAV patients, those with higher chance to develop valvular calcifications and aortic stenosis.

STIFFNESS ARTERIOSA E CONTENUTO MINERALE OSSEO IN UNA COORTE DI DONNE IN POST-MENOPAUSA

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Introduzione. Le malattie cardiovascolari e l'osteoporosi sono entrambi cause comuni di morbidità e mortalità. Fino a poco tempo fa queste due patologie erano considerate indipendenti; da alcuni anni è stato evidenziato come l'osso ed il sistema vascolare condividano diverse caratteristiche strutturali, sia a livello cellulare, che molecolare. È stato dimostrato come nei pazienti con osteoporosi sia frequente il riscontro di calcificazioni vascolari che rappresentano un importante fattore di rischio cardiovascolare. Con il nostro gruppo di lavoro abbiamo valutato la presenza di correlazioni tra grado di mineralizzazione ossea e compromissione vascolare in donne post-menopausali.
Materiali e Metodi. Abbiamo studiato una coorte di donne post-menopausali (n=35); età =64.3±9.4 anni) afferite agli ambulatori della UOC Medicina 1 per una valutazione del rischio osteometabolico e risultate essere osteopeniche o osteoporotiche all'esame DXA. Come controllo abbiamo preso in esame un gruppo di donne sane di pari età. Le donne di entrambi i gruppi sono state sottoposte ad un esame ecocolordoppler dei tronchi sovra-aortici con ecografo Esaote Mylab dotato di software per la misurazione automatica del QIMT e dei parametri di stiffness arteriosa (PWV, AI α e β index).
Risultati. Nelle donne con osteoporosi/osteopenia abbiamo riscontrati una correlazione inversa tra la Bone Mineral Density (BMD) e la stiffness arteriosa. Tale correlazione ha raggiunto la significatività statistica sia per la BMD del collo femorale (R= -0.30; p<0.05) che a livello del femore totale (R=-0.33; p<0.05).

Un trend simile è stato osservato anche per quanto riguarda la relazione fra BMD e IMT.
Nel gruppo di donne sane non abbiamo riscontrato associazioni significative tra parametri di rigidità vascolare e BMD.
Conclusione. I risultati di questo studio preliminare evidenziano, nelle pazienti affette da osteopenia/osteoporosi, una correlazione inversa fra BMD e Pulse Wave Velocity.Questi dati sembrano confermare l'esistenza di un cross-talking fra tessuto osseo e vasi.

METABOLOMICS BY NUCLEAR MAGNETIC RESONANCE IDENTIFIES PATIENTS WITH HIGH RISK OF DEATH WITHIN TWO YEARS AFTER A CARDIOVASCULAR EVENT: THE CASE OF THE AMIFLORENCE II STUDY

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Background. Risk stratification and management of patients with acute coronary syndromes (ACS) is challenging. Aim of this study was to evaluate the possible role of metabolomics in the prognostic stratification of ACS patients.
Methods. 918 patients (345 females, 633 males, median age 74) were enrolled; among these 146 died (negative outcome), whereas 832 showed a positive outcome within 2 years from the cardiovascular event. Patients serum samples were analyzed via high resolution Proton Nuclear Magnetic Resonance and the obtained spectra were used to characterized the metabolic profiles of the two cohorts of patients. Multivariate statistics and a Random Forest classifier were used to create a prognostic model for the prediction of death within 2 years from the cardiovascular event.
Results. In the training set (n=80 positive outcomes, n=80 negative outcomes), metabolomics showed significant differential clustering, with a good separation of the two outcomes cohorts. A prognostic risk model predicted death with sensitivity, specificity, and predictive accuracy of 78.5% (95%CI 77.7-79.3%), 69.9% (95%CI 69.2-70.5%) and 74.3% (95%CI 73.6- 74.8%), respectively, and an area under the ROC curve of 0.846. These results were reproduced in an independent test set (n=752 positive outcomes, n=66 negative outcomes), obtaining 67.3% sensitivity, 86.4% specificity and 84.8% predictive accuracy. The known prognostic factors age, sex, previous CABG, previous PCI, heart failure, atrial fibrillation, cerebrovascular disease, diabetes, creatinine concentration, Killip class, and acute coronary syndrome classification were compared with the NOESY1D RF risk score, calculated on the test set, in univariate and multivariate regression analyses. In the univariate analysis many of prognostic factors were statistically associated with the outcomes, but the RF score shows the p-value by far more significant (p=2.65e-12). Moreover, in the multivariate regression only the age, the Killip class and the RF score still remain statistically significant, demonstrating their independence with respect to the other variables.

USE OF EVOLOCUMAB IN A PATIENT WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction. Homozygous familial hypercholesterolemia (FH) is a rare (1:160.000-360.000) but serious disorder with a substantial reduction in low-density lipoprotein receptor (LDLR) function, severely elevated low-density lipoprotein cholesterol (LDL-C), cardiovascular disease, and often death in early decades of life. Response to conventional lipid-lowering agents is modest. Novel monoclonal antibodies to proprotein convertase subtilisin/kexin 9 (PCSK9) have shown ability to substantially reduce LDL-C in heterozygous familial hypercholesterolemia. We evaluated the efficacy and safety of evolucumab in a patient with homozygous familial hypercholesterolemia in clinical practice.
Materials and Methods. A 49 years old homozygous FH patient with a missense mutation in the LDLR gene (Gly352Asp) and with severe cardiovascular disease (3 vessels coronary artery disease at the age of 29 followed by a triple bypass grafting, aortic mechanical valve replacement for severe stenosis) was followed by our lipid clinic. His pre-treatment LDL-C level was 420 mg/dl. At the time of the study his lipid-lowering therapy comprised atorvastatin (80 mg) plus ezetimibe (10 mg) daily in association with LDL-apheresis performed every tenth day. During the study the patient was monitored for 168 days with three different regimens:
1) 42 days with LDL-apheresis + atorvastatin/ezetimibe;
2) 61 days with same therapy plus subcutaneous 420 mg evolocumab every 30 days;
3) 65 days with 420 mg evolocumab every 15 days. Blood for laboratory testing was obtained under fasting conditions in pre-, post-apheresis and intermediate time point (5 days after LDL-apheresis). Laboratory tests included: total cholesterol, LDL-C, HDL-C, triglycerides, Lp(a), ApoB, ApoAI, AST, ALT, CPK, PCR. Evolocumab was administered on site by trained study staff.

Results. The patient was monitored in the early 42 days and the mean LDL-C levels were 165 mg/dl in pre-apheresis, 77 mg/dl in post-apheresis, 136 mg/dl at the intermediate time point. The first administration of evolocumab was followed by the elevation of liver enzymes 2 times the upper limit of normal. The amplitude of this elevation resulted lower in the following administrations. No elevation was observed for CPK. During the administration of evolocumab every 30 days, a reduction in mean LDL-C was observed: 149 mg/dl (n.s.) in pre-apheresis, 64.5 mg/dl (p<0.01) in post-apheresis, 118 mg/dl (p<0.01) at the intermediate time point. When evolocumab was administered every 15 days, mean LDL-C were 164 mg/dl (n.s.) in pre-apheresis, 63 mg/dl (p<0.01) in post-apheresis, 123 mg/dl (p=0.01) at the intermediate time point. By calculating the cumulative load in the three periods, the mean LDL-C was 129 mg/dl in the first period, 113 mg/dl in the second period (13% reduction), 117 mg/dl in the third period (9% reduction). The combined effect of different doses of evolocumab on LDL-C level was an 11% reduction.
Discussion. This is the first report describing the efficacy of an anti-PCSK9 antibody in a homozygous FH patient treated with lipid-lowering drugs and on LDL-apheresis. The selection of the

patient for therapy with evolocumab was based on the assumption that his LDLR mutation was receptor-defective. An increase in liver enzymes was observed after the administration but the drug was well tolerated. The lipid profile was significantly improved by evolocumab 420 mg administered every 30 days (13% LDL-C reduction) or every 15 days (9% LDL-C reduction) but the patient was still far from his LDL-C target (<70 mg/dl). Our experience reinforce the importance for a strict follow up to monitor efficacy and safety of evolocumab in homozygous FH patients.

ASSOCIATION BETWEEN LP (A) AND SMALL DENSE LDL IN MENOPAUSAL WOMEN WITHOUT METABOLIC SYNDROME

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The association between Lipoprotein (a) (Lp [a]) and small dense LDL (sd-LDL) has been evaluated in 129 menopausal women, without Metabolic Syndrome (MS), living in the area of Naples (Italy) participating to a large (N=5.062) epidemiological Study from 1993 (Progetto Atena). Lp (a) was measured by an ELISA, solid phase two-site enzyme immunoassay, using polyclonal antibodies raised against purified Lp(a) (Mercodia Diagnostics, Uppsala, Sweden), LDL particle separation was performed by Lipoprint System: seven LDL subfractions were obtained and LDL score (% of sd-LDL particles) calculated. Women with elevated Lp a (above 50 th percentile of studied population) have increased LDL score compared with those below 50 th percentile of studied population (p=0.032 Mann Whitney). The association between Lp (a) and sd-LDL was evaluated taking into account different adjustment models. Women with elevated levels of Lp a (above the 50th percentile) show the following OR of having LDL score (above the 75 th percentile): 3.22, 95% Confidence Interval =1.07 - 9.74, p=0.038; adjusted for age and BMI; 3.13, 95% Confidence Interval= 1.02 - 9.61, p=0.046; adjusted for age, BMI and Apo B; 3.23, 95% Confidence Interval =1.03 - 10.14, p=0.044; adjusted for age, BMI and Systolic Pressure. In this group of menopausal women without MS, Lp (a) and small dense LDL give additional information in the risk assessment for atherosclerotic cardiovascular disease.

THE PREDIMED SCORE: INDEX OF ADHERENCE TO THE MEDITERRANEAN DIET IN THE GENERAL POPULATION: THE PLIC STUDY

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Introduction. Interventional randomized studies have demonstrated the association between adherence to the Mediterranean diet and a reduced risk of cardiovascular events.
The purposes of the study are:

1) describe the general population's eating habits and the level of adherence to the Mediterranean diet (by calculating the PREDIMED score: Primary Prevention of Cardiovascular Disease with a Mediterranean Diet) and their change in a 10 years' follow-up;
2) assess whether this adhesion and its change are associated with determinants of cardiovascular risk and subclinical atherosclerotic damage.

Materials and Methods. The study included 1398 subjects (575 men and 823 women, mean age 57 (49-62) years) which was calculated the PREDIMED score at baseline and after a median follow-up of 11 (11-11) years (by two independent dieticians without know the identity and the patient's clinical picture). The information of the medical, pathological and pharmacological history were collected and were assayed major biochemical parameters; the carotid intima-media thickness (CCA-IMT) was determined by ultrasonography on both visits. In order to assess the degree of correlation between the score PREDIMED and the actual intake of macronutrients, weekly intakes of carbohydrates, proteins, fats (saturated and unsaturated) were assessed in a subgroup of 325 subjects.
Results. The analysis conducted on 325 subjects (128 men and 197 women, median age 55 (47-63)) has shown that individuals with high adherence to the Mediterranean diet (PREDIMED >8) have a reduced daily intake of saturated fat, equal to the consumption of proteins; this results in an increased calories intake, in light of an increased consumption of carbohydrates, compared to subjects with reduced PREDIMED score. Among the 1398 subjects, the score was significantly increased in men at baseline (p=0.001) and it was not different between the subjects in primary prevention than patients with previous cardiovascular event (p=0.176); however, older subjects had a higher adherence to the Mediterranean diet, reflecting a reduced daily intake of fat in the diet. There were no statistically significant differences between the scores and the biochemical parameters, CCA-IMT (p=0.067) and anthropometric parameters (except for systolic blood pressure, increased in subjects with scores above the average, p=0.006).
At the end of the observational period, we observed that 283 subjects had maintained the level of adherence to the Mediterranean diet, 609 had increased the score while 506 had reduced it. Parallel to the score variation, anthropometric and cardio-metabolic parameters did not change. The score was not associated with a higher annual progression of CCA-IMT (p=0.616).
Conclusion. The PREDIMED score proves to be a useful index of daily intake of macronutrients, though its variation over time does not reflect the change of clinical and cardio-metabolic parameters useful for cardiovascular risk stratification.

FAMILIAL HYPERCHOLESTEROLEMIA: DEVELOPMENT OF A RESEARCH AND DIAGNOSTIC APPROACH TO THE DISEASE

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Background. Familial hypercholesterolaemia (FH) is a common genetic cause of premature coronary heart disease (CHD) due to life-long elevated plasma LDL cholesterol levels. FH is caused by mutations in LDLR, APOB and PCSK9 genes found in 80%, 5%, and 1%, respectively, of FH subjects. However, many subjects with primary hypercholesterolemia did not demonstrate functional mu-

tations in any of these genes. In a substantial proportion of FH patients without a known mutation, their high LDL-C concentrations might have a polygenic cause. Aims of this work are:
1) to characterize at molecular level by Sanger sequencing of LDLR gene 11 consecutive patients with clinically suspected FH evaluated by Duch score;
2) to optimize the diagnostic workup in FH patients by developing a panel of genes known to be involved in hypercholesterolaemia to be analyzed by next generation sequencing (NGS), and to evaluate whether this approach allows an improvement in time and costs of genetic diagnosis, hence determining a better management of subjects for secondary and primary prevention.
Methods. FH subjects are identified using clinical and biochemical features: high LDL-C (>190 mg/dl), triglycerides (>300 mg/dl), Lp(a) (>800 mg/L); typical physical stigmata such as tendon xanthomata, xanthelasma, and arcus corneae; personal history of early cardiovascular disease; and family history of early cardiovascular disease or marked hyperlipidemia applying the Dutch Lipid Clinic Network criteria. Traditional FH genetic characterization was performed by an initial direct sequencing of LDLR gene followed by MLPA analysis of big deletions or duplications. For NGS approach, the panel of genes associated to FH was assembled according to the most recent literature data.
Results. Four pathogenetic mutations (three missense and one splicing mutation) were identified in four out of eleven patients analyzed. The 3 missense mutations (c.102 C>A p.Cys34X; c.1783 C>T p.Arg595Glu and c.1775 G>A p.Gly592Glu) were previously identified in FH patients and were estimated to be pathogenetic by the major prediction in silico tools (Polyphen-2, HOPE, SNP&GO and SIFT). The splicing mutation (c.1358+2 C>T) has never been reported in literature and affects the donor splicing site of intron 9 of the gene and its effect was evaluated to be damaging by Human Splicing Finder tool. MLPA analysis of negative patients, in particular those with large region of homozigosity in LDLR gene, didn't allow to identify any big insertion or deletion.
Moreover, we developed a panel of 39 genes (LDLR, APOB, PCSK9, LDLRAP1, APOE, APOA2, APOA5, LIPI, ABCA1, EPHX2, PPP1R17, GHR, ITIH4, BTN2A1, NPC1L1, ABCG5, ABCG8, APOA1, APOC3, APOA4, LIPC,LPL, PON1, CETP, LRP1, SREBF1, SCARB1, SREBF2, GCKR, CREB3L3, APOC2, CELSR2, SLC22A1, HFE, MYLIP, ST3GAL4, NYNRIN, CH25H, INSIG2) involved in FH or other familial dyslipidemias for targeted NGS approach. This approach enables us to significantly reduce the time of the analysis (one month for 39 genes evaluation/patient versus 15 days for the analysis of one gene needed with Sanger technology) as well as a reduction in costs (400 € for NGS analysis versus 300-1000€ for Sanger sequencing of one-three major associated genes). The NGS approach allow us to identify monogenic as well as polygenic forms of FH. The designed panel allows also the genetic diagnosis of other familial dyslipidemias (e.g. hypertrigliceridemias).
Conclusions. In conclusion, this study confirms that LDLR analysis allows the diagnosis in around 40% of patiens with suspected FH. Considering the cost-effectivness of the analysis, this study shows the significative advantages of using a NGS approch for the study of FH. The analysis of a large panel of genes allow us a better understanding of the molecular bases of dyslipidemias. Moreover, NGS approach by increasing genetic diagnosis performance will improve: patient's management and cascade screening to early identify patients in the same family; the possibility of increase adherence to diet and pharmacological treatment identifying early individuals at very high risk of premature CAD or preclinical atherosclerosis.
(LIPIGEN participating center)

RISK AWARENESS IN PROSALUTE, A NEW COMMUNITY PROGRAM FOR CARDIOVASCULAR HEALTH IN MILAN, ITALY

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Background. Awareness of the own cardiovascular (CV) risk is a prerequisite to follow preventive actions. Risk awareness requires health literacy and self-knowledge, and both depend on many context-specific factors. ProSALUTE is an experimental CV health program for the medium/low-income and multi-ethnic community of the Ponte Lambro district, Milan, Italy (n=3600 adults). CV risk domains are assessed through an assisted questionnaire and objective anthropometric and laboratory measures are obtained to estimate CV risk and to propose personalized interventions.
Objective. To assess the accuracy of the personal CV risk perception and the influence of knowledge of own risk factor levels on the accuracy.
Methods. Data from the first 100 participants to ProSALUTE were analyzed. The Framingham Risk Score (FRS) was used to estimate 10-y CV risk category. The question "What do you think is your risk of having cardiovascular problems in the next 10 years?" (5 categories, from very low to very high) was posed to estimate CV risk perception. The questions "Do you know your usual values of blood pressure/cholesterol/triglycerides/glucose" were posed as index of risk factor awareness. Cohen's Kappa coefficients were used to analyze concordance between estimated and perceived CV risk categories.
Results. 59%, 23% and 15% of the subjects had a low, intermediate and high FRS, respectively. Risk perception and FRS categories agreed in 41.2% and differed by 1 and by 2 categories in 46.4% and 12.4% of the subjects, respectively. Among subjects with high FRS, only 20% perceived being so. Concordance between FRS and perceived risk overall was scarce (K=0.0745). Knowing 0-1 or 3-4 own risk factor values only modestly affected extent of concordance (K=0,094 vs. K=0,2219, p=0,067).
Conclusion. In our context, perception of personal CV is inaccurate and scarcely influenced by risk factors knowledge. Actions to enhance health literacy are needed to improve risk awareness.

EARLY ADOPTERS OF A FREE-ACCESS PRIMARY CARDIOVASCULAR PREVENTION PROGRAM IN A MULTIETHNIC COMMUNITY IN NORTH ITALY

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Background. Primary cardiovascular prevention programs (PPP) may be more effective by addressing disadvantaged people. Yet, their participation in PPP is low. Shift to multi-ethnicity and deprivation in populations call for inclusive strategies to reduce CVD.

Purpose. To assess the relationship between socioeconomic status (SES) and adherence to a PPP among residents of a multiethnic European community in Milan.
Methods. 688 randomly selected residents (both genders, 40-65 y.o.) from a Milan's suburb were invited by mail to participate in a free-hospital-based PPP. Promotion was performed in 5 languages. Early Adopters (EA=146), who replied within one month, were compared with Not-yet Respondents (NR, n=542) in terms of immigrant or native status, schooling level, work category, age and gender.
Results. Gender and age were similarly distributed in both groups. As compared with NR, EA were featured by: less immigrants (27% vs 36%, p=0.03), higher schooling (>12y=14% vs 5%, 8 to 12y=50% vs 30% and <8y=36% vs 65%; p12y=83% vs 38%, p=0.01, 8 to 12y=54% vs 36%, p=0.048 and <8y=24% vs 18%, p=ns).
Conclusions. In a PPP in Milan, a lower participation of immigrants than natives was strictly related to their lower SES, thus excluding immigrant-specific barriers. Further strategies are needed to enhance participation to PPPs of people at low SES.

PROSALUTE: A NEW COMMUNITY PROGRAM FOR CARDIOVASCULAR HEALTH

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The largest benefit of Community programs for cardiovascular health may be obtained by addressing high-risk, disadvantaged and traditionally hard-to-reach groups. Effective actions include health promotion, timely screening of modifiable risk factors, application of evidence-based targets and interventions, broad access to heart-friendly environments/facilities and dissemination of favorable social norms. Thus, community prevention is a multifaceted task that requires multidisciplinary collaboration. A suitable program should be tailored to the specific social context and make the most of local resources to improve access, adherence and continuity, as well as sustainability.
ProSalute (ProHealth) is an experimental model of primary CV prevention for the prevalently low-income and multiethnic community of Ponte Lambro (n=3600 adults), the neighborhood where the coordinating hospital (Centro Cardiologico Monzino, Milan, Italy) is located. CV risk domains assessed through an assisted questionnaire include family and personal history, life-style and psychological and socioeconomic status. A 2-years pilot phase (n=600, age 45-65y) started on May 2015.
Original program features:
1) Personal invitation to foster participation of hard-to-reach groups.
2) Running by specialists (nurse, internist, nutritionist, psychologist, social worker) from an academic hospital.
3) Involvement of a nurse as case manager and motivational-interviewer
4) Personalized intervention according to individual global risk and specific risk factors.
5) Short term modules of care for specific problems (smoking cessation, control of depression, nutritional counseling, pharmacological control of risk factors).
6) Networking with representatives of the Community to plan communication and community preventive actions (walking groups, etc).
7) Nudge of participants to utilize environmental, organizational and professional local resources useful to sustain a healthy life-style.

Outcomes will be assessed at 6 and 12 months using indexes of adherence, life-style improvement, risk reduction and costs. According to the results, this model could be transferred and adapted to other contexts in Italy and abroad.

HEALTH SYSTEM BARRIERS AND FACILITATORS TO MEDICATION ADHERENCE FOR THE SECONDARY PREVENTION OF CARDIOVASCULAR DISEASE: A SYSTEMATIC REVIEW

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Background. Secondary prevention of cardiovascular disease (CVD) is cost-effective, but uptake is suboptimal. Understanding barriers and facilitators to adherence to secondary prevention of CVD at multiple health system levels may inform policy.
Objectives. To conduct a systematic review of barriers and facilitators to adherence/persistence to secondary CVD prevention medications at health system level.
Methods. Included studies reported effects of health system level factors on adherence/persistence to secondary prevention medications for CVD (coronary artery or cerebrovascular disease). Studies considered at least one of: beta-blockers, statins, angiotensin-renin system blockers, and aspirin. Relevant databases were searched from 1 January 1966 until 1 October 2015. Full texts were screened for inclusion by two independent reviewers.
Results. Of 2246 screened articles, 25 studies were included (12 trials, 11 cohort studies, 1 cross-sectional study and 1 case-control study) with 132140 individuals overall (smallest n=30, largest n=63301). Three studies included upper middle-income countries, one included a low middle-income country, and 21 (84%) included high-income countries (9 in the USA). Studies concerned established CVD (n=4),cerebrovascular disease (n=7) and coronary heart disease (n=14). Three studies considered both persistence and adherence. Quantity and quality of evidence was limited for adherence, persistence and across drug classes. Studies were concerned with governance and delivery (n=19, including 4 trials of fixed dose combination therapy, FDC), intellectual resources (n=1), human resources (n=1) and health system financing (n=4). Full prescription coverage, reduced copayments, FDC and counselling were facilitators associated with higher adherence.
Conclusions. High-quality evidence on health system barriers and facilitators to adherence to secondary prevention medications for CVD is lacking, especially for low-income settings. Full prescription coverage, reduced copayments, FDC and counselling, may be effective in improving adherence, and are priorities for further research.
This work has been presented at the World Congress of Cardiology 2016, Mexico City.

RUOLO DELLA SFINGOSINA 1-FOSFATO E DEL SUO RECETTORE S1P3 NEL TRASPORTO INVERSO DEL COLESTEROLO

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La sfingosina 1-fosfato (S1P) svolge molteplici funzioni nel sistema cardiovascolare, potenzialmente rilevanti per l'ateroprotezione, ma dal meccanismo molecolare ancora sconosciuto. Lo scopo di questo studio è quello di dimostrare che l'attività anti-aterogena di S1P endogena è legata, almeno in parte, ad una modulazione del metabolismo lipidico. A tal fine ci siamo avvalsi di un modello sperimentale innovativo, costituito da topi che sovraesprimono il recettore S1P3 specificamente nei macrofagi (topi S1P3-Lyz). Nei macrofagi peritoneali murini (MPM) di questi animali è stato valutato l'efflusso di colesterolo, esponendo le cellule ad HDL o plasma murino. Il trasporto inverso del colesterolo (RCT) in vivo è stato quantificato iniettando in topi C57BL/6 (WT) MPM radiomarcati e arricchiti di colesterolo provenienti da topi WT o S1P3-Lyz.
Gli MPM sovraesprimenti S1P3 mostrano un aumento significativo dell'espressione genica e proteica del trasportatore lipidico ABCG1, rispetto ai macrofagi controllo. Coerentemente, in MPM S1P3-Lyz l'efflusso di colesterolo è maggiore, rispetto a MPM controllo, in cellule esposte a plasma (3.18%±0.32 vs 2.05%±0.53; $p<0.05$). Dopo incubazione degli MPM con LDL acetilate, le cellule S1P3-Lyz evidenziano una maggior capacità di efflusso lipidico, rispetto ai controlli, sia verso HDL (8.47%±0.63 vs 5.93%±0.46; $p<0.001$) sia verso concentrazioni crescenti di plasma (0.1%-2%; differenza statisticamente significativa in tutte le condizioni). L'RCT in vivo è incrementato nei topi iniettati con macrofagi provenienti da topi S1P3-Lyz, come dimostrato dall'aumento della quantità di 3H-colesterolo ritrovata nel plasma (0.99%±0.32 vs 0.60%±0.12; $p<0.05$), nel fegato (2.66%±0.41 vs 1.99%±0.35; $p<0.01$) e nelle feci (0.99%±0.19 vs 0.66%±0.10; $p<0.01$) rispetto ai topi iniettati con MPM WT.
Questi risultati rappresentano la prima evidenza di uno stretto legame tra S1P e metabolismo cellulare del colesterolo e possono contribuire a chiarire il ruolo di S1P nell'aterogenesi, al fine di proporre un nuovo approccio per la diagnosi e il trattamento delle patologie cardiovascolari su base aterosclerotica.

EVOLOCUMAB IN ASSOCIAZIONE AL TRATTAMENTO CON AFERESI LIPOPROTEICA IN PAZIENTE CON IPERCOLESTEROLEMIA FAMILIARE ETEROZIGOTE INTOLLERANTE ALLE STATINE. CASE REPORT

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Donna di 49 anni, HeFH (mutazione gene LDLR) intollerante alle statine (mialgie/miosite ed episodio di rabdomiolisi da atorvastatina nel 2010). Affetta inoltre da S di Cogan. Ipercolesterolemia nota dall'adolescenza, profilo lipidico in assenza di terapia ipolipemizzante: colesterolo-tot=522 mg/dl, LDL-c=434, HDL-c=84, trigliceridi=120, Lp(a)=11 mg/dl.
All'EO: peso kg 68, BMI 27,3, PAO 120/84 mmHg. Non xantomi, nè gerontoxon. Non soffi vascolari. In terapia con ASA, Metilprednisolone, metotrexato, pantoprazolo, ac clodronico.
Eco TSA:Bilateralmente, piccole calcificazioni ateromasiche puntiformi dell'arteria carotide interna ed esterna. TAC coronarica:Calcium Score secondo Agatston: 46.9
Da dicembre 2015 avviato evolocumab 140 mg ogni 2 settimane ottenendo una riduzione assoluta del colesterolo-LDL di 73 mg/dl mantenuta per 21 settimane (valori medi di colesterolo-LDL =361 mg/dl). Da Maggio 2016 è stato associato trattamento con aferesi lipoproteica (sistema HELP) con una progressiva riduzione del colesterolo-LDL (LDL-c preaferesi al 1°, 2° e 3° trattamento rispettivamente di 361 → 256 → 214 mg/dl e successiva stabilizzazione a valori medi preaferesi di 204 mg/dl, post-aferesi di 40 mg/dl).
Questi dati suggeriscono un effetto sinergico della terapia con evolocumab in associazione all'aferesi lipoproteica. Ulteriori studi sono auspicabili per confermare questa osservazione.

INDICE DEGLI AUTORI