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Periodicità Trimestrale

Scopi

Il "Ĝiornale Italiano dell'Arteriosclerosi" (GIA), è un periodico di aggiornamento che nasce come servizio per i medici, operatori sanitari e studenti di medicina e delle professioni sanitarie, con l'intenzione di rendere più facilmente disponibili informazioni e revisioni critiche nel campo dell'arteriosclerosi e delle malattie ad essa correlate. Lo scopo della rivista è quello di

assistere il lettore fornendogli: a) revisioni critiche di argomenti di grande rilevanza nel campo dell'arteriosclerosi sia per quanto riguarda gli aspetti di base che gli aspetti clinico-applicativi;

- b) quesiti relativi agli argomenti trattati per una verifica di auto apprendimento;
- c) opinioni di esperti qualificati sui nuovi sviluppi delle conoscenze sull'arteriosclerosi;
- d) lavori originali relativi ad aspetti di ricerca sanitaria nell'ambito dell'arteriosclerosi e delle malattie ad essa correlate.

TIPOLOGIA E STRUTTURA DEGLI ARTICOLI

GIA accetta le seguenti categorie di contribuiti: lavori originali, rassegne, casi clinici e forum dei lettori. Titolo e, se previsti, parole chiave e sommario dovranno essere sia in italiano che in inglese.

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I lavori originali saranno sottoposti a processo di "peer review". La lunghezza del testo non deve superare le 4.000 parole (esclusa la bibliografia) ma incluso l'abstract, con un massimo di 4 figure o tabelle. Il frontespizio dovrà contenere:

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Bibliografia

Citazione di articoli su riviste: Es. 1: Austin MA, Hutter CM, Zimmern RL, Humphries SE. Familial hypercholesterolemia and coronary heart disease. Am J Epidemiol 160: 421-429, 2004. Es. 2: Humphries SE, Whittall RA, Hubbart CS et al. Genetic causes of familial hypercholesterolemia in patients in the UK: a relation to plasma lipid levels and Coronary heart disease risk. J Med Genet 43: 943-949, 2006

Citazioni di capitoli di libri Assmann G, von Eckardstein A, Brewer H. Familial analphalipoproteinemia: Tangier disease. In "The metabolic and molecular bases of inherited disease", Scriver CR, Beaudet AL, Sly WS, Valle I, eds, 8th ed. New York, McGraw-Hill, 2001; 2937-60.

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Al termine del testo è opportuno inserire un capitolo dedicato alle prospettive future con particolare riferimento agli aspetti clinico-applicativi.

Glossario: È uno strumento di comunicazione fortemente raccomandato. Esso dovrebbe contenere una concisa ma esauriente spiegazione dei termini "nuovi o meno comuni" utilizzati nella rassegna. Qualora l'autore lo ritenga utile, al glossario può essere allegata una o più "finestre esplicative" dedicate ad argomenti a cui si fa riferimento nella rassegna e che non sono discussi in sufficiente dettaglio nel corpo del testo.

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Questionario di auto-apprendimento: Per ogni rassegna il questionario dovrà contenere 5-10 domande con risposta a scelta multipla.

Casi clinici

Si riferisce alla presentazione di un caso clinico, preparato su richiesta da medici esperti, che ha lo scopo di rafforzare standard di comportamento clinico, diagnostico e/o terapeutico, basati sulle evidenze.

Forum su Medicina, Scienza e Società

Si tratta di articoli brevi o lettere all'editore (1.500 parole) sollecitati ad esperti, riguardanti commenti e/o opinioni su temi di particolare attualità. Il testo non dovrà superare le 1.500 parole. Non è richiesto un sommario. Le voci bibliografiche non devono superare il numero di 10 e devono essere riportate come indicato per le rassegne.

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Il testo dell'articolo deve essere predisposto utilizzando il programma Microsoft Word per Windows o Macintosh. I dischetti devono riportare sull'apposita etichetta il nome del primo autore, il titolo abbreviato dell'articolo, il programma di scrittura e la versione, ed il nome del contenuto/file.

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PRESENTAZIONE DEL NUMERO

Focus

Colesterolo e demenza

In assenza di terapie specifiche, la prevenzione rappresenta l'unico presidio per contrastare con efficacia il deterioramento cognitivo, che si manifesta con l'età. È noto che la demenza nella sua forma sporadica, non determinata geneticamente, è una malattia alla cui comparsa concorrono molteplici fattori di rischio, sia genetici che ambientali, molti dei quali potenzialmente modificabili. Il *focus* di questo numero è dedicato specificamente al coinvolgimento del colesterolo nei processi neurodegenerativi che sottendono al decadimento cognitivo. In particolare l'attenzione viene focalizzata sul rapporto tra farmaci ipocolesterolemizzanti e demenza, con riferimento specifico a statine e inibitori del PCSK9.

Nuovi farmaci

Nuovi ipoglicemizzanti e protezione cardiovascolare

L'aterosclerosi e le sue complicanze rappresentano la causa prevalente di malattia e di morte del paziente diabetico. L'armamentario terapeutico del diabete si è arricchito recentemente di nuovi farmaci, glifozine e analoghi di GLP 1, dotati non solo di effetto ipoglicemizzante, ma soprattutto di protezione cardiovascolare. Viene presentata una revisione ragionata sugli effetti cardiovascolari osservati negli studi randomizzati controllati, soffermandosi in particolare sui meccanismi fisiopatologici di protezione. Con l'avvento di questi farmaci, le più recenti linee guida nazionali e internazionali raccomandano di personalizzare la terapia del diabete sulla base della presenza o meno di malattia cardiovascolare.

Fisiopatologia della placca

Tolleranza immunitaria e aterosclerosi

La revisione è dedicata a coloro che vogliono approfondire il *trait-d'union* tra infiammazione (Virchow) e lipidi circolanti (Anitschkow) nella formazione, sviluppo e rottura della placca. In particolare, nell'articolo vengono esaminate le componenti del sistema immunitario coinvolte nella patogenesi e nella progressione della placca, con riferimento all'infiltrazione delle lipoproteine contenti apoB nel sub-endotelio, alla trasformazione dei macrofagi in cellule schiumose, ai linfociti T autoreattivi con coinvolgimento del sistema immune. La comprensione di questi meccanismi può fornire nuovi approcci di terapia e prevenzione dell'aterosclerosi e delle sue complicanze.

Medicina, Scienza e Società

Università e sistema sanitario

Si tratta di un rapporto problematico, che non sempre garantisce l'efficace integrazione tra attività assistenziale e didattica nelle strutture sanitarie che ospitano una scuola di medicina. L'articele, con lo stilo della lettera all'editore, vuole sussitare un dibattite sull'esperienza dell'au

L'articolo, con lo stile della lettera all'editore, vuole suscitare un dibattito sull'esperienza dell'autore nella gestione di una prestigiosa scuola di medicina. 3

FOCUS

COLESTEROLO E DEMENZA: UN LEGAME COMPLESSO Cholesterol and dementia: a controversial link

LUCIA FAROTTI^{1,2}, NICOLA SALVADORI^{1,2}, PAOLO CALABRESI¹, LUCILLA PARNETTI^{1,2}

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SUMMARY

Cognitive impairment is common among elderly adults, and dementia represents the main cause of disability in this population. Neurodegeneration due to Alzheimer disease (AD) underlies the majority of dementia, and cerebrovascular disease is the second-most common cause. Unsurprisingly, prevention of cognitive decline is a worldwide public health issue. Cognitive impairment, dementia and AD are multifactorial conditions with several potentially modifiable risk factors including vascular and lifestyle factors. Among them, hypercholesterolemia is an established risk factor. Multi-domain interventions include diet, physical activity and cognitive stimulation. With regard to pharmacological agents, statins and PCSK9 inhibitors are the most promising drugs. In this scenario, it is worth of note that potential risks of adverse neurological effects of intensive lipid-lowering treatment have been hypothesized, as cholesterol is a component of the central nervous system. Epidemiological data have supported a possible association between the use of statins and the risk of dementia, with controversial evidences. Recently, a systematic review and meta-analysis showed that use of statins is significantly associated with a reduced risk of all-caused dementia, AD and mild cognitive impairment. Similarly, the use of monoclonal antibodies inhibiting PCSK9 raised concerns about their potential neurological side effects, as PCSK9 has a role in neuron differentiation, apoptosis and migration. Results from the EBBINGHAUS trial showed no impaired cognitive functions in patients treated with evolocumab compared with controls. In conclusion, lowering cholesterol is of utmost importance in cognitive impairment prevention. The use of available drugs is recommended, even if long-term observations are needed.

Keywords: Cholesterol, dementia, Alzheimer's disease, statins, PCSK9 inhibitors.

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Introduzione

Il deterioramento cognitivo è una condizione comune nella popolazione anziana. La demenza, l'espressione più severa del deterioramento cognitivo che comporta anche la dipendenza dell'individuo sul 5

piano funzionale, rappresenta la principale causa di disabilità nella popolazione anziana e colpisce attualmente circa 50 milioni di persone in tutto il mondo (1). Ci si aspetta che tale numero raggiunga i 130 milioni di individui nel 2050, se la prevalenza per età dovesse rimanere invariata. L'individuazione e la correzione dei fattori di rischio modificabili offre pertanto la possibilità di limitare il numero di persone affette da demenza.

Ci sono numerose cause di deterioramento cognitivo e di demenza: si parla di demenza degenerativa primaria quando l'eziologia è neurodegenerativa, di demenza secondaria quando tale condizione è conseguenza di una o più patologie pre-esistenti. La più comune forma di demenza è rappresentata dalla malattia di Alzheimer (Alzheimer's disease, AD), che è anche la più frequente tra le malattie neurodegenerative. La malattia cerebrovascolare (cerebrovascular disease, CVD) rappresenta la seconda causa più comune di demenza nella popolazione adulta. Studi neuropatologici e di neuroimaging mostrano che in molti casi la demenza può presentare, più propriamente, un'eziologia a genesi mista (sia neurodegenerativa che vascolare), con una particolare prevalenza nei soggetti ultraottuagenari; questo, quindi, rende la demenza mista di fatto la forma più comune nella popolazione anziana (2-4).

Ad oggi, non esistono terapie *disease-modifying* per combattere con efficacia le malattie neurodegenerative che conducono a demenza. Tuttavia, l'attendibilità della diagnosi condiziona profondamente le prospettive dello sviluppo di strategie di prevenzione secondaria e terziaria. Secondo i più recenti criteri diagnostici per l'AD, la malattia è caratterizzata da una lunga fase preclinica asintomatica, in cui i sintomi cognitivi sono assenti, seguita da una fase di deterioramento cognitivo lieve (Mild Cognitive Impairment, MCI) che può poi evolvere a franca demenza, la fase finale e più severa (5). Analogamente, sono attualmente in via di sviluppo linee guida per la standardizzazione della classificazione diagnostica all'interno dell'ampio spettro clinico ed eziologico rappresentato dal *vascular cognitive impairment*, cioè il deterioramento cognitivo legato a CVD (6).

Complessivamente, questi nuovi criteri diagnostici hanno l'obiettivo di facilitare la diagnosi precoce negli individui a rischio di sviluppare demenza, e che potrebbero beneficiare di interventi in grado di ritardare o evitare l'insorgenza di disturbi cognitivi severi e disabilitanti.

Fattori di rischio modificabili

La possibilità di prevenire la demenza è supportata da una grande quantità di studi osservazionali, i quali hanno dimostrato che la demenza, e più nello specifico l'AD (nella sua forma sporadica, cioè non geneticamente determinata), sono patologie multifattoriali ed eterogenee, determinate da diversi fattori di rischio sia genetici che ambientali. Molti di questi fattori sono potenzialmente modificabili e offrono quindi la possibilità di effettuare prevenzione primaria (4). Diversi studi hanno evidenziato che fattori di rischio vascolare quali ipertensione, ipercolesterolemia e obesità nell'età adulta (*midlife*). diabete mellito e malattie cardio- e cerebrovascolari (4) portano ad un aumento del rischio di sviluppare AD e demenza in generale. La crescente attenzione verso le fasi pre-sintomatiche e pauci-sintomatiche delle malattie sottostanti le diverse forme di demenza ha incoraggiato lo sviluppo di strumenti per stimare il rischio di sviluppare demenza,

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sotto forma di scale per l'attribuzione di punteggi e l'ottenimento di uno score finale. Gli score che si basano sui comuni fattori di rischio dell'età adulta potrebbero individuare meglio i soggetti predisposti, che non hanno ancora sviluppato la patologia cerebrale. La maggior parte degli score a disposizione è in grado di predire lo sviluppo di demenza entro i successivi 5-10 anni (7). Pochi sono invece quelli disegnati per valutare il rischio di demenza entro i successivi 20 anni, e di questi l'unico utilizzato in trials sulla prevenzione è il Cardiovascular Risk Factors, Aging and Dementia (CAIDE) risk score (Tabel*la 1*). Il rischio di progressione entro 20 anni, sulla base del CAIDE risk score, è riportato in Tabella 2. Fissando un cut-off ≥9. sensibilità e specificità sono risultate rispettivamente 0.77 e 0.63; il valore predittivo negativo è di 0.98 (8).

Ruolo del colesterolo

L'ipercolesterolemia è un fattore di rischio noto per CVD. Esistono inoltre evidenze di un coinvolgimento del colesterolo nei processi neurodegenerativi che sottendono l'AD, in particolare nella formazione delle placche di β -amiloide (A β) che caratterizzano la malattia (9). Elevati livelli di colesterolo sono stati messi in correlazione con un'aumentata produzione e deposizione di A β (9). In condizioni fisiologiche, la proteina precursore dell'amiloide (Amyloid Precursor Protein, APP) è prodotta in grande quantità dai neuroni e metabolizzata attraverso due vie distinte, una di tipo amiloidogenico ed una non amiloidogenica. L'enzima β-site APP-cleaving enzyme 1 (BACE 1) è coinvolto nel clivaggio extracellulare di APP e rappresenta una tappa fondamentale nella formazione di peptidi amiloidogenici (10) Studi su modelli animali hanno evidenziato che

Tabella I - CAIDE risk score

Fattore di rischio		Score
Età	<47 anni	0
	47-53 anni	3
	>53 anni	4
Scolarità	≥10 anni	0
	7-9 anni	2
	<7 anni	3
Sesso	F	0
	М	1
Pressione arteriosa	≤140 mmHg	0
	>140 mmHg	2
ВМІ	≤30 kg/m²	0
	>30 kg/m²	2
Colesterolo totale	≤6,5 mmol/l	0
	>6.5 mmol/l	2
Attività motoria	Sì	0
	No	1

Tabella 2 - Rischio di sviluppare demenza entro 20 anni secondo il CAIDE risk score.

CAIDE risk score	Rischio di demenza a 20 anni
0-5	1,0%
6-7	1,9%
8-9	4,2%
10-11	7,4%
12-15	16,4%

un elevato contenuto di colesterolo nei neuroni è associato ad aumentati livelli di BACE1 nell'ippocampo (11). Tra i fattori di rischio genetici per la forma sporadica di AD, un ruolo centrale è svolto dalla presenza dell'allele ε 4 dell'apolipoproteina E (APOE) (10).

In particolare, la presenza di uno o più alleli apoE ε 4 è associata ad ipercolesterolemia e ad un profilo lipidico pro-aterogenico (12). L'ApoE media inoltre il rilascio neuronale di colesterolo nel sistema nervoso centrale; l'allele ε 4 risulta meno efficiente della forma ε 3 nel trasportare il colesterolo cerebrale (13).

Studi osservazionali hanno evidenziato che i soggetti con un'elevata aderenza ad un profilo dietetico salutare hanno un ridotto rischio di sviluppare deterioramento cognitivo e AD. La dieta mediterranea è uno dei pattern dietetici più studiati. Le caratteristiche fondamentali includono l'uso di frutta e verdura, grassi insaturi, pesce e prodotti a base di cereali (14). Parallelamente alla dieta, una regolare attività fisica è stata associata ad una riduzione del rischio di declino cognitivo in numerosi studi longitudinali. Tale associazione è stata osservata per l'attività motoria nell'età adulta, ma si osserva un beneficio anche quando l'attività motoria si mantiene o addirittura l'esercizio viene incrementato nell'età avanzata (14). È stato ipotizzato anche un ruolo del training cognitivo in età avanzata quale strumento di prevenzione per lo sviluppo di demenza, ma gli attuali risultati non sono incoraggianti (14).

Strategie di prevenzione farmacologiche

L'intervento farmacologico sui fattori di rischio modificabili e la sua efficacia nel trattamento della demenza è chiaramente un argomento molto ampio. In questa revisione, l'attenzione viene focalizzata sul rapporto tra trattamento farmacologico dell'ipercolesterolemia e demenza, con particolare riferimento a due classi farmacologiche: le statine e gli inibitori di PCSK9.

Statine

Le statine sono farmaci che inibiscono la sintesi del colesterolo endogeno inibendo la Idrossimetilglutaril-CoA (HMG-CoA) reduttasi.

Nonostante gli evidenti benefici sul rischio cardio- e cerebrovascolare, negli ultimi anni sono state sollevate delle perplessità in merito ai potenziali rischi/ benefici in riferimento al deterioramento cognitivo in soggetti anziani che fanno uso di statine. I dati epidemiologici hanno fornito in tal senso evidenze controverse (15), tanto che, nel 2012, la U.S. Food and Drug Administration (FDA) ha segnalato un possibile ruolo dell'uso di statine addirittura nel favorire lo sviluppo di deterioramento cognitivo (16). Diversi possibili fattori possono aver contribuito a questi risultati eterogenei. In primo luogo, le statine potrebbero mostrare effetti neuroprotettivi limitatamente alle fasi più precoci di malattia (17, 18). In secondo luogo, la durata del trattamento e la dose somministrata potrebbero aver influenzato gli outcome (18, 19). Inoltre, è possibile che alcuni medici non prescrivano statine in pazienti con un pre-esistente deterioramento cognitivo per un ipotetico scarso beneficio legato ad una ridotta aspettativa di vita (20, 21). Infine, il genotipo ApoE potrebbe influenzare l'associazione tra l'uso di statine e l'incidenza di demenza: secondo un recente studio, le statine potrebbero avere particolare efficacia in pazienti con AD omozigoti per ApoE £4 (22).

Chu e coll. hanno recentemente pubblicato una review sistematica/meta-analisi sul rapporto tra statine e rischio di demenza (23). Dall'analisi di 16 studi è risultato che i soggetti trattati con statine presentavano una minore probabilità di sviluppare demenza rispetto a coloro che non venivano trattati (rischio ridotto del 15,1%). Analogamente, i partecipanti di 6 studi sottoposti al trattamento con statine avevano una minore probabilità di sviluppare MCI rispetto ai soggetti non trattati (rischio ridotto del 26,3%). Per quanto riguarda più specificamente l'AD, nei 14 studi presi in considerazione i partecipanti trattati con statine mostravano una minore probabilità di sviluppare la malattia rispetto ai non trattati (rischio ridotto del 28,1%). Sorprendentemente, l'analisi dell'uso di statine sull'insorgenza di demenza vascolare non ha mostrato differenze statisticamente significative tra il gruppo di pazienti trattati e i non, forse a causa dell'esiguo numero di studi disponibili (23).

L'esatto meccanismo con cui le statine esercitano una funzione protettiva nei soggetti adulti non è ancora stato chiarito. ma potrebbe includere diverse vie fisiopatologiche. In primo luogo, evidenze in modelli animali hanno documentato che le statine sono capaci di diminuire i livelli di colesterolo e di ridurre la formazione della β -amiloide (24) e dei grovigli neurofibrillari (25). Un ulteriore meccanismo da prendere in considerazione è il possibile effetto anti-infiammatorio delle statine a livello cerebrale. Le statine riducono infatti la produzione di citochine pro-infiammatorie - quali l'interleuchina (IL)-1β, IL-6 e il tumor necrosis factors-gamma (TNFgamma) - indotta dalla β-amiloide nell'ippocampo (26). Le statine potrebbero infine esercitare degli effetti indipendenti dal colesterolo (effetti pleiotropici) intervenendo sulla disfunzione endoteliale, la quale gioca un ruolo centrale nell'avvio dei processi infiammatori legati all'aterosclerosi (27).

Ulteriori studi sono necessari per valutare gli effetti delle statine sulla base delle loro caratteristiche chimiche (idrofiliche e lipofiliche), del sesso, dell'etnia e del genotipo APOE dei pazienti.

Inibitori del PCSK9

La scoperta e la caratterizzazione della proproteina della convertasi subtilisina/ kexin tipo 9 (proprotein convertase subtilisin/kexin type 9, PCSK9) ha rivoluzionato le conoscenze relative al metabolismo lipidico e ha permesso la più grande innovazione terapeutica nell'ambito delle dislipidemie dai tempi dell'introduzione delle statine. PCSK9 è un enzima codificato dal gene PCSK9 sul cromosoma 1, espresso principalmente nel fegato, ma anche in molte altre cellule e tessuti. Una volta secreto nel plasma, il PCSK9 lega il recettore delle lipoproteine a bassa densità (low-density lipoprotein receptor, LDLR) sulla superficie degli epatociti. incrementando quindi la sua degradazione nel sistema endosoma/lisosoma, prevenendo il suo recupero e determinando una riduzione della clearance del colesterolo LDL (LDL-C).

Recentemente, la FDA e la European Medicines Agency hanno approvato 2 anticorpi monoclonali *(monoclonal antibodies, mAbs)* in grado di inibire il PCSK9: evolocumab e alirocumab. Tali farmaci hanno permesso di ottenere una riduzione dei livelli di LDL-C fino al 60-70% sia in monoterapia che in combinazione con altri trattamenti ipolipemizzanti, determinando un significativo beneficio dal punto di vista cardiovascolare, superiore a quello raggiunto con il solo impiego di statine ed ezetimibe (28, 29).

La possibilità di ottenere livelli molto bassi di LDL-C con gli inibitori di PCSK9 ha sollevato delle perplessità su possibili effetti avversi di tipo neurologico analogamente a quanto descritto per le statine. Alla base di tali perplessità c'è la consapevolezza che il colesterolo è un componente fondamentale del sistema nervoso, nonché diverse considerazioni sul possibile ruolo del PCSK9 nella neurogenesi (30), nella migrazione neuronale (31) e nell'apoptosi (32). Studi su roditori hanno dimostrato un'aumentata espressione di PCSK9 in specifiche aree del sistema nervoso centrale durante lo sviluppo embrionale (30). PCSK9 sembra inoltre modulare i livelli

di LDLR nelle aree cerebrali che hanno subito un evento ischemico (33). È stato ipotizzato infine anche un ruolo di PCSK9 nella patogenesi dell'AD, dal momento che PCSK9 contribuisce all'eliminazione della forma intermedia, non-acetilata, di BACE1 (34).

Malgrado un suo presunto ruolo nella fisiologia del sistema nervoso, l'assenza del PCSK9 in topi *knockout* o in esseri umani con mutazioni *loss-of-function* del gene PCSK9 non ha evidenziato alcun legame con alterazioni neurologiche, e in particolare, con deficit cognitivi (35, 36).

L'analisi dei dati relativi alla fase 2 e 3 di trial clinici randomizzati sui mAbs ha mostrato una più elevata incidenza di eventi avversi neuro-cognitivi in soggetti trattati con inibitori del PCSK9, sebbene l'incidenza complessiva sia bassa (<1%) e non legata al grado di riduzione dei livelli di LDL-C. E' significativo tuttavia che nei primi trials tali eventi avversi erano per lo più self-reported, senza alcuna valutazione cognitiva oggettiva, e nella maggior parte degli studi non veniva riportata alcuna informazione sullo stato cognitivo dei pazienti alla baseline. Ne consegue che la attendibilità di questi risultati sia dubbia e motivo di dibattito (37).

Il trial EBBINGHAUS (Evaluating

RIASSUNTO

Risk Subjects) è stato il primo trial prospettico specificamente disegnato per rispondere alla domanda se il trattamento con inibitori di PCSK9 avesse un impatto negativo sul profilo cognitivo. Rispetto ai controlli, i pazienti trattati con evolocumab non hanno evidenziato alcuna compromissione ai test cognitivi. Particolarmente interessante è il fatto che non sia stata osservata alcuna compromissione cognitiva nei pazienti che raggiungevano livelli

estremamente bassi di LDL-C (38).

PCSK9 Binding Antibody Influence on

Cognitive Health in High Cardiovascular

Conclusioni

Il controllo dei fattori di rischio modificabili è di estrema importanza nella prevenzione del deterioramento cognitivo e della demenza. In questo scenario, un ruolo centrale è svolto dal controllo dell'ipercolesterolemia. L'uso di statine non ha evidenziato effetti avversi sulla sfera cognitiva, rivelandosi un efficace strumento di prevenzione. Analogamente, gli attuali dati sull'inibizione del PCSK9 attraverso anticorpi monoclonali non evidenziano rischi di sviluppare deficit cognitivi. Ulteriori studi sugli effetti a lungo termine contribuiranno ad ampliare le nostre conoscenze in merito.

Il deterioramento cognitivo è comune tra la popolazione anziana e la demenza rappresenta la principale causa di disabilità. La più comune forma di demenza degenerativa è rappresentata dalla malattia di Alzheimer (AD), mentre la malattia cerebrovascolare è seconda per frequenza. Non è pertanto sorprendente che la prevenzione del deterioramento cognitivo sia considerata una questione di salute pubblica di interesse mondiale. Il deterioramento cognitivo, la demenza e l'AD sono condizioni multifattoriali a cui contribuiscono numerosi fattori di rischio modificabili, inclusi i fattori vascolari e lo stile di vita. Di questi, l'ipercolesterolemia è un fattore ormai noto. Interventi multi-dominio includono la dieta, l'attività fisica e la stimolazione cognitiva. Relativamente ai trattamenti farmacologici, le statine e gli inibitori di PCSK9 sono quelli più promettenti. In questo contesto, sono stati ipotizzati dei possibili effetti avversi di tipo neurologico derivanti dalla riduzione dei livelli di colesterolo, dal momento che esso è un componente fondamentale del sistema nervoso. Dati epidemiologici hanno supportato una possibile associazione tra l'uso di statine e il rischio di sviluppare demenza, con evidenze controverse. Recentemente, una review sistematica/meta-analisi ha evidenziato che l'uso di statine è associato ad una riduzione significativa del rischio di sviluppare demenza, AD e deterioramento cognitivo lieve. Analogamente, l'uso di anticorpi monoclonali anti-PCSK9 ha sollevato delle domande in merito a possibili effetti collaterali di tipo neurologico, dal momento che PCSK9 sembra avere un ruolo nella differenziazione, l'apoptosi e la migrazione neuronale. I risultati dal trial EBBINGHAUS hanno escluso la presenza di deterioramento cognitivo nei pazienti trattati con evolocumab rispetto ai controlli. In conclusione, ridurre il colesterolo è di fondamentale importanza nella prevenzione del deterioramento cognitivo. In merito ai trattamenti farmacologici, ulteriori dati sugli effetti a lungo termine sono necessari per ampliare il panorama delle nostre conoscenze.

Parole chiave: Colesterolo, demenza, malattia di Alzheimer, statine, inibitori di PCSK9.

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NUOVI FARMACI

NUOVI IPOGLICEMIZZANTI E PROTEZIONE CARDIOVASCOLARE New hypoglycemic drugs and cardiovascular protection

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SUMMARY

The risk of cardiovascular disease (CVD) (myocardial infarction, stroke, peripheral vascular disease) is twice in type 2 diabetes (T2D) patients compared to non-diabetic subjects. Furthermore, cardiovascular disease (CV) is the leading cause of death in patients with T2D.

In the last years several clinical intervention studies with new anti-hyperglycaemic drugs have been published and they have shown a positive effect on mortality and cardiovascular risk in T2D patients. In particular, these studies evaluated sodium/glucose-2 cotransporter inhibitors (SGLT2i) and GLP-1 receptor agonists (GLP-1RA).

In secondary prevention, it was demonstrated that SGLT2i and GLP-1RA drugs reduce CV events and mortality, and new guidelines consider these drugs as first-choice (after metformin) in T2D treatment; the mechanisms involved are not yet fully understood, but they appear to be both "glycemic" and "extra-glycemic".

In this revision we will examine the principal results of the clinical trials on SGLT2i and GLP-1RA, their clinical relevance in term of treatment of type 2 diabetes, and we will discuss how these drugs may exert their cardiovascular protective effects.

Keywords: Cardiovascular disease, heart failure, type 2 diabetes, sodium/glucose-2 cotransporter inhibitors (sglt2i), GLP-1 receptor agonists (glp-1ra).

Rischio cardiovascolare nel diabete

Le persone con diabete di tipo 2 (DT2) hanno un rischio di malattie cardiovascolari (CVD) (infarto miocardico, ictus, malat-

Indirizzo per la corrispondenza

Prof. Marco Giorgio Baroni, Sezione di Endocrinologia, Dipartimento di Medicina Sperimentale, Sapienza Università di Roma E-mail: marco.baroni@uniroma1.it tia vascolare periferica) superiore di due o più volte rispetto a soggetti non diabetici, e la malattia cardiovascolare costituisce la principale causa di morte nei pazienti con DT2 (1). Gli studi clinici d'intervento (2-5) hanno costantemente dimostrato che l'abbassamento di HbA1c nei pazienti con DT2 ha solo un modesto (2, 3) o nessun effetto (4, 5) sulla riduzione del rischio cardiovascolare (CV). Al contrario, la correzione dei fattori di rischio CV tradizionali, ad esempio pressione sanguigna e colesterolo, riduce marcatamente il rischio di CVD e la mortalità nei pazienti con DT2.

L'iperglicemia, infatti, è un debole fattore di rischio per CVD (5, 6) e i grandi trials d'intervento (2-4) mirati al trattamento intensivo della glicemia non sono riusciti a ridurre significativamente il rischio CV e la mortalità (*Figura 1A*), soprattutto in studi di prevenzione secondaria. Inoltre, negli studi di prevenzione primaria come UKPDS (5) e Veterans Affairs Diabetes Trial (VADT) (7), sono stati necessari 10 anni per osservare un beneficio CV associato al miglioramento del controllo glicemico. Anche trials più recenti con nuove classi di farmaci come gli inibitori delle dipeptidil-peptidasi 4 (DPP4) o le nuove insuline non sono riusciti a dimostrare effetti positivi sulla riduzione di mortalità e rischio CVD (*Figura 1B*). Va ricordato che l'FDA (Food and Drug Administration) negli USA impone di studiare gli effetti cardiovascolari e di mortalità di tutti i nuovi farmaci per il diabete, dopo l'esperienza negativa osservata nello studio del rosiglitazone (8). Tutti i nuovi farmaci devono essere studiati in popolazioni ad alto rischio cardiovascolare, per dimostrare la sicurezza del farmaco.



Figura I - Riduzione della Glicemia: Trattamento Intensivo vs Standard. Mortalità da tutte le cause (A) Effetto su MACE* di Pioglitazione, DPP4-i e Insulina (B).

Solo lo studio Proactive con il pioglitazione (9) ha dimostrato un effetto positivo sugli *end-point* secondari (*Figura 1B*), che era il 3 point MACE (Major Cardiovascular Events) costituito da infarto, stroke e mortalità CV. Tuttavia il disegno dello studio, che aveva inserito anche la malattia vascolare periferica tra gli end-point primari, ha impedito che il risultato fosse significativo.

Un intervento multifattoriale, invece, mirato alla riduzione di diversi fattori di rischio CV. si è dimostrato efficace nella riduzione degli eventi CV e nella mortalità nel diabete (10). La spiegazione risiede probabilmente nel comune denominatore di molti dei fattori di rischio CV, quali ipertensione, dislipidemia e adiposità addominale, che è l'insulino-resistenza. Farmaci che hanno effetto solo sulla riduzione della glicemia, come le sulfaniluree, l'insulina e i DPP4-i, senza avere effetti sugli altri fattori di rischio, sono destinati a fallire nella riduzione del rischio CV. In questo senso è spiegabile il risultato, anche se parziale, del pioglitazone (9), che agendo sull'insulino-resistenza e correggendo alcune delle sue componenti (come ipertensione e dislipidemia, oltre al controllo glicemico), è riuscito a ridurre il numero di eventi nei pazienti trattati.

Negli ultimi anni, con grande sorpresa della comunità scientifica, sono stati pubblicati una serie di studi clinici di intervento con nuovi farmaci per il diabete che hanno dimostrato un effetto positivo sulla mortalità e sul rischio cardiovascolare nel diabete. Questi studi hanno riguardato in particolare gli effetti sul rischio CV dei farmaci inibitori del cotrasportatore sodio/ glucosio-2 (sodium–glucose cotransporter 2 o SGLT2) e degli analoghi del GLP1 (glucagon-like peptide-1).

Inibitori del cotrasportatore Sodio/ Glucosio-2 (Sodium–Glucose Cotransporter 2 SGLT2I)

In condizioni normali il rene è in grado di riassorbire tutto il glucosio filtrato, e lo fa attraverso due cotrasportatori sodio/ glucosio, l'SGLT2 nel tubulo prossimale e l'SGLT1 nel suo tratto più distale. Circa il 90% del glucosio filtrato viene riassorbito dagli SGLT2, e il restante 10% dagli SGLT1. Il risultato netto è l'assenza di glicosuria e





il recupero del glucosio filtrato (Figura 2). Nel diabete si è osservato che la capacità riassorbitiva degli SGLT2 è persino superiore ai soggetti normali, risultando quindi in una maggiore attività e un ulteriore aumento della glicemia. È stato ipotizzato che attraverso l'inibizione di questi cotrasportatori si potesse "forzare" una perdita di glucosio nelle urine, riducendo guindi la glicemia plasmatica. Gli studi clinici hanno effettivamente dimostrato la grande efficacia anti-iperglicemica di questi farmaci (11). Come tutti i nuovi farmaci. anche per gli inibitori dell'SGLT2, detti anche gliflozine, è stato necessario effettuare studi clinici sul rischio cardiovascolare. Il primo studio ad essere pubblicato è stato L'EMPAREG-CV outcome (12), studio di intervento con l'inibitore dell'SGLT2 Empagliflozin contro placebo e trattamento standard, effettuato in una coorte di 7.020 pazienti con DT2 ad alto rischio cardiovascolare (con pregressi eventi o con multipli fattori di rischio CV). Il dato dello studio EMPAREG è stato sorprendente: si è osservata una riduzione della morte cardiovascolare del 38%, delle ospedalizzazioni per scompenso cardiaco del 35%, e della morte da tutte le cause del 32%. Mai nessun trial nel diabete aveva dato risultati così significativi, tra l'altro l'effetto degli SGLT2i si osservava già nei primi 3 mesi di trattamento, suggerendo un meccanismo non esclusivamente collegato alla riduzione glicemica. L'Hba1c, infatti, mostrava un differenza di solo -0.5% tra i due bracci, differenza che si osservava solo dopo 8-12 mesi dall'inizio del trattamento, insufficiente a dimostrare una causalità.

Poco dopo lo studio EMPAREG, è stato pubblicato lo studio CANVAS, Canagliflozin contro placebo e trattamento standard (13), in una coorte di più di 10.000 soggetti con DT2 ad alto rischio cardiovascolare. Anche nello studio CANVAS si è dimostrato un effetto significativo di protezione cardiovascolare, con una riduzione del 14% del *end-point* primario (3 point MACE) e del 33% delle ospedalizzazioni per scompenso cardiaco. Non sono risultati significativi i singoli end-point, come infarto o stroke, e neanche la mortalità per tutte le cause.

Da segnalare che sia nell'EMPAREG sia nel CANVAS risultava significativo anche l'effetto di nefro-protezione, valutato come ridotta progressione di albuminuria o di mantenimento del eGFR.

Ai risultati di questi studi clinici randomizzati si sono aggiunti dati di "real world" che hanno confermato gli effetti positivi sulla riduzione del rischio CV nel diabete. La studio CVD-REAL (14) è stato effettuato in più di 300.000 pazienti con diabete di tipo 2, in larga maggioranza (87%) senza pregressi eventi CV o elevato rischio CV, raccogliendo i dati dalle cartelle cliniche in 6 nazioni comprendenti USA e 5 stati europei. In questa coorte si è confermato l'effetto protettivo degli SGLT2 inibitori (erano utilizzati Dapagliflozin, Empagliflozin e Canagliflozin) rispetto alla terapia con altri antidiabetici orali. I risultati erano molto simili allo studio EMPAREG (12), con una riduzione del 30-40% in mortalità e scompenso cardiaco, e del 20-30% in infarto e stroke. Questi effetti si osservavano sia in pazienti senza rischio cardiovascolare sia in quelli (pochi) con precedenti eventi CV. A questi dati si sono aggiunti quelli dello studio EASEL (15), un altro studio "real-world" che ha confrontato gli inibitori degli SGLT2 con terapie standard in pazienti con DT2. Anche nello studio EASEL si è osservata una riduzione del 43% di scompenso cardiaco e mortalità. Da notare che anche in questo studio sono stati impiegati tutti e tre gli inibitori del SGLT2 (Dapagliflozin, Empagliflozin e Canagliflozin), suggerendo che gli effetti osservati sono probabilmente di classe e non specifici dei singoli SGLT2i. Infine, è prevista a breve la pubblicazione dello studio clinico randomizzato DECLARE-TIMI 58 (16), che valuterà gli effetti di Dapagliflozin sugli eventi cardiovascolari. Dati preliminari sembrano indicare che anche in questo caso si osserva una riduzione degli eventi CV.

È quindi evidente che gli inibitori degli SGLT2 sono in grado di modificare il rischio cardiovascolare nei pazienti con DT2, certamente in prevenzione secondaria ma forse, almeno dai dati di "*realworld*", anche in prevenzione primaria. La questione aperta riguarda invece quali siano i meccanismi, posto che la riduzione della glicemia non sembra il meccanismo centrale della riduzione del rischio CV.

Meccanismi di protezione cardiovascolare degli inibitori degli SGLT2

Sono diversi i meccanismi ipotizzati che possono portare alla riduzione del rischio cardiovascolare nei soggetti trattati con inibitori degli SGLT2; in generale possono essere divisi in metabolici e cardioemodinamici (*Tabella 1*).

Tra i possibili effetti metabolici non-glicemici ascrivibili agli SGLT2i hanno ricevuto particolare attenzione gli effetti sulla lipolisi indotta dagli SGLT2i. Si è osservato, infatti, che nei soggetti trattati con SGL-T2i c'era un aumento della mobilizzazione degli acidi grassi (FFA) dal tessuto adiposo. Le possibili cause di questa mobilizzazione di FFA sono la necessità di utilizzare nuovi substrati in presenza di glicosuria e un aumento del glucagone. L'aumento del glucagone, in particolare, è dovuto ad effetti diretti degli inibitori degli SGLT2. È stato dimostrato che le alfa cellule del pancreas possiedono i co-trasportatori SGLT2, la cui inibizione determina un aumentato rilascio di glucagone. A sua volta il glucagone determina lipolisi e utilizzo di corpi chetonici. L'aumento della lipolisi favorisce un aumento della spesa energetica e consumo delle riserve adipose, che risulta in perdita di peso (in media 3-5 kg). Inoltre, l'aumento della chetogenesi epatica favorisce l'utilizzazione degli acidi grassi, con effetti positivi sulla steatosi epatica e sull'infiammazione (*Tabella 1*).

Gli effetti diretti cardio-emodinamici degli inibitori degli SGLT2 sono stati chiamati in causa per spiegare gli effetti positivi sul rischio cardiovascolare, in particolare sullo scompenso. Andando a guardare in dettaglio i risultati dei trial clinici si è visto, infatti, che molti degli effetti positivi erano determinati dalla riduzione dello scompenso cardiaco (-38%), una complicanza molto frequente nel DT2 (17).

Gli effetti diretti cardiaci degli inibitori degli SGLT2 sono molteplici (*Tabella 1*), e includono un miglioramento del precarico

Effetti metabolici	Effetti cardiaci diretti		
T. Adiposo: aumento della mo- bilizzazione degli acidi grassi (FFA) e riduzione dell'infiamma- zione degli adipociti. Perdita di peso (circa 3-5 Kg)	Miglioramento del precarico ventricolare (secondario a na- triuresi e diuresi osmotica) e del post-carico attraverso la ridu- zione della pressione		
Pancreas: aumento della secre- zione di glucagone che determi- na aumento della lipolisi e upta- ke FFA nel fegato	Miglioramento del metabolismo cardiaco		
Fegato: aumento glicogenolisi, gluconeogenesi, uptake FFA e chetogenesi	Inibizione dello scambio sodio- idrogeno nel miocardio		
Cuore: Scambio substrati con aumento dell'uso di FFA e che- toni ("superfuel") e riduzione uso di glucosio. Diminuzione grasso epicardico	Riduzione della fibrosi e della necrosi cardiaca		
	Modificazione della produzione di adipochine e citochine e del grasso epicardico		

Tabella I - Possibili meccanismi coinvolti n	iegli el	ff∈tti	car-
diovascolari e metabolici degli SGLT2i.			

ventricolare (secondario a natriuresi e diuresi osmotica) e del post-carico attraverso la riduzione della pressione, miglioramento del metabolismo cardiaco, inibizione dello scambio sodio-idrogeno, riduzione della fibrosi cardiaca e modificazione della produzione di adipochine e citochine e del grasso epicardico.

Alcuni di questi meccanismi sono molto interessanti. Ad esempio, l'attivazione del feedback tubulo-glomerulare, mediato dall'aumento del sodio che arriva alla macula densa dovuto all'inibizione del cotrasporto sodio/glucosio, e conseguente riduzione dell'attivazione del sistema renina-angiotensina, sembra determinare una contrazione del volume e dell'edema cardiaco (18). La riduzione di sodio e di volume determina anche effetti positivi sulla pressione arteriosa, che è risultata ridotta di 4 mmHg in media in tutti gli studi sugli SGLT2i (19). Entrambi questi effetti sono molto precoci, e potrebbero contribuire a spiegare la rapidità di effetto sul rischio CV osservato nei trials clinici (12, 13).

Per quanto riguarda la modulazione del trasporto sodio/idrogeno nei cardiomiociti, è noto che nello scompenso cardiaco associato al diabete è presente un aumento del trasportatore NHE (Na+/ H+ exchanger). Questo aumento determina un'elevazione delle concentrazioni di sodio e calcio, che possono contribuire alla disfunzione cardiaca. Dati recenti dimostrano un'affinità tra gli inibitori degli SGLT2 e il sito legante il sodio del NHE-1, con blocco del trasporto di sodio (20). A dimostrazione del potenziale effetto positivo dell'inibizione del NHE, in passato un inibitore selettivo del NHE, il Cariporide (21), aveva dimostrato di ridurre il rischio di infarto in pazienti sottoposti a bypass cardiaco, anche se i decessi erano aumentati per sanguinamento cerebrale, per cui il farmaco è stato ritirato.

Gli effetti sul metabolismo lipidico e sull'assetto adipo-citochinico può essere, almeno in parte, ricondotto agli effetti sulla lipolisi discussi in precedenza. Si è osservata infatti una riduzione del grasso epicardico (fattore di rischio CV), in modelli animali trattati con SGLT2i (22), e nei pazienti trattati con SGLT2i, si sono osservati aumento di adiponectina e riduzione di TNF-alfa, con miglioramento dell'insulino-sensibilità e riduzione dell'infiammazione (23).

In conclusione, gli inibitori degli SGLT2 hanno effetti cardiovascolari e renali che sono indipendenti dalla riduzione del glucosio, e che possono spiegare la riduzione del rischio cardiovascolare osservata nei pazienti con DT2 trattati con SGLT2i. Le osservazioni sugli effetti non-glicemici, in particolare quelli sullo scompenso cardiaco, hanno portato ad avviare una serie di *trial* mirati anche in pazienti senza DT2. Inoltre, sono in corso studi meccanicistici sulla NAFLD (steatosi epatica non-alcoolica), ipertensione e patologie vascolari periferiche per chiarire meglio tutti gli aspetti riguardanti gli inibitori degli SGLT2.

Gli analoghi del Glucagon-Peptide -1 (GLP-1)

Gli studi clinici d'intervento che hanno dimostrato un effetto positivo sulla mortalità e sul rischio cardiovascolare nel diabete hanno riguardato anche gli agonisti del recettore del GLP-1 (GLP-1RA). I GLP-1RA sono farmaci che mimano l'azione del GLP-1 endogeno e migliorano l'omeostasi glucidica attraverso diversi meccanismi (*Figura 3*): 1) stimolazione della secrezione di insulina:

- 1) sumoiazione dena secrezione di insumia,
- 2) inibizione della secrezione di glucagone;
- soppressione diretta e indiretta della produzione endogena di glucosio;
- 4) riduzione dell'appetito;
- 5) aumento della sensibilità all'insulina

come conseguenza della perdita di peso;

6) ritardo dello svuotamento gastrico, con conseguente riduzione dell'iperglicemia post-prandiale.

Nei pazienti con DT2 è stata dimostrata una ridotta produzione di GLP-1 in risposta al pasto, e i numerosissimi trials effettuati con GLP-1RA hanno tutti mostrato una grande efficacia nel controllo glicemico, a cui si è sempre associata una significativa perdita di peso. Le formulazioni attualmente disponibili vengono somministrate per iniezione sottocutanea e vengono classificati in agonisti a breve durata d'azione (lixisenatide), a durata d'azione intermedia (liraglutide) e a lunga durata d'azione (exenatide, dulaglutide, semaglutide e albiglutide). Sono farmaci ormai largamente utilizzati nella cura del DT2 poiché abbassano la glicemia con riduzione di peso, e presentano basso rischio di ipoglicemia (24). Inoltre, il trattamento con i farmaci GLP-1RA ha effetti favorevoli su diversi fattori di rischio cardiovascolare, come il peso corporeo stesso e la pressione arteriosa (25).

Sono stati condotti 5 trial di sicurezza cardiovascolare con i farmaci GLP-1RA: ELIXA (lixisenatide) (26), LEADER (liraglutide) (27), SUSTAIN (semaglutide) (28), EXSCEL (exenatide a rilascio prolungato) (29) ed HARMONY (albiglutide) (30) (*Tabella 2*).

Lo studio ELIXA, condotto su 6.068 soggetti con pregressi eventi cardiovascolari trattati con lixisenatide o placebo in aggiunta alla terapia pre-esistente, ha mostrato piena sicurezza ma effetti cardiovascolari neutri: l'HR per MACE (morte cardiovascolare, infarto e ictus non fatale) era 1.02 (95% CI 0.89-1.17, p=n.s.). In questo studio l'HR per scompenso cardiaco è risultato 0.96 (95% CI 0.75-1.23, p=NS) (26) (*Tabella 2*).

Nello studio LEADER, condotto su quasi 10.000 pazienti con DT2 con pregressa malattia cardiovascolare e lieve insufficienza renale, l'aggiunta di liraglutide invece che di placebo alla terapia pre-esistente è risultata associata a una riduzione significativa degli eventi che costituivano l'endpoint primario (MACE) del 13% (HR

Trial	Farmaco	N. pazienti	Mace Hazard Ratio (95% Cl)	Morte CV Hazard Ratio (95% CI)	Morte da tutte le cause Hazard Ratio (95% CI)
Elixa	Lixisenatide	6.068	1.02 (0.89-1.17) >	0.98 (0.78-1.22) ->	0.94 (0.78-1.13) ->
Leader	Liraglutide	9.340	0.87 (0.78-0.97)	0.78 (0.66-0.93)	0.85 (0.74-0.97) ↓
Sustain	Semaglutide	3.297	0.74 (0.58-0.95)	0.98 (0.65-1.48) >	1.05 (0.74-1.50) ->
Exscel	Exenatide a rilascio prolungato	14.752	0.91 (0.83-1.00) ->	0.88 (0.76-1.02) ->	0.86 (0.77-0.97) ↓
Harmony	Albiglutide	9.463	0-78 (0.68-0.90) ↓	0·93 (0.73-1.19) ->	0·95 (0.79-1.16) ->

Tabella 2 - Principali risultati dei trial di outcome cardiovascolare con GLP-1RA.

0.87, 95% CI 0.78-0.97, p=0.01 per superiorità). Il periodo di *follow-up* mediano è stato di 3.8 anni e la differenza di HbA1c fra i due gruppi di trattamento era di circa 0.4%. Inoltre, nei pazienti trattati con liraglutide si è osservata una riduzione del 22% della morte cardiovascolare (HR 0.78, 95% CI 0.66- 0.93, p=0.007) (27) (*Tabella 2*).

Nello studio SUSTAIN, che ha coinvolto 3.297 con DT2 e con pregressa malattia cardiovascolare (58.8%) o ad alto rischio cardiovascolare, l'aggiunta di semaglutide (0.5 o 1 mg alla settimana) invece che di placebo alla terapia standard, è risultata associata a una riduzione degli eventi che costituivano l'*endpoint* primario (MACE) del 26% (HR 0.74, 95% CI 0.58-0.95, p=0.02 per superiorità). Il periodo di follow-up mediano è stato di 2.1 anni, e la differenza di HbA1c rispetto al placebo era di -0.7 % per il gruppo trattato con semaglutide 0.5 mg e -1.0% per il gruppo trattato con semaglutide 1 mg (28) (*Tabella 2*).

Lo studio EXSCEL, condotto su 14.752 pazienti (73% con pregressa malattia cardiovascolare), ha mostrato che l'aggiunta di exenatide LAR invece che di placebo alla terapia pre-esistente era associata a una non-significativa riduzione degli eventi che costituivano l'endpoint primario (MACE) del 9% (HR 0.91; 95% CI 0.83-1.00; p< 0.001 per non-inferiorità e p=0.06 per superiorità). Il periodo di follow-up mediano è stato di 3.2 anni, e la differenza di HbA1c fra i due gruppi di trattamento era di 0.53%. Tuttavia, il trattamento con exenatide LAR era associato a una riduzione del 14% della mortalità per tutte le cause (HR 0.86; 95% CI 0.77-0.97; p=0.016) (29) (Tabella 2).

Infine, lo studio HARMONY ha coinvolto 9.463 pazienti che sono stati seguiti per 1 anno e 6 mesi. I pazienti con DT2 e malattie cardiovascolari sono stati divisi in due gruppi: ad uno è stata effettuata un'iniezione settimanale con albiglutide in aggiunta alla terapia standard, mentre al secondo gruppo è stato somministrato placebo in aggiunta alla terapia standard. I dati hanno evidenziato una riduzione significativa del 22% del rischio di infarto, ictus e morte per cause cardiovascolari nel gruppo trattato con il nuovo farmaco. L'*outcome* primario composito si è verificato in 338 (7%) su 4.731 pazienti nel gruppo trattato con albiglutide e in 428 (9%) su 4.732 pazienti nel gruppo placebo (HR 0.78; 95% CI 0.68-0.90), mostrando che l'albiglutide era superiore al placebo (p <0.0001 per la non inferiorità; p = 0.0006 per superiorità) (30) (*Tabella 2*).

E stato inoltre osservato in tutti questi trials che il trattamento con agonisti del recettore del GLP-1 induce un significativo calo ponderale (31) e una significativa riduzione della pressione arteriosa (28). Inoltre, i dati provenienti dagli studi di *outcome* cardiovascolare LEADER e SU-STAIN indicano che gli agonisti del recettore del GLP-1, in particolare liraglutide e semaglutide, sono efficaci nella prevenzione della comparsa e della progressione della nefropatia diabetica, sebbene rimanga ancora da chiarire in quale misura questo effetto sia mediato dal miglioramento del controllo glicemico (27, 28).

Un studio abbastanza recente di 18 mesi in "*real-life*" ha mostrato che il trattamento con liraglutide in aggiunta a metformina determina un miglioramento di numerosi fattori di rischio cardio-metabolici. Dallo studio, infatti, è emersa una riduzione significativa dello spessore medio-intimale carotideo nei pazienti con sindrome metabolica trattati con liraglutide. Poiché lo spessore medio-intimale carotideo è un marker surrogato di aterosclerosi precoce e subclinica, la sua riduzione dopo utilizzo di liraglutide sembra essere congruente con gli emergenti effetti cardiovascolari di questo farmaco (32). Nel complesso questi dati suggeriscono che i farmaci GLP-1RA possiedono un effetto protettivo sul piano cardiovascolare ed effetti favorevoli su altri fattori di rischio cardiovascolare, come per esempio il peso corporeo e la pressione arteriosa.

Nonostante i numerosi dati emersi dagli studi, i meccanismi attraverso i quali alcuni farmaci agonisti del GLP-1R siano in grado di determinare una riduzione dei tassi di MACE rimangono ancora poco chiari. In ogni caso, l'evidenza che il trattamento con GLP-1RA sia in grado di ridurre il tasso di eventi cardiovascolari nel paziente con DT2 ha indotto a collegare l'attività dei farmaci GLP-1RA alla cardioprotezione (33): il GLP-1 è in grado di ridurre i valori pressori, migliorare la funzione endoteliale e aumentare la contrattilità del miocardio.

Meccanismi di protezione cardiovascolare degli analoghi del GLP-1

Studi sperimentali condotti sia in modelli animali sia nell'uomo hanno dimostrato che il GLP-1 endogeno presenta effetti positivi su diversi parametri di rischio cardiovascolare, tra i quali la funzione endoteliale e, di conseguenza, anche sui livelli pressori (34). Questi effetti sono stati confermati anche con i farmaci agonisti recettoriali del GLP-1.

L'ipertensione arteriosa è un fattore noto di rischio cardiovascolare nei pazienti affetti da DT2 ed è stato osservato che il trattamento con GLP-1RA è associato a un significativo abbassamento della pressione sistolica (35). Riduzioni significative della pressione sistolica sono state osservate dopo solo 2 settimane di trattamento con liraglutide e si manifestano prima che si verifichi un significativo calo ponderale (36). I meccanismi con cui i GLP-1RA riducono la pressione arteriosa non sono completamente chiari. Si ipotizza che pos-

sano essere legati ad un miglioramento della funzione endoteliale e ad un effetto natriuretico (37, 38). In uno studio, Kim e collaboratori, hanno dimostrato la presenza di recettori per il GLP-1 a livello degli atrii cardiaci, e hanno osservato che il GLP-1 è in grado di attivare questi recettori promuovendo il rilascio del fattore natriuretico atriale (ANF) che, come è noto, possiede effetti natriuretici e di riduzione dei livelli di pressione arteriosa. Gli autori di questo studio mostrano che gli effetti di vasodilatazione arteriosa vengono mediati da una sostanza rilasciata dagli atrii del cuore stimolati dal GLP-1 e non dal GLP-1 stesso, e che questa sostanza è l'ANF. In base a questa teoria, viene quindi definito un nuovo asse multi-organo in grado di regolare i livelli di pressione arteriosa: il punto di partenza è l'intestino che produce GLP-1, che agisce sulle cellule cardiache provocando il rilascio di ANF, il quale a sua volta agisce sui vasi arteriosi inducendo vasodilatazione, e a livello renale determina anche un aumento dell'eliminazione urinaria di sodio (natriuresi) (39).

Studi su modelli animali hanno anche dimostrato che i recettori per GLP-1 sono espressi sui cardiomiociti e sulle cellule endoteliali (40) e che il trattamento con GLP-1 nativo possiede effetti cardioprotettivi: aumenta infatti il recupero funzionale, l'utilizzazione di glucosio e la vitalità del tessuto miocardico dopo danno da ischemia/riperfusione e favorisce la vasodilatazione e di conseguenza il flusso coronarico (40). In un modello di topi non diabetici in cui veniva indotto infarto del miocardio dopo occlusione coronarica, il trattamento con liraglutide riduceva la rottura del cuore e l'area infartuale, e migliorava significativamente la funzione contrattile e la sopravvivenza (41). Inoltre, il trattamento con liraglutide conferiva cardioprotezione e aumentava la sopravvivenza di topi diabetici con infarto del miocardio indotto da occlusione coronarica rispetto al trattamento con metformina (41).

Lo stress ossidativo è un altro meccanismo coinvolto nel danno cardiaco. In uno studio di Laviola e collaboratori è stata valutata la capacità del GLP-1 di contrastare gli effetti tossici dello stress ossidativo nelle cellule progenitrici cardiache isolate dal cuore umano. Queste cellule sono in grado di proliferare indefinitamente e di differenziarsi nelle principali linee presenti nel tessuto miocardico maturo (cardiomiociti, cellule endoteliali, cellule muscolari lisce), rappresentando quindi un importante indicatore della capacità del cuore di reagire e di ricostituirsi in seguito a danno indotto dall'ischemia o da altre cause. In presenza di un aumento delle specie reattive dell'ossigeno, le cellule progenitrici cardiache vanno incontro ad apoptosi con riduzione del potenziale rigenerativo del miocardio. Questo studio ha dimostrato che il GLP-1 contrasta l'effetto pro-apoptotico del perossido d'idrogeno e aumenta la sopravvivenza delle cellule progenitrici cardiache umane. Tali effetti del GLP-1 si realizzano attraverso la capacità di contrastare il meccanismo intracellulare che media l'apoptosi indotta dal perossido di idrogeno e che coinvolge l'attivazione della stress-chinasi JNK (42). Questi dati, osservati in cellule umane, potrebbero essere alla base del possibile effetto protettivo nei confronti degli eventi cardiovascolari maggiori attribuito alle terapie con incretine.

Nell'uomo diverse evidenze suggeriscono che il GLP-1 è in grado di esercitare effetti positivi sulla funzione endoteliale e sulla funzione cardiaca in vivo: l'infusione endovenosa di GLP-1 induce un miglioramento della vasodilatazione flusso-mediata di circa il 50% in soggetti diabetici di tipo 2 coronaropatici (37). In un altro studio condotto in pazienti con insufficienza cardiaca, in classe NYHA III e IV, sottoposti a infusione con GLP-1 per 5 settimane, è stato dimostrato un significativo miglioramento della frazione di eiezione del ventricolo sinistro, del test dei 6 min di cammino



e del Minnesota Living with *Heart Failure Quality of Life score* (43). Altri studi hanno dimostrato una riduzione del danno cardiaco indotto da infarto acuto del miocardio con elevazione del tratto ST in seguito a trattamento con exenatide durante le procedure di rivascolarizzazione (44, 45) e un miglioramento della frazione di eiezione in soggetti con infarto acuto del miocardio con elevazione del tratto ST trattati con liraglutide per 7 giorni (46).

In conclusione, sia i dati sperimentali provenienti da modelli cellulari e animali, sia i dati provenienti dagli studi condotti sull'uomo, dimostrano che la somministrazione di GLP-1 e dei suoi analoghi è in grado di determinare numerosi effetti positivi sia a livello endoteliale che cardiaco, indipendentemente dall'azione ipoglicemizzante, e in associazione con effetti metabolici che determinano una riduzione dei fattori di rischio cardiovascolari "classici" (obesità, ipertensione, dislipidemia) (*Figura 3*).

Conclusioni e prospettive future

I risultati di tutti questi importanti studi clinici randomizzati in prevenzione cardiovascolare sia primaria sia secondaria avevano già indotto la Società Italiana di Diabetologia a redigere un documento di consenso relativo agli effetti di prevenzione cardiovascolare e di rallentamento della progressione della malattia renale dimostrato da alcuni dei farmaci della classe dei GLP-1RA e degli SGLT2i (47).

Negli ultimi mesi, nelle nuove linee guida delle due principali società scientifiche internazionali di diabetologia (American Diabetes Association-ADA ed European Association for the Study of Diabetes-EA-SD) sono state inserite due importanti novità riguardo l'algoritmo terapeutico del DT2 e la scelta dei farmaci più appropriati per le diverse tipologie di pazienti (48); tali indicazioni erano state già introdotte nel documento Standard di Cura SID/AMD 2018 (49). Secondo queste linee guida, nel momento in cui la metformina, che resta il farmaco di prima scelta, non sia più sufficiente come unico trattamento farmacologico per raggiungere e mantenere un controllo me-

Glossario

ADA: American Diabetes Association ANF: Fattore Natriuretico Atriale **CANVAS:** Canagliflozin Cardiovascular Assessment Study CV: cardiovascolare **CVD-Real:** Cardiovascular Events Associated with SGLT-2 Inhibitors Versus Other Glucose-Lowering Drugs **CVD:** malattia cardiovascolare **DECLARE-TIMI 58:** Dapagliflozin Effect on Cardiovascular Events DPP4-i: inibitori delle Dipeptidil-peptidasi 4 DT2: diabete di tipo 2 EASD: European Association for the Study of Diabetes EASEL: Evidence for Cardiovascular Outcomes with Sodium Glucose Cotransporter 2 Inhibitors in the Real World ELIXA: Evaluation of Lixisenatide in Acute **Coronary Syndrome EMPAREG:** the Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients-Removing Excess Glucose EXSCEL: Exenatide Study of Cardiovascular Event Lowering Trial FDA: Food and Drug Administration FFA: acidi grassi liberi GLP-1RA: agonisti del recettore del GLP-1 HARMONY: Albiglutide and cardiovascular outcomes in patients with type 2 diabetes and cardiovascular disease **LEADER:** Liraglutide Effect and Action in Diabetes: **Evaluation of Cardiovascular Outcome Results** MACE: Major Cardiovascular Events NAFLD: steatosi epatica non-alcoolica SGLT2i: inibitori del cotrasportatore sodio/glucosio-2 SUSTAIN: Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes

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tabolico adeguato, è necessario indirizzare la scelta verso i farmaci anti-iperglicemici più sicuri, quelli cioè che non causano ipoglicemia. A differenza del precedente consenso EASD/ADA che metteva sullo stesso piano tutte le terapie disponibili, i nuovi *statement* suggeriscono di preferire classi terapeutiche più moderne e sicure (inibitori di DPP-4, gliflozine, GLP-1RA, pioglitazone) rispetto alle sulfaniluree.

La seconda indicazione è la caratterizzazione dei pazienti in relazione alla presenza o meno di malattia cardiovascolare in atto, ai fini di personalizzare la terapia: dopo fallimento della metformina, nei pazienti con pregressi eventi cardiovascolari, è raccomandabile l'uso di farmaci dei quali sia dimostrata l'efficacia nella prevenzione secondaria degli eventi cardiovascolari (pioglitazone, empagliflozin, canagliflozin, liraglutide).

Sulla base di questi dati, nelle scelte farmacologiche successive alla terapia con metformina i nuovi *statement* "dividono" i pazienti in soggetti con malattia cardiovascolare accertata e soggetti senza malattia cardiovascolare accertata. I primi sono candidati alla terapia con SGLT2i e GLP-1RA.

Infine, gli effetti "extraglicemici" osservati per i farmaci SGLT2i e GLP-1RA sono serviti a iniziare studi clinici mirati, ad esempio, al trattamento dello scompenso cardiaco in pazienti senza diabete o al trattamento dell'obesità, della NA-FLD o dell'ipertensione. In questo modo sarà possibile capire la reale portata degli effetti diretti cardiovascolari di queste classi di farmaci.

RIASSUNTO

I soggetti con diabete di tipo 2 (DT2) presentano un rischio di sviluppare malattie cardiovascolari (CVD) (infarto miocardico, ictus, malattia vascolare periferica) doppio rispetto a soggetti non diabetici. La malattia cardiovascolare (CV) rappresenta la principale causa di morte nei pazienti con DT2. Nell'ultimo periodo sono stati pubblicati numerosi studi clinici di intervento con nuovi farmaci anti-iperglicemici che hanno dimostrato un effetto positivo sulla mortalità e sul rischio cardiovascolare nei pazienti con DT2. Questi studi hanno riguardato in particolare i farmaci inibitori del cotrasportatore sodio/glucosio-2 (SGLT2i) e gli agonisti del recettore del GLP-1 (GLP-1RA). In prevenzione secondaria i farmaci SGLT2i e GLP-1RA si sono dimostrati efficaci nel ridurre gli eventi CV e la mortalità, e le nuove linee guida propongono questi farmaci come prima scelta (dopo la metformina) nella terapia del DT2; i meccanismi coinvolti non sono ancora del tutto chiari, ma sembrano essere sia "glicemici" che "extra-glicemici".

In questa rassegna esamineremo i risultati dei principali trials clinici con SGLT2i e GLP-1RA, la rilevanza clinica rispetto al trattamento del diabete di tipo 2, e discuteremo i possibili meccanismi attraverso i quali questi famaci determinano i loro effetti protettivi cardiovascolari.

Parole chiave: Malattia Cardiovascolare (cvd), scompenso cardiaco, diabete di tipo 2 (dt2), inibitori del cotrasportatore sodio/glucosio-2 (sglt2i), agonisti del recettore del glp-1 (glp-1ra).

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FISIOPATOLOGIA DELLA PLACCA

TOLLERANZA IMMUNITARIA E ATEROSCLEROSI Immune tolerance and atherosclerosis

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SUMMARY

Cardiovascular diseases, such as myocardial infarction (MI) and stroke, are one of the most prevalent causes of morbidity and mortality worldwide. Atherosclerosis, a pathological process that affects the arterial walls of blood vessels, is the main reason for the onset of these diseases. Atherosclerosis is a systemic inflammatory disease characterized by the accumulation of lipid particles, cells of the immune system, endothelial cells and components of the extracellular matrix, in the subendothelial regions leading to the narrowing of the arterial lumen and the formation of atherosclerotic plaques. These plaques, also called atheromas, can break, leading to reduced blood flow to thrombosis and ischemia. The treatment of these lesions can be performed with surgical techniques such as angioplasty and stenting. In addition to these techniques it is possible to intervene already in the early stages of the onset of atherosclerosis thanks to anti-inflammatory treatments. In fact, clinical observations support a prominent role of systemic inflammation in the pathogenesis of this disease and for this reason treatments have been developed that limit inflammation and have a considerable benefit in the management of various cardiovascular disorders. Moreover, atherosclerosis is currently considered an immune-mediated process given the involvement of many components of the immune system both in the pathogenesis and in the progression of plaque: macrophages that turn into foam cells, autoreactive T cells, autoantibodies, autoantigens and secreted cytokines from cells at sites of inflammation. The involvement of the immune system in the pathogenesis of atherosclerosis offers the possibility of new methods to treat or prevent the development of this disease. Many studies have indicated that immunomodulation and especially the induction of immunological tolerance may have a protective and therapeutic role in atherosclerosis.

Keywords: Tolerance, atherosclerosis, macrophages, regulatory T cells, immunomodulation.

Il sistema immunitario nell'aterosclerosi

Nel 19 ° secolo, Rudolf Virchow descrisse l'aterosclerosi come *"entzündung"* ossia infiammazione (1). Solo nel 1913,

Indirizzo per la corrispondenza

Marco Gargaro Università degli Studi di Perugia, Dipartimento di Medicina Sperimentale E-mail: marco.gargaro@unipg.it Nikolai Anitschkow dimostrò che un'alimentazione ricca di colesterolo promuove l'aterosclerosi (2). Tuttavia, recentemente è stato delineato un profilo autoimmunitario di tale patologia in cui giocano un ruolo chiave anche i fattori genetici. Studi di associazione *genome-wide* (GWAS) hanno identificato diversi polimorfismi a singolo nucleotide (SNP) che sono associati ad un aumentato rischio di disfunzione dell'arteria coronaria (3), tra i quali lo SNPs nel *lo*- 27

cus 9p21 che implica un malfunzionamento del signaling dell'IFN γ (4).

Il complesso processo di aterosclerosi ha origine nel momento in cui si verifica l'accumulo nello spazio sub-endoteliale o "intima" di apolipoproteine B contenenti lipoproteine (apoB LP), alterando il diametro del lume delle arterie e di conseguenza il flusso sanguigno (5). Le lipoproteine così intrappolate subiscono modificazioni biochimiche da parte di proteasi e lipasi, portando alla formazione di specie reattive dell'ossigeno e LDL ossidate (oxLDL) che acquisiscono le caratteristiche di damageassociated molecular patterns (DAMPs), i quali a loro volta provocano l'attivazione di una risposta immunitaria innata (6). La risposta immunitaria innata inizia poiché l'accumulo delle oxLDL nell'intima provoca l'espressione di molecole di adesione come VCAM-1 da parte delle cellule endoteliali (7, 8) che richiamano monociti e leucociti. In questi siti la sottopopolazione di monociti Ly6Chi si differenzia in macrofagi che acquisiscono un fenotipo infiammatorio e rappresentano la principale popolazione cellulare della placca. Il continuo accumularsi di lipoproteine porta alla trasformazione dei macrofagi in foam cells. Tali cellule, intrappolate all'interno dell'intima arteriosa, perdono la loro capacità migratoria e creano un'area centrale nella placca che consiste di cellule apoptotiche e necrotiche, cristalli di colesterolo e altro materiale extracellulare (9). I macrofagi nelle placche esprimono vari recettori Toll-like (TLR) che riconoscono i DAMPs (10, 11), ma anche batteri, tossine e glicoproteine virali (12). L'attivazione dei TLR attiva MYD88 che porta alla secrezione di citochine pro-infiammatorie quali IL-1ß e IL-18 (13). Anche i cristalli di colesterolo contribuiscono all'induzione di un fenotipo pro-infiammatorio dei macrofagi grazie alla loro capacità di attivare i domini NACHT,

LRR e PYD dell'inflammasoma NLRP3 che a sua volta fa rilasciare IL-1 β (14, 15). Inoltre, IL-1β stimola le cellule muscolari lisce a secernere IL-6 la quale segnala la produzione di proteina C reattiva (PCR) da parte del fegato. Anche le cellule dell'immunità adattiva hanno un ruolo cruciale nella formazione, nella stabilità e nella rottura della placca. Dopo l'attivazione delle cellule dell'immunità innata (macrofagi e cellule dendritiche) si sviluppa anche la risposta immunitaria adattativa che si contraddistingue per l'accumulo di cellule T helper (Th), cellule B e per una riduzione delle cellule T regolatorie (T_{reg}) (16). In particolare, si è osservato che la risposta T mediata nell'aterosclerosi presenta un alterato equilibrio tra cellule T effettrici (Th1, Th17) e cellule T_{reg} . I linfociti Th1, secernendo IFNy, promuovono un'ulteriore attivazione dei macrofagi infiammatori (M1) e contrastano la formazione del cappuccio fibroso della placca degradando il collagene e inibendo la proliferazione delle cellule muscolari lisce. Invece le cellule Th17, secernendo IL-17A, promuovono la stabilità della placca favorendo il rilascio e la deposizione di collagene da parte delle cellule muscolari lisce con conseguente aumento della formazione del cappuccio fibroso della placca (17, 18). Questi effetti portano a placche vulnerabili che possono rompersi e causare eventi trombotici. Le cellule T_{reg} e Th migrano nelle lesioni aterosclerotiche in via di sviluppo e modulano il microambiente infiammatorio locale, in gran parte influenzando i fenotipi dei macrofagi. Al contrario, i fenotipi dei macrofagi risolutivi o infiammatori possono spostare l'equilibrio della cellula T della placca verso i fenotipi di T_{reg} e Th, rispettivamente. L'alterazione dell'equilibrio tra le cellule T_{reg} e Th potrebbe riflettersi sulla loro plasticità fenotipica e ciò comporterebbe la ri-differenziazione tra feno-

tipi regolatori e infiammatori. L'equilibrio T_{reg}-Th può anche essere influenzato dalle condizioni metaboliche sistemiche della placca. Anche altre cellule immunitarie, inclusi neutrofili e gli aggregati neutrofilipiastrine, NK (Natural Killer), mastociti e eosinofili sono presenti all'interno della lesione aterosclerotica, ma il loro ruolo non è stato ancora definito (16). Oltre alle cellule immunitarie, si verifica l'accumulo nell' intima anche dei miofibroblasti. Queste cellule sono una sorgente di matrice extracellulare che favoriscono la risposta infiammatoria e il danno vascolare (19). In condizioni fisiologiche, dopo il processo infiammatorio, i macrofagi e altre cellule immunitarie secernono molecole in grado di ridurre il processo infiammatorio e favoriscono il riparo tissutale. Una compromessa risoluzione della lesione arteriosclerotica favorisce l'infiammazione cronica che promuove la progressione delle placche e, in particolare nell'uomo, favorisce fenomeni trombotici occlusivi che culminano in eventi cardiovascolari (20-22).

Ruolo dei macrofagi infiammatori nella progressione della placca aterosclerotica

Le funzioni dei macrofagi possono variare ampiamente in base a un numero di variabili e fattori tra cui l'ambiente ("nicchia tissutale") (23, 24), il metabolismo intracellulare (25), i metaboliti del microbiota intestinale (26) e fattori genetici ed epigenetici, inclusi RNA non codificanti (27-30). Tutti questi fattori sono in grado di direzionare le funzioni dei macrofagi verso un fenotipo pro o anti-infiammatorio. In generale, i macrofagi infiammatori M1 svolgono processi che promuovono la progressione dell'aterosclerosi, mentre i macrofagi M2 (risolutivi) svolgono funzioni che possono sopprimere la progressione

della placca o promuoverne la regressione (31). Con la secrezione di citochine, proteasi e altri fattori, i macrofagi infiammatori aumentano l'espansione cellulare delle lesioni e causano cambiamenti morfologici della placca che possono scatenare la rottura della placca e la trombosi acuta. Due cambiamenti chiave promossi da macrofagi infiammatori sono la necrosi della placca e l'assottigliamento del cappuccio fibroso. Al contrario, i macrofagi M2 svolgono funzioni associate alla stabilizzazione della placca. Queste funzioni comprendono: la rimozione delle cellule morte (efferocitosi), che stabilizzano le placche prevenendo la necrosi cellulare post-apoptotica; la secrezione di collagene che può formare una cicatrice protettiva sulla lesione e la produzione di proteine e lipidi che riducono l'infiammazione e promuovono la riparazione dei tessuti. La proporzione relativa di macrofagi con diversi marcatori fenotipici varia a seconda della regione della placca. Ad esempio, i macrofagi infiammatori sono abbondanti in regioni di placca che tendono a rompersi e sono quelli in grado di secernere metalloproteinasi di matrice, MMP2 e MMP9, che potrebbero contribuire alla rottura della placca (32). Al contrario, le placche che appaiono più stabili per la presenza di cappucci fibrosi più spessi e aree di necrosi più piccole, si trovano ad essere arricchite di macrofagi M2 importanti nella regressione di placca e sono in grado di contenere la risposta infiammatoria nel sito lesionato (33).

Ad oggi, rimangono ancora ignoti i meccanismi alla base dell'eterogeneità dei macrofagi nei vari stadi della progressione e della regressione dell'aterosclerosi. Un importante contributo alla proliferazione dei macrofagi infiammatori è dato dal tipo di monociti infiltranti l'endotelio che determinano il loro cambiamento fenotipico. La proliferazione dei macrofagi si verifica

nelle placche avanzate (34, 35), ma i meccanismi e le conseguenze della proliferazione dei macrofagi lesionali rimangono in gran parte sconosciuti. In un recente studio è stata valutato l'effetto della carenza del gene Zfp148 (zinc finger DNA binding protein 148), che codifica per una proteina che sopprime l'espressione della proteina P53, nelle cellule ematopoietiche di topi Apoe^{-/-} (36). In questi topi vi è una diminuzione della proliferazione dei macrofagi lesionali e una marcata diminuzione dei marker di infiammazione e dell'area complessiva della lesione in modo dipendente da P53. Sono stati proposti diversi meccanismi che guidano la proliferazione dei macrofagi nell'aterosclerosi, compresa l'attivazione della segnalazione del recettore scavenger di classe A, l'attivazione del GTPase Ras e la perdita della funzione dell'inibitore della chinasi ciclina-dipendente 2A (CDKN2A) (37, 38). Riguardo all'ipotesi di Ras, uno studio recente ha dimostrato che il trattamento con le statine, inibitori della reduttasi HMG-CoA, bloccando l'attivazione di Ras nei macrofagi lesionali, riduce la loro proliferazione e l'infiammazione della placca (39). Inoltre, la modulazione fenotipica dei macrofagi lesionali può variare anche in base alle condizioni ambientali delle lesioni. Per esempio, l'accumulo di colesterolo in queste cellule può innescare diverse risposte infiammatorie che influenzano la membrana plasmatica attraverso l'attivazione di recettori infiammatori, guali recettori TLR (40). L'accumulo di colesterolo nei macrofagi attiva l'inflammasoma, portando ad un aumento della produzione di IL-1β proaterogenica.

Recenti dati di letteratura hanno dimostrato che la plasticità dei macrofagi nel processo aterosclerotico è dovuta anche a cambiamenti epigenetici nelle prime fasi della differenziazione monociti-macrofagi. Ad esempio, l'esposizione dei monociti alla lipoproteina a bassa densità ossidata (LDL) rende le cellule più responsive ai ligandi dei recettori TLR2 e TLR4 (41). Questa risposta è stata associata ad una modifica epigenetica dell'istone H3K4 nei promotori di molti dei geni infiammatori. Un altro studio ha dimostrato che gli inibitori HDAC3 favoriscono l'espressione dei geni ABCA1 e ABCG1 in macrofagi coltivati *in vitro*, e che la delezione HDAC3 in topi *Ldlr*^{-/-} alimentati con *Western Diet*, sopprime l'aterosclerosi e promuove un ridotto sviluppo dei macrofagi infiammatori (42).

L'equilibrio tra i linfociti T effettori e regolatori condiziona lo sviluppo della placca aterosclerotica

Come per i macrofagi, anche il fenotipo delle cellule T può cambiare in risposta a fattori ambientali che possono alterare le loro capacità di funzionare come cellule regolatrici o infiammatorie. È noto che le cellule T_{reg} hanno proprietà soppressive nella risposta immune, ma in molte malattie autoimmuni assumono un ruolo infiammatorio e patogenetico (43). E stato dimostrato che durante lo sviluppo della lesione aterosclerotica i linfociti T_{reg} diminuiscono ma non quelli effettori (44). Questo fenomeno è associato al fatto che le cellule T_{reg} sono in grado di trasformarsi in cellule con funzioni infiammatorie come dimostrato da recenti studi effettuati su modelli murini. Infatti, recenti studi hanno dimostrato che l'ambiente della placca aterosclerotica e l'ipercolesterolemia condizionano la capacità soppressiva delle T_{reg} che acquisiscono funzioni infiammatorie simili a quella delle cellule Th1. Cellule CD4+CD25+IFNy+FoxP3+Tbet+, denominate Th1-Treg si accumulano nelle lesioni aterosclerotiche di topi Apoe^{-/-}. La conversione in cellule Th1-Treg, per contro, non si verifica nei topi C57BL/6 non aterosclerotici (45). Queste cellule mostrano funzioni regolatorie alterate in vitro e una ridotta espressione di diversi geni legati alla funzione immunosoppressiva tipici delle T_{reg} . Indipendentemente da questo studio, altri ricercatori hanno dimostrato che il 40% di cellule T CD4+ nell'aorta aterosclerotica di topi Apoe^{-/-} hanno un fenotipo CCR5⁺ FoxP3⁺ T-bet⁺ e producono IFN-γ e TNF- α (46). Queste cellule, chiamate FoxP3⁺ CCR5⁺ CD25⁺, promuovono l'aterosclerosi in esperimenti di trasferimento adottivo e non sopprimono la proliferazione cellulare delle cellule T effettrici. È interessante notare che, per la delezione del fattore di trascrizione T-bet, le cellule Foxp3⁺ perdono il loro fenotipo immunosoppressivo e non sono più in grado di prevenire l'autoimmunità (47). Questa apparente contraddizione nelle funzioni osservate delle cellule T_{reg} che esprimono T-bet potrebbe indicare che lo sviluppo di cellule T_{reg} infiammatorie che esprimono l'IFNy è legato alle condizioni uniche dell'ipercolesterolemia sistemica o al microambiente della placca. Oltre alla plasticità delle T_{reg} , non va sottovalutata quella degli T effettori che in molte condizioni patologiche possono differenziarsi, all'interno dei tessuti infiammati, verso fenotipi più patogenetici durante la progressione della malattia. Per esempio, sotto l'influenza di citochine infiammatorie innate, le cellule Th17 protettive perdono la capacità di produrre IL-10 e diventano capaci di produrre IL-22 e IFNy acquisendo un fenotipo patogenetico (48). Questa conversione di cellule Th17 in cellule effettrici patogenetiche è promossa da IL-1 β , IL-6 e IL-23, citochine note per essere espresse nelle lesioni aterosclerotiche. Il contributo delle cellule Th17 allo sviluppo della lesione aterosclerotica rimane ancora incerto sulla base degli studi effettua-

ti su modelli murini, ma le cellule T che producono IL-17 e IFNy sono state identificate nelle arterie coronariche umane di pazienti con aterosclerosi (49). Sebbene i linfociti T non accumulino grandi quantità di colesterolo nell'ipercolesterolemia, al contrario dei macrofagi, l'esposizione a oxLDL può indurre profondi effetti sulle funzioni delle cellule T. Data l'importanza del fegato come sito per l'induzione della tolleranza delle cellule T periferiche e dato il suo ruolo centrale nella gestione del colesterolo e nella sintesi delle lipoproteine. è interessante considerare come le cellule T epatiche rispondano ai cambiamenti nell'omeostasi del colesterolo. L'ipercolesterolemia nei topi Ldlr/- alimentati con Western Diet è in grado di aumentare il numero di cellule T_{reg} intraepatiche e l'espressione di TGF-β, citochina che guida il differenziamento delle cellule Treg e principale mediatore dell'immunosoppressione (50). Esperimenti di trasferimento adottivo hanno dimostrato che queste cellule T_{reg} epatiche si ritrovano nell'aorta aterosclerotica. Inoltre, l'ipercolesterolemia induce la differenziazione delle cellule T epatiche Th1 o Th17 a seconda della presenza o assenza dei recettori LDL. Questi dati suggeriscono che i cambiamenti sistemici nel metabolismo delle lipoproteine e nel colesterolo alimentare potrebbero cambiare il rapporto tra i sottogruppi di cellule T_{reg} e Th generati nel fegato, il che cambierebbe i rapporti delle cellule che entrano nelle placche.

Inoltre, i meccanismi mediante i quali l'ipercolesterolemia potrebbe influenzare la differenziazione delle cellule T_{reg} o T_{eff} molto probabilmente implicano effetti legati alle cellule dendritiche (DC). Queste cellule si trovano nelle regioni soggette a aterosclerosi prima dello sviluppo della lesione e assorbono rapidamente le oxLDL (51). Le DC mantengono le loro funzioni

di presentazione dell'antigene dopo essere diventate foam cells (52) e rispondono all'accumulo di colesterolo aumentando la produzione di citochine che guidano la differenziazione dei sottogruppi di cellule Th (53). Tuttavia, sembrano esserci anche effetti diretti del colesterolo sulle cellule T. Ad esempio, le cellule T di topi carenti del trasportatore di colesterolo ABCG1 (ATP-binding cassette (ABC) transporters) mostrano aumentate risposte proliferative alla stimolazione del TCR e la delezione selettiva delle cellule T di ABCG1 determina un aumento della differenziazione delle cellule T_{reg} dalle cellule T naive (54). L'attivazione delle cellule T richiede l'assemblaggio del recettore-antigene e di altre molecole di segnalazione in zattere lipidiche ricche di colesterolo. che è un prerequisito per la formazione di sinapsi immunitarie con cellule presentanti l'antigene. Pertanto, alterazioni nel colesterolo cellulare potrebbero influenzare l'attivazione delle cellule T a causa dei cambiamenti nella formazione della zattera lipidica. È stato dimostrato che la funzione antitumorale delle cellule T CD8⁺ incrementa in topi in cui il contenuto di colesterolo non esterificato nella membrana cellulare è elevato. Pertanto, l'impatto immunologico netto dei cambiamenti nel colesterolo delle cellule T sembra variare a seconda del compartimento intracellulare interessato e del sottogruppo delle cellule T.

Aterogenesi e immunotolleranza

La malattia vascolare aterosclerotica rimane la principale causa di morte nel mondo industrializzato e la sua incidenza dovrebbe aumentare nei prossimi 20 anni (55, 56). Negli ultimi anni sta emergendo l'idea di aterosclerosi come patologia immuno-mediata e per questo la comprensione dei modi in cui l'immunità innata e adattativa influenzano tale patologia sta continuato a evolversi insieme alle aree emergenti nella ricerca immunologica.

Nel presente lavoro, abbiamo enfatizzato soprattutto la dinamica e l'eterogeneità dei macrofagi e delle cellule T e il modo in cui questi tipi cellulari influiscono sull'equilibrio tra progressione e risoluzione dell'infiammazione della placca. Queste aree emergenti di indagine hanno sollevato nuove domande, ma hanno anche convalidato gli attuali sforzi per modificare gli approcci terapeutici esistenti e sviluppare nuove strategie. L'osservazione della patogenesi dell'aterosclerosi come reazione immunologica agli LP apoB nella parete arteriosa enfatizza l'importanza fondamentale e il successo delle terapie plasmatiche mirate ad abbassare i livelli di apoB-LP, incluse statine e inibitori di PCSK9 (Proprotein Convertase Subtilisin/Kexin Type) (57). Abbassando la concentrazione di LP di apoB nel sangue si ha una riduzione della loro infiltrazione nelle lesioni in via di sviluppo e in questo modo queste terapie riducono il carico di DAMP e antigeni che causano risposte immunitarie innate e adattive nell'arteria. Inoltre, l'ipercolesterolemia può promuovere l'attivazione aterogenica delle cellule immunitarie circolanti e dei loro precursori prima ancora che entrino nella lesione. Se gli LP di apoB ricchi di colesterolo fossero abbassati al di sotto di una determinata soglia, si arresterebbe la progressione della placca e ciò indurrebbe la regressione. Tuttavia, questo potenziale non è stato raggiunto a causa di una serie di problemi, un esempio dei quali è l'effetto contrastante dell'insulino-resistenza indotta dall'obesità. La resistenza all'insulina è un potente fattore di rischio per la cardiopatia ischemica a causa dei suoi legami con la dislipidemia e l'infiammazione (58, 59).

Terapie mirate contro le citochine, come gli antagonisti del TNF o dell'IL-17, hanno trasformato il trattamento delle malattie autoimmuni, tra cui l'artrite reumatoide e la psoriasi. Queste malattie comportano un aumento del rischio cardiovascolare, ma non è chiaro se le terapie anti-citochine utilizzate per ridurre la patologia autoimmune influenzino direttamente l'infiammazione e la progressione della placca. Lo studio CANTOS (Anti-inflammatory Outcomes Study), recentemente completato, ha sperimentato un anticorpo IL-1β-bloccante (Canakinumab). Questo è il primo studio clinico sull'efficacia di una terapia anti-citochina somministrata esplicitamente per le malattie cardiovascolari (60). Rispetto al placebo, è stato dimostrato che il trattamento di oltre 10.000 pazienti con Canakinumab riduce il rischio di infarto miocardico, ictus e morte cardiovascolare del 15%. Il trattamento con Canakinumab ha ridotto significativamente le concentrazioni di PCR e IL-6 nel siero, ma non ha avuto effetti sui lipidi, suggerendo che la riduzione del rischio cardiovascolare era direttamente correlata agli effetti anti-infiammatori.

Esiste anche la prova che le condizioni metaboliche possono indurre cambiamenti epigenetici nei macrofagi e quindi portare a una forma di memoria immunitaria innata (61, 62) che potrebbe promuovere la progressione della lesione. A questo proposito è stato dimostrato l'effetto di inibizione dell'espressione genica dell'infiammazione, attraverso l'utilizzo di modulatori epigenetici fisiologici (ad esempio inibitori delle proteine del bromodominio e del motivo extra-terminale, BET), che sono in grado di ridurre l'aterosclerosi (63, 64).

Molti studi preclinici hanno convalidato il concetto di trattare l'aterosclerosi con vaccinazioni terapeutiche che tollerano gli antigeni che guidano le risposte pro-aterogenetiche delle cellule T. Recenti progressi in questo campo includono l'identificazione di epitopi peptidici apoB100 che legano le glicoproteine complesse di istocompatibilità (MHC) del topo o della classe II umana e la dimostrazione che la vaccinazione di topi che esprimono molecole MHC in grado di legare il peptide riduce la formazione di lesioni aterosclerotiche (65, 66). Queste evidenze sugge-

Glossario

- **GWAS:** Genome Wide Association Study. Studio prospettico effettuato in larga scala che consiste nell'indagare tutti (o quasi tutti) i geni di diversi individui di una popolazione al fine di determinare le variazioni geniche tra gli individui in esame. In seguito, si associano le differenze osservate ad una malattia.
- **SNP:** *Single Nucleotide Polymorphism.* Sono polimorfismi a singolo nucleotide, ovvero variazioni di un singolo nucleotide (Adenina, Citosina, Timina, Guanina) presenti in determinati tratti di DNA e costituiscono strumenti fondamentali per variati studi di genetica.
- **DAMP:** Damage-associated molecular patterns. Molecole rilasciate da cellule danneggiate chiamate anche "profili molecolari associati al danno" che sono riconosciute dall'immunità innata (macrofagi e cellule dendritiche) che quindi viene quindi stimolata ad eliminare le cellule danneggiate.
- NLRP3: L'inflammasoma *Nod-like receptor pyrin domain containing protein 3* (NLRP3) è un complesso proteico multimerico formato da NLRP3, dalla proteina adattatrice ASC e dalla caspasi 1. Stress, patogeni e sostanze tossiche sono in grado di attivare l'inflammasoma, portando all'attivazione della caspasi1 e alla successiva maturazione dell'interleuchina-18 (IL-18) e interleuchina-1β (IL-1β).

riscono che la modulazione dell'sistema immunitario induce meccanismi di protezione mediati dall'induzione delle cellule T_{reg} (66) o dall'inibizione dell'attivazione dei macrofagi attraverso l'uso di anticorpi monoclonali anti-PCSK9 che bloccano l'interazione delle LDL con il proprio recettore (65).

Conclusioni

L'immunomodulazione presenta un grande potenziale per il trattamento dell'aterosclerosi nell'uomo, sebbene finora sia stata testata solo in modelli animali. Studi sperimentali e dati osservazionali hanno fornito gli elementi essenziali per l'identificazione di potenziali *target* per il trattamento dell'aterosclerosi. La riduzione dell'infiammazione può essere raggiunta attraverso l'inibizione di mediatori dell'infiammazione oppure attraverso l'induzione dell'immunotolleranza. Nonostante i risultati incoraggianti derivati da studi retrospettivi osservazionali con inibitori dei mediatori dell'infiammazione (anti-TNF-α nell'artrite reumatoide), diversi trials clinici hanno evidenziato la scarsa attività di queste molecole nel trattamento dell'aterosclerosi. Infatti, in molti di questi studi si è osservato un incremento dei rischi associati al trattamento anti-infiammatorio rispetto ai benefici, tra questi ritroviamo il trattamento con inibitori della COX-2 e anti-IL-12/IL-23 p40. Questi dati enfatizzano la complessità della risposta infiammatoria nell'aterosclerosi. Pertanto, un'approfondita conoscenza sui meccanismi cellulari e molecolari che si verificano in ogni stadio dell'aterosclerosi potrebbero essere utili per predire come specifici trattamenti anti-infiammatori possono condizionare l'inizio e la progressione della patologia.

RIASSUNTO

Le malattie cardiovascolari, quali infarto del miocardio (MI) e *ictus*, rappresentano una delle cause più prevalenti di morbilità e mortalità in tutto il mondo. L'aterosclerosi, processo patologico che colpisce le pareti arteriose dei vasi sanguigni, rappresenta il principale motivo dell'insorgenza di queste malattie. L'aterosclerosi è una malattia infiammatoria sistemica caratterizzata dall'accumulo di particelle lipidiche, cellule del sistema immunitario, cellule endoteliali e componenti della matrice extracellulare, nelle regioni sub-endoteliali che portano al restringimento del lume arterioso e alla formazione delle placche aterosclerotiche. Queste placche, dette anche ateromi, possono rompersi portando alla riduzione del flusso sanguigno, alla trombosi e all'ischemia. Il trattamento di queste lesioni può essere eseguito con tecniche chirurgiche come angioplastica e stenting. Oltre a queste tecniche è possibile intervenire già nelle prime fasi dell'insorgenza dell'aterosclerosi grazie a trattamenti anti-infiammatori. Infatti, osservazioni cliniche sostengono un ruolo di primo piano dell'infiammazione sistemica nella patogenesi di questa malattia e per tale ragione sono stati sviluppati trattamenti che limitando l'infiammazione hanno un notevole beneficio nella gestione di diversi disturbi cardiovascolari. Inoltre, attualmente l'aterosclerosi è considerato un processo immuno-mediato dato il coinvolgimento di molti componenti del sistema immunitario sia nella patogenesi che nella progressione della placca: macrofagi che si trasformano in cellule schiumose, linfociti T autoreattivi, autoanticorpi, autoantigeni e citochine secrete dalle cellule nei siti di infiammazione. Il coinvolgimento del sistema immunitario nella patogenesi dell'aterosclerosi offre la possibilità di nuovi metodi per trattare o prevenire lo sviluppo di questa malattia. Molti studi hanno indicato che l'immunomodulazione e soprattutto l'induzione della tolleranza immunologica possono avere un ruolo protettivo e terapeutico nell'aterosclerosi.

Parole chiave: Tolleranza, aterosclerosi, macrofagi, cellule T regolatorie, immunomodulazione.

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MEDICINA, SCIENZA E SOCIETÀ

UNIVERSITÀ E SISTEMA SANITARIO: RICERCA DI UN RAPPORTO NUOVO TRA ASSISTENZA E DIDATTICA University and Health System: search for a new relationship between assistance and teaching

ELMO MANNARINO

Già Presidente della Scuola di Medicina e Chirurgia, Università degli Studi di Perugia

Il Docente universitario di materia clinica, sia che si tratti di Professore Ordinario, Associato o Ricercatore, normalmente svolge attività assistenziale nell'Azienda Ospedaliera nella quale insiste l'Università di appartenenza. La situazione è però differente nelle diverse Regioni e sedi universitarie. Gli universitari svolgono i loro compiti assistenziali o in un Policlinico Universitario o in una Azienda mista Università-Ospedale o in una Azienda Ospedaliera che ha soltanto stipulato rapporti convenzionali con l'Università locale di riferimento. In genere il clinico universitario, pur avendo istituzionalmente compiti di didattica e di ricerca, svolge la sua attività assistenziale insieme al collega ospedaliero che ha un diverso stato giuridico

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e che appartiene ai ruoli del Servizio Sanitario Nazionale. La coabitazione, trattandosi di personale appartenente a differenti Istituzioni è ovviamente complessa e non sempre facile da gestire, soprattutto dove non è stata costituita un'Azienda mista Università-Ospedale. Basti pensare al diverso trattamento economico tra il dipendente universitario convenzionato e quello ospedaliero, peraltro differente tra le varie Regioni. Solitamente l'Universitario che fa non solo assistenza al malato, ma anche didattica e ricerca, non riceve una retribuzione adeguata ai due lavori svolti, essendo l'assegno integrativo per l'attività assistenziale sottoposto ad un riassorbimento progressivo con l'avanzamento di carriera. Altra evidente discrepanza è rappresentata dalla constatazione che, nonostante sia previsto che il Professore Universitario per poter svolgere pienamente i suoi compiti debba avere un incarico di Struttura Semplice o almeno di Alta SpecializzazioNella quasi totalità delle Regioni è palese il ruolo marginale svolto dalle Scuole/Facoltà di Medicina nella gestione aziendale, alle quali non è solitamente consentito di entrare nella programmazione ospedaliera, pur svolgendo compiti assistenziali fondamentali per il funzionamento del sistema. Per questo motivo è evidente la scarsa attenzione riservata alle esigenze didattiche e di ricerca da parte delle Aziende Sanitarie nelle quali svolge attività assistenziale la componente universitaria.

Altro punto critico dei rapporti tra Scuole di Medicina e Aziende Sanitarie è quello delle Scuole di Specializzazione che, per la formazione degli specialisti, si avvalgono di strutture ospedaliere. Gli Specializzandi sono spesso coinvolti nelle attività assistenziali in assenza di regolamenti precisi e senza una chiara definizione dei compiti e delle loro esigenze formative. Si tratta di situazioni assai differenti nelle varie Regioni e nelle diverse Università, pur avendo il titolo di specializzazione lo stesso valore in tutto il territorio nazionale. Occorre rilevare come, a tutt'oggi, sia non del tutto semplice affidare a dipendenti ospedalieri, anche particolarmente qualificati nella pratica clinica, l'insegnamento di una disciplina nelle Scuole di Specializzazione in assenza dell'adeguata produzione scientifica richiesta, mettendo talora in discussione la sopravvivenza stessa della Scuola in quella sede universitaria per mancanza di docenti della disciplina. Purtroppo la valutazione delle capacità cliniche non ha ancora la necessaria considerazione neppure nella abilitazione nazionale per i diversi livelli di docenza universitaria. Forse l'istituzione da parte delle nostre Università del ruolo di "Clinical Professor", come già avviene in altri Paesi, potrebbe

ovviare a queste difficoltà, contribuire al riconoscimento della buona pratica clinica e a salvare qualche Scuola di Specializzazione. È osservazione comune il difficile accesso degli Specializzandi ad alcune strutture assistenziali o il loro non corretto impiego nelle strutture ospedaliere, con conseguente ricadute negative sulla loro formazione. Molto spesso la necessità di strutture assistenziali adeguate per una qualificata formazione universitaria pre- e postlaurea non coincide con le esigenze funzionali ed organizzative dell'Ospedale che ospita la Scuola di Medicina.

Le attività di formazione non rientrano quasi mai nelle valutazioni ospedaliere e conseguentemente nella definizione del budget da assegnare alle varie strutture. Il carico didattico e di ricerca svolto dalla componente assistenziale universitaria non ha di fatto alcun riconoscimento nella discussione ed allocazione delle risorse dell'Azienda. Nella valutazione della performance si utilizzano indicatori assistenziali - ad esempio degenza media, numero di prestazioni, etc. - che non considerano in alcun modo didattica e ricerca. Un esempio da imitare potrebbe essere quello degli Istituti di Ricovero e Cura a Carattere Scientifico (IRCCS) nei quali viene valutata e tenuta nella debita considerazione anche l'attività di ricerca. Questo costituirebbe sicuramente uno stimolo e una valida motivazione per la componente ospedaliera a svolgere attività di ricerca clinica.

In molte sedi universitarie la coabitazione Università-Ospedale è oggi problematica per la vigente confusione normativa che non regola in maniera chiara e soddisfacente i compiti assegnati alle due istituzioni, fondamentali per il funzionamento ottimale del Sistema Sanitario Nazionale. Ne consegue una innegabile penalizzazione soprattutto per i Docenti Universitari che sono costretti a mantenere elevati livelli

di performance non solo in ambito clinico, ma anche in quelli della didattica e della ricerca. Momenti che spesso non trovano il necessario raccordo con le necessità assistenziali dell'ospedale. Specialmente in quelle Regioni dove non è stato definito il "Patto di Intesa tra Università e Regione", pure previsto dalla Legge. Di fatto, l'Università non ha ruolo formale e conseguentemente sostanziale nella governance delle strutture sanitarie. Ad esempio la nomina dei Direttori Generali delle Aziende, dove non esistono Aziende integrate Università/Ospedale, è di fatto unilaterale, come pure la individuazione del numero e delle funzioni delle Strutture Semplici, non tenendo presenti le esigenze della didattica e della ricerca. Ciò è causa inevitabilmente di conflittualità tra le varie componenti assistenziali con ricadute negative sulla performance sia universitaria che ospedaliera. Non esiste tra l'altro una normativa univoca che regolamenti in tutto il territorio nazionale la retribuzione per le prestazioni assistenziali effettuate per soggetti esterni dal personale universitario.

La presenza di un organico misto ospedaliero e universitario in una stessa struttura assistenziale rende di fatto difficile la coabitazione anche per legittimi interessi contrapposti, non solo per il diverso trattamento economico, il diverso stato giuridico e le diverse valutazioni, ma anche perché talora inibisce assurdamente l'attribuzione di apicalità dirigenziali ad una delle componenti che operano in quella stessa sede. Tutto questo sulla base della pregiudiziale etichettatura universitaria o ospedaliera della struttura senza tener conto delle effettive esigenze assistenziali, della pianificazione strategica e soprattutto della qualità oggettiva dei professionisti indipendentemente dalla loro appartenenza all'Università o al Sistema Sanitario.

Per ovviare alle difficoltà citate e per una più efficace integrazione delle attività assistenziali svolte dalle Università nell'ambito del Sistema Sanitario Nazionale, non sembra ulteriormente dilazionabile, dove non sia stato ancora fatto, l'attuazione di tutti i punti previsti dai diversi protocolli di intesa tra Regioni e Università, ma soprattutto la costituzione di Aziende miste Università/Ospedale in tutte le strutture sanitarie che ospitano una Scuola di Medicina. È altresì necessario che le Autorità preposte al governo dell'Università e del Sistema Sanitario, sulla base dell'esperienza maturata in questi anni, rivedano e attualizzino l'intero quadro normativo che regolano i complessi rapporti tra queste due istituzioni, fondamentali per la crescita culturale del Paese e la salute della collettività.

CONGRESSO NAZIONALE S. S. A. BOLOGNA 25/27.11.2018

ABSTRACT



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FH SELECTIVE SCREENING IN PEDIATRICS: A PILOT STUDY

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Introduction. Familial Hypercholesterolemia (FH) is the most common genetic disorder leading to very high cholesterol levels and premature coronary heart disease (CHD). FH is underdiagnosed and undertreated worldwide. An early detection in childhood is useful to establish an adequate management in order to prevent CHD.

Materials and Methods. We selected, in collaboration with three Coronary Care Units in the Province of Cuneo, Piedmont (Cuneo, Mondovì, Savigliano) and the Internal Medicine and Endocrinology Departments of Cuneo Hospital, families at cardiovascular risk on the basis of a positive history of CHD and/or the presence of severe hypercolesterolemia in parents and/or grandparents. We evaluate cholesterol levels in children/adolescents belonging to these families through a capillary blood test (Afinion, Alere srl-Abbott). Children presenting TC levels ≥90° percentile were submitted to fasting venous blood sample collection to evaluate the lipid profile. FH was diagnosed according to Lipigen and Simon Broome Register Criteria and DNA analysis are in progress

Results. Sixteen families were recruited in the study. All the families presented a positive CHD family history (43% premature CHD). We evaluated 23 subjects (M/F 14/9), aged 9.6±4.5 vs, BMI 17.5±3.8 kg/m² (4 subjects were overweight and 1 obese). Five subjects (M/F 4/1, age 7±4.5 ys) presented elevated TC levels at the capillary blood test (TC 275.4±31.0 mg/dl). The lipid profile analysis confirmed the preliminary data: TC 288±24.2 mg/dl, HDL-C 51.8±2.9 mg/dl, TG 93(42- 127) mg/dl, LDL-C 221.5±32.5 mg/dl. Eighteen subjects presented normal TC levels at the capillary blood test (145.6±22 mg/dl).

Conclusion. This pilot study evidenced the relevance of an adequate screening program for FH diagnosis since childhood. Children showing a positive family history of premature CHD and/or dyslipidaemia with TC >290 mg/dl in their relatives should undergo biochemical analysis to optimize FH detection and treatment.

IN VITRO STUDIES TO IDENTIFY AND SORT EXOSOMES BASED ON THEIR SIZE AND LIPID COMPOSITION. PRELIMINARY RESULTS

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Extracellular vesicles (EVs) are a group of membrane-derived structures released by cells, divided into two main groups: microvesicles (size 50-1000nm), and exosomes (size 50-150 nm). EVs circulate in biological fluids and transport and deliver lipids, proteins and nucleic acids to distant cells, and contribute to homeostasis, diseases development and progression. In this in vitro study, we utilized a lymph-node melanoma metastatic cell line (LM-16).

1. The most used EV isolation method is differential centrifugation (DC). By using an algorithm that takes in consideration g-force, time, viscosity of the medium and density of desired particles, to better define the properties of different EVs populations, we pelleted 4 different fractions (from 300 to 50 nm). After DC we processed the different pellets with Gaschromatography (lipid analysis) and Nanosight and TEM (distribution and dimensional characterization). Our data suggest that a decrease in EVs population with bigger diameter is counterbalanced by a decrease in relative percentage of saturated fatty acids (from 44.05% for 300nm to 71.44% for 50 nm), counterbalanced by a decrease in unsaturated ones (from 44.89% to 23.66%).

2. Since EVs are mainly made of cholesterol and phospholipids we can assume that lipid alteration can influence their functions, size and composition. We are evaluating if Simvastatin, an HMG-CoA reductase inhibitor, can alter EVs features. After 6-days incubation with 0.75 µM simvastatin and 24-hrs in serum free medium, we isolated EVs with DC. Our preliminary data shows that simvastatin increases EVs size (from 171.30 to 201.2 nm). In future we will utilize trehalose to prevent EVs aggregation and we will try to find new biomarkers to validate our method. We plan also to analyze lipid EVs composition and of their parental cells in order to understand if these alterations can lead to the biosynthesis of dysfunctional EVs to find new approaches for tumor and atherosclerosis treatment.

GUT MICROBIOTA. CAROTID VASCULAR DAMAGE AND MAJOR CARDIOVASCULAR **RISK FACTORS IN THE GENERAL POPULATION: WHICH ASSOCIATION?**

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Introduction. Increasing evidence indicates that alterations in Gut Microbiota (GM) composition might influence development of atherosclerosis and cardiovascular disease (CVD) (1-2), via activation of the immune system, alteration of cholesterol metabolism, and production of bacterial metabolites. The purpose of the study is to analyze GM in a large cohort, representative of the general population to relate intestinal bacterial community profiling to the presence of carotid vascular damage and to other major risk factors for CVD, and to find new diagnostic/prognostic biomarkers. Methods. GM composition of 345 subjects from a population-based study (PLIC, "Progressione delle Lesioni Intimali Carotidee" project), was assessed through genomic DNA extraction from fecal samples and V3-V4 regions of the 16S rRNA gene sequencing by HiSeq 2500 Illumina platform, generating 250 bp paired end reads. At least 30'000 high-quality reads per sample were obtained and analyzed using QIIME; taxonomy was assigned using Greengenes

database. Carotid vascular damage was assessed by ultrasound, defining presence of atherosclerotic plaque for focal or diffusive arterial wall thickening over 1.3 mm.

Results. We significantly highlighted a relevant separation between individuals with or without carotid vascular damage (p=0.016, unweighted Unifrac distance), implying relevant species diversity in the microbiota derived from both groups. In particular, subjects with atherosclerotic plaque, a significant increasing of pro-inflammatory genera Escherichia and a trend toward reduction of Roseburia (previously identified in subjects without chronic inflammatory conditions) was outlined. GM profiling correlated with multiple cardio-metabolic risk factors (gluco-metabolic/lipid profile, blood pressure, lifestyle habits), even in multivariate adjusted models.

Conclusions. We have provided new insights into how GM dysbiosis is related to the presence or absence of carotid vascular damage and cardio-metabolic risk factors even in the general population characterized by low CV risk. Analysis on other samples from geographical distant cohort is planned. The future challenge will be to determine:

- a) whether specific bacterial species/metabolites are the cause or consequence of the disease, through planned transcriptomic analysis;
- b) determine causal biomarkers (trimethylamine N-oxide, TMAO) interaction.

GLYCOLYSIS INHIBITION WITH NOVEL COMPOUNDS SUPPRESSES ANGIOGENESIS

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Angiogenic signals induce endothelial cells (ECs) to switch their metabolism to being highly glycolytic. Clinical evidence has shown that the formation of new vessels destabilizes atherosclerotic plaques. Glycolysis modulation by inhibition of the key enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB3) is a possible therapeutic approach to reduce angiogenesis. We studied the effects of two novel self-synthesized phenoxindazole analogues inhibitors of PFKFB3 (PA-1 and PA-2) on glycolysis, cell proliferation, migration, and capillary tube formation in the human EC lines HUVEC and EA.hy926. Angiogenesis was assessed on matrigel and angiogenesis-related marker expression was measured by real-time PCR or western blot. PA-1 and PA-2 inhibit PFKFB3 (IC50 4 and 3 nM, respectively) both at gene and protein levels. 3PO, a known inhibitor of glycolysis, does not bind to nor inhibit PFKFB3. All compounds significantly reduce lactate release and increase intracellular lactate levels.

PA compounds downregulate the expression of lactate/monocarboxylate transporters MCT1 and MCT4, while 3PO slightly upregulates the latter. PA-1 and PA-2 suppress capillary tube formation in both cell types, while 3PO does not have any effect in EA.hy926 and induces it in HUVECs. PA-1 and PA-2 markedly inhibit EC migration, proliferation and wound closing capacity. PA compounds downregulate the expression of markers of migration and angiogenesis (CCL5, VCAM-1, VEGFA and VEGFR2). 3PO induces the expression of ICAM-1, VCAM-1 and VEGFR2, probably explaining the differences we observed in capillary tube formation. Interestingly, 3PO suppresses several processes involved in the angiogenic process although it does not interact with PFKFB3, which implies for off-target effects. These findings suggest that glycolysis inhibition regulates EC migration, proliferation and capillary-like structure formation and this exerts a multitarget anti-angiogenic activity. Hence, PFKFB3 inhibition with PA compounds could be a promising therapeutic approach to promote plaque stability. This project has been funded by the European Union's Horizon 2020 Marie Skłodowska-Curie grant (#67552).

GENETIC DIAGNOSIS, ACHILLES TENDON ULTRASONOGRAPHY AND DUTCH LIPID CLINIC NETWORK SCORE OF FAMILIAL HYPERCHOLESTEROLAEMIA: PRELIMINARY DATA FROM THE UNIVERSITY OF PADUA OUTPATIENT LIPID CLINIC

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Aims. Ultrasonography (US) is used to detect Achilles tendon (AT) xanthomas in patients (pts) with Familial Hypercholesterolaemia (FH). Our study focused on potential associations between FH genotype, clinical phenotype and ultrasonographic (US) AT findings, evaluating the contribution of AT US to identify individuals with an FH-causing mutation.

Subjects and Methods. Genetic screening, clinical and biochemical parameters were analysed in 116 pts with possible, probable or definite clinical diagnosis of FH according to the Dutch Lipid Clinic Criteria (DLCNS); 71 pts underwent bilateral AT US; ultrasonographic xanthomas (USX) were defined as the presence of AT thickness >6.15 mm in at least one of the AT and/or presence of hypoechogenic formations.

- **Results.**
- 85 pts were positive for LDL gene receptor mutation (35 carriers of null allele (NA) and 50 of defective (DEF) allele) and 31 with no known mutations (NM) for FH. Presence of xanthomas and gerontoxon, total and LDL-cholesterol (NA vs DEF vs NM: 326.5+97.7 mg/dl, 316.9+93.9 mg/dl, 211.1+76.3 mg/dl, p<0.000) at diagnosis were significantly higher in NA pts than other subgroups. AT thickness was significantly different among the three groups (p<0,005) and 78.2%, 72.4% and 31.6% had USX in NA, DEF and NM carriers respectively (p=0.002).
- 2) Among the 52 pts positive for FH-causing mutations: 36 pts had definite clinical diagnosis of FH by the DLCNS, while in 16 pts the diagnosis was either possible (5%) or probable (23%). Among these 16 pts, one had clinically evident xanthomas while 10 had USX. Thus, in nine pts the presence of USX was clinically undetected and thereby not considered for DLCNS calculation.

Conclusions.

- Genotypic functional characterization is associated with different phenotypic clinical features, AT thickness and presence of US xanthomas.
- AT ultrasonography may help reclassifying as definite FH, patients with DLCN score of possible/probable FH.

ULTRASONOGRAPHIC DETECTION OF XANTHOMAS IN ACHILLES TENDONS OF SUBJECTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Aim. To analyse the diagnostic value of the Achilles tendon (AT) ultrasonography to identify xanthomas compared with clinical examination in subjects with Heterozygous Familial Hypercholesterolemia (HeFH).

Methods. We enrolled 101 subjects with HeFH between the age of 18 and 70 years old from January 2016 to October 2017. Medical history, clinical examination, complete lipid profile, and Dutch Lipid Clinic Network (DLCN) score were collected; all the subjects were submitted to bilateral AT ultrasonography to evaluate AT thickness, echo-structure and presence of hypo-echogenic formations. Subjects with previous tendon ruptures were not included.

Results. 101 subjects (63 F, and 38 M) with a mean age of 48.0±15.5 years and a mean DLCN score of 10.1 ± 4.9 . 10.2% of subjects presented xanthomas at clinical examination (XC) with an echographic pattern compatible with xanthoma (XUS), 51% presented XUS but not XC, and 38% had neither XUS or XC. AT thickness was positively associated with LDL cholesterol at the diagnosis (r=0.411, p<0.001), and this association was maintained in a multivariate logistic regression analysis adjusted for age, sex and lipid lowering therapy (OR 1.020, 95%CI 1.004-1.035; p=0.012).

Conclusions. We have confirmed that ultrasonography is more sensitive than clinical examination to identify xanthomas, and it could represent, with medical history and clinical examination, a useful method to reach an early diagnosis of HeFH.

ENGINEERED REGULATORY T CELL ADOPTIVE THERAPY AS A NOVEL TOOL FOR THE TREATMENT OF ATHEROSCLEROSIS

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Aim. Loss of anti-inflammatory activity of regulatory Tcells (Treg) has been associated to immunoinflammatory diseases, including atherosclerosis. Therefore, the use of Treg-Adoptive Cell Therapy (ACT) is emerging as a therapeutic strategy to specifically modulate impaired immune responses. Although ACT has produced encouraging results in animal models, a main limitation remains the possibility to specifically target a selected tissue. Our aim was to develop and test a plaque-homing selective Treg-ACT in animal models.

Methods. Treg were retrovirally (IRES-EGFP vector) transfected with chemokine receptors or an empty vector and i.v. injected (2x105 GFP+ cells/mouse) in male 8-week WTD LDLR KO. Homing of transfected Treg to atherosclerotic plaque, its progression and composition was analysed by flow cytometry and histology.

Results. The chemokine CX3CL1 is selectively expressed in the aorta, but not in other tissues (lymph nodes, spleen and liver) of 8-week WTD LDLR KO, contrary to CCL2, usually associated with inflammation during atherosclerosis. Therefore, we compared homing of CCR2- and CX3CR1-transfected Treg to the aorta. While migration of CCR2-transfected Treg was not tissue selective, CX3CR1-Treg showed a specific homing to atherosclerotic plaques (2,5% of GFP+/lived cell compared to 0.6%) with similar homing in lymph nodes and spleen. Next we investigated whether CX3CR1-Treg reduce atherosclerosis by performing plaque analysis 4 weeks after ACT. Altough levels of plasma cholesterol were similar, CX3CR1- compared to control-Treg treated mice showed decreased plaque area and macrophage infiltration, and increased stability.

Conclusion. CX3CL1 is selectively expressed in atherosclerotic plaque and the CX3CL1/CX3CR1 axis may represent a pathway to target selective migration to the atherosclerotic plaque. Over-expressing CX3CR1 appears a promising ACT to promote selective homing of Treg into the plaque thus limiting atherosclerosis progression.

MOLECULAR CHARACTERIZATION OF MUTATIONS CAUSING LIPOPROTEIN LIPASE DEFICIENCY

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Background and Aims. Type 1 hyperlipoproteinemia is a rare autosomal recessive disorder most often due to loss of function mutations in the lipoprotein lipase gene (LPL), resulting in severe hypertriglyceridemia and recurrent pancreatitis episodes.1,2 In this work we studied the molecular mechanism beyond two already known mutations in LPL gene.

Methods. Two patients with hypertriglyceridemia were selected from the Lipid Clinic in Vienna. The first patient was compound heterozygous for c.680T>C (p.V200A) and c.1139+1G>A. The second patient compound heterozygous for c.953A>G (p.N291S) and c.1019-3C>A.LPL gene was sequenced and postheparin plasma samples (ex vivo) were used to test LPL activity. In vitro experiments were performed in HEK293T cells transiently transfected with wild type or mutant LPL plasmids. Cell lysate and media were used to evaluate LPL production, secretion and activity by western blot analysis and LPL enzymatic assay, respectively.

Results and Conclusions. Immunoblot analysis and LPL activity measurement in plasma samples showed a faint LPL band and a strong reduction of LPL activity in both patients. In vitro experiments, showed a 10% reduction in LPL synthesis in both V200A and N291S mutant cells whereas LPL secretion was significantly reduced (80%), only in V200A. No LPL activity was detected in both

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mutants. Our data show that V200A is a mutation that alter LPL production, secretion and activity whereas cells transfected with N291S showed normal levels of LPL protein but absent enzyme activity. We describe novel pathogenic LPL mutation leading to virtual absence of LPL activity and protein.

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LP (A) AND CARDIOVASCULAR EVENTS IN A GROUP OF MEDITERRANEAN WOMEN (PROGETTO ATENA). PRELIMINARY REPORT

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Introduction. Association between Lp (a) and cardiovascular disease (CVD) was evaluated in a group of 196 women participating to Progetto Atena (N=5062), a study on the etiology of major chronic diseases in women (nested case-control study).

Methods. At the time of first visit (baseline visit) blood samples were collected and stored in liquid nitrogen (-196°C). Women at time of recruitment were free of cancer and cardiovascular disease. After 25 years from baseline visit Lp (a) was detected, on serum stored at time of baseline visit, by solid phase two-site enzyme immunoassay (ELISA). The women were divided into a control group (group 1, N=105) and in a group (group 2, N=91) who had cardiovascular events after the initial visit.

Results. No statistically age differences resulted between group 1 (55.6 ± 7.8 yrs) and group 2 (56.8 ± 7.3) (p=0.266). In the two group no statistical significance was found in the principal cardiovascular risk factor Total Cholesterol, HDL, Triglycerides, Body Mass Index, Waist Circumference). LDL Cholesterol was higher in the group 2 (168.5 ± 51.0 mg/dL) vs group 1 (154.6 ± 47.3) (p=0.05). Women with elevated Lp (a), in the IV quartile of Lp (a) distribution, showed an increased risk of having CVD compared with those in the I quartile: IV vs I quartile (O.R.=2.34; p=0.038), adjusted for age, Systolic blood pressure, Body mass index and LDL-Cholesterol.

Conclusions. These findings indicate that women who had CVD after baseline visit had elevated Lp (a) (>20 mg/dL of studied population) at baseline visit. Elevated Lp (a) was related to future cardiovascular event independently of major cardiovascular risk factor in this group of women participating to Progetto Atena. Screening and treatment of elevated Lp (a) must take into account when clinician estimated cardiovascular risk specially in women.

A CROSSTALKING BETWEEN SARCOPENIA, OSTEOPOROSIS AND ATHEROSCLEROSIS.

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Background. In recent years there has been a growing interest in evaluating the relationships between sarcopenia, bone mineral density (BMD) and fragility fracture risk.

Aim. The aim of the study was to evaluate the prevalence of sarcopenia in an older population and the correlation between sarcopenia and BMD. Methods: In 117 healthy subjects (20 males and 97 females) aged between 60 and 80 years (72,5 \pm 6,1 years), we evaluated the presence of sarcopenia on the basis of EWGOP consensus: muscle mass (Skeletal Mass Index- SMI) with DXA, muscle strength with handgrip and physical performance with Short Physical Performance Battery (SPPB). We measured BMD at the lumbar spine (BMD-LS), femoral (neck: BMD-FN, Total: BMD-FT) and whole body (BMD-WB) with DXA (Prodigy, GE Lunar). In all subjects we were measured calcemia, phosphoremia, creatinine and 25OHD. In addition, ultrasonographic examination of the epiaortic vessels in each patient was performed by measuring IMT and presence of atheromatous plaques according to the European Carotid Surgery Trial (ECST) method and was assessed quality intima-media thickness (QIMT) and pulse wave velocity (PWV) with a Mylab-70 ultrasound instrument.

Results. Men were more sarcopenic according to SMI. We found no significant differences between the genders in terms of muscle strength or SPPB. One third of the patients had sarcopenia with no significant differences between men and women, while the prevalence of osteoporosis was significantly higher in women. The values of sarcopenia showed a significant correlation with BMD (r=0,38; p<0,01) at the level of all skeletal sites. T-score values of sarcopenic patients was reduced at all levels obtaining statistical significance only at the femoral level (p<0,01). The prevalence of fractures was greater in sarcopenic patients than in non-sarcopenic patients. Muscle mass and muscle strength assessed with handgrip were found to be an independent predictor of BMD in males but not in females. Dividing patients into two groups based on presence of sarcopenia (30 patients with sarcopenia and 59 patients without) we observed that the values of IMT were higher in patients with sarcopenia (1,01±0,31 vs 0,90±0,23) with statistical significance (p<0,05). In addition the values of QIMT were significantly higher in patients with sarcopenia with statistical significance (0,6±0.1 vs 0.8±0.1, p<0,05). Moreover, we found that the values of PWV were higher in patients with sarcopenia (9,1±2,5 vs 8,7±1,9) even though the difference didn't reach statistical significance.

Conclusion. Our data confirm that an evaluation of the muscle tissue, measured by the lean appendicular mass and the muscle strength and physical performance, is important as well as the study of bone mineral density (BMD). In addition these data show an a correlation of sarcopenia with atherosclerosis and consequently the higher cardiovascular risk in sarcopenic patients.

A PEDIATRIC-TAILORED APPROACH TO DETECT CHILDREN AND ADOLESCENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction. To the best of our knowledge, no validated scores to detect pediatric patients with familial hypercholesterolemia (FH) are available so far. Revised-Dutch-Clinic-Network-Score criteria (rDLCNSc) are worldwide used to detect adult patients with FH and are often experimentally adapted to pediatric age (PEDrDLCNc). PEDrDLCNc include 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 higher than 8 qualify for definite FH.

Matherials and Methods. The aim of this study is to evaluate the efficacy of PEDrDLCNc to select pediatric patients eligible for DNAmutational -analysis for FH in patients referring to our Lipid Center. In a 24months-period, 110 patients were referred to our Lipid Center for hypercholesterolemia. PEDrDLCNc were calculated for each patient. 28/110 (26%) showed a score of 6 or higher and underwent genetic analysis for LDL-R mutation. 4 tests are still ongoing. Parents' median age (years) was 43 (mother) and 46 (father).

Results. 14/28 (50%) of our 28 tested patients had a mutation of LDL-R gene; 25/28 (89%) had positive family history for hypercholesterolemia; 2/28 (7%) had positive pCAD in parents/first-degree relatives; 8/28 (28%) had pCAD in second-degree relatives. Out of the 14 patients with positive genetic test, 2/14 (14%) had pCAD in first-degree relatives, 7/14 (50%) had pCAD in second-degree relatives and 13/14 (93%) had positive family history for lipid disorders. Conclusions. PEDrDLCNc is a useful tool to detect patients eligible for FH molecular diagnosis, though it should be more targeted to pediatric age. Almost all patients with positive genetic test have a positive family history for lipid disorders. This item seems to be the most important one in anamnestic evaluation. 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.

IDENTIFICATION OF PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA BY USE OF CLINICAL FEATURES AND GENETIC INVESTIGATIONS (LIPIGEN STUDY*)

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Background. Dutch Lipid Clinic Network Score (DLCSN) is a validate score for clinical diagnosis of Familial Hypercholesterolemia (FH). About 90% of patients with DLCSN ≥ 6 has a pathogenic variant of genes implicated in FH (1). The aim of our study was investigating the prevalence of mutations not only in patients with a "definite" (DLCNS >8) or "probable" (6-8) clinical diagnosis, but

also in subjects with "possible" FH (DLCNS 3-5). The secondary aim was to identifying any clinical features that alone may be indicative of positive genetic confirm.

Methods. We recruited individuals with DLCNS ≥3, without secondary causes of dyslipidemia. Genetic analyses to detection major mutation of genes implicated in monogenic hypercholesterolemia (LDL receptor, ApoB, PCSK9, ApoE, LDLRAP1, STAP1) were performed by Progenika from 2012 to 2016 and by FererIn-Code since 2017.

Results. We analyzed genetic reports from 76 subjects enrolled from 2012 to 2017. Their mean DLCNS was 7.4 (range 3-16). Of these, 51 (67,1%) had an FH genetic variant. Subjects with DLCNS >8 were 29 (39,2%) and 20 individual had a genetic mutation confirmed; 25 subjects had a DLCNS 6-8 (genetic mutation confirmed; 25 subjects) and 22 (27.5%) had a DLCNS 3-5 (14 had a genetic mutation). Logistic regression analysis showed a correlation of positive genetic analysis with DLCNS (p<0.05). No clinical characteristics of DLCNS 3-5 showed LDL-receptor mutation; one patient also had two other mutations, on ApoB and on LDLRAP1, classified as of uncertain significance for FH.

Conclusions. DLCNS is still useful in the suspicion of FH, although an amount of patients with genetic mutation has scores of 3-5. Studies with larger sample are needed to better define its role and to identify any suggestive clinical features, especially for patients with low DLCNS.

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ASSOCIATION BETWEEN GLOBAL CARDIAC CALCIFICATION (GCCS) AND OSTEOPOROSIS

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Background. Epidemiologic and clinical data have suggested the existence of a biologic linkage between bone and vascular system. Osteoporosis and atherosclerosis are two prevalent major health care concerns that frequently coexist. Several studies reported correlation between lower values of Bone Mineral Density (BMD) and cardiovascular events. Furthermore literature suggests that cardiac calcification (measured with Global Cardiac Calcium Score, GCCS) is associated with cardiovascular events and mortality.

Aim. This study aimed to evaluate if cardiac calcium deposit was correlated with BMD.

Methods. In 36 subjects assessed for bone fracture risk (mean age 72±5,7 years) we measured Bone Mineral Density (BMD) at lumbar spine (BMD-LS) at femur (Neck: BMD-FN; Total: BMD-FT) and we assessed with echocardiography a global cardiac calcium score (GCCS).GCCS is a semi-quantitative score, that was applied assigning points for calcification in the aortic root and valve, mitral annulus and valve and sub-mitral apparatus, and points for restricted leaflets mobility (1).

Results. The results show that there is a significant inverse correlation between BMD-FN and BMD-FT with GCCS (r=-0,285, p<0,05 and r=-0,376, p<0,05 respectively). No significant correlation was found between BMD-LS and GCCS.Dividing patients into two groups based on presence of bone fragility fractures (13 patients with fragility fractures and 23 patients without) we observed that the value of GCCS was higher in patients with bone fragility fractures (2,54±1,3 vs 2,30±1,5) but not statistically significant. Moreover, dividing patients on the basis of presence of sarcopenia (9 patients with sarcopenia and 27 patients without sarcopenia) we found that the values of GCCS was higher in patients with sarcopenia (3,0±1,4 vs 2,1±1,4) even though the difference didn't reach statistical significance.

Conclusion. Our data suggest link between osteoporosis and cardiac calcification. The burden of cardiac calcium seems to be higher in patients with fragility fractures. These findings confirm that osteoporotic patients have an higher risk of cardiac and vascular calcification and this confirm the greater risk of cardiovascular events in osteoporotic patients.

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ROLE OF NON ALCOHOLIC STEATOSIS IN CAROTID ATHEROSCLEROSIS

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There is a growing evidence in literature for a close relationship between non-alcoholic hepatic steatosis (NAFLD) and cardiovascular morbidity and mortality. However, it has not been clarified whether the increase in cardiovascular risk is due to the presence of cardiovascular risk factors associated with the metabolic syndrome, commonly found in patients with NAFLD, or if NAFLD itself is a mediator of atherosclerosis (ATS). The aim of the study was to investigate the correlation between hepatic steatosis and carotid ATS, observable in its early stages with the increase of miointimal thickness (IMT). In this study 110 patients, referred to the clinic of metabolic disease, were divided into two groups based on the presence or absence of fatty liver on the ultrasound examination of the abdomen (70 patients with steatosis of which 42 men and 28 women and a control group of 40 patients without steatosis). Ultrasonographic examination of the epiaortic vessels in each patient was performed by measuring IMT and possible presence of atheromatous plaques according to the European Carotid Surgery Trial(ECST) method. We found an IMT of 1.32±0.68 mm in patients with steatosis and 1.14±0.76 in non-steatose subjects with a statistically significant difference (p<0.01). The presence of atheromatous carotid plaques was 40.5% in patients with steatosis and 20.5% in the control group with a statistically significant difference (p<0.01). The patients with hepatic steatosis were then ruled according to the degree of steatosis (grade I, II and III) observing that increasing hepatic steatosis severity was accompanied by an increase in the IMT values, however without reaching statistical significance (p=0.1).Likewise, there was a positive, but not statistically significance, relationship between the severity of NAFLD and the burden of ATS. The results confirm the association between the presence and the degree of NAFLD and the carotid ATS, and this supports the hypothesis that hepatic steatosis can be considered a cardiovascular risk factor.

HDL3 MODULATES THE PHENOTYPIC SWITCH INDUCED BY CIGARETTE SMOKE IN VASCULAR SMOOTH MUSCLE CELLS

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Background and Aims. Up to 50% of foam cells in human atherosclerotic plaque originates from smooth muscle cells (SMCs). Upon cholesterol loading, SMCs modify their phenotype from contractile to synthetic, with a decrease of the expression of typical SMC markers (a-actin, calponin) and an increase of pro-inflammatory markers (Mac-2). Moreover, cholesterol loading reduces the expression of myocardin, a SMC specific-transcriptional factor involved in SMC differentiation, and that of its modulator, miR-143/145. On the contrary, cholesterol increases the expression of Klf4, a myocardin repressor. Data recently published by our group showed that these changes are prevented by HDL3 only in the presence of the Abca1 membrane transporter. The phenotypic behavior of SMCs is modulated also by cigarette smoke (CS), an important atherosclerotic risk factor. To characterize the role of Abca1 and HDL3 in this process, we evaluated HDL3 effects on CS-induced phenotypic changes in SMCs expressing or not Abca1. Methods. SMCs, isolated from the aortae of wild-type (WT) and Abca1 knock-out (KO) mice, were incubated with cigarette smoke condensate (CSC).Results: CSC incubation promotes SMCs phenotypic switch by downregulating the expression of a-actin and calponin and by increasing the expression of Mac-2, Abca1, Abcg1. HDL3 prevents this effect by returning the values to baseline in WT cells, whereas the effect of HDL3 is completely lost in Abca1 KO cells. Myocardin and Klf4 expression is respectively reduced and increased by CSC treatment, indicating this pathway as a possible mechanism underlying the phenotypic modulation of SMCs. Conclusions. Our results indicate that CSC, like cholesterol, induces the SMCs phenotypic switch from contractile to synthetic. HDL3, by interacting with Abca1, modulates the Klf4-myocardin axis and prevents the CSC-induced phenotypic switch in SMCs. In Abca1 KO cells, the preventive effect of HDL3 is completely lost, confirming that the presence of Abca1 is mandatory for the protective activity of HDL3.

EFFECTS OF PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) **INHIBITORS ON CLINICAL OUTCOMES:** A META-ANALYSIS OF RANDOMIZED **CONTROLLED TRIALS**

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Background and Aim. Since the role of proprotein convertase subtilisin/kexin type 9 (PCSK9) in LDL receptor degradation was discovered, several pharmacological approaches to inhibit this protein and lower plasma LDL cholesterol (LDL-C) have been developed, such as monoclonal antibodies (mAbs), which are able to reduce plasma LDL-C up to 60% in different hypercholesterolemic patients. The aim of this meta-analysis was to investigate the effects of PCSK9 antibodies treatment on different clinical outcomes in randomized controlled trials (RCTs).

Methods. PubMed/MEDLINE and EMBASE were searched from inception to May 2018. The main inclusion criteria were: (1) English language, (2) phase 2 or 3 RCT, (3) comparing PCSK9 mAbs with placebo, (4) with effects on outcomes reported and (5) with duration longer than 8 weeks. Mantel-Haenszel method was used to estimate the pooled odds ratios (ORs) with the 95% confidence interval (CI). A continuity correction in case of rare events was also applied. Between-study heterogeneity was tested by Cochrane's Q test and measured with the I2 statistics.

Results. Twenty-six RCTs were selected, comprising more than 60,000 patients. The analysis showed that the exposure to anti-PCSK9 did not lead to a significant reduction in mortality from all causes and cardiovascular (CV) disease. An 18% reduction (OR=0.82, 95% CI: 0.77-0.87) of CV events and a 5% reduction in incidence of serious adverse events (OR=0.95, 95% CI: 0.91-0.99) were observed in subjects exposed to mAbs compared to the control group (exposed to placebo, on top of statin). A 5-years followup adjusted analysis confirmed the results and highlighted even more the benefit of a long-term exposure to PCSK9 mAbs.

Conclusions. This updated meta-analysis confirmed the efficacy of different PCSK9 antibodies in reducing incidence of cardiovascular events across different type of patients.

IL-18 AS A POSSILE LINK BETWEEN PHYSICAL INACTIVITY AND INSULINE RESISTANCE

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Introduction. According to the WHO, unhealthy dietary habits and physical inactivity, along with alcohol consumption and use of tobacco, are the pillars that support the development and maintenance of chronic diseases. Physical inactivity, and partic-

ularly sedentary lifestyle, has belatedly caught the attention of the scientific community, compared to other harmful behaviors. Physical inactivity is crucial in the development of overweight and obesity, but there is increasing evidence that physical inactivity and a sedentary lifestyle can increase the prevalence and severity of metabolic and cardiovascular diseases, independently from body weight and composition. Low grade systemic inflammation is one of the best known processes involved in pathogenesis of metabolic and cardiovascular diseases and IL-18 has recently been recognized as a potential actor in the development of atherosclerotic plaque and insulin resistance. The aim of this study was to evaluate the association between physical activity and sedentary behavior, insulin resistance and IL-18 plasma levels.

Materials and Methods. We enrolled 451 subjects (age >60 years) with preserved motor performance and good health conditions (low prevalence of chronic diseases). Subjects underwent blood sampling, bio-impedance analysis for body composition evaluation and assessment of cardiorespiratory fitness with UKK 2 km walking test and/or cycle ergometer (stress test). All subjects filled a questionnaire concerning weekly physical activity (weekly METS) and daily sedentary time (screen time). Variables with a non-normal distribution were log-transformed. Gender differences in the population were assessed with t-test. The association between variable of interest was assessed with bivariate Pearson correlation. Multiple linear regression analysis was performed (method Stepwise) in order to identify independent predictors of IL-18 levels and insulin resistance.

Results. IL-18 was significantly higher in males compared to females, (400.6 (320.8-503.0) vs 322.4 (263.1-405.5) pg/ml, p<0.001). We observed a significant association between IL-18, markers of systemic inflammation (CRPhs. c. Pearson 0.273, p<0.001) and insulin-resistance (HOMA-IR, c. Pearson 0.283, p,0.001), in both sexes. Moreover, only in males, we observed a significant association between IL-18 levels and markers of adiposity (BMI, WC, FM respectively with c. Pearson 0.205, 0.233, 0.230, all p<0.005). We also tested the correlation between IL-18 and lifestyle indicators in males: IL-18 values were positively associated with daily screen time (0.179, p=0.013) and inversely correlated with VO2 max and UKK fitness index (respectively c. Pearson -0.190, p=0.007 and c. Pearson -0.220, p=0.005). In order to identify the independent predictors of IL-18 in male, we built a linear regression model with IL-18 as the dependent variable. Both UKK fitness index and screen time resulted as independent predictors of IL-18 levels with opposite effect (UKK fitness index: beta coefficient -0.216, p=0.006; screen time: beta coefficient 0.216, p=0.006). Similarly, we performed a linear regression analysis entering HOMA index as the independent variable, in the whole population and age, WC, FM, VO2 max, UKK fitness index, weekly METS, daily screen time and IL-18 as dependent variables. IL-18, FM together with WC resulted as independent predictors of insulin-resistance (respectively, beta coefficient 0.210, 0.270, 0.224, all p<0.005).

Conclusions. Our data confirm the association between IL-18 and insulin-resistance. IL-18 also shows, at least in the male population, a direct association with adiposity and measures of sedentariness, and an inverse association with physical activity and cardio respiratory fitness. Interestingly, there is an independent and opposite effect of physical activity and screen time on IL-18 levels, highlighting as sedentary time has a specific role in affecting cardiovascular and metabolic risk. In conclusion, it is likely that IL-18 plays a role in the complex interaction between lifestyle, adiposity and cardiovascular risk. Further studies are needed to clarify the possible sex-specific difference in the regulation of IL-18 concentrations.

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PREVALENCE OF MICRO -AND MACROVASCULAR COMPLICATIONS IN ITALIAN SUBJECTS AFFECTED BY TYPEII DIABETES MELLITUS

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Introduction. The prevalence of Diabetes Mellitus is rising year after year worldwide. About 1 in 11 adults is affected by Diabetes in the world. The persistence of elevated blood glucose levels can lead to the development of serious chronic complications: diabetic retinopathy, nephropathy, neuropathy (microvascular), coronary artery disease, peripheral arterial disease and ischemic stroke (macrovascular).

Aim of the Study. The aim of the study is evaluating the prevalence of microvascular and macrovascular complications in a Diabetic population of South Italy.

Materials and Methods. The study was conducted analyzing retrospectively a large database which contains informations related to more than 10000 patients afferent to twelve medical practitioners which work cooperatively in the same city. We evaluated: the prevalence of diabetic patients, the presence of CV risk factors (arterial pressure, smoking, lipidic profile, abdominal circumference, obesity) and the presence of chronic complications.ResultsThe total number of the subjects analized in the informatic platform was 10638. 800 of 10638 were affected by TypeII Diabetes Mellitus, M=320,F=480(age 59±18.8years). Patients affected by obesity were 68%, patients affected by hypertension were 68.75%, patients affected by hypercholesterolemia were 70%. Patients with neuropathy were 41.375%, while those who showed retinopathy were 36%. Subjects with CAD were 40 patients (5%), with Ischemic Stroke 12 (1.5%), with Peripheral obstructive chronic arteriopathy 96 (12%). Conclusion. This study evaluated the prevalence of Type II Diabetes Mellitus and its complications in a population of South Italy showing its great increase compared to data from international and national studies. A correct life-style, a strict glycemic control, followed by a strict preassure control, lipidic control and the use of adeguated drugs is recommended in diabetic patients to prevent chronic metabolic disorders.

SERUM LIPOPROTEIN (A) PREDICTS ACUTE CORONARY SYNDROMES IN PATIENTS WITH SEVERE CAROTID STENOSIS

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Background and Aim of the Study. Lipoprotein (a) [Lp (a)] is a well established independent risk factor for the development and progression of atherosclerosis and cardiovascular disease 1,2, but the cut-off values of lp (a) above which cardiovascular risk increases and so its pathophysiological and prognostic role remain unclear, mainly because of the published data are limited. The aim of this pilot study was to explore the role of Lp(a) in predicting the development of acute coronary syndrome (ACS) and its relationship with atherosclerotic plaque components in high-risk patients. **Materials and Methods.** We included 180 patients with severe carotid artery stenosis undergoing endarterectomy. According to the most recent scientific evidences 3, we selected the Lp(a) cut-off value of 10 mg/dl to predict 24-month follow-up ACS. In addition, the relationship between the levels of lp (a) and the different components of atherosclerotic plaque was assessed.

Results. At baseline, no significant differences were found in the two groups of patients [Lp (a) <10 mg / dl or >10 mg / dl] with regard to demographic characteristics, ongoing drug therapy and laboratory parameters, with the exception of higher levels plasma fibrinogen and D-dimer and the most frequent presence of chronic coronary artery disease in subjects with high levels of Lp (a). The main finding of our study was the association between high serum Lp(a) levels (>10 mg/dl) and the increased risk of developing an ACS at 2-year follow-up. With the histopathological analysis of carotid plaques, the only significant finding was a slight increase in the content of smooth muscle cells in the upstream regions of the plaques in subjects with Lp(a) >10 m/dl.

Conclusions. Our results suggest a possible predictive role of Lp(a) for the development of ACS in patients with advanced carotid atherosclerosis, and the cut off of 10 mg/dl could be an appropriate value to identify patients with an increased cardiovascular risk.

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SILENCING OF HISTONE DEACETYLASE 3 AT DIFFERENT STAGES OF DIFFERENTIATION: EPIGENETIC EFFECTS ON ADIPOCYTE METABOLISM

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Introduction. Obesity is a condition characterized by uncontrolled expansion of adipose tissue mass resulting in pathological weight gain. Histone deacetylases (HDACs) have emerged as crucial players in epigenetic regulation of adipocyte metabolism. Previously, our group demonstrated that selective inhibition of class I HDACs using MS-275 improves white adipocyte functionality and promotes browning of C3H/10T1/2 mesenchymal cells differentiated into adipocytes. These effects are observed in db/db and diet induced obesity mice, two models of obesity and type 2 diabetes, and in mice with adipose-selective inactivation of HDAC3.

Aim. The molecular basis of class I HDACs action in adipose tissue are not deeply characterized and it is not known whether the effects of their inhibition are exerted on adipocyte precursors or mature adipocytes. Therefore, the aim of the present work is to explore the molecular mechanism of class I HDAC action in adipocytes by evaluating the effects of HDAC3-specific silencing at different stages of differentiation.

Material and Methods. HDAC3 was silenced in C3H/10T1/2 mesenchymal stem cells at different stages of differentiation into adipocytes. shRNA targeting HDAC3 was used to generate the knock-down model.

Results. HDAC3 silencing at the beginning of differentiation enhanced adipocyte functionality by amplifying the expression of genes regulating differentiation (Pparg, Cebpa), oxidative metabolism (Acadm), browning and mitochondrial activity (Ucp1, Cox7a1). However, no differences in terms of gene expression were found when HDAC3 silencing occurred in terminally differentiated adipocyte. Therefore, HDAC3 early silencing resembles the effects of MS-275 chemical inhibition in vitro and in vivo. Furthermore, we demonstrated MS-275 upregulates Pparg and Ucp1 genes by increasing histone H3 lysine 27 acetylation on their enhancer regions. Thus, early epigenetic events mediated by class I HDAC inhibition/silencing are crucial to imprint adipocyte precursors towards the above-mentioned metabolic phenotype. Our data suggest that these effects are exerted on adipocyte precursors. Supported by grants from MIUR Progetto Eccellenza, FP7 NR-NET PITN-GA-2013-606806 and CARIPLO Foundation 2015-0641.

ROLE OF PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) IN THE FUNCTIONALITY OF CARDIAC MITOCHONDRIA

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Background. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a 692-amino acid glycoprotein that belongs to the family of proprotein convertases. It is secreted into the circulation by the liver, interacts with several receptors of the LDL-R family but also with CD36 and favors their degradation. Viceversa increased expression of receptors of the LDL-R family was shown to favor lipid accumulation, accumulation that in the heart results in myocyte apoptosis, myocardial fibrosis, impaired mitochondrial function and cardiac dysfunction.

Aim. This project is aimed at testing the hypothesis that PCSK9 might regulate lipoprotein and fatty acid metabolism in the heart, thus modulating mitochondria physiology, heart metabolism and cardiac function.

Methods. 2-months old WT, PCSK9 KO and Albumin CRE PCSK9 conditional KO (lacking PCSK9 production in the liver) male mice were fed for 20 weeks with SFD (Standard Fat Diet - 10% Kcal fat). MRI has been performed on these mice and the hearts have been collected. Mitochondrial respiration was investigated under resting conditions and following maximal coupling and uncoupling conditions in all mice models. A metabolomic analysis have been performed and changes in the profile of mitochondrial proteins were tested by western blotting.

Results. The lack of PCSK9 in cardiac cells is associated with a reduced ATP production as well as a reduced total energy charge (ATP; 15,07±1,49Vs9,412±1,161 and Energy charge; 14,03±1,238Vs9,439±1,161, p<0.05) indicating an altered metabolism that can lead to mitochondrial damage. Metabolomics data shows that PCSK9 KO and Albcre-PCSK9 KO mice have less glucose 6-P, ribose-5P and erythrose-4P compare to WT, alterations that are known to be associated with an increased glycolytic flow. These observations are paralleled by a reduction of the Krebs cycle intermediates in PCSK9 KO and AlbCRE-PCSK9 KO animals compared to WT. Western Blot analysis of protein lysates showed a reduced expression of key proteins of the electron transport chain and an increased expression of LDL receptor in both PCSK9 KO and AlbCRE-PCSK9 KO models. From the Oxygen Consumption data we have seen that the reduced expression of the electron transport chain complexes is associated with a reduced oxygen consumption and mitochondrial respiration. The heart of PCSK9 KO mice are also subjected to a morphological change such as an increased thickness of the left ventricular wall during systole.

Conclusion. Our data suggest that the lack of circulating PCSK9 is associated with altered lipids metabolism in the heart that associate with altered morphology and functionality. This preliminary data supports the need for an extensive characterization of this animal model.

SONOGRAPHIC EVALUATION OF ACHILLES TENDON IN FAMILIAL AND POLYGENIC HYPERCHOLESTEROLEMIA: A CASE-CONTROL STUDY

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Introduction. Familial Hypercholesterolemia (FH) is a monogenic metabolic disease in which defects in LDL binding or internalizing cause hypercholesterolemia and premature atherosclerosis. Its diagnosis relies on physical examination and biochemical, anamnestic and genetic data (some of which included into a comprehensive score, e.g. the Dutch Lipid Clinic Network Score [DLS]). Aim of this study was to evaluate the role of a simple and standardized ultrasound testing of Achilles tendon thickness, in the diagnosis of Familial Hypercholesterolemia (FH), assessing its correct classification capacity (CCC).

Patients and Methods. In a case-control study we enrolled 60 males and 60 females, 18-70 years old, with genetically confirmed heterozygous FH, who addressed a single Lipid Clinic. Each one of these patients was sex and age-matched with subjects affected by polygenic hypercholesterolemia (PH, n=120) and normolipemic controls (n=120). All these individuals underwent bilateral ultrasound measurement of Achilles tendon thickness (ATT, 2 cm from heel) and the presence of echo-structural characteristics relating to Achilles tendon xanthomatosis (ATX) was recorded.

Results. Increased ATT, by sex and aged specific cut-off values, showed a diagnostic sensibility of 72% and specificity of 79% in diagnosing FH. In the whole population the CCC of this method, examined by ROC curve analysis, was fairly good (AUC, 0.78) even though less than that calculated by DLS alone (not including the genetic score). In the subgroup of patient with intermediate probability of disease (DLS, 4-5) combining DLS with sonographic measurements improved diagnostic accuracy over DLS alone (sensibility 96%, specificity 87% vs. 74% and 60%, respectively). Focal anechoic pattern in the context of increased ATT was related to ATX and detected only in FH group (sensitivity 23% and specificity 100%). Therefore ATX proved pathognomonic for this genetic condition while it was not significantly associated with a history of premature CHD (RR 2.00, p=n.s.).

Conclusions. Our investigation confirms the excellent applicability and cost-efficacy (compared to the genetic analysis) of ultrasound Achilles tendon evaluation in FH diagnosing, above all in those subjects in whom DLS probability is intermediate.

PARAOXONASE-1 (PON-1) AND LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 (LP-PLA2) ACROSS LIPOPROTEIN SUBCLASSES IN TYPE 2 DIABETES SUBJECTS.

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Paraoxonase-1 (PON1) and Lipoprotein-associated Phospholipase A2 (Lp-PLA2) are two lipoprotein-associated enzymes influencing oxidative stress and therefore involved in the pathogenesis of several human diseases including type 2 diabetes mellitus (T2DM) and atherosclerosis. The activity of both enzymes is conditioned by the composition of lipoprotein carriers. It's well known that inT2DM there are significant changes in lipid profile and in the distribution of the subclasses of lipoproteins. Thus, it becomes relevant to understand the interaction of PON1 and Lp-PLA2 with subclasses of lipoproteins in T2DM.We evaluated serum levels of PON1-Arylesterase, PON1-Lactonase and Lp-PLA2 activities and lipoprotein subclasses in 202 non-diabetic subjects (Controls) and 92 T2DM outpatients.Arylesterase, but not Lactonase or Lp-PLA2 activities, was independent and inversely associated with TD2M (Odds Ratio=3.389, 95% Confidence Interval 1.069-14.756). Regarding distribution in lipoprotein subclasses, we observed that arylesterase activity was independently related with large HDL-C and small intermediate density lipoprotein cholesterol (IDL-C) in Controls while, along with Lactonase, it was related with small low density lipoprotein cholesterol (LDL-C), all IDL-C subspecies and very low density lipoprotein cholesterol (VLDL-C) in T2DM (p<0.05 for all). Lp-PLA2 showed significant correlation with small LDL-C, large IDL-C and VLDL-C only in T2DM group. Our study we observed that T2DM subjects has lower levels of PON1-Arylesterase activity compared to Controls and that during T2DM there could be a shift of PON1 and Lp-PLA2 towards the more pro-atherogenic lipoprotein subclasses. The possibility of a link between the two observed phenomena required further investigations.

CURRENT IMPACT OF DIABETES ON IN -HOSPITAL SURVIVAL AND COMPLICATION OUTCOMES IN PATIENTS HOSPITALIZED FOR STROKE – THE VASD-OUTCOME STUDY

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Background. The prevalence of diabetes mellitus (DM) and accompanying cardiovascular complications is increasing worldwide. The treatment strategies for both DM and cerebrovascular accidents (CVA) have considerably improved in the recent past, thus possibly softening the detrimental effect of DM in patients with CVA. However, the availability of updated data on the current epidemiology of DM in patients hospitalized for CVA is relatively limited.AIM - To estimate the prevalence of DM in a large contemporary cohort of individuals admitted for CVA and to investigate the independent impact of DM and hyperglycemia on the in-hospital survival and complication outcomes.

Experimental Design. We assessed the prevalence of DM, previously known (known-DM) or newly-diagnosed (new-DM), in all patients (n=542, males 51.5%) with primary admission for stroke at the Stroke Unit of the Neurology Department at the Verona University Hospital from 1/1/2015 to 12/31/2016. DM was ascertained according to previous diagnosis, glucose-lowering medications at admission/discharge or random plasma glucose \geq 200 mg/dL. The association of DM with in-hospital death, infectious/cardiorespiratory complications, duration and costs of hospitalization was tested in all patients and separately in known-DM and new-DM. Similar analyses were applied by stratifying the whole cohort according to quartiles of plasma glucose level at hospital admittance.

Results. Prevalence of DM was 21.03% (78.1% known-DM: 21.9% new-DM 27.2%). In-hospital death rate was 10.5% (n=57 death events). Compared to non-diabetic patients, those with DM showed an increased risk of death (15.8 vs. 9.1%; crude-OR 1.87 95%CI, 1.03-3.41) and complications (48.2 vs. 33.3%; crude-OR 1.87, 1.22-2.86). DM diagnosis was associated with higher hospitalization costs (mean±SEM, 4,087±127 vs. 4,123±318 €, p=0.88) and longer hospital stay (14±1.06 vs. 8.80±0.39 days, p=0.72), although these differences did not reach statistical significance. The total number of death events occurred within the Stroke Unit. When the analyses were restricted to the subgroup of "survivors", a significantly longer hospital stay was observed in patients with DM (2.32±1.02 vs. 0.98±0.19 days, p=0.038). After multivariable adjustment (age, sex, e-GFRMDRD, hemorrhagic stroke, lipid-lowering or anti-hypertensive therapy), DM remained an independent predictor of in-hospital complications (adj-OR 1.87, 1.22-2.86), but not mortality (adj-OR 1.79, 0.92-3.46). All study outcomes did not display significant differences between new-DM vs. known-DM. After stratification of the whole cohort according to the admittance plasma glucose levels, hyperglycemia resulted as predictor of in-hospital death and complications, independent of DM status, age, sex, e-GFRMDRD, alteplase medication and ongoing glucose-lowering, lipid-lowering or anti-hypertensive medication, with patients falling in the highest quartile (plasma glucose ≥118 mg/dL) displaying an increased risk of death (3.25, 1.40-7.54) and complications (2.30, 1.24-4.23), as compared to those comprised in the lowest tier (<86 mg/dL, reference). After stratification of the whole cohort according to age, those comprised in the highest tertile (age \geq 81 years) displayed a risk of death significantly increased (adj-OR 3.25, 1.40-7.54; n=34 events), as compared to the younger subgroup falling in the lowest tier (<72 years, ref.). The opposite was observed with respect to the in-hospital complications endpoint, with younger individuals displaying a considerably higher risk, than those at older age (adj-OR 3.13, 1.42-6.9).

Conclusions. These data highlight that the occurrence of DM in patients admitted for stroke is extremely high in frequency still nowadays and it carries an excess burden of mortality and complications. Hyperglycemia per se shows an incremental risk for adverse clinical outcomes, thus underscoring the urgent need for strategies to anticipate DM diagnosis and promptly set a proper therapy in patients at high cardiovascular risk.

ASSOCIATION BETWEEN TSH AND NON -ALCOHOLIC FATTY LIVER DISEASE IN THE OVERWEIGHT-OBESE POPULATION

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Background. Non alcoholic fatty liver disease (NAFLD) is a spectrum of disorders characterized by accumulation of fat in the liver in the absence of other recognized causes of fatty. It affects up to 30% of the population in Western countries. Furthermore increased increased risk of incident T2DM by approximately twofold and an approximate twofold increased risk of CKD were reported. It was previously described a correlation between thyroid dysfunction, especially hypothyroidism and subclinical hypothyroidism, and Steatosis. Thyroid gland play an important role in the regulation of diverse metabolic processes, promoting hyperlipidemia and obesity, thus contributing to NAFLD. However, it is still unclear whether TSH even in the euthyroid conditon is associated with Steatosis in the overweight-obese population.

Methods. In this cross-sectional study were enrolled 149 individuals (age 18-65 y) with BMI $\geq 25 \text{ kg/m}^2$ who underwent a liver steatosis assessment by CAP (Controlled Attenuation Parameter) measurement, referred to Clinical Nutrition Unit at the "Magna Graecia" University in the last year. Liver Transient Elastography was used to quantify liver steatosis by CAP assessment. Ten successful measurements were performed on each patient (Success rate: 60%). The ratio of the IQR of liver stiffness to the median (IQR/MLSM) was calculated as an indicator of variability. The final CAP score (ranged from 100 to 400 decibels per meter (dBm-), was the median of individual measurements. For each patients, several the laboratories test were assessed. We excluded those who had chronic hepatitis B and/or C virus infection, alcohol abuse, impaired liver function, presence of autoimmune disease, Type 1 and 2 diabetes mellitus, fasting glucose >110 mg/dL, tryglicerides 200 mg/dL, TSH >5 mIU/L or <0.3 mIU/L.

Results. After multivariable analysis, the CAP was associated with WHR (p<0.001), tryglicerides (p=0.006), BMI (p=0.023) and TSH tertiles (p=0.03). Participants in tertile I (low TSH values) had a lower Controlled Attenuation Parameter than those in tertile II (p<0.001) and tertile III (p<0.001).

Conclusion. We found a positive association between Controlled Attenuation Parameter and TSH values: highest levels of TSH, within the reference ranges, were associated with highest CAP values. Thyroid function, even within reference range of thyroid function tests, is significantly correlated with CAP Score in overweight-obsese. Further studies on the involvement of thyroid hormone in NAFLD may help to understand its underlying mechanisms and to identify at-risk patients reducing the onset and possible progression of NAFLD and its potential complications.

IDENTIFICATION OF GENETIC VARIANTS IN METHIONINE METABOLISM GENES IN PATIENTS WITH MARFAN SYNDROME OR RELATED DISORDERS CHARACTERIZED BY NEXT GENERATION SEQUENCING ANALYSIS

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Background. Previous data from literature evidenced the role of homocysteine and MTHFR C677T polymorphism in influencing the severity of cardiovascular manifestations in patients with Marfan syndrome (MFS). In this study, we investigated the influence of genetic variants in genes related to methionine metabolism (CBS, MTHFR, MTR, MTRR, MMADHC), identified in patients admitted to the Referring Center for MFS and Related Disorders through next generation sequencing (NGS) target gene approach, on cardiovascular manifestations severity.

Methods. A NGS target gene panel including 94 genes involved in MFS and related connective tissue disorders in differential diagnosis has been used to investigate the genetic profile of 21 patients suspected to be affected by MFS, or bicuspid aortic valve with aortic diltation [median age (range) 41 (13-70) yrs; 63.6% males].

Results. NGS target gene approach permitted the detection of 12 missense genetic variants in the 5 genes related to methionine metabolism: 4 variants in MTHFR gene (rs1801133, rs1801131, rs72552099, rs35737219), 2 in MTR gene (rs1805087, rs121913579), 5 in MTRR gene (rs1532268, rs1801394, rs10380, rs162036, rs140944718), and rs61750442 variant in MMADHC gene. Increased minor allele frequency (MAF) for MTHFR rs1801133 and rs35737219, MTRR rs10380, rs162036 and rs140944718 with respect to MAF reported from literature in the Caucasian population was observed. Statistically significant difference in Z score

values of aortic diameters at the sinus of Valsalva according to Devereux RB et al. 2012 between carriers and non-carriers of MTRR rs1801394 polymorphism (NM_002454.2:c.66A>G, NP_002445.2:p. Ile22Met) has been found [3.01 (1.77-6.20) vs 1.9 (0.1-6.0) p=0.036). SIFT and Polyphen evaluation showed a deleterious effect of this variant.

Conclusions. Investigation of genes related to methionine metabolism by NGS approach identified genetic variants able to modulate severity of cardiovascular manifestations in MFS and related disorders. Further evaluation of these variants in a larger population could allow to confirm this datum.

LARGE GENES PANEL TARGETED HIGH-THROUGHPUT SEQUENCING IN DETERMINING MONOGENIC AND POLYGENIC NATURE OF FAMILIAL HYPERCHOLESTEROLEMIA IN RESEARCH AND DIAGNOSIS

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Background. Familial hypercholesterolaemia (FH) is an autosomal dominant genetic disorder characterized by elevated plasma levels of low density lipoprotein-cholesterol (LDL-C), which can lead to premature cardiovascular disease. FH is caused mainly by mutations in LDLR, APOB and PCSK9 genes. However, many subjects with primary hypercholesterolemia did not demonstrate functional mutations in any of these genes.

Aim. Of this work is to sequence, through a targeted highthroughput sequencing (HTS) strategy, 55 genes, involved in FH or other alterations of lipid profile, in patients with suspected FH. The design also allow to genotype all variants included in the Talmud et al. (2013) low-density lipoprotein cholesterol gene score and in statins pharmacogenetics. We would evaluate whether this approach allows an improvement in diagnosis of genetic dyslipidemias and in research of novel associated genes, polygenic profiles impact and pharmacogenetic approaches.

Methods. Subjects with suspected FH are identified using the most common diagnostic algorithm, the Dutch Lipid Clinic Network Score. We analyzed 30 clinically probable or definite FH patients. DNA libraries were prepared using Agilent Haloplex HS Enrichment System and the sequencing was performed using Illumina MiSeq Reagent Kit v3. Sequencing results were analyzed using both Agilent SureCall software and a pipeline developed by the bioinformatics group of our Department. The possible pathogenicity of variants was evaluated using six different in silico tools. Results. In 16 out of 30 patients, we found major mutations that can explain their lipid profile. The remaining patients showed a burden of polymorphisms at high and low/very low allele frequency in different genes that in part represent, based on some studies, known risk factors for or atherosclerotic disease that could confirm a polygenic predisposition to the disease. We started the segregation analysis in families of two patients. In one family the role of mutations in ABCG5 gene in lipid profile alteration was demonstrated. Furthermore, our data suggested that the simultaneous evaluation of genes involved in lipid-lowering drugs pharmacogenetics could improve a personalized management of patients with an earlier achievement of the therapeutic target also avoiding adverse effects.

Conclusions. Our results showed the significant advantages of using an HTS approach for the diagnosis and study of FH. Indeed, with our specific 55 genes panel we can not only screen the 3 major genes involved in FH, but we can also define the role of other major genes (e.g, ABCG5) and have a larger view about the patient's polygenic predisposition to the lipid disorders. The expansion of the case study will allow to confirm the hypothesis that familial hypercholesterolaemia can also be caused by an accumulation of common small-effect LDL-C-raising alleles. Finally, the screening of the genes involved in lipid-lowering drugs pharmacogenetics could allow to apply an early personalized therapy.

PCSK9: A ROLE IN THE PROGNOSTIC STRATIFICATION OF SEPTIC PATIENTS?

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Background. PCSK9 plays a critical role in regulating circulat-

ing cholesterol levels, through the reduction of the membraneassociated LDL receptor. In experimental models plasma PCSK9 levels were increased in sepsis. At normal levels, PCSK9 has no influence upon hepatocyte bacterial endotoxin clearance, but as levels rise, there is a progressive inhibition of clearance. It has been shown that decreased PCSK9 function increases survival of septic patients. Aim of the study was to assess prognostic value of early assessment of PCSK9 levels in septic patients.

Methods. 263 patients were enrolled in a prospective analysis aiming to find reliable biomarkers for early sepsis diagnosis. Patients admitted to High-Dependency Unit of Emergency Department with diagnosis of severe sepsis/septic shock were eligible. We evaluated vital signs and laboratory data at admission (T0), at 6 hours (T6) and after 24 hours (T24); Sequential Organ Failure Assessment (SOFA score) was calculated at T0,T6 and T24. Primary end-points were 7- and 28-day mortality.

Results. Mean age of the study population was 74±14 years, 58% male gender; mean SOFA score at admission was 5.3±2.7. The most frequent infection source was respiratory (45%). Day-28 mortality was 25%. PCSK9 normal values are lower than 250 ng/ ml. In septic patients, at every evaluation point, PCSK9 level was significantly higher than normal values reported in previous studies (T0 661±405ng/mL, T6 687±417ng/mL, T24 718±430 ng/mL). There was no significant difference between patients with Gram+ or Gram-infection (T0: 641±493 vs 701±406 ng/mL; T6: 652±433 vs 769±389 ng/mL; T24: 690±397 vs 811±501 ng/mL, all p=NS). Only at T0 non-survivors by day-28 showed a significantly lower level than survivors (549±437 vs 696±390 ng/mL, p=0.016); all other evaluations were comparable regardless of the outcome, both considering day-7 and day-28 mortality rate. An Analysis for Repeated Measures between T0 and T24 levels did not show any significantly different trend between day-7 (T0: 668±389 vs 623±492; T24: 702±388 vs 843±669 ng/mL) and day-28 (T0: 696±390 vs 549±437; T24: 718±392 vs 719±553 ng/mL) survivors and non-survivors (all p=NS). There was no correlation between SOFA score values and PCSK9 levels at all evaluation (non-parametric correlations: all p=NS). We divided the study populations in two subgroups according to the level of T0 SOFA score (≤ and >5): PCSK9 levels were comparable regardless the severity of sepsis-induced organ damage (T0: 696±381 vs 614±444; T6: 716±417 vs 631±421; T24: 709±413 vs 716±470 ng/mL, all p=NS). Finally we compared PCSK9 levels in patients with T0 lactate level \leq and >2: even in this analysis we did not find any significant difference (T0: 672±485 vs 642±365; T6: 689±449 vs 664±373; T24: 711±392 vs 698±452 ng/mL, all p=NS). **Conclusions.** PCSK9 levels were significantly increased in septic patients. However, the levels did not show any significant association with indexes of hypoperfusion and with the severity of organ damage, as well as with the short- and medium-term mortality rate.

STATIN-INDUCED MYOPATHY: DIFFERENT STRATEGIES FOR MANAGEMENT AND DIFFICULT CHALLENGE TO REDUCING CARDIOVASCULAR RISK

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Background. Statin-related myopathy is a common problem in clinical practice, especially due to the matter to reach LDL-C target and reduced patients' compliance with therapy.

Aim. To evaluate patients with statin-induced myopathy characteristics and the behavior in washing-out and rechallenging therapy. **Methods.** We retrospectively reviewed data from 1240 hypercholesterolemic patients visited at our Center between 2006-2015.

Results. Among 714 patients treated with statins, 113 (15,8%) presented muscle-related symptoms: 103 stopped the previous treatment; 89 of them (78.8%) agreed to do a rechallenge, 14 (12.4%) did not. After wash-out, 12 (10.6%) were still grieved for muscle symptoms, whereas 19/89 patients who did a rechallenge re-presented the same problems. CPK values among patientspresenting muscle-symptoms were elevated (M>450;F>400 mg/dL) in 14 subjects (12.4%) but were >4xULN (myositis) only in 4 of them. Nobody had rhabdomyolysis. The most used statin in symptomatic patients was atorvastatin (43=38.0%), following by simvastatin (35=31.0%) and rosuvastatin (24=21.2%); few patients (10=8.8%) took fluvastatin, lovastatin or pravastatin but no significant relation was found and this can simply reflect the market trend. Subsequently, in the rechallenge/down-titration phase, attempting to maintaining a good efficacy/tolerability, statins more frequently used have been rosuvastatin (28=31.5%), simvastatin+ezetimibe (21=23.6%) orpravastatin (20=22.4%); 12 patients (13.5%) disagreed and continued with other Lipid-lowering drugs (monacolin K, fenofibrate). Once stopped the first statin, only 31 out of 113 patients (27.4%) reached the LDL-goal recommended by SCORE System. In particular 68 patients (60.2%) had a familial dyslipidaemia and only 14 (20.6%) had reached the LDL-C target. Moreover, patients with very-high cardiovascular risk were 17 (15.0%) but only 1 had LDL-C levels <70 mg/dl.

Conclusions. Statin-induced myopathy causes a delay and a hurdle to the reducing of LDL-C levels, especially in higher risk categories. Such patients should benefit from alternative therapeutic options.

THE WALKING ELDERLY

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Background. The hypertension is very common condition in elderly patients. Some studies demonstrate the preservation of muscle mass and physical performance improve survival in the elderly. The aim of the present study was to assess the relationship between reduced physical mobility and survival in elderly hipertensive patients.

Methods. We have observed the data stored in our hypertension treatment center for patients aged from 80 to 96 years. In our study we analyzed data from 120 elderly patients affected by hypertension; we evalueted the presence of co-morbidities that reduce walking. We divided patients into A Group and B Group. A Group: (60 patients) presence of co-morbidities or conditions that reduce walking (arthritis, peripheral neuropathy, episodes of past falls, fear of falling). B Group: (60 patients) normal walking. the patients performed the walking speed test.We analyzed the incidence at 5 years in the 2 groups of acute events (stroke, ima, acute renal failure), death. Dosages of standard therapies for Hypertension such as ACE-inhibitors or beta-blockers and calcium channel blockers were used.Patients affected by secondary hypertension, smoking habit, alcohol consumption, dementia, diseases of the central nervous system a clinical history of CVD, liver or kidney advanced diseases were excluded.

Results. We found a significant association between reduced ambulation and the incidence of acute events (p<0.003). We found a significant association between reduced ambulation and the incidence of death in 5 years (p<0.004). Increased walking time was related to a high incidence of adverse events and death in Group A. **Conclusions.** Our results are in agreement with experimental evidences, suggesting that preserved walking, and preserved muscle mass plays an important role in the development of vascular damage and complications in elderly hypertensive patients. Reduced walking significantly increases the incidence of complications and death at 5 years in the elderly hypertensive patient

METABOLOMIC SIGNATURE OF ANGIOPOIETIN-LIKE PROTEIN 3 DEFICIENCY IN FASTING AND POSTPRANDIAL STATE

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Objective. Angiopoietin-like 3 (ANGPTL3) is a promising therapeutic target for lowering atherogenic lipid levels and decreasing cardiovascular disease risk. We aimed to determine detailed metabolic effects of ANGPTL3 deficiency in fasting and postprandial state. **Material and Methods.** The study population consisted of individuals carrying S17X loss-of-function (LOF) mutation in AN-GPTL3 gene (N=6 homozygous and N=32 heterozygous carriers) and controls (N=38). We used nuclear magnetic resonance metabolomics to quantify 225 metabolic measures. We compared metabolic signatures of LOF carriers and controls in fasting state and after an energy-rich meal.

Results. In fasting, ANGPTL3 deficiency resulted in similar reductions in magnitude for low density lipoprotein cholesterol (LDL-C, β =-0.74 per SD change, SE=0.16, P=2.5x10-5) and triglyceride-rich lipoproteins (cholesterol in very low density lipoprotein, b=-0.75, se=0.16, P=7.1x10-6. Further, beta-hydroxybutyrate, a biomarker of hepatic fatty acid beta-oxidation, was elevated (β =0.55, SE=0.17, P=0.002). Absolute levels of both cholesterol and triglycerides were substantially lowered in most lipoprotein subclasses, with the relative proportion of triglycerides being increased and cholesterol proportion reduced in triglyceride-rich lipoproteins. Homozygous ANGPTL3 LOF carriers showed blunted postprandial response in triglyceride-rich lipoproteins and several fatty acid measures, and more pronounced increases in ketone bodies when compared to controls.

Conclusions. In addition to overall triglyceride and LDL-C lowering effects, ANGPTL3 deficiency results in reduction of cholesterol content in triglyceride-rich lipoproteins. Further, ANGPTL3 LOF carriers had elevated ketone body production, suggesting enhanced fatty acid β -oxidation. The metabolic profile in individuals representing human knockout of ANGPTL3 reinforce ANGPTL3 as a promising therapeutic target for decreasing cardiometabolic risk.

A DIFFICULT CASE OF CHRONIC AND PROGRESSIVE ISCHEMIC ARTERIOPATHY IN A YOUNG PATIENT WITH HIGH LDL CHOLESTEROL AND LIPOPROTEIN (A) DESPITE MAXIMUM STANDARD LIPID-LOWERING THERAPIES

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Background. Patients who didn't reach their LDL-C target were so far forced to undergo invasive and expensive treatments such as LDL-Apheresis. PCSK9 inhibitors represent a revolution for these patients.

Aim. To evaluate efficacy and tolerability of evolocumab 140mg (E140) Q2W in a 54-yo patient in secondary prevention for multiple episodes of MI/ACS/UA again and again treated by PCI and placement of stents from the age of 49, despite maximum tolerated regimen of standard lipid lowering therapies (LLT).

Case report. The first time we visited him, patient presented BMI=27, DLS=6, LDL-C=144 and Lp(a) 104mg/dL; LDL-C score according to FerrerInCode (Lipigen project) =1.23 (values >0.73 are associated with high probability of polygenic cause) (1). His history showed LDL-C values consistently >100-120 mg/dl and Lp(a) >100 mg/dl despite rosuvastatin 20 mg/die plus ezetimibe 10mg/die (maximum tolerated LLT). In October 2017, we intro-

duced E140 mg Q2W on top of his LLT, checkin lipid and hepatic profile firstly once a month and then every three months. He quickly reached his LDL-C target (lowest LDL-C value =5 mg/dl) and Lp(a) =21 mg/dl. For the first time in the last five years he stabilized his coronary atherosclerosis symptoms, without needing revascularization procedures and so gaining a good quality of life (QoL) and a significant save of money for public health. Adherence and tolerability have been excellent. Laboratory data (LDL-C between 10-20 mg/dL) remained almost constant during the observation period so that we could reduce statin and suspend ezetimibe. The average reduction of LDL-C continued to be higher than 90%.

Conclusions. A long-term observation will be necessary to evaluate efficacy and tolerability of this treatment over time but, to date, E140 has shown, in this difficult case, a surprising LDL-and Lp(a) reduction, much greater than that reported in literature (2), a stabilization of symptoms with a very good result regarding QoL and relevant money saving.

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EFFICACY AND TOLERABILITY OF PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 INHIBITORS IN PATIENTS AFFECTED BY FAMILIAL HYPER-CHOLESTEROLEMIA AND / OR AT HIGH CARDIOVASCULAR RISK NOT REACHING TARGET WITH STANDARD THERAPIES: A REAL-LIFE OBSERVATIONAL STUDY

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Background. PCSK9-i have recently been authorized in Italy for the treatment of HeFH, high-risk and statin intolerant patients not reaching target with standard therapies.

Aim. Evaluate efficacy and tolerability of PCSK9-i in a real-life setting in 23 patients followed at our Center.

Method. As usual, PCSK9-i were administered subcutaneously Q2W using pre-filled syringes on top of the maximum tolerated standard therapies.

Results. 8 of 23 patients received evolocumab 140, 10 of them alirocumab 75 and 5 alirocumab 150. Only three didn't take background statin for intolerance. Two patient were treated since 2012 and 6 since 2016 because participating in phase 3 trials with alirocumab. The others started treatment between July 2017 (PC-SK9-i approved in Abruzzo) and May 2018. Minimum treatment evaluation time was 3 months.Mean LDL-C reduction was -49%

over the results achieved with standard therapies. One patient surprisingly increased baseline values (perhaps due to incorrect administration or to an unknown PCSK9 gene's mutation) and two (brothers with LDL-R 2HeFH, encoded among the HoFH) were poor responders: by removing these 3 subjects from the analysis we observed a -60% LDL-C reduction, in line with literature data (1, 2). There was a significant TC reduction and HDL-C increase but no significant changes of triglyceride. 17 patients out of 23 (73,9%) reached their LDL-C target; 6 patients did not, but 3 of them (the 2 brothers 2HeFH and one in secondary prevention) got a reduction (-20% for 2HeFH and -53% for high-risk pt. respectively) as expected for their categories (3, 4); 2 were poor responders (-25%) perhaps because they didn't take statins as background therapy. There were no significant difference regarding efficacy and tolerability between Evolocumab and Alirocumab.

Conclusion. Our results are similar to the few real-life available data (5, 6). Longer-term real-life studies are still needed to evaluate efficacy, tolerability and safety of these drugs.

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DYSLIPIDEMIA AND MULTIPLE RISK SCORES IN HYPERTENSIVE PATIENTS: A REPORT FROM A "HYPERTENSION EXCELLENCE CENTRE" OF THE EUROPEAN SOCIETY OF HYPERTENSION

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Introduction. Hypertension is the first risk factor for death in adults and the evaluation of global cardiovascular (CV) risk is mandatory. The aim of our study was to focus on dyslipidemia in a population of hypertensive patients referred to a "Hypertension Excellence center" of ESH only for the management of high blood pressure. Moreover, we analyzed CV risk according to different

CV risk scores, in order to compare dyslipidemia and lipid control among several international guidelines.

Material and Methods. Retrospective observational study on 1219 consecutive outpatients referred to our hypertension center and studied by ambulatory blood pressure monitoring (ABPM). Medical history, anthropometric measurements, laboratory test and ABPM parameters were evaluated. LDL cholesterol (LDLc) was considered "controlled" according to 2016 ESC/EAS guide-lines, 2013 AHA/ACC guidelines, 2014 NICE guidelines and 2016 CCS guidelines.

Results and Conclusion. Mean age: 56.5±13.7 years with male prevalence (55.6%). Overweight/obese patients (OV/OB) were 70.2%. OV/OB had higher glycaemia [96 (88-107) vs 89 (83-99) mg/dl; p<0.001] and a higher risk of having diabetes mellitus (13.7% vs 5.3%; p<0.001; OR:2.8). OV/OB had an atherogenic dyslipidemia characterized by lower HDL (51.2±14.2 vs 58.5±14.9 mg/dl; p<0.001) and higher non-HDL cholesterol (148.8±40.5 vs 140.9±36.3 mg/dl; p=0.006). While 41.6% of all patients had controlled BP. only 28.5% achieved LDLc controlled according to 2016 ESC/EAS guidelines. Surprisingly, the higher the CV risk, the lower was the LDLc control (p<0.001). There was poor concordance between the guidelines analyzed. More than 30% of patients that were uncontrolled based on 2016 ESC/EAS guidelines resulted controlled based on 2013 AHA/ACC and 2014 NICE guidelines. Dyslipidemia is still neglected in hypertensive patients, especially in patients with higher CV risk. OV/OB had a "double-trouble" lipid profile. Physicians should pay more attention to this lipid-linked CV risk factor in clinical practice for a correct CV prevention in hypertensive patients.

IDENTIFICATION OF PUTATIVE FH SUBJECTS AMONG PATIENTS WITH CORONARY ARTERY DISEASE

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Introduction. Familial Hypercholesterolemia (FH) is a common autosomal co-dominant disease due to pathogenic mutations in LDLR, APOB and PCSK9 genes. FH is characterized by high LDL-C levels from birth and an increased risk of coronary artery disease (CAD) that can be, at least partially, reversed through lipidlowering therapy (LLT). Prompt identification of FH among CAD patients could be extremely useful for intensification of therapy and cascade screening.

Methods. Data of patients (≤65 y/o) subjected to percutaneous transluminal coronary angioplasty (PTCA) in 2014 and 2015 were extracted from interventional cardiology database. Data of pharmaceutical prescriptions in 2013, 2014 and 2015 were obtained for identified patients. For each patient, determinations of Total Cholesterol, LDL-C, HDL-C, and Triglycerides were extracted from laboratory database (1 year before and after the PTCA episode). Dutch Lipid Clinic Network Score (DLCNS) for FH was calculated by using LDL-C values and family and clinical history of premature CAD.

Results. 657 subjects (554 males/103 females, age 27-65) were identified, 15.4% of which on LLT for more than one year at the time of PTCA. A valid LDL-C value (either basal or corrected for statin therapy) was obtained for 583 subjects and mean (±SD) LDL-C was 139.4 (±48.1) mg/dl. Values of LDL-C≥155 mg/dl were present in 30.9% of subjects. Calculation of DLCNS revealed that

22.8% of subjects were, at least, possible FH (DLCNS \geq 3 and LDL-C \geq 155), 7.7% had a DLCNS \geq 5 and 2.9% were probable/definite FH (DLCNS \geq 6).

Conclusion. Our findings were in accordance with results from other studies and demonstrated that it is possible to give an accurate estimation of putative FH patients by combining different databases. Subjects with DLCNS25 should be genetically screened for FH. The application of this strategy to routine clinical setting would allow to identify FH patients and to develop cascade screening in relatives for primary prevention of CAD.

MOLECULAR CHARACTERIZATION OF THREE PATIENTS WITH DOUBLE HETEROZYGOUS, COMPOUND HETEROZYGOUS AND HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction. Familial Hypercholesterolemia (FH) is a genetic disorder that results in premature coronary artery disease (pCAD) due to lifelong exposure to elevated LDL-C levels. Heterozygous FH (1:200-250) is caused by one pathogenic variant in one of the major FH candidate genes (LDLR, APOB, PCSK9). Homozygous FH (1:200000-300000) is caused by biallelic pathogenic variants and, if untreated, can cause severe pCAD in the first decades of life.

Methods. An automatic alert was generated by clinical laboratory in case of LDL-C ≥250 mg/dl. This approach allowed to identify three patients with severe hypercholesterolemia. Patient 1 was 38 y/o male with a known hypercholesterolemia, past statin therapy suspended for muscle pain and no family history of pCAD. Total cholesterol and LDL-C were 331 and 261 mg/dl, Dutch Lipid Clinic Network Score (DLCNS) was 6. Patient 2 was a 56 y/o female with severe hypercholesterolemia since adolescence and a positive family history of pCAD. Total cholesterol and LDL-C were 329 and 253 under 40 mg/daily simvastatin, DLCNS was 8. Patient 3 was a 42 y/o male with inflammatory bowel disease and a positive family history for pCAD. Total cholesterol and LDL-C were 427 and 381 mg/dl, DLCNS was 10. He recently suffered from unstable angina. Genetic testing for FH was performed in all patients.

Results. Patient 1 was a double heterozygous FH, being a carrier of missense mutation in LDLR (p.Val523Met) and APOB (p.Arg3558Cys) respectively. Patient 2 was a compound heterozygous FH having two missense mutations in two different LDLR alleles (p.Pro181Arg and p.Pro685Leu). Patient 3 was a true homozygous FH having a biallelic missense variant in LDLR (p.Gly592Glu).

Conclusions. Automatic alert by clinical laboratory is an useful tool to identify patients with a severe, possibly monogenic, hyper-cholesterolemia. Molecular characterization can drive the therapeutic choice and allows cascade screening in relatives.

PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 AND VASCULAR CALCIFICATION UNDER UREMIC CONDITION

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Introduction. Vascular calcification represents a main risk factor of cardiovascular events in patients with chronic kidney disease (CKD). Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) levels correlates with the presence of calcific aortic valve stenosis and carriers of the PCSK9 R46L loss-of-function variant have a low calcific aortic valve stenosis.

Aim. We investigated a possible role of PCSK9 on aortic calcification by using a uremic rat model of vascular calcification and in vitro cultured human smooth muscle cells (hSMCs) overexpressing PCSK9.

Results. Sprague-Dawley rats were fed a standard diet (n=10) or uremic diet containing 0.5% adenine (n=10) for 6 weeks. The uremic condition was documented by increased urine volume (26 ml/ day vs 58 ml/day), plasma creatinine (25.7 µM vs 208 µM) and phosphate levels (2.64 µM vs 6.11 µM). High phosphate concentration was associated to aortic calcification determined by measuring aorta Ca2+ concentrations (0.34 mg/g tissue vs 2.48 mg/g tissue) and by Von Kossa staining. This pathological condition was associated to a significant increase of total cholesterol (from 75.3 mg/ dL to 107.6 mg/dL) and PCSK9 levels (from 40.1 ng/ml to 109.7 ng/ml). Higher expression of PCSK9 was also observed in kidney (+4.8 fold) and liver (+1.5 fold). The overexpression of PCSK9 in hSMCs (from 0.02 ng/ml to 11.3 ng/ml) induced a significant increase of extracellular calcification in response to 5 days exposure to 2.4 mM PO4- (+39% compared to control hSMCs), while PO4- reduced the release of PCSK9 from hSMCs (-33.6%) and the mRNA expression levels (-43%) together with significant reduction of mRNA expression of LDL receptor and HMG-CoA reductase.

Conclusions. The present study indicates a direct role of PCSK9 on vascular calcification associated to a CKD condition. Further analysis will attempt to identify the molecular mechanism of this action and to study the effect of monoclonal antibodies anti PCSK9.

EFFECT OF BERBERINE AND SILYMARIN ON SERUM LIPIDS AND FASTING PLASMA GLUCOSE: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL STUDIES

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Aim. We aimed to assess the impact of a combination of berberine and silymarin on serum lipids and fasting plasma glucose through a systematic review of literature and meta-analysis of the available randomized, double-blind, placebo-controlled clinical studies (RCTs).

Methods. A systematic literature search in SCOPUS, PubMed-Medline, ISI Web of Science and Google Scholar databases was conducted up to 2nd September 2018, in order to identify RCTs assessing changes in plasma concentrations of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and fasting plasma glucose (FPG) during treatment with berberine and silymarin in combination. Two review authors independently extracted data on study characteristics, methods and outcomes. Quantitative data synthesis was performed using a random-effects model.

Results. We identified 5 eligible RCTs, with 497 subjects overall included. Berberine and silymarin combination treatment exerted a positive effect on CT [weighted mean difference (WMD): -25.3 95%CI(-39.2, -11.4) mg/dL; p<0.001], TG [WMD: -28 95%CI(-35.3, -20.6) mg/dL; p<0.001], LDL-C [WMD): -29.1 95%CI(-39.7, -18.6) mg/dL; p<0.001] and FPG [WMD: -7.5 95%CI(-13, -1.9) mg/dL; p=0.008]. **Conclusions.** The present findings suggest that the coadministration of berberine and silymarin is significantly associated with an advantageous improvement in lipids and glucose profile, suggesting the possible use of this nutraceutical combination in order to promote the cardiovascular health.

SAFETY OF RED YEAST RICE SUPPLEMENTATION: A META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Background. Recently, concerns regarding the safety of red yeast rice (RYR) have been raised after the publication of some case reports claiming toxicity. Since the previous meta-analyses on the effects of red RYR were mainly focused on its efficacy to improve lipid profile and other cardiovascular parameters, we carried out a

meta-analysis on safety data derived from the available randomized controlled clinical trials (RCTs).

Methods. Several databases were searched, with no language restriction, from inception to August 20th, 2018. Primary outcomes were musculoskeletal disorders (MuD). Secondary outcomes were non-musculoskeletal adverse events (Non-MuD) and serious adverse events (SAE). Subgroups analyses were carried out considering the intervention (RYR alone or in association with other nutraceutical compounds), monacolin K administered daily dose (\leq 3, <3-5 and >5 mg/day), follow-up (>12 or \leq 12 weeks), with statin therapy or statin-intolerance and type of control treatment (placebo or statin treatment). Fixed effects meta-regression analyses including some potential independent moderator variables (i.e. age at baseline and monacolin K daily dose) were planned to explore heterogeneity.

Results. Data were pooled from 52 RCTs comprising 110 treatment arms, which overall included 8503 subjects, with 4421 in the RYR arm and 4287 in the control one. Monacolin K administration was not associated with increased risk of MuD (OR=0.94, 95%CI 0.53, 1.65). Moreover, meta-analysis showed reduced risk of Non-MuD (OR=0.59, 95%CI 0.50, 0.69) and SAE (OR=0.54, 95%CI 0.46, 0.64) versus control. Subgroups analyses confirmed the high tolerability profile of RYR. All the results were robust in the leave-one-out sensitivity analysis. Meta-regression analyses did not suggest an increased risk for RYR-associated MuD, Non-MuD and SAE depending on age. Furthermore, increasing daily doses of monacolin K were negatively associated with increasing risk of Non-MuD (slope: -0.10; 95%CI: -0.17, -0.03; two-tailed p<0.01).

SEX-BASED DIFFERENCES IN ADHERENCE TO STATIN THERAPY

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Introduction Several studies demonstrated

Introduction. Several studies demonstrated sex disparities not only in drug response, but also in drug use. The aim of this study was to estimate sex-based influence on adherence to statin therapy and evaluate whether potential determinants have a different impact between males and females.

Methods. We conducted a retrospective observational study, using the healthcare utilization databases of the Italian Lombardy Region. All residents aged≥40 with a first prescription (index date, ID) of statin from 2002/01/01 and 2009/12/31 were selected. We evaluated adherence to therapy in the first year since the ID as Medication Possession Ratio (MPR), with optimal adherence level for values ≥ 0.80 . To analyze the relation between optimal adherence and influencing factors, log-binomial models stratified by sex were applied to calculate relative risks (RR) and 95% confidence intervals (95%CI).

Results. In our cohort, 764,342 new statin users were identified (31.0% with a single prescription). Among subjects with more than one prescription (n=527,485), 48.4% were females; mean age was 65.4 ± 9.9 years for women and 62.3 ± 10.2 years for men.

The mean MPR was higher for men $(0.64\pm0.28 \text{ vs } 0.58\pm0.28, p<0.0001)$. Even stratifying by age, men were likely to be more adherent. The presence of diabetes, comorbidity (Charlson score ≥ 1) and the use of high-potency statins were associated with a higher probability of optimal adherence in both sexes. Treatment with antihypertensive therapy or a previous cardiovascular event were associated with a greater probability of being adherent in men than in women (RR 0.87; 95%CI 0.87-0.88 vs RR 0.93; 95%CI 0.92-0.93 and RR 0.80; 95%CI 0.80-0.81 vs RR 0.87; 95%CI 0.86-0.88, respectively).

Conclusions. Among statin users, women showed a lower adherence level than men, regardless of age. Further studies are required to identify the most suitable interventions to increase adherence to therapy, improving health status and ensuring a more effective public resource management.

ROLE OF MMP9 GENE POLYMORPHISM IN GENETIC SUSCEPTIBILITY FOR CAROTID ARTERY DISEASE

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Introduction. Is well known that Matrix metalloproteinases are involved in atherosclerosis processes and plaque vulnerability and our previous studies showed an association between carotid artery disease (CAS) and single nucleotide polymorphisms (SNP) MMP9 gene. We evaluate three single nucleotide polymorphisms (SNPs) of the metalloproteinase 9 gene (MMP9), rs17576, rs3918256, rs3918249, according to literature evidence.

Materials and Methods. Polymorphisms were studied in n=814 patients with CAS and n=814 apparently healthy subjects with Real Time Technology.

Results. Polymorphisms are in Hardy-Weinberg equilibrium both in patients and controls. The percentage of individuals carrying the T allele of the polymorphism rs3918249 in CAS patients is significantly higher than that observed in the controls (CC=40.8%, CT=43.9%, TT 15.3% vs CC=58.7%, CT=34.8%, TT=6.5%, p<0.0001). For the polymorphism rs3918256 we observed that: AA patients =58.2%, GA=36.5%, GG=5.2% vs. controls AA=45.2%, GA=42.8%, GG=11.9%, p<0.0001, showing an higher percentage of G allele carriers in the controls. The genotypic distribution of the third polymorphism, rs17576 was AA=56.9%, GA=35.7%, GG=7.4% in patients vs AA=48.0%, G=44.0%, GG 8.0% in controls, p<0.0001. At the multivariate logistic regression (adjusted for traditional cardiovascular risk factors), the carriers of the allele rs3918249 T showed a significant and independent risk for CAS [OR=2.266 (95% CI 1.64-2.50), p<0.0001]; the carriers of the allele A rs3918256 showed a protective association against the onset of CAS [OR=0.59 (95% CI 0.45-0.78), p<0.0001]; the carriers of the allele G rs17576 showed only a trend of the protective association observed in the univariate analysis, [OR=0.77 (95% CI 0.59- 1.007), p=0.084]. Also, at multivariate regression analysis the condition of being carriers of a number ≥5 risk alleles is associated with an increased risk of occurrence of SC [OR=3.5 (95% CI 2,3-5.2), p=0.0001].

Conclusion. Our data showed a possible role of the rs3918249 MMP9 gene polymorphism inin CAS' onset, and a protective role of the rs rs3918256 polymorphism as for rs17576. Also, the risk conferred by the alleles T rs3918249, A rs3918256 and A rs17576 is increased in the presence of a "Burden" of risk alleles.

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

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Introduction. Statin-associated muscle symptoms (SAMS) are one of the main reasons for statin non-adherence and/or discontinuation, contributing to adverse cardiovascular outcomes. However, a definitive diagnosis of SAMS is difficult because symptoms are subjective, influenced by comorbidities, and there is no "gold standard" diagnostic test.

Aim and Methods. The PROSISA (Project on Statin Intolerance SISA) is an observational, retrospective and multicenter study aimed at assessing the prevalence of statin intolerance due to muscolar symptoms in dyslipidemic patients on statin treatment followed by Italian lipid clinics, between 1st January 2006 and 31st December 2015. Anamnestic data, biochemical values before and during statin therapy, and the potential occurence of muscular symptoms have been collected.

Results. In the first six months, the PROSISA database accounted for 6429 patients (mean age 66.7 ± 12 years; 53.9% males) on statins. During the statin therapy, 787 patients (12.2%), mainly treated with simvastatin (30.6%), rosuvastatin (28.3%) and atorvastatin (27.1%), reported muscular symptoms: myalgia (74.2%), cramps (25.8%), fatigue (18.8%), myositis (1.4%) and rhabdomyolysis (0.8%). Among them, 375 patients underwent dechallenge, with disappearance of muscular symptoms in 87.2% of cases, while overall 503 patients underwent rechallenge (237 with change of statin/dose reduction without interruption of therapy) mainly with rosuvastatin (32.6%) and simvastatin (30.4%) at low dosage, with reappearance of muscular symptoms in 151 patients. Among them, the muscle symptom mainly reported was myalgia (76.2%), while no cases of myositis and rhabdomyolysis were reported.

Conclusion. This preliminary analysis offers a general outlook of SAMS. The percentage of patients in whom intolerance has been confirmed by dechallenge/rechallenge is between 26-30%, emphasizing the need to deepen the assessment beyond the simple occurrence of muscle symptoms for a definitive diagnosis of SAMS and discontinuation of treatment. The final results will be the cornerstone to obtain a complete overview of identification and management of SAMS in Italy.

EFFECTS OF ANGPTL3 IN C2C12 MUSCLE CELLS: RESULTS OF A PRELIMINARY STUDY

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Background. ANGPLT3 protein is known to be involved in the inhibitory regulation of extracellular lipases (LPL and HL). Thereby, its absence has been shown to increases the lipolytic degradation of TG-rich lipoproteins. Recently, we have reported that ANGPTL3 may also influence fatty acid (FFA), which are important substrates for energy production. In particular, we found that ANGPTL3 can influence lipolysis in 3T3-L1 adipocytes throughout a biphasic action strictly depending on the presence or absence of β -adrenergic stimulation. If this mechanism is also acting in muscles tissue is not known. Therefore, our primary purpose was to evaluate the effects of ANGPTL3 in isolated muscle cells. This would allow to improving our knowledge about the role of ANGPTL3 as also a regulator of energy substrate utilization.

Methods. Differentiated C2C12 cells will be treated by ANG-PTL3 (100nM) and beta-agonist isoproterenol(100nM). Proteins and genes expression involved in intracellular lipolytic pathways will be investigated at the different time (30,60,90 minutes) by a combination of biochemical measures, Western blot, and RT-qPCR analyses.

Results. In comparison with control, the treatment of muscle cells with ANGPTL3 and beta-agonist isoproterenol (ISO) caused a reduction in the level of AMPK protein expression (after 30 minutes and 60 minutes respectively; p<0.05). ANGPTL3-treated, as well as ISO-treated cells, showed increased gene and protein expression of ERK1/2, also escalated markedly levels of ERK1/2 phosphorylation form (pERK).

Conclusions. In the present study, our data demonstrated by multiple technical approaches the effectiveness of ANGPTL3 on the intracellular signaling pathway in C2C12 muscle cells.

FAMILIAL HYPERCHOLESTEROLEMIA: 10-YEARS EXPERIENCE OF GENETIC SCREENING

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Introduction. Heterozygous Familial Hypercholesterolemia (HeFH) is the most common autosomal dominant disorder associated with high levels of LDL cholesterol (LDL-c) and increased cardiovascular risk. The genes involved in FH are LDLR, APOB and PCSK9 while LDLRAP1 is involved in a rare recessive form of FH. Homozygous FH (HoFH) is caused by mutation at homozygous status or of 2 mutations at compound heterozygous status and can lead to cardiovascular accidents since the childhood.

Patients and Methods. We enrolled 638 subjects with clinically diagnosis of FH, of whom 467 were unrelated. The LDLR, PCSK9, LDLRAP1 and part of exons 26 and 29 of the APOB genes were se-

quenced. MLPA was performed to identify large rearrangements in the LDLR gene.

Results. The genetic screening revealed mutations in 362 unrelated FH patients; in particular we found 18 patients compound heterozygotes and 3 patients homozygotes for mutations in LDLR gene. The cascade screening in family members of 79 unrelated patients revealed a mutation rate of 100%. We observed that LDL-c gradually increased from patients without mutations, to heterozygous patients with missense mutations, to heterozygous patients with a null mutation and to the HoFH patients, while the HDL cholesterol gradually decreased. Multivariate logistic regression analysis showed that LDL/HDL ratio and TC levels were significantly associated with presence of mutation (OR 1.40; 95%CI: 1.09-1.81; P<0.001 and OR 1.01; 95%CI: 1.00-1.02; P<0.0001 respectively). Total cholesterol. LDL-c and LDL/HDL ratio were statistically higher in HoFH respect to HeFH patients, while HDL cholesterol levels were lower in HoFH respect to HeFH patients. All these difference were statistically significant with a p<0.001.

Conclusions. Our screening revealed a mutation in 77.5% of patients, of which 6% are HoFH with a frequency of 1:300.000 in Campania Region. The evaluation of the mutation type and correlation genotype-phenotype could be useful for the patient management.

MANAGEMENT OF HYPERCOLESTEROLEMIC SUBJECTS AT HIGH CARDIOVASCULAR RISK WITH PCSK9 INHIBITORS: EXPERIENCE IN A LIPID CLINIC

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Background. Monoclonal antibodies anti-PCSK9 (Alirocumab and Evolocumab) have been recently approved by Food and Drug Administration (FDA) and European Medicines Agency (EMA) and are available in Italy for the treatment of patients with familial hypercholesterolemia (FH) and for secondary prevention in subjects with high cardiovascular (CV) risk on top of standard lipid lowering therapy (LLT) or in mono therapy in statin intolerant patients.

Objectives and Methods. The aim of this study was to evaluate the efficacy and safety of Alirocumab and Evolocumab in 50 patients (35 FH and 15 High CV Risk) in a real life setting. 33 subjects (27 FH and 6 High CV Risk) were treated with Alirocumab (75 mg or 150 mg based on LDL-C at baseline) and 17 subjects (8 FH+9 HCR) obtained Evolocumab (140 mg). Patients have self administered monoclonal antibodies subcutaneously every two weeks. Clinical and biochemical parameters, anthropometric measures, medical history, background LLT and side effects were evaluated over a period of 2 years.

Results. Patients treated with Alirocumab and Evolocumab exhibit a substantial and sustained reduction of the LDL-C plasma levels by a mean of more than 50% (LDL-C decrease variability between 46.2% and 77.3%) and LDL-C targets <100 mg/dl or <70 mg/dl were reached in all subjects. No significant change was observed in liver function tests. Injection site local reactions such skin erythema, pain, swelling and bruise were reported in 20% of patients but these adverse events progressively reduced over the subsequent drug administrations. No flu-like symptoms have been reported.

Conclusions. This study confirms the efficacy and safety of treatment with Alirocumab and Evolocumab both in FH and high CV risk patients attending a Lipid clinic.

PREVALENCE OF STATIN INTOLERANCE IN A COHORT OF OUTPATIENTS IN A LIPID CLINIC

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Background. Statins represent the gold standard in the management of dyslipidemia and are very effective in the prevention of cardiovascular diseases. They are generally safe and well tolerated however some patients experience statin intolerance issues. The prevalence of intolerance varies widely. Statin-associated muscle symptoms (SAMS) are one of the principal reasons for statin nonadherence and/or discontinuation, contributing to adverse cardiovascular outcomes.

Objective and Methods. The aim of this study was to evaluate the prevalence of statin intolerance in a group of 550 outpatients (232 males and 318 females) in primary and secondary prevention examined between 2006 and 2017 at the Lipid Clinic at the University Hospital in Palermo. Anthropometric measures, clinical and biochemical parameters, statin therapy and adverse events (AEs) were evaluated.

Results. In our cohort the prevalence of statin intolerance was of 19%. The main AEs registered were myalgia (51.7%), muscle weakness (19.5%), cramps (16.5%) and CPK increase (9%). 3 subjects suffered rabdomyolysis. Statin intolerant subjects were mainly females (59,3%), hypothyroidism, type 2 diabetes and liver steatosis were more frequent among intolerant subjects (21.4% vs 7.2%, p value 0.002.22.3% vs 4.02%, p value 0.003 and 16.5% vs 3.8%, p value 0.002, respectively). No changes of liver and kidney function tests were registered. In most cases subjects were "switched" to other statins (76.7%) and 23.3% of intolerant patients underwent to therapy discontinuation.

Conclusions. In this study the most common statin related side effect associated with therapy non-adherence was myalgia. Statin intolerance is a significant problem worldwide and appears to be growing. A step-by-step approach, including careful examination of all other precipitating factors that may increase the risk of statin intolerance, might be useful for preventing treatment discontinuation.

EFFECT OF LEGUME CONSUMPTION ON CARDIOMETABOLIC RISK FACTORS

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Background and Aims. Numerous studies have evaluated dietary factors as conditions which can reduce cardiometabolic risk factors. In particular legumes, which are part of the Mediterranean diet, have many antioxidant properties and in particular it has been shown that they can act by reducing some risk factors for metabolic syndrome. Therefore, the aim of our study was to clarify whether the intake of legumes could reduce some cardiometabolic risk factor, in particular blood triglyceride levels.

Methods. In this cross-sectional study, which is part of a project on the Mediterranean diet, financed by Ministry of Health and conducted between February 2013 and December 2014 at the U.O.C Nutrizione Clinica. 149 consecutive elderly individuals were enrolled with follow-up time of 11±5 months. Subjects with evidence of chronic-degenerative, endocrine, cardiovascular diseases and subjects who consumed excessive alcohol (>20 g/day) were excluded. All participants underwent an anthropometric evaluations and biochemical evaluation. In addition, all participants underwent a dietary intake assessment by a seven-day food record. For the purposes of the statistical analysis, the Δ of the triglyceride blood values between baseline and 1-year follw up was calculated. A T test was performed for independent samples to compare the averages between the groups. A Roc curve was used to identify a daily intake cut-off of legumes predicting the reduction in triglyceride levels. All statistical analyzes were performed using SPSS 20.0.

Results. The mean age was 69 ± 3 aa (63% women), IMC 29 ± 4 , the mean intake of legumes was 10 ± 12 (adjusted for 1000/Kcal). ROC curve demonstrates that intake of 7.4 g/day/1000 Kcal of legumes has a sensitivity of 70% and a specificity of 60% to predict the reduction of triglycerides.

Conclusion. The results of this study are extremely important because they demonstrate for the first time the link between the reduction of triglycerides after one year and a higher intake of legumes. The intake of about 8 g/day (3-4 servings per week) would lead to a reduction in the levels of triglycerides. These results should be encouraged to perform further studies to better clarify how these dietary factors can influence cardiometabolic risk factors in the broader objective of achieving "low cost" prevention of cardiometabolic diseases.

PREVALENCE AND HEALTHCARE COSTS OF CORONARY ARTERY AND PERIPHERAL ARTERIAL DISEASE IN ITALY

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Background. Coronary Artery Disease (CAD) and Peripheral Arterial Disease (PAD) have an atherosclerotic basis and their concomitant manifestation is commonly reported. The aim of the present study was to evaluate prevalence and annual health care costs of patients with CAD, PAD or both in Italy.

Materials and Methods. Data has been obtained reviewing the health care database of a North-Eastern Italian Region (Veneto Region) recorded between 01/01/2017 and 31/12/2017. We included adult patients (≥18 years) with diagnosis of CAD (at least one prescription of nitrates and/or CAD diagnosis at hospital discharge), and diagnosis of PAD (at least one prescription of antiplatelet drugs with concomitant prescription of anti-hypertensive

agents or lipid-lowering agents and/or PAD diagnosis at hospital discharge). Patients were characterized during the period 2009-2016 for comorbidities and in year 2016 for drug use. Treatment adherence was calculated using the Medication Possession Ratio. Healthcare cost analysis included drugs and hospitalization costs. Results. Over 4.9 million health-assisted subjects, 8.8% (430,254, mean age 74.2 years) received a diagnosis of CAD or PAD. Among them, 41.2% (177,157, mean age 78.8) had a PAD, 30.4% (130.853, mean age 74.2) had a CAD while 28.4% (122.244, mean age 74.7) had both diseases. Diabetes mellitus was observed in 10% of patients, heart failure in 11.8% while the 30.9% experienced a previous cardiovascular event. The cardiovascular drugs more frequently prescribed were anti-hypertensives agents (85%), antiplatelet drugs (58.7%) and lipid-lowering drugs (53%). Among patients treated with antiplatelet drugs, 36.2% were adherent to the treatment. The total mean annual healthcare cost per patient was €3,238.4 (€2,047.8, €3.390.0, €4,801.6 for patients with PAD, CAD or both, respectively). The total cost was 4.5-fold higher when compared to the mean annual healthcare of general population living in the area, 2.4-fold higher when considering comparable age and sex.

PPAR ALPHA ACTIVATION RESCUES ACQUIRED LYSOSOMAL ACID LIPASE DEFICIENCY IN FATTY LIVER

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Aim. Lysosomal acid lipase (LAL) is responsible for the hydrolysis of cholesteryl esters and triglycerides in the lysosomal compartment. Patients with NAFLD often show reduction of LAL activity in absence of LIPA gene mutations. Aim of the study was to characterize LAL deficiency in NAFLD and to investigate a possible therapeutic approach in vitro and in vivo.

Methods. 164 patients with biopsy-proven NAFLD were compared to 50 dyslipidemic patients with a similar incidence of metabolic syndrome but no fatty liver and to 30 controls. LAL activity was measured on dried blood spots (DBS) and liver biopsies. LAL expression and activity was tested in HepG2 cells treated with fatty acids, in the presence or absence of a PPAR-alpha agonist. A pilot study with fenofibrate on NAFLD patients was performed.

Results. LAL activity on DBS was reduced in NAFLD patients compared to dyslipidemic patients and controls. LAL activity was inversely correlated with the degree of fibrosis, but the correlation was completely lost after correction for platelet count. LAL activities measured on DBS and liver tissues were strongly correlated. Hepatic reduction of LAL activity was not due to decreased LAL expression. HepG2 cells treated with fatty acids also displayed a reduction of LAL activity with no changes of LAL expression. The activation of PPAR-alpha before or after fatty acid loading was able to prevent and correct LAL reduction in HepG2 cells. In NAFLD patients, fenofibrate for 1 month tended to increase LAL activity.

Conclusions. NAFLD is associated with an acquired LAL deficiency that is likely due to a by-product inhibition of fatty acids and not to other concomitant metabolic alterations. PPAR-alpha agonists could increase LAL activity in NAFLD patients by stimulating fatty acid oxidation.

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CLINICAL SIGNIFICANCE OF GENDER DIFFERENCES IN OLDER PEOPLE WITH METABOLIC SYNDROME

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Objectives. Metabolic syndrome (MetS) is defined as a cluster of risk factors that, if associated, increase the risk to develop cardiovascular disease (CVD) and type 2 diabetes mellitus (DM2). It is important to understand the impact of MetS in geriatric patients, and to assess the major gender differences, regarding both the impact of individual components and the role the syndrome plays on CV risk and comorbidities. Aims of the study are to analyse gender differences, poorly investigated in geriatric patient with MetS, and to assess whether these differences could affect the standard treatment options, often controversial in the elderly.

Methods. A total of 1382 outpatients attending our Cardiogeriatric Clinic were studied. MetS was defined in accordance with the "Harmonized definition" (2009). In all evaluated patients we used a "multidimensional geriatric assessment" including: pharmacological and medical history, clinical examination, blood tests, anthropometric parameters and the main geriatric syndromes, focusing on depression. Results. MetS prevalence was 27,8% (27,3% in males, 28,2% in females), with a higher prevalence in women aged 85 and older compared with men in the same age group. Being a woman was found to be an independent risk factor to develop MetS (OR 2,56; p=0,001). In males, the most common component was high blood sugar levels (32,4%), while in females, was elevated waist circumference (46,9%). Interestingly 93,2% of the sample with MetS has hypertension. Even if males with MetS have a higher CV risk (39,3%) compared to women (25,3%), no differences were observed between treatment with CV drugs between genders.

Conclusions. Main differences between genders concern women >85, the prevalence of CVD, which is higher in men and dizziness (higher in women). Remarkably, the prevalence of depression is deeply associated with MetS in women. Increasing awareness in physicians about CV prevention in postmenopausal women was also observed.

LIPOPROTEIN (A) LEVELS PREDICTS MORTALITY AND HAEMORRHAGIC TRANSFORMATION IN ISCHEMIC STROKE PATIENTS TREATED WITH THROMBOLYSIS: RESULTS FROM THE MAGIC STUDY

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Background and Purpose. Lipoprot ein(a) [Lp(a)] is endowed with both proatherosclerotic and prothrombotic properties: promotes smooth muscle cell proliferation, endothelial cell adhe-

sionmolecule expression, foam cell generation, and endothelial dysfunction. Elevated Lp(a) levels are significantly associated with higher incidence of ischemic stroke.

Aim. We aimed to investigate whether Lp(a) levels taken before recombinant plasminogen activator (rtPA) administration predict adverse clinical outcomes in tPA t reated ischemic stroke patientsof the MAGIC Study.

Methods. Blood sampl es were taken before and 24-h after rt-PA from 327 patients (mean age 68 years, median NIHSS 11). Prert-PA values and delta median values [(24-h biomarker - pre-rt-PAbiomarker)/(pre-rt-PA biomarker)] of Lp(a) were analyzed in relation to: 1) 3-month death, and 2) symptomatic hemorrhagic transformation.

Results. Pre-tPA levels of Lp(a) were significantly different in patients who died with respect to survivors [141(92-258) vs 90(44-175) mg/L, p=0.010]. Similarly, Lp(a) values were significantlyhigher in patients with than without haemorrhagic transformation [138(72-263) vs 91(44-175) mg/L, p=0.049]. Adjusting for age, sex, glycemia, baseline NIHSS, history of atrial fibrillation, orcongestive heart failure, history of inflammatory diseases or infections occurred within the last 7 days before stroke onset, pre-tPA Lp(a), in addition to pre-tPA A2M and deltaMMP-9 remained significantly and independently associated with 3 month-death [OR (95% CI): pre-tPA Lp(a): 2.63(1.26-5.48), p<0.010; A2M: 1.63(1.18-2.25), p=0.003; deltaMMP9:1.72(1.20-2.47), p=0.003]. At logistic regression analysis, after adjustment for major clinical determinants of outcomes, in addition to deltaMMP-9, pre-tPA Lp(a) levels remain a significant and independent determinant of hemorrhagic transformation [OR (95% CI) Lp(a): 1.78 (1.04-3.03), P=0.035; deltaMMP9: 1.48(1.08-2.102), p=0.014].

Conclusion. Our findings suggest that in addition to A2M and deltaMMP-9 also high levels of Lp(a) are significant an d independent markers of mortality and hemorrhagic transformation. Lp(a) may be used to improve prediction of unfavourable outcomes in the clinical setting of ischaemic stroke patients treated with thrombolytic therapy.

PROGNOSTIC VALUE OF SEPSIS-INDUCED COAGULATION ABNORMALITIES: AN EARLY ASSESSMENT IN THE EMERGENCY DEPARTMENT

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Background. Coagulation abnormalities are frequent in patients with sepsis and coagulation activation contributes to multiple organ failure as a result of microvascular thrombosis. Several papers reported an increased mortality rate in patients with a more pronounced coagulation dysfunction in the early days of their ICU course, but few data exist about the prognostic role of abnormal coagulation parameters measured in the first 24 hours after Emergency Department admission.

Aims. To evaluate if the measurement of parameters of increased coagulation activation at ED admission could add prognostic information to the routine assessment of septic patients based on indices of hypoperfusion and parameters of organ failure.

Methods. This report utilized a portion of the data collected in a

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prospective study, with the aim to identify reliable biomarkers for an early sepsis diagnosis. In the period November 2011-December 2016, we enrolled 268 patients, admitted to our High-Dependency Unit from the ED with a diagnosis of severe sepsis/septic shock. Study-related blood samplings were performed at ED-HDU admission (T0), after 6 hours (T6) and 24 hours (T24): D-dimer (DD), thrombin-antithrombin complex (TAT) and prothrombin fragment F1+2 (F1+2) levels were analyzed. The primary end-points were day-7 and in-hospital mortality.

Results. Day-7 mortality rate was 16%. DD (T0: $4661\pm4562 \mu g/ml$ vs $3190\pm7188 \mu g/ml$, p=0.001; T6: $4498\pm4931 \mu g/ml$ vs $2822\pm5623 \mu g/ml$, p=0.003; T24 2905±2823 $\mu g/ml$ vs $2465\pm4988 \mu g/ml$, p=0.08) and TAT levels (T0 29±45 vs 22±83; T6 21±22 vs 15±35; T24 16±19 vs 13±30, all p<0.05) were higher among non-survivors compared with survivors. We defined an abnormal coagulation activation (COAG+) as Ddimer>1300 µg/ml and TAT>7 ng/ml (median value in our study group). At T0 and T6 COAG+ patients had higher SOFA score (T0: $5.8\pm2.9 vs 4.9\pm2.4$, p=0.020, T6: $7.4\pm3.3 vs 5.6\pm2.5$, p=<0.001) and lactate level (T0: $3.8\pm4.1 vs 2.3\pm2.0$, p=0.001; T6: $3.3\pm3.7 vs 1.7\pm1.2$, p=0.002). At T0, COAG+ patients showed a higher day-7 mortality rate (HR 2.50; 95%CI 1.15-5.45, p=0.021), even after adjustment for SOFA score and lactate level.

Conclusions. Presence of abnormal coagulation activation at ED admission yielded an independent prognostic association with an increased short-term mortality rate.

TRANSCRIPTOMICS ANALYSIS OF CEREBRAL THROMBI AND PERIPHERAL VENOUS BLOOD: A PROMISING RESOURCE FOR EVALUATING PATHOPHYSIOLOGY AND OUTCOMES IN ACUTE ISCHEMIC STROKE

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Background. The thrombotic/thromboembolic occlusion of a cerebral artery leads acute ischemic stroke (AIS) which is a leading cause of morbidity and mortality. Nowadays the election therapies for AIS are represented by rapid restoration of cerebral blood supply by intravenous thrombolysis and mechanical thrombectomy. Previous data showed that analysis of clots (e.g histological analysis of thrombus composition) can be useful in understanding AIS pathophysiology mechanisms and outcomes as clots composition and characteristics may influence the effective recanalization of occlusive thrombi in such arteries.

Aims. Of this study were 1) to investigate the global gene expression profile from RNA extracted from cerebral thrombus and peripheral blood in patients with AIS in order to identify specific genes and markers that might help to distinguish causes of ischemic stroke and/or determinants of clinical outcomes and 2) to compare the gene expression profiles obtained from clot and systemic component.

Method. Twenty-one consecutive AIS patients (7 males, 14 women) [Mean Age \pm SD 77.9 \pm 10.8] undergoing endovascular treatment were collected and followed-up for at least 3 months. Blood samples were collected before and 24 h after thrombectomy in tubes

with anticoagulants (plasma EDTA and citrated), as well as in tubes without anticoagulant (serum) and liquid for RNA stabilization. The thrombus obtained during thrombectomy was collected in RNA later. RNA was extracted by PAX gene blood miRNA kit. The global gene expression was assessed by Affymetrix technology using GeneChip Human Trascriptome 2.0 Array allowing the analysis of 44,699 genes, >285,000 full-length transcripts coverage. Results. The analysis highlighted 1,712 out of 2,653 probe sets identifying annotated genes. Among expressed genes in thrombi. of greater interest, we observed metalloproteinases 11 and 24, androgen receptor, ubiquitin conjugating enzyme E2B, and interferon regulatory factor 8 and 4 previously involved in activation of neural stem cells under physiological and regenerative conditions, synapse reorganization after traumatic brain injury and neuronal response to cerebral ischaemic-reperfusion injury. Gene onthology analysis showed that, besides the expected alteration of biological processes and molecular functions related to regulation of apoptosis, cell death and catabolic processes, the expressed genes in cerebral thrombi are involved in biological processes and molecular function and pathways related to: neuronal response to stress; neuron differentiation, development, organization, and maturation; cerebral cortex development.

Conclusion. Preliminary results suggest thrombus global gene expression profiling might be a promising resource to investigate causes of ischemic stroke and/or determinants of clinical outcomes.

HELPER-DEPENDENT ADENOVIRAL VECTOR EXPRESSING MLDLR/MTF FUSION PROTEIN UNDER THE CONTROL OF A MUSCLE SPECIFIC PROMOTER FOR THE TREATMENT OF FH

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Familial hypercholesterolemia (FH) is a genetic Hyperlipidemia most commonly due to mutations in the low-density lipoprotein receptor (LDLR) gene that result in high levels of LDL-cholesterol (LDL-C) with consequent development of xanthomas, atheromas and increased risk of premature cardiovascular complications. Several therapeutic options are available for FH patients. The conventional therapy is based on the use of high-dose statins; however, when this approach is not effective, patients often rely on more invasive interventions such as LDL apheresis. The need for additional effective therapeutic options makes gene therapy an attractive approach to reduce LDL-C in FH patients with a single vector administration. Adenoviral vectors (Ad) are highly efficient gene transfer vectors; in particular Helper dependent (HD-Ad) are characterized by long-lasting high-levels of transgene expression with limited toxicity. We have recently developed a safe and effective gene therapy strategies based on a HD-Ad vector for the expression of a circulating fusion protein composed by the extracellular portion of the murine LDL receptor (mLDLR) fused in frame with the entire murine transferrin (mTf). The chimeric protein binds and remove circulating LDLs from the bloodstream by receptormediated endocytosis through the interaction with the transferrin receptor (TfR). We administered this vector intravenously in LDLR-deficient mice and observed expression of the protein with a consequent improvement of lipid profile and reduction of aortic atherosclerotic lesions. In order to develop a safer strategy for clinical applications, we have recently generated a HD-Ad vector encoding the fusion protein under the control of a muscle specific promoter (HD-AdmCK-mLDLR/mTf) for intramuscular delivery. We evaluated the ability of our vector to drive the expression of the fusion protein in the C2C12 myoblast cell line and we are currently characterizing transgene expression and activity after intramuscular delivery in LDLR deficient mice.

POSTTRANSCRIPTIONAL REGULATION OF PCSK9 EXPRESSION BY MORUS ALBA, ACTIVE COMPONENT OF LOPIGLIK®, IN HEPG2 CELL LINE: ITS ROLE IN LDL-CHOLESTEROL UPTAKE

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Background and Aim. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a pivotal regulator of low-density lipoprotein cholesterol (LDL-C) plasma levels. In a previous work, we observed that the treatment of dyslipidemic subjects with an innovative nutraceutical combination containing: Red yeast rice (Monacolin K 3.3 mg), Berberis aristata cortex extract (Berberine 531.25 mg) and Morus alba leaves extract (1-Deoxynojirimycin 4 mg) (LopiG-LIK® - Akademy Pharma) did not alter the plasma levels of PCSK9. Thus, the aim of the present study was to define the effect of the LopiGLIK® nutraceutical combination on genes involved in cholesterol homeostasis, including PCSK9, and thus the molecular mechanism underlying the hypocholesterolemic effect of Morus alba leaves extract.METHODS: HepG2 cell line were incubated with Red yeast rice (RYR; 50 µg/ml) Berberis aristata cortex extract (BCE; 40 µg/ml) and Morus alba leaves extract (MLE; 1 mg/ml), alone or in combination, for 24 h. Their effects on PCSK9 expression (western blot), secretion (ELISA), transcription (Luciferase Promoter assay and RT-qPCR) and on LDL-uptake (flow cytometry) were then determined.

Results. As expected, RYR significantly induced both the 74kDa pro-PCSK9 (+3.1 fold) and the 62KDa active form (+2.1 fold), PCSK9 secretion (+9.8%±1.2%), PCSK9 mRNA (1.89±0.11 fold), and its promoter activity (+2.4±0.5 fold). In contrast, BCE reduced PCSK9 expression (-37.0%, pro-PCSK9 and -64.3%, active form), PCSK9 secretion (-64.8%±16.2%), mRNA levels (+85.0%±0.5%), and promoter activity (+63.0%±11.8%). Interestingly, MLE determined a concentration-dependent inhibition of mRNA PCSK9, with a maximal reduction at 1mg/ml (-54.8%±0.7%). The same concentration of MLE reduced PCSK9 expression levels (pro-PCSK9: -17.1%; active form: -59.3%), and its release into the cultured media (+3.8%±23.6%). MLE did not alter the PCSK9 promoter activity, suggesting a different mechanism of action compared to BCE. The same analyses were performed on additional SREBP-genes as

HMG-CoAR, LDLR and FAS. At transcripts levels, RYR significantly induced both HMG-CoAR and FAS; conversely, BCE reduced them. MLE behaved as BCE did, decreasing both genes (-50%). Surprisingly, no significant variation in LDLR mRNA levels have been detected upon RYR treatment, whilst BCE partially reduced them. At the protein levels, both RYR and BCE induced LDLR expression by 2.8 and 1.5 fold, respectively. No effects on LDLR expression have been seen upon MLE treatment. The combination of BCE (40 µg/ml), RYR (50 µg/ml) and MLE (1mg/ml) reduced the PCSK9 mRNA (-77.3±0.8%), the PCSK9 expression (pro-PC-SK9: -96.6%: active form: -93.3%), the extracellular protein levels (-74.4%±14.9%), and the PCSK9 promoter activity (-76.0%±9.6%). In addition, the combination reduced both HMG-CoAR and FAS mRNAs, whilst a slight significant induction of LDLR was observed at protein level. MLE was able to positively modulate the LDL-DiO uptake (+3.0±0.01 fold), strengthening the RYR and BCE positive action when in combination.

Conclusion. BCE and MLE actively counteract the induction of PCSK9 by RYR, an effect that could explain the unchanged plasma levels of PCSK9 in patients treated with LopiGLIK[®]. In addition, the use of MLE, for glycemic control, together with RYR and BCE, positively increased LDL-DiO uptake by HepG2, supporting the rational of using this nutraceutical combination for controlling both hyperlipidemic and hyperglycemic conditions.

LONG-TERM EXPOSURE TO AIR POLLUTION RAISES CIRCULATING LEVELS OF PCSK9 IN OBESE INDIVIDUALS

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Background. Exposure to airborne particulate matter (PM) has been consistently associated with early death and increased morbidity, particularly raising the risk of cardiovascular disease (CVD). Obesity, one of the leading CVD risk factors, increases susceptibility to the adverse effects of PM exposure. The proprotein convertase subtilisin/kexin type 9 (PCSK9) has been related to a large number of CV risk factors, e.g. atherogenic lipoproteins, arterial stiffness, and platelet activation.

Objective. To evaluate, in a cohort of susceptible obese subjects, the effects of PM10 exposure on PCSK9 circulating levels and how these may be associated with the CV Framingham Risk Score (FRS).

Methods. In 500 obese subjects, participating in the cross-sectional SPHERE (Susceptibility to Particle Health Effects, miR-NAs and Exosomes) study, we evaluated the effects of long- and short-term PM10 exposure on circulating PCSK9 levels. Results. In the studied individuals (BMI: $33.3\pm5.2 \text{ kg/m}^2$) with an annual average PM10 exposure of $40.12\pm4.71 \text{ µg/m}^3$, PCSK9 levels were 248.7±78.6 ng/mL. In univariate analysis, PM10 exposure (annual average) was associated to PCSK9 levels (β =1.83, SE=0.75, p=0.014) as well as to lipoproteins, i.e. LDL-C (β =0.33, SE=0.10, p=0.0008), non-HDL-C (β =0.37, SE=0.09, p<0.0001), TGs (β =0.11, SE=0.05, p=0.0165) and HDL-C (β =0.71, SE=0.24, p=0.0034). Interestingly, in a multivariable linear regression model, the association between PCSK9 and PM10 was observed only for carriers of lower concentrations of interferon (IFN)- γ , whereas it was lost in pres-

ence of higher IFN- γ levels. PCSK9 levels were positively associated with the Framingham Risk Score, which was raised by 15.8% for each 100 ng/mL rise of PCSK9.

Conclusions. Obese individuals with less inflammation, i.e. low IFN- γ appear to be hypersusceptible to PM10 exposure, as expressed by raised PCSK9 concentrations.

GENETIC CHARACTERIZATION OF A FAMILY WITH CLINICAL SUSPICION OF HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction. Homozygous familial hypercholesterolaemia (HoFH) is a rare and life-threatening disease characterized clinically by plasma cholesterol levels >500 mg/dL, extensive xanthomas, marked premature and progressive atherosclerotic cardiovascular disease. HoFH has shown to be caused by mutations in both alleles of the gene encoding the LDL receptor (LDLR).

Patients and Methods. The case report is based on a family from Campania Region; the index case was a 44-year-old woman with xanthomas, total cholesterol (TC) levels of 240 mg/dl and LDL cholesterol (LDLc) of 186 mg/dl after maximal therapy with statins and ezetimibe, with suspicion of HoFH. Her 39-year-old brother also showed a similar lipid profile; furthermore two nephews aged 16 and 18 showed marked hypercholesterolemia. The genetic screening included the analysis of LDLR, PCSK9 and part of exons 26 and 29 of the APOB genes. MLPA was performed to identify large rearrangements in the LDLR gene.

Results. Our genetic screening revealed that index case was heterozygous for the mutation c.2312-3C>A in the intron 15 of LDLR gene. The cascade screening performed in the brother and in two nephew of the proband, revealed heterozygosity for the same mutation. We studied other 7 families with the same mutation, observing in all a very severe phenotype (baseline TC 431±112 mg/dl, LDL-c 349±100 mg/dl); After genetic test the index case and her brother were treated with PCSK9 inhibitor, reaching therapeutic target, while the two young nephew start early statin therapy.

Conclusions. The clinical suspicion of HoFH is not confirmed by genetic test that revealed the presence of only one mutation in LDLR; the c.2312-3C>A is the most frequent splicing mutation causing FH in our region and is associated to a severe phenotype, that could be suggest the presence of a double mutations in LDLR. The genetic screening is useful to establishment the best terapeutic treatment.

ASSOCIATION BETWEEN SMALL DENSE LDL PARTICLES AND CARDIOVASCULAR EVENTS IN A GROUP OF MEDITERRANEAN WOMEN (PROGETTO ATENA). PRELIMINARY REPORT

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Association between small dense LDL (sd-LDL) and cardiovascular disease (CVD) was evaluated in a group of 193 women participating to Progetto Atena (nested case-control study). At the time of first visit blood samples were collected and stored in liquid nitrogen. After 25 years from baseline visit LDL particle separation was performed, on serum stored at time of baseline visit, by Lipoprint System: 7 LDL subfractions (LDL 1 to LDL 7) were obtained, mean LDL particle size and LDL score (% of sd-LDL) were calculated. Analysis was performed in 193 patients. The rest of 107 patients are currently undergoing biochemical determinations. Women were divided into a control group (group 1, N=102) and in a group (group 2, N=91) who had cardiovascular events after the initial visit. LDL score was higher in women with cardiovascular disease as compared to control women (6.5±8.6 vs 7.7±13.2 mg/dL) (p=0.386); mean LDL size was lower in group 2 than group 1 (26.8±0.5 vs 26.9±0.4 nm) (p=0.409). Cholesterol LDL resulted statistically different, group 1 (152.5±44.4 mg/dL) vs group 2 (168.5±51 mg/dL) (p=0.021). In multivariate analysis adjusted for age, LDL, Systolic blood pressure and BMI, LDL score value above the 95th percentiles (25.0 mg/dL) was found to be associated to cardiovascular disease but statistical significance was not reached (p=0.270), due to small sample size. No statistically age differences resulted between two groups. These preliminary data indicate that in women who had CVD after baseline visit, LDL score is higher and mean LDL size lower than control women. High concentrations of sdLDL are associated to future cardiovascular events in this group of Mediterranean women participating to Progetto Atena. Further studies are envisaged to investigate the specific contribution of LDL subfractions to cardiovascular risk particularly in women.

EFFECTS OF BARIATRIC SURGERY ON CARDIOVASCULAR RISK SCORE IN DIABETIC SUBJECTS

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Introduction. In obese patients with type 2 diabetes (T2DM) bariatric surgery is the most effective strategy to produce durable weight loss, diabetes remission and reduction of cardiovascular (CV) risk.

Objective. To evaluate the effect of bariatric surgery on CV risk in obese patients with T2DM using the "Progetto Cuore "(PC) risk score.METHODS: We studied 134 obese T2D patients, M/F 50/84, age 50.4±8.6 years, BMI 45.6±7.4 kg/m², who underwent to bariatric surgery: laparoscopic gastric banding (LGB, N=10), Roux-en-Y gastric bypass (RYBP, N=91) and sleeve gastrectomy (SG, N=33) during the period 2005-2016. In all patients the CV risk factors were collected at baseline and after 12 months of follow-up. We applied the PC risk score to predict the 10-year risk of CV events.

Results: 12 months after surgery body weight loss was 26.4 \pm 8.9% and diabetes remission occurred in 79.9% of participants (6/10 of LGB, 71/91 of RYBP and 30/33 of SG). Comparing with baseline, 10-year risk of CV events at follow-up decreased according to PC score (Δ PC - 38,3%, p<0,001). The most substantial risk reduction was observed in subjects with diabetes remission (Δ PC p<0,001) and in those with higher 10-year risk at baseline(Δ PC% p=0,017). In the LGB group we observed a smaller risk reduction than other two groups (Δ PC% p=0,029).

Conclusions. Bariatric surgery is associated with a significative reduction of 10-year CV risk according to PC score. Risk reduction varies according to subjects' characteristics and surgical technique.

ASSOCIATION BETWEEN DECREASING ESTIMATED GLOMERULAR FILTRATION RATE AND RISK OF CARDIAC CONDUCTION DEFECTS IN PATIENTS WITH TYPE 2 DIABETES

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Background. To examine the association between of decreasing estimated glomerular filtration rate (eGFR) or abnormal albuminuria and risk of cardiac conduction defects in patients with type 2 diabetes mellitus (T2DM).

Methods. We examined a hospital-based sample of 923 patients with T2DM discharged from our Division of Endocrinology over the years 2007-2014. Standard electrocardiograms (ECGs) were performed in all patients; eGFR was estimated by using the chronic kidney disease epidemiology collaboration (CKD-EPI) equation, and albuminuria was measured by an immuno-nephelometric method on a morning spot urine sample.

Results. A total of 253 (27.4%) patients had some type of cardiac conduction defects on ECGs (defined as at least one heart block among first-degree atrio-ventricular block, second-degree block, third-degree block, left bundle branch block, right bundle branch block, left anterior hemi-block or left posterior hemi-block). Prevalences of patients with eGFRCKD-EPI<30, eGFRCKD-EPI 59-30 ml/min/1.73 m² and abnormal albuminuria (i.e. urinary albuminto-creatinine ratio >30 mg/g) were 7.0%, 29.4% and 41.3%, respectively. After adjustment for established cardiovascular risk factors, diabetes-related variables and potential confounders, there was a significant, graded association between decreasing eGFR values and risk of cardiac conduction defects (adjusted-odds ratios of 2.05 [95% CI 1.2-3.5], 2.85 [95% CI 1.6-5.1] and 3.62 [95% CI 1.6-8.1] for eGFRCKD-EPI 89-60, eGFRCKD-EPI 59-30 and eGFRCKD-EPI <30 ml/min/1.73 m², respectively). Conversely, abnormal albuminuria was not associated with an increased risk of conduction defects (adjusted-OR 1.09, 95% CI 0.7-1.6).

Conclusions. Decreasing eGFR is independently associated with an increased risk of persistent cardiac conduction defects in patients with T2DM.

ASSOCIATIONS BETWEEN HIGHER PLASMA CERAMIDE LEVELS AND LOWER PERCENTAGE OF POST-STRESS MYOCARDIAL PERFUSION IN PATIENTS WITH ESTABLISHED OR SUSPECTED CORONARY ARTERY DISEASE UNDERGOING MYOCARDIAL PERFUSION SCINTIGRAPHY

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Background. We recently found that distinct plasma ceramides are closely associated with stress-induced myocardial perfusion defects in patients with established or suspected coronary artery disease (CAD) undergoing myocardial perfusion scintigraphy (MPS). However, it is currently not known whether plasma ceramides are also associated with a reduction of percentage of poststress myocardial perfusion in these patients.

Methods. We measured six previously identified high-risk plasma ceramide species [Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/20:0), Cer(d18:1/22:0), Cer(d18:1/24:0) and Cer(d18:1/24:1)] in 167 consecutive patients with established or suspected CAD, who performed a stress myocardial perfusion scintigraphy (MPS) for clinical indications. Plasma ceramides were measured by a targeted liquid chromatography-tandem mass spectrometry assay both at baseline and after MPS. Multivariable linear regression analysis was undertaken to examine the associations (standardized B coefficients) between plasma ceramides and the percentage of post-stress myocardial perfusion after adjustment for multiple cardiovascular risk factors.

Results. Seventy-eight patients had stress-induced myocardial defects on MPS (mainly located in the antero-apical wall). Of the six measured plasma ceramides, basal levels of Cer(d18:1/18:0) (B=-0.182, p<0.05), Cer(d18:1/20:0) (B=-0.224, p<0.05), Cer(d18:1/22:0) (B=-0.163, p<0.05) and Cer(d18:1/24:1) (B=-0.20, p<0.05) were inversely associated with the percentage of post-stress antero-apical wall perfusion. Notably, these significant associations persisted after adjustment for age, sex, smoking, hypertension, dyslipidemia, diabetes, previous CAD, left ventricular ejection fraction and type of stress testing. Similar results were also observed for post-stress plasma ceramides.

Conclusions. Higher circulating levels of specific ceramides, both at baseline and after stress, were independently associated with a lower percentage of post-stress antero-apical wall perfusion in patients with established or suspected CAD referred for clinically indicated MPS.

TOPIRAMATE PROTECTS APOE-DEFICIENT MICE FROM KIDNEY DAMAGE WITHOUT AFFECTING PLASMA LIPIDS

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Topiramate is an anticonvulsant drug also prescribed for migraine prophylaxis that acts through several mechanisms of action. Several studies indicate that topiramate induces weight loss and a moderate reduction of plasma lipids and glucose. Based on these favourable metabolic effects, aim of this study was to evaluate if topiramate could modulate atherosclerosis development and protect target organs of dysmetabolic conditions. Thirty apoE-deficient mice were divided into three groups and fed for 12 weeks a high fat diet (Control) or the same diet containing topiramate at 0.125% (T-low) and 0.250% (T-high). Body weight, water and food intake were monitored throughout the study. Plasma lipids and glucose levels were measured and a glucose tolerance test was performed. Atherosclerosis development was evaluated in the whole aorta and at the aortic sinus. Histological analysis of liver, kidney and adipose tissue was performed. Topiramate did not affect weight gain and food intake. Glucose tolerance and plasma lipids were not changed and, in turn, atherosclerosis development was not different among groups. Topiramate did not modify liver and adipose tissue histology. Conversely, in the kidneys, the treatment reduced the occurrence of glomerular lipidosis (on average, more than 11 glomeruli affected in Control vs 1 to 3 glomeruli affected in the treated groups) and tubular necrosis (30% in Control. 10% in T-low and 0% in T-high) and by decreasing foam cells accumulation and reducing the expression of inflammatory markers. Blood urea nitrogen levels were also reduced by treatment (-18% in T-low and -25% in T-high). Our results indicate that topiramate does not affect atherosclerosis development, but preserves kidney structure and function. The study suggests that topiramate could be investigated in drug repurposing studies for the treatment of glomerular lipidosis.

EVALUATION OF HDL FUNCTIONALITY AND DIETARY-DERIVED METABOLITES IN CALCIFIC AORTIC STENOSIS

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Aim. Calcific aortic valve disease (CAVD) is the most prevalent valvular heart disease. Its pathogenesis has not been elucidated clearly. In this study, we aimed to investigate the link between HDL function, evaluated as cholesterol efflux capacity, and the disease severity. In addition, we assessed the levels of some dietary and gut microbiota-derived metabolites and investigated their relationship with CAVD severity.

Methods. Subjects with moderate- severe calcific aortic stenosis (CAS) (n=60), aortic sclerosis (ASc) (n=49) and age- and sexmatched control subjects (n= 48) were included in this study. Severity of CAVD determined by echocardiographic, computed tomographic and histopathological examinations. High- density lipoprotein cholesterol efflux capacity (HDL-CEC) was assessed by a radioisotopic technique. Serum choline, betaine and gut microbiota- derived metabolite levels were measured using ultra-performance liquid chromatography- tandem mass spectroscopy.

Results. Patients with moderate- severe CAS displayed significantly lower total HDL-CEC compared to both control (p= 0.011) and ASc (p= 0.015) groups. Moreover, total HDL-CEC was negatively correlated with parameters suggested to have prognostic implications in AS, such as peak aortic jet velocity (AVmax) (r=-0.302, p=0.002), aortic valve calcium (AVC) score (r=-0.332, p=0.010), coronary artery calcium (CAC) score (r=-0.355, p=0.005). Total HDL-CEC was also found to be negatively correlated with plasma choline levels (r=-0.210, p=0.031). In addition, total HDL- CEC were found to be independent associates of AVmax (β: -0.419, SD: 0.139, p=0.005). Patients with moderate - severe CAS had significantly higher plasma levels of choline when compared to both control (p<0.001) and ASc (p=0.006) groups. Betaine and TMAO levels did not differ among groups (p>0.05). Higher quartiles of plasma choline were also found to be associated with AVC score (p<0.001), absolute left ventricular global longitudinal strain (p= 0.012) and with mitral annular calcium (MAC) score (p=0.013).

Conclusion. In this work we have demonstrated that HDL functionality, reflected with HDL - CEC, is independently associated with CAVD severity. We have also shown a relationship between presence and severity of CAVD and the plasma levels of dietaryderived metabolites. These findings suggest that HDL function and choline and betaine as well, may be a novel determinant of CAVD besides traditional risk factors.

GERANYLGERANIOL PREVENTS THE SIMVASTATIN-INDUCED PCSK9 EXPRESSION: ROLE OF THE SMALL G PROTEIN RAC1

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Statins are the gold standard therapy for the prevention and treatment of dyslipidemia, their hypolipidemic effect is related to the increased expression of the LDLR on the surface of the hepatocytes. The biological target of statin is the Hydroxyl-methyl glutaryl- Co enzyme A reductase (HMGCR), which is the rate limiting step of cholesterol biosynthesis; in response to their action, the intracellular pool of free sterols decreases and the transcription factors sterol responsive element binding protein (SREBP) undergoes proteolytic activation resulting in the induction of the LDL receptor (LDLR), HMGCR and of PCSK9. Interestingly the levels of PCSK9 increase as soon as 24 hours after a single dose of atorvastatin. By inhibiting the HMGCR, statins also reduce the amount of nonsteroidal isoprenoid compounds; in the early phase of treatment in fact plasma levels of mevalonate (MVA) drop up to 70% within 1-2 hours while a reduction of LDL-C starts to be detectable only after 24 h and becomes substantial after 6-7 days. Considering the correlation between the timing of PCSK9 upregulation and MVA decrease following statin treatment, we hypothesized that the prenylation of the small G-proteins, like Rac1 and RhoA, is a crucial step for the activation of PCSK9 transcription. In the CaCo-2 cell line 24 hours incubation with Simvastatin 40 µM induced both PCSK9 mRNA (10.7±3.2-fold change) and protein (2.2±0.3fold change). The induction of PCSK9 mRNA was partially, but significantly, prevented by the co-incubation with mevalonate (MVA), farnesol (FOH) and geranylgeraniol (GGOH), while this completely prevented upregulation of secreted PCSK9, evaluated by ELISA assay. Simvastatin also reduced by -35.7±15.2% the Rac1-GTP levels, while no changes were observed on RhoA- and Cdc42-GTP. This effect was also prevented by co-incubation with MVA or GGOH. We then performed RT-qPCR analysis for PCSK9 mRNA after imparing the activity of Rac1 in a number of ways. First, we took advantage of a small molecule (Raci) that is able to interfere with the interaction between Rac1 and Tiam1, and thus with the exchange between GDP to GTP. We also transfected the dominant negative mutant N17Rac1, which leads to expression of Rac1 in either a nucleotide free state or in its inactive stat. Finally, we used a siRNA against Rac1 to knock-down its expression. Each of these strategies to reduce Rac1 activity led to upregulation of PCSK9, similarly to simvastatin treatment. Rac1-GTP is involved in the nuclear translocation of STAT proteins such as STAT3, which has been shown to suppress the transcription of SREBP-1 and genes involved in the de novo lipogenesis, including PCSK9. Thus, we hypothesize that STAT3 was the downstream regulator of PCSK9 transcription by simvastatin. Indeed, in the presence of simvastatin or of the Rac inhibitor, the nuclear translocation of STAT3 was significantly reduced, which in turn could activate PCSK9 transcription by SREBP. In conclusion, this study reveals a direct role of Rac1 on simvastatin-mediated PCSK9 expression via the reduction of STAT3 nuclear translocation. Those results further support the rationale for a combination therapy of statins and PCSK9 inhibitors, especially in patients with an acute myocardial infarction that receive statin in secondary prevention.

ADHERENCE TO CARDIOVASCULAR MEDICATION IN A CARDIOVASCULAR DISEASE PREVENCTION CENTER

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Chronic diseases demands long term drugs administration and need constant evaluation to dedicate out-patient clinic. Adherence to the therapy is a corner stone to achieve the target to prevent cardiovascular diseases. So, treatment failure, caused by poor medication adherence, leads to frequent re-hospitalizations, poor outcome and increased health care costs. To evaluate drug adherence we can use validate tests; the Morisky medication adherence scale is a commonly used adherence screening tool. It is composed of 4 ves/ no questions about past medication use patterns and is thus quick and simple to use during drug history interviews.We evaluated the adherence to therapy of 245 outpatients (age \geq 18 years), consecutively visited in our cardiovascular disease prevenction center unit (Lipid Center Cosenza), from 1 January to 30 July 2018. All of there were in cardiovascular treatments also in multiple drugs therapy. We used of the self reported Morisky score as a screening tool for identifying patients adherence to medications. The self report adherence in the patients begin treated was 75.9% (score 3-4) and only 59 of the 245 patients (24.1%) were categorized as non-adherent. Among non adherent patients (51% female), 38.9% were in monotherapy, while 42% took more than one drug. The 20.3% of non-adherent patients reported an adverse drug reaction. In conclusion, the data collected in our clinic show a good therapeutic adherence. Adherence can be improved with surveillance and counselling activities by doctor, nurse and pharmacist during the subsequent planned out-patient follow-up.

POTENTIAL IMPACT OF ACC/AHA GUIDE-LINES FOR MANAGEMENT OF ARTERIAL HYPERTENSION IN THE CHOISE OF THERAPY IN CLINICAL ASSET

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The 2017 ACC/AHA guidelines (GL) for management of arteria hypertension has changed classification and treatment of blood pressure (BP) recomanding to begin BP drug treatment at ≥130/80 mmHg for patients with an estimated 10 years ASCVD risk ≥10%. This can has a deep impact on the rapeutical activity. We wanted to evaluate the percentage of patients reaching the suggested new goal and the number of drugs required to reach it. Two hundred sixty hypertensive outpatients, with an estimated 10 years ASCVD risk ≥10%, consecutively refered to our dedicated clinic, were evaluated. All of them were drug treated from more than three mounts. Of these, 41.2% were female the mean of age was 62 years. According to 2017 ACC/AHA GL, 40.7% did not reached a good BP control, instead, considering the new 2018 European GL, 27.7% did not reached BP <140/90 mmHg. The median drug prescribed was 2.15 for all patients, 2.9 for patients with BP ≥140/90 mmHg, 2.33 for patients with BP between $\geq 130/90$ and < 140/90 mmHg. In conclusion, the new ACC/AHA GL create a greater number of not at target hypertensive patients and this can cause an important effect on the use of pharmacological therapy. In our study, 43% of patients, at therapeutical goal with the 2018 european GL, became now not at target and need more anthypertensive drugs.

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SAFETY OF STATINS AND MONITORING **OF DRUG-RELATED MYOPATHY** IN A DEDICATE CLINIC

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Statins are a widely used group of cholesterol-lowering agents with a favorable benefits for cardiovascular disease. It's known that statins can have potential adverse effects (AEs). The frequency of AEs, due to statins, is not well known. In fact, there is a wide variability between AEs reported in clinical randomized trial and the date available in post marketing settings. In recent years, pharmacovigilance has the important aim of monitoring the incidence of all adverse reactions to drugs. Unfortunately not in all countries, the reporting reaches standard levels set by the WHO. For these reasons, the Italian Agency of the Drugs (AIFA) has been promoting and financing regional projects with multidisciplinary groups to increase spontaneous reporting. We started a survey program finalized to report all adverse events from drugs observed in our Cardiovascular Prevention Clinic. We evaluated all of the 350 outpatients (≥ 18 years), consecutively visited in our dedicated clinic for prevenction of cardiovascular diseases (Lipid Center Cosenza). All of patients were in cardiovascular treatment with drugs. A total of 30 ADRs were observed; 29 were non-serious. In particular, 4 myopathy events (3 simvastatin and 1 lovastatin) were reported (13.3%) and included in the national pharmacovigilance network (RNF). Muscular symptoms were reported by 3.4% of all statin users. Incidence of myalgia was found to be higher in the age group between 45 and 54 years old and males experienced more ADRs than females. In conclusion, we reported, regarding statins use, a lower number of AES, compared to other post-marketing study.

THE IMPACT OF THE MEDITERRANEAN DIET ON PLASMA LDL-C LEVELS IN FH CHILDREN

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Introduction. Dyslipidemia plays a major role in cardiovascular disease (CVD) particularly when Familial Hypercholesterolemia (FH) occurs. Eating habits have positive influence on the clinical outcome but the effect of the dietary counseling on FH children is not well known. The aim of the study was to investigate the Mediterranean Diet impact on lipid profile with particular focus on dietary fat quality.

Material and Methods. Fifty FH children (4-18 years), not undergoing phytosterols or statin therapy, were included in the study. Dietary intake was recorded twice, at baseline and after dietary advices, by food week diaries. The recommended dietary pattern (Mediterranean Diet) was normocaloric and characterized by wide consumption of cereals, legumes, fish, plant foods, nuts, olive oil as the main source of fat, moderate consumption of dairy products and eggs, a small amount of meat, particularly red meat. Food diaries have been analyzed to estimate nutrients intake. Plasma lipid levels were evaluated at baseline and after three month since the diet counseling.

Results. Among 50 FH children, 38 followed dietary recommendations while 12 did not. In the compliant group, after dietary advice, the mean intake of carbohydrate, protein, total fat, saturated fat (SFA), unsaturated fats was 53.7±4.52, 15.0±1.88, 30.6±4.4, 8.17±1.78, 18.8±2.8 E%, respectively, while the mean fiber intake was 12.6±2.51g. Compared to baseline, LDL-C levels in compliant FH children were reduced by 9% while in the non-compliant group LDL-C levels increased by 8.9%.

Conclusions. The lipid profile improvement here found could be correlated to the low SFAs intake and the assumption of unsaturated fatty acids, fibers as well as other bioactive compounds. Furthermore the synergy among the nutrient-rich foods in this pattern can be favorable. This study demonstrates the efficacy of the Mediterranean Diet in FH children.

DOES LIPOPROTEIN(A) PREDICT EARLY ONSET OF CARDIOVASCULAR DISEASE IN PEDIATRIC FAMILIAL HYPERCHOLESTEROLEMIA?

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Aim. Lipoprotein(a) [Lp(a)] is a marker that predicts atherosclerotic cardiovascular disease (ASCVD) in adults with familial hypercholesterolemia (FH) in combination with low-density lipoprotein cholesterol (LDL-C) level, but its role for children with FH is debated. The aim of this study is to examine Lp(a) values in a sample of pediatric patients affected by FH.

Materials and Methods. Retrospective review of pediatric patients with FH identified LDL-C, Lp(a), and family history of AS-CVD

Results. We are collecting data on our population of pediatric patients affected by FH. Twenty-four children (17 female, five males, median age at diagnosis y 8.3) from 21 families were identified right now; in 12/24 patients Lp(a) was high. Among these, 7/12 had a family history of ASCVD. In contrast, 5 patients with normal Lp(a) had a family history of ASCVD too. In a recent study, Zawacki et al. suggested that children with FH in addition to high Lp(a) may be at higher risk for ASCVD than their LDL-C alone would suggest. Follow up is in progress and data are limited. Anyway, we observed an unclear relationship.

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PHTHALATES EXPOSURE AS DETERMINANT OF ALBUMINURIA IN TYPE 2 DIABETES SUBJECTS: A CROSS-SECTIONAL STUDY

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Background. Albuminuria is an independent risk factor for cardiovascular (CV) disease. Epidemiological studies reported an association between exposure to phthalates, a group of environmental contaminants, and states of higher CV risk like obesity and type 2 diabetes (T2D). No studies have so far addressed the presence of these compounds in urines of T2D individuals with different degrees of renal function.

Materials and Methods. Concentrations of three phthalate urinary metabolites (mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP) and mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP)), were quantified by UHPLC-ESI-QTOF in a morning urine sample of 209 T2D subjects (75.5% normoalbuminuric, 19.7% microalbuminuric, 4.8% macroalbuminuric). Data were normalized for urinary creatinine and related to clinical parameters, adjusting for confounders by univariable and multivariable regression models.

Results. Participants were 67.8 ± 12.4 years old; 59% males. T2D duration was 10.5 ± 9.7 years, HbA1c was $7.18\pm1.31\%$; 29% were insulin-treated. Creatinine-adjusted urinary concentrations of MEHP, MEOHP, MEHHP (detected in 95% of the subjects) were 7.53 [4.84-12.60] µg/g, 3.04 [1.03-5.14] µg/g and 10.70 [7.02-17.40] µg/g. No difference emerged according to age, sex, BMI, T2D duration, smoking, BP, HBA1c and eGFR, even after adjusting for confounders. Exposure to MEHP (p<0.02) and MEOHP (p<0.04) was higher in micro/macroalbuminuric than in normoalbuminuric individuals; 4th vs 1st quartile of MEHP and MEOHP showed higher risk of albuminuria (MEHP: OR 4.83 [CI 1.45-16.06], p<0.03; MEOHP: OR 3.29 [CI 1.08-10.04], p<0.04). MEOHP was higher (p=0.034) in subjects with CV events, while MEHP showed a trend (p=0.061).

Conclusion. These findings point out for the first time an association between phthalates exposure and AER in T2D subjects independent of GFR, representing a potential early hallmark of widespread vascular damage. Though considering the limitations of a cross-sectional study, a long-term exposure to these contaminants might mark a higher CV risk, implying the need for prospective studies addressing the pathophysiologic mechanisms underlying such association.

USE OF LOW-DENSITY LIPOPROTEIN CHOLESTEROL GENE SCORE TO DEMONSTRATE POLYGENIC FAMILIAL HYPERCHOLESTEROLEMIA: A CASE-CONTROL STUDY

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Background/Objective. Familial hypercholesterolemia (FH) is an autosomal dominant disorder caused by mutations in LDLR, APOB, or PCSK9 in approximately 80% of the cases. It has been shown that mutation negative FH patient may be carrier of the polygenic form of familiar hypercholesterolemia (pFH). pFH can be detected based on LDL cholesterol (LDL-C)-associated single nucleotide polymorphisms (SNPs).

Methods. To address whether polygenic forms may explain hypercholesterolemia in FH mutation negative patients, we gathered data from 125 mutation-negative (FH-) and 295 mutation-positive FH patients (FH+), as well as from 933 controls and genotyped the patients for 6 GLGC SNPs and compared the 6-SNP genetic score among patient groups and controls.

Results. Mutation-negative FH patients had significantly higher 6-SNP (0.69 ± 0.20) score than controls or mutation-positive FH patients (0.62 ± 0.21 and 0.58 ± 0.24 , p<0.001 respectively). We observed lower scores in mutation-positive FH patients compared with controls and mutation negative subjects (p=0.037 and p<0.001, respectively). Plasma levels of LDL-C showed to be associated with patients' genotyping as FH+ subjects had higher levels of LDL-C compared with FH- and controls (262.2 ± 84.9 , 200.8 ± 48.3 and 116.1 ± 37.7 , respectively) and the comparison between groups was statistically significant (p<0.001).

Conclusion. The present study demonstrates the utility of SNP score analysis for identifying polygenic FH in a cohort of clinically diagnosed FH patients by showing that small-effect common SNPs may cumulatively elevate LDL-C levels. In addition, the genetic risk score can be used also as a marker of the severity of hyper-cholesterolemia in FH patients.

LIVER STEATOSIS AND CAROTID INTIMA-MEDIA THICKNESS IN ADULTS. TRENDS FROM THE FOIE GRAS PROJECT IN SOUTHERN ITALY STUDYING THE ADHERENCE TO MEDITERRANEAN DIET AND PHYSICAL ACTIVITY

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Background. Nonalcoholic fatty liver disease (NAFLD) is the emerging health problem worldwide putting populations at increased risk of progressive liver damage, failure and neoplasia, and cardiovascular risk. The worrisome rise of the metabolic syndrome is also affecting subjects at a younger age but might be prevented by healthy lifestyles. AIM: To check the potential association between degree of liver steatosis, carotid intima-media thickness (CIMT), and lifestyles in adult subjects.

Methods. 60 subjects were consecutively enrolled at the Division of Internal Medicine "A. Murri", University of Bari 'Aldo Moro', Southern Italy, and grouped according to the presence of ultrasonographic liver steatosis (grade 0=absent; 1=homogeneous liver brightness, 2=proximal liver brightness+distal hypoechogenicity; 3=proximal liver brightness+distal hypoechogenicity without visible diaphragm line). CIMT was also measured ultrasonographically (Noblus® Hitachi, 3.5, 7.5 MHz Probe, Italy). Questionnaires were used to quantitatively assess and subgroup one-year Mediterranean Diet adherence (score 0-18, low=0-6, sufficient=7-12, high=13-18; Sofi et al. 2014) and weekly physical activity levels (Metabolic Equivalent Tasks, METs; 1 MET=3.5 mL/kg/min of oxygen consumption or 1.5

Kcal/Kg/hr; Saint-Maurice et al. 2018), as markers of compliance to healthy lifestyles.

Results. NAFLD subjects (n=29, 16M, 13F) and healthy subjects (n=31, 14M, 17F) participated. NAFLD subjects (mean steatosis score 1.54±0.12) were older (age 48.5±2.28 yrs, range 26-69 vs. 29.7±1.89 yrs, range 18-62, P<0.0001), more overweight-obese (BMI 31.3±1.19 kg/m²; 93% overweigh-obese vs. 22.4±0.72 kg/ m², 23% overweight-obese, P<0.0001) and with thicker CIMT (0.91±0.03 mm vs. 0.59±0.02 mm. P<0.0001) than healthy subjects. The grade of steatosis increased with body size, while percent of subjects with abnormal CIMT (≥1.0 mm) was 39% and 0% in NAFLD and healthy subjects, respectively (P<0.0001). Dietary adherence scored "sufficient" and was comparable in both groups (NAFLD 10.4±0.51 [median 10.5] vs. healthy 9.7±0.56 [median 9.0], P=NS). Percent of subjects scoring sufficient-high dietary adherence was 76% (NAFLD) and 84% (healthy), P=NS. Weekly physical activity levels were low and equally comparable (NAFLD 1461.8±357.6 METs [median 630] vs. healthy 1906.0±237.7 METs [median 1620]. P=NS) while percent of inactive subjects (i.e., <675 METs) was 52% and 26% (NAFLD and healthy subjects, respectively, P<0.001) with similar sitting times: NAFLD 253±30.5 min/ day vs. healthy 311±28.3 min/day, P=NS.

Conclusion. In adults, liver steatosis is associated with increased CIMT, pointing to increasing cardiovascular risk. This trend develops on a background of an apparent "sufficient" adherence to Mediterranean diet but poor physical activity levels. Thus, even in a typical Mediterranean area, lifestyles deserve active and urgent educational programs to counteract the universal rising trend of NAFLD.

LOW DENSITY LIPOPROTEIN RECEPTOR PLAYS A FUNDAMENTAL ROLE IN THE ACTIVATION OF CD8+ T LYMPHOCYTES

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Aim. Cholesterol is essential for the correct function and proliferation of immune cells. The aim of this study was to evaluate whether the uptake of low-density lipoprotein by the LDL-receptor (LDLR), plays a role in the activation of T lymphocytes, that are important players in the maintenance of the chronic response associated to atherosclerosis.

Methods. Immunophenotypic analysis and cytokines production evaluation in T lymphocytes from WT and LDLR KO mice, after in vitro (anti-CD3/anti-CD28 stimulation, mixed lymphocytes reaction) and in vivo (vaccination, homeostatic proliferation) activation through flow cytometry, gene expression analysis and protein quantification.

Results. LDLR increases after in vitro activation of CD8+ T cells. By using LDLR KO mice, we found that LDLR deficiency impairs CD8+ proliferation in vitro both after anti-CD3/anti-CD28 stimulation and after allogenic stimulation (proliferated cells: 1764±38 WT, 1144±64 LDLR KO p<0.01). Furthermore, INFγ production was reduced in LDLR KO CD8+ Tcells (13.9±1% WT, 8.4±0.6% LDLR KO p<0.01). Similarly, in vivo antigen-specific activation (vaccination with oval

bumin) resulted in a reduced proliferation and cytokines production (*JIFN*_Y p<0.001, *JIL13* p<0.01, *Jperforin* p<0.05) in CD8+ of LDLR KO mice. On the contrary, during homeostatic proliferation, a non-antigen-driven in vivo assay, WT and LDLR KO CD8+ T cell showed similar proliferation and cytokines production. Next, we investigated whether these effects were caused by an impaired T cells activation and indeed we found a reduced expression of CD69 (WT 61.6±6.1%, LDLR KO 41.8±8.5%, p<0.01), a marker of early activation, and decreased phosphorylation of Akt, a downstream molecule of the TCR that is rapidly phosphorylated after CD4+ and CD8+ T cell activation. Defects in signalling can be the consequence of altered lipid rafts formation, membrane portions enriched in cholesterol. Indeed, we observed a significant reduction of neutral lipids (MFI nile red WT 12520±2071, MFI LDLR KO 9639±272, p<0.05) in CD8+ T cells of LDLR KO mice after activation in vitro which was paralled by reduced Ctxb expression, a marker of lipid rafts.

Conclusions. Our results show that LDL receptor plays a critical role for immunometabolic responses in CD8+ T lymphocytes. In particular, its absence alters proliferation, activation and cytokines production in antigen-driven mechanism. LDL receptor in lymphocytes could therefore represent a checkpoint linking cellular cholesterol metabolism to adaptive immune response.

REVISED AMERICAN ACADEMY OF PEDIATRICS GUIDELINES ON PEDIATRIC HYPERTENSION AND CAROTID STIFFNESS IN OBESE CHILDREN

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Background. A clinical practice guideline with new pediatric hypertension definitions has been recently published. These guidelines include reference tables of normative blood pressure values and new definitions of elevated BP and hypertension.

Aim. Of the present study was to evaluate implications of these revised AAP pediatric hypertension guidelines on the association with carotid stiffness in a group of obese children.

Subjects and Methods. 300 consecutive obese children from 5 to 15 years evaluated for obesity in a specialistic setting care, were invited to participate in a study to investigate their cardiovascular markers of atherosclerosis. All children underwent an ultrasound carotid arterial examination. Carotid arterial stiffness was calculated using the following formula: β = (natural logarithm systolic blood pressure - natural logarithm diastolic blood pressure)/(systolic diameter - diastolic diameter)/diastolic diameter. Increased carotid stiffness was defined by a value >2.71, corresponding to the 90th percentile of lean children studied in a previous research and to the 50th percentile of obese children included in the study. Results. In a logistic regression analysis stage 2 hypertension was significantly associated with increased carotid stiffness (OR 2.4, 95% CI 1.2 - 4.9; p=0.015) even after adjustment for the main cardiovascular risk factors (age, gender, BMI, LDL-cholesterol, HDL- cholesterol, triglycerides, glucose, HOMA).

Conclusion. The new pediatric clinical practice guidelines for high blood pressure have changed the clinical approach to identification of hypertension. Using these new criteria we report that in obese children with stage 2 hypertension there was an association with stiffer carotid arteries. However, in obese children with elevated blood pressure or stage 1 hypertension this association was not demonstrated. These results facilitate identification of obese children already affected by subclinical atherosclerosis and enable therapeutic interventions as early as possible to interrupt the pathological process.

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

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Aims. PCSK9 inhibitors (PCSK9i) have proven effective and safe for reducing LDL cholesterol (LDL-C) in clinical trials. We aimed at evaluating efficacy and safety of PCSK9i in the real-life setting of the lipid clinic in Modena.

Patients and Methods. 35 consecutive patients who started treatment with PCSK9i (evolocumab 17; alirocumab 18) between Feb.2016 and Jun.2018 were followed-up for a median period of 20[6-116] weeks. Baseline and on-PCSK9i therapy clinical data and lipid profile were registered.

Results. Baseline patients characteristics were as follow: men/ women 23/12; median age 60[26-76] years; 26 with familial hypercholesterolemia (FH): 25 on secondary cardiovascular prevention: 5 with total statin intolerance; 5 with type 2 diabetes (T2D); 7 on LDL-C apheresis. PCSK9i significantly reduced LDL-C by -63[-77 - -27]% (p<0.001); 83% of patients reached LDL-C therapeutic target. Lp(a) levels were also significantly reduced by -39[-73 - +21]% (p<0.001), but only 5 out of 13 patients with high baseline Lp(a) levels reached <50 mg/dl. PCSK9i LDL-C lowering efficacy was independent from baseline characteristics (FH vs. non-FH; primary vs. secondary cardiovascular prevention; presence vs. absence of T2D) and from the type of PCSK9i. LDL-C apheresis was discontinued in 86% of patients. Patients with total statin intolerance showed a less significant reduction in LDL-C and were less likely to reach LDL-C therapeutic target. No significant changes in glycemia, CPK and aminotransferases were seen during follow-up. Three patients experienced adverse events (1 local skin reaction, 1 diffuse skin rash, 1 rhinopharyngitis) and none discontinued the treatment. Two patients experienced cardiovascular events (1 coronary and 1 carotid revascularization) during follow-up.

Conclusions. PCSK9i are effective and safe in attaining LDL-C therapeutic target in our real-life cohort of high-risk patients. Open clinical issues are: management of high-risk patients with total statin intolerance or high Lp(a) levels; PCSK9i long-term safety; PCSK9i efficacy on cardiovascular outcomes.

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

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Aims. Despite every patient with familial hypercholesterolemia (FH) is at increased lifetime risk for cardiovascular events, the risk is heterogeneous and risk stratification is challenging. Fatty liver (FL), a very common cause of chronic liver disease, is associated with an increased risk of cardiovascular disease (CVD). We aim at determining the prevalence and the predictors of FL, and at evaluating whether FL is associated with increased prevalence of CVD in FH patients.

Patients and Methods. In this pilot study, all adult patients with a clinical and/or molecular diagnosis of definite FH (Dutch Lipid Clinic Network - DLCN score >8) with available liver ultrasound data, who regularly attended our lipid clinic in Modena, were retrospectively enrolled. Main study measures were detection of FL (by liver ultrasound) and CVD (by patient history and chart review).

Results. 78 patients were enrolled in this study; men (n=39) and women (n=39) were equally represented, median age was 53 [18-92] years, 44.9% (n=35) were past or current smokers, 64.1% (n=50) were overweight/obese, 6.4% (n=5) had type 2 diabetes, and 48.7% (n=38) had arterial hypertension. The prevalence of FL was 43.6% (n=34). Patients with FL were significantly more likely to have overweight/obesity (82.4% vs. 50.0%, p=0.003), type 2 diabetes (14.7% vs. 0.0%, p=0.009) and arterial hypertension (64.7% vs. 36.4%, p=0.013) than those without FL. They also had significantly higher values of BMI (p=0.001), waist circumference (p<0.001), triglycerides (p=0.007) and alanine aminotransferase (p=0.008). The prevalence of FL increased across the number of factors of the metabolic syndrome (p=0.012). Age, sex, LDL cholesterol and DLCN score were not significantly different between FH patients with and without FL. 18 (23.1%) FH patients had a history of CVD. Of these, 16 (20.5%) had coronary heart disease (CHD) (myocardial infarction, angina or coronary revascularization procedures), 5 (6.4%) had cerebrovascular disease (ischemic stroke, transient ischemic attack or carotid revascularization procedures). and 4 (5.1%) had peripheral vascular disease (symptomatic lower extremity peripheral arterial occlusive disease or lower extremity revascularization procedures); 4 patients (5.1%) had CVD in multiple sites. FH patients with FL showed a significantly higher prevalence of CVD than their counterpart without FL (35.3% vs. 13.6%, p=0.024); the association between FL and CVD was largely conveyed by CHD (CHD: 32.4% vs. 11.4%, p=0.023; cerebrovascular disease: 8.8% vs. 4.5%, p=0.444; peripheral vascular disease: 8.8% vs. 2.3%, p=0.193; multiple-site CVD: 8.8% vs. 2.3%, p=0.193). In univariate logistic regression analysis, FL was significantly associated with CVD (crude OR 3.5, 95%CI 1.1-10.5; p=0.029) and CHD (crude OR 3.7, 95%CI 1.2-12.1; p=0.028). Together with FL, age (p=0.007), smoking status (p=0.003), arterial hypertension (p=0.008), fasting glucose (p=0.012), HDL cholesterol (p=0.041) and LDL cholesterol (p=0.001) were significantly associated with CVD, whereas sex, BMI, waist circumference, metabolic syndrome, Lp(a) and DLCN score were not. The association between FL and CVD remained significant even after adjustment for age, sex and smoking status (adjusted OR 4.3, 95%CI 1.1-16.6; p=0.037), but it was weakened after further adjustment for BMI and metabolic syndrome factors. **Conclusions.** FL, mainly attributable to nonalcoholic fatty liver disease (NAFLD), is extremely common in FH patients and is associated with a higher prevalence of CVD, particularly CHD. Future larger and prospective studies are needed to determine whether NAFLD may be considered as an independent non-traditional cardiovascular risk factor and may help in the risk stratification of FH patients.

EFFICACY AND SAFETY OF PCKS9 -INHIBITORS IN A COHORT OF DYSLIPIDEMIC PATIENTS

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Introduction. The use of PCSK9-inhibitors represents the new frontier in the management of hypercholesterolemia. Studies of efficacy have shown a reduction in LDL-C levels up to 60%. The aim of the work was to evaluate the reproducibility in real-life of the efficacy, durability and safety data of anti-PCSK9.

Methodology. Treatment with Alirocumab (75-150 mg sc every 14 days) or Evolocumab (140 mg sc every 14 days) was initiated in 27 subjects with not on target LDL-C levels according to ESC guidelines/EAS 2016. Heterozygous FH was present in 15 subjects (55%); among these, 5 were in primary prevention, 7 in secondary prevention and 3 were intolerant to statins. 12 subjects (45%) had a form of polygenic hypercholesterolemia and were at very high CV risk or in secondary prevention; among these, 7 were intolerants to statins. All subjects were treated with ezetimibe 10 mg/day. The serum levels of total cholesterol, HDL cholesterol and triglycerides were measured before and at 2 (n=27), 6 (n=19) and 12 (n=14) months since start of treatment. Data are expressed as m + SD. Data were analyzed with Student's T distribution for simple data or for paired data as appropriate.

Results. After two months of therapy, LDL-C levels were reduced by an average of 53.9% compared to baseline (from 195±78 mg/dL pre-treatment to 87±58 mg/dL, p<0.005), while there were no statistically significant differences among LDL-C at 2 months and LDL-C at 6 and 12 months of therapy (87.4±73 and 98±74 mg/dL, respectively) (p=ns). Similar results were obtained for non-HDL cholesterol levels: 224±82 mg/dL pre-treatment vs 107±71 mg/dL at 2 months (p<0.005 vs baseline) and 113±73 mg/dL after 12 months (p<0.005 vs basal). A significant reduction of triglycerides was observed after two months of treatment (131±48 mg/dL at baseline vs 99±25 mg/dL at 2 months, p<0.005), at 6 months (99.7±34 mg/dL, p=0.023), but not at 12 months (109±39 mg/dL, p=ns). No significant changes in HDL-C levels were observed before and after the introduction of anti-PCSK9 therapy: 48±10 mg/dL at baseline vs 47±9 mg/dL at 2 months (p=ns), 48±9.5 mg/dL at 6 months (p=ns) and 51±8 mg/dL at 12 months (p=ns). The baseline LDL-C in the group of subjects intolerant to statins (n=10) was 187.1±74 mg/dL and 199.8±81 mg/dL in subjects receiving statins (n=17) (p=ns). After 2 months of treatment the LDL-C values were reduced to 100.6±54 mg/dL in the subjects intolerant to statins (-46.2%) and to 79 ± 60 mg/dL (-60.4\%) in the subjects taking statins, without statistically significant differences between the two groups (p=ns). No significant side effects were found. Compliance to treatment was 100%.

Discussion. These data confirm the efficacy of PCSK9 inhibitors in the reduction of LDL and non-HDL cholesterol values. The reduction of LDL-C levels is similar in subjects treated with statins and ezetimibe and in subjects intolerant to statins who take only ezetimibe. The maximal effect is achieved after two months of treatment and is maintained over time. 85% of subjects achieved LDL-C target values, a difficult result to reach until few months ago. The absence of significant side effects argues in favor of the safety of treatment with the anti-PCSK9 antibodies.

PERFORMANCE EVALUATION OF THE DUTCH LIPID CLINIC NETWORK SCORE USING AN ITALIAN DATABASE OF FAMILIAL HYPERCHOLESTEROLEMIA PATIENTS

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Background. Familial hypercholesterolemia (FH) is a common genetic condition characterised by high plasma levels of lowdensity lipoprotein cholesterol (LDL-C) from birth and increased risk of premature coronary heart disease. Although it is a severe disorder, FH is currently under-diagnosed. The Dutch Lipid Clnic Network (DLCN) score is commonly used by healthcare professionals as tool that guides FH diagnosis in the setting of general medicine. Aims To evaluate the performance of the DLCN score, understanding whether missing information may affect the identification of FH subjects.

Methods. Patients are enrolled in the LIPIGEN study, an Italian integrated network aimed at improving the identification of patients with genetic dyslipidaemias. The analysis was carried out in all mutation-positive patients, aged 18 years and older, who underwent clinical evaluation and had available information on LDL-C levels.

Results. The DLCN score was applied on 1377 adults (mean age 42.9±14.2 years) with genetic diagnosis of FH, resulting in 28.5% of the sample classified as probable FH and 37.9% as definite FH. In total, 43.4% had at least one missing data out of 8, and about 10.0% had at least 4 missing data. When analyzed based on the type of missing data, a higher percentage of subjects with at least 1 missing data in the clinical history (54.6%) or physical examination (40.4%) was classified as possible FH (DLCN score 3-5). Moreover, it has been proved that using real or estimated pre-treatment LDL-C levels may significantly modify the DLCN score, highlighting an overestimation of the number of patients with definite FH diagnosis when pre-treatment LDL-C levels are calculated.

Conclusions. Although the DLCN score is a useful tool for the FH diagnosis, it may be limited by the complexity to retrieve all the information, suggesting a crucial role of the clinical judgement in the identification of FH subjects.

GALECTIN-3 SERUM LEVELS AND ADVANCED ATHEROSCLEROTIC PLAQUES: CORRELATION WITH PLAQUE PRESENCE AND NUMBER OF VESSELS

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Introduction. Atherosclerosis is a leading cause of cardiovascular morbidity and mortality worldwide.Inflammation drives the formation, progression, and complication of atherosclerotic plaques. The plaque complications lead to plaque erosion, plaque rupture and thrombus formation. We aimed to investigate the accuracy of circulating Gal-3 in predicting advanced atherosclerotic plaque presence and to evaluate the relationship of circulating Gal-3 both with intra-plaque Gal-3 and plaque characteristics.

Materials and Methods. Advanced human carotid plaques from 99 patients undergoing carotid endarterectomy were histologically classified as type Va (fibroatheroma), type Vb (mainly calcific) and type VI (complicated lesion) according to American Heart Association guidelines. Additional characteristics such as percentage of fibrosis, calcifications, necrosis and number of vessels were also recorded for each plaque. Gal-3 was measured in serum samples from patients and 78 healthy controls by immunometric assays. In the subgroup of 40 patients Gal-3 was directly detected in the plaques by immunohistochemistry.

Results. Gal-3 serum levels were significantly higher in patients than in controls (19.8 ± 5.8 vs 14.0 ± 3.6 ng/mL, p<0.0001). Analysis of ROC curves confirmed the discriminating power of the marker with an AUC of 0.806 (p<0.0001). At multivariate logistic regression Gal-3 serum levels remain associated with plaque presence independently from age, sex and LDL-c with an odd ratio of 1.26 (1.13-1.41, p<0.0001). No differences were found between Gal-3 serum levels among the different plaque types. No correlation was observed between serum and intra-plaque Gal-3 levels. A significant association between Gal-3 plaque levels and the number of vessels present in the atherosclerotic plaque was observed (p=0.002).

Conclusion. Our data show that Gal-3 is a good marker discriminating between subjects with and without advanced atherosclerotic plaque independently from age, sex and LDL-c. The association between Gal-3 and number of vessels suggests that Gal-3 could act as a pro-angiogenic factor.

A CASE OF PREMATURE ATHEROSCLEROSIS AND IPERCHOLESTEROLEMIA LOW RESPONSIVE TO THERAPY: WHEN SUSPECT SITOSTEROLEMIA

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Introduction. Sitosterolemia is a rare disorder of lipid metabolism that is inherited in an autosomal recessive pattern, characterized by several plant sterols in blood. Signs and symptoms consist in large xanthomas can cause pain, difficulty with movement, joint stiffness and blood abnormalities that occasionally are the only signs of the disorder, resulting in hemolytic anemia, stomatocytes and macrothrombocytopenia.

Aim. Report a case of sitosterolemia characterized by ipercholesterolemia, tendom xantomas, premature atherosclerosis and minor thrombocytopenia.

Results. We report the case of 57-year-old man diagnosed with hypercholesterolemia and macrothrombocytopenia. He presented with lipid profile suggestive for familial hypercholesterolemia (FH), with total cholesterol 320 mg/dl e LDL 260, tendinous xanthomas, xanthelasmas and early onset atherosclerotic cardiovascular disease (DLCNS 12); hemorrhagic diathesis (epistaxis in childhood, hematuria and post-traumatic ecchymosis during anticoagulant/antiplatelet therapy for mechanical valve); light thrombocytopenia (PTS 116 x10.9/L), anemia (Hb 127 g/L), increase of LAD 332 U/L with low level of haptoglobin, normal bilirubin, negative direct and indirect Coombs test, normal platelets aggregation and immunophenotype test. Furthermore the genetic screening for FH resulted negative. He presented increased plasma sitosterol levels 123,7 ug/ml (v.n. 0,7-7,0) and the hypothesis of sitosterolemia was confirmed by genetical and molecular analysis that shown the presence of a homozygous mutation of the gene ABCG8, exon, c.320C/G (p.S107X). The sterol absorption inhibitor ezetimibe was associated to rosuvastatin therapy and diet low in shellfish sterols and plant sterols (vegetable oils, margarine, nuts, seeds, avocados, and chocolate), with reduction of total cholesterol (-30,6%) and LDL-C (-42,4%) with reduction of xanthomas. Conclusion. Sitosterolemia is characterized by a phenotype similar to FH. Considering the severe premature atherosclerosis with high CHD risk and the possibility of abnormal hematologic findings with haemorragic diathesis is important to consider this differential diagnosis to start as soon as possible the most appropriate therapy.

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MEASURING EFFEROCYTOSIS IN ATHEROSCLEROSIS: NEW INSIGHTS FROM TRANSGENIC MOUSE MODELS

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Background and Aims. Macrophage apoptosis may play opposite roles in atherosclerosis, limiting plaque progression in early lesions, while worsening the disease at late stages. Late apoptotic cells produce proatherogenic inflammatory responses and efferocytosis, the adequate clearance of apoptotic bodies and cells by macrophages, is fundamental to hamper the atherosclerotic plaque progression. Indeed, an impaired efferocytotic process leads to wide necrotic cores and extensive pro-inflammatory response, thus increasing plaque vulnerability. Studying efferocytosis' efficacy in atherosclerotic lesions could help to evaluate plaque progression and eventually develop proefferocytic therapies. To address this issue, we applied a peculiar method for evaluating apoptosis and efferocytosis in vivo in athero-prone transgenic mouse models.

Materials and Methods. LDLR-/- mice on cholesterol-rich diet were irradiated and transplanted with bone marrow isolated from transgenic mice overexpressing sphingosine 1-phosphate receptor 1 (S1P1) in myeloid lineage (S1P1-Lyz and S1P1-F4/80) or control mice. After sacrifice, aortic root cryosections were analyzed through confocal microscopy using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) to identify apoptotic bodies/cells and monocyte/macrophage-2 cytosolic marker (MOMA-2) to stain macrophage positive area. TUNEL signals associated with macrophage cytosol were classified as efferocytosis, while free TUNEL signals as apoptosis. Both apoptotic and efferocytic signals were normalized to the respective lesion area (mean±SD weighted on mm²).

Results and Conclusions. Apoptosis rate was significantly higher in controls compared to S1P1-F4/80 and S1P1-Lyz mice (16.11±13.98 vs 7.14±3.98 and 5.11±1.64, respectively). Conversely, efferocytosis significantly increased in S1P1-F4/80 and S1P1-Lyz compared to control group (6.26±3.85 and 11.17±2.36 vs 2.46±2.20, respectively). These data confirm previous in vitro studies pointing to an antiapoptotic role for S1P-S1P1 axis. Accordingly, our in vivo evaluation of both apoptosis and efferocytosis is an efficient method that could be useful in analysing atherosclerotic lesion progression and plaque stability.

EFFECT OF PERIODONTAL DISEASE **ON GLUCOREGULATORY HORMONES** IN INDIVIDUALS WITH MORBID OBESITY

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The link between periodontitis (PD) and obesity is well known: however, the influence of PD on the glucoregulatory hormone pattern has never been explored. We measured clinical periodontal parameters (probing pocket depth, PPD, bleeding on probing, BOP, clinical attachment level, CAL) in 110 severely obese, non-diabetic individuals. Insulin, glucagon, GLP-1 and GIP were assessed after three days of standardized diet.47 subjects had periodontitis (PD+) and 63 did not (PD-). PD+ showed a mean number of teeth of 26, 30.3% of gingival sites with PPD>4mm, 55.2% of BOP sites and a mean CAL loss of 4.1mm, suggestive of a generalised tissue loss. hsCRP was above the normal range in both groups (4.31±3.80 vs 3.67±2.62 mg/l, p=0.29). PD+ showed higher IL-6 levels (3.4±3.9 vs 5.2±4.1 pg/ml, p=0.02); IL-1β did not differ. No relationship emerged between cytokines and hormones. Compared with PD-, PD+ had higher glucagon (26.60[25.22] vs 3.93[7.50] ng/l, p<0.0001) and GIP levels (10.56[13.30] vs 6.43[8.43] pmol/l, p<0.001), and lower GLP-1 (11.78[10.07] vs 23.34[16.80] pmol/l, p<0.0001). Insulin did not differ. In PD+, after adjustment for confounders, PPD was directly related to glucagon (β =0.424, p=0.002) and inversely to GLP-1 (β =-0.159, p=0.044). In PD+, CAL and PPD were related with glucagon (r=0.404, p=0.003 and r=0.456, p<0.001 respectively). In a multiple regression analysis, such relationship held for PPD (β =0.431, p=0.002); when adding incretins in the model, glucagon and GLP-1 were both associated (full model r2=0.30). In comparing different stages of PD, glucagon was related with the severity of periodontitis (16.17±9.95, 25.25±4.34, 39.24±4.45 ng/l from stage I to III; p=0.03). We describe here for the first time an impaired incretin axis combined with a relative hyperglucagonemia in obese non-diabetic subjects with PD and subclinical inflammation; this hormonal pattern might contribute to deteriorate their glucose tolerance, partially explaining the higher risk of diabetes observed in these patients.

MICROARRAY ANALYSIS OF LIVER GENE EXPRESSION IN APOA-I MILANO KNOCK-IN MOUSE MODELS

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Atherosclerosis, the dominant cause of CVD, is a vascular sitespecific chronic inflammation initiated in response to retained and modified lipids within arterial wall that can lead to clinically significant endpoints. Several epidemiological studies have shown that there is an independent and inverse relationship between circulating HDL-C levels and CVD. However, it has been proven difficult to successful reduce CVD risk with drugs increasing HDL-cholesterol. Moreover, it was reported that upon acute-phase response, HDL of both patients and animals not only lose some of normal functionality, but also gain atypical functions. The natural variant of human apoA-I, i.e. the apoA-I Milano is the result of a point mutation, with an arginine to cysteine substitution at position 173. Carriers exhibit hypertriglyceridemia with markedly reduced HDL and apoA-I plasma levels, a condition generally associated with a high risk of premature coronary disease. Evaluation of the cardiovascular status in apoA-IM carriers, compared with control subjects from the same kindred, did not reveal any evidence of increased CVD. Aim of this study was the identification of pivotal molecules that to a large extent differentiate two mouse models expressing this natural variant. In our study two different mouse models have been generated and used, i.e. the hA-IM k-in (hA-IM), and the hA-IM k-in overexpressing human apoA-II (hA-IM/AII) mice. This aim has been accomplished by using the Affimetrix GeneChip Mouse Gene ST system. The results obtained from the DAVID platform showed 1965 differentially regulated genes, 955 downregulated (from -1 to -3.3 fold change) and 1010 upregulated (from 1 to 4.6 fold change), in hA-IM compared to hA-IM/AII mice. Among them, we found genes related to metabolic and inflammatory pathways, for example: the coagulation cascade (alpha-2-macroglobulin) and the melanogenesis pathway (elongation enzymes). These preliminary data, requiring validation, indicate that hA-IM could be involved in several pro and anti-atherogenic pathways.

MYOCARDIAL OVEREXPRESSION OF ANKRD1 RESULTS IN SINUS VENOSUS DEFECTS AND LEADS TO ADULT DIASTOLIC DYSFUNCTION

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Increased Ankrd1 levels linked to gain of function mutations have been correlated to selective congenital heart disease onset and adult cardiomyopathy occurrence in humans. 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. The link between increased ankrd1 level and cardiac structural and functional disease onset is not understood. To get insight into this problem, two "gain of function" mouse models by overexpressing both ankrd1WT and ankrd1T116M, 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.Both transgenic mice were characterized by impaired cardiac remodeling, which strongly affects the developing sinoatrial region and leads to sinus venosus defects. Transgenic mice survived to adulthood but developed left atrial enlargement accompanied by severe diastolic dysfunction. Isolated myofibrils from embryonic to adult hearts presented progressive and differential alterations in contractile parameters, indicating a functional shift towards stiffer and hyper-contractile phenotype. At the molecular level, these changes are accompanied by dynamic alterations in titin isoforms ratio. Embryonic and neonatal transgenic cardiomyocytes present irregular shape and sarcomeric disorganization, which progresses into sarcomeric loss and mitocondrial damage in adult ventricular but not atrial cardiomyocytes. Our data indicate that genetic mutations leading to increased ankrd1 levels can lead both to congenital heart disease and adult cardiomiopathy via a common cellular mechanisms, ie the progressive alteration of cardiomyocyte contractile and functional properties. Ankrd1 is a critical sensor-signaling molecule, which finely modulates cardiomyocyte stiffness/ function/properties during development and postnatal life.

A CASE OF HYPOBETALIPOPROTEINEMIA DUE TO A NOVEL APOB GENE PATHOGENIC VARIANT AFFECTING SPLICING

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Background. Familial hypobetalipoproteinemia (FHBL) is a dominant monogenic disorder characterized by a significant reduction of plasma LDL-C. The main candidate genes involved in FHBL are APOB, ANGPTL3, and PCSK9. The APOB-linked FHBL1 may be associated with liver steatosis and chronic diarrhea, depending on the specific genotype involved.

Aim of the Study. Genetic characterization by NGS of a patient thought to have FHBL.

Results. A 17 y-old obese boywas referred to our Lipid Clinic for a long-standing history of very low plasma cholesterol levels associated with episodes of abdominal pain and diarrhea. At the age of 11y, during a hospitalization, several clinical and instrumental examinations were performed: abdominal echography documented hepatomegaly with diffuse steatosis without focal lesions, while hematological parameters were normal with the exception of plasma lipids (total cholesterol 72, LDL-C 15.5, HDL-C 50, triglycerides 33 mg/dL). At the age of 17 v after admission to our Lipid Clinic blood samples were collected for DNA analysis and the evaluation of other biochemical parameters. The only reported symptoms were intermittent episodes of diarrhea especially following the consumption of fatty meals. Genetic analysis showed that the proband was heterozygous for a splice site variant in intron 3 of APOB gene (c.237 +1G>A) predicted to be pathogenic in silico. An APOB minigene construct harboring the mutation, generated in vitro two abnormal transcripts producing an identical truncated apoB protein (p.Val80Ilefs*10). In addition to the APOB pathogenic variant, the NGS analysis revealed variants in other genes which might contribute to hypobetalipoproteinemia.

Conclusion. This report highlights the importance of genetic diagnosis to explain a rare phenotype of hypobetalipoproteinemia and further confirms that a broader gene analysis performed through NGS might help in defining more accurately the link between each single genotype and a specific clinic phenotype.

GENETIC BACKGROUND OF HDL DEFICIENCY DIFFERENTLY AFFECTS MONOCYTE PHENOTYPE AND FUNCTION

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Aim. In epidemiological studies HDL-cholesterol showed a robust inverse relationship with coronary heart disease. Besides their role in promoting cholesterol efflux from macrophages, HDLs can contribute to atheroprotection limiting the inflammatory responses during atherogenesis. Since monocytes can contribute in creating a proinflammatory milieu, in this study we aim to evaluate the effect of genetic HDL deficiency on pure monocyte phenotype and functionality.

Methods and Results. Monocytes were isolated from fresh blood in carriers of genetic hypoalphalipoproteinemia due to mutations in LCAT (n=12), ABCA1 (n=10) and APOA1 (n=9) and from controls (n=9) matched for age, gender and BMI.Exclusion criteria were known cardiovascular disease, renal disease and diabetes. While no difference in the monocyte subset distribution was found between carriers and controls, expression of the integrin CD11c, as assessed by flow cytometry, was nearly half in mutant LCAT (Δ MFI 220±56 vs 415±38 p=0.032) and consistent in all monocyte subset. According to the decrease in CD11c, only monocyte in LCAT deficiency displayed a reduction in transendothelial migratory capacity ex vivo compared to control monocyte (39.7±1.92 vs 48.0±1.83 percentage of migrated monocytes p=0.006). Monocytes were 24-hour challenged with LPS and Pam3Cys as toll-like receptor ligands and showed comparable cytokine production in ABCA1 and APOA1 genotype. On the contrary, monocyte isolated from LCAT deficient subjects proved to be less efficient in producing IL-8 and IL1-β upon stimulation compared to matched controls. A sub-analysis in LCAT mutations carriers was performed, revealing a gene-dose dependent effect, for all the parameters considered. No difference in circulating levels of hsCRP, MCP-1, TNF- α and IL-6 was appreciated.

Conclusions. Genetic HDL deficiency due to ABCA1 or APOA1 mutations does not affect monocyte phenotype and functionality. Conversely, LCAT deficiency seems to reduce the pro-inflammatory potential, contributing to increase the atheroprotective role of HDL often seen in this disease.

MICE WITH HUMANIZED LIVER FOR STUDYING EPIGENETIC MARKS INVOLVED IN REGULATION IN CARDIOMETABOLIC DISEASES

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Introduction. The HUMAN project aims to identify the genetic basis of cardiometabolic diseases using mouse models with humanized liver bearing allelic variants associated to disease state. To overcome the differences between human and mouse metabolism, the HUMAN consortium developed mouse models that closely resembles human metabolism. This study aims to characterize the epigenome landscape of histone marks in humanized mouse livers and correlate it to the metabolic phenotypes. The focus is on the different distribution of the histone 3 lysine 27 acetylation (H3K27ac) mark of active enhancers and histone 3 lysine 4 trimethylation (H3K4me3) mark of active promoters in livers from donors with allelic variants for TCF7L2 and, moreover, on the comparison with murine hepatocytes.

Methods. Chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) of H3K27ac and H3K4me3 histone marks.

Results. The histone mark H3K27ac showed higher enrichment on regulatory region of PPARa (Peroxisome proliferatoractivated receptor alpha), even though not statistically significant (p=0,4385), in mice with protective allele (30%) vs. mice with risk allele (22%). Histone mark H3K27ac was more enriched in human PPARa of mice humanized with TCF7L2 protective alleles (30%) compared to the homologous PPARa region in murine hepatocytes (18%, p=0,0406). These data correlate with the increased expression of FGF21, target of PPARa, in humanized livers compared to mouse livers. The enhanced expression of FGF21 may explain the higher oxidative metabolism observed in mice with humanized liver.

Conclusion. ChIP-seq protocol allowed to investigate changes in epigenetic markers in mice with humanized livers bearing a protective allelic variant of TCF7L2 compared to murine hepatocytes. Our results indicate that mice with humanized livers have an enhanced oxidative metabolism. These data suggest a possible role of the PPAR α -FGF21 axis in the higher oxidative capacity observed in mice with humanized livers. [Supported by grant EU FP7-602757 HUMAN].

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INTERLEUKIN-11 EXERTS ANTIATHEROGENIC EFFECTS IN VITRO AND IN VIVO

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Background and Aims. Interleukin-11 (IL-11) is a stromal cell derived cytokine, highly expressed by smooth muscle cells (SMCs), heart, skin and testis. It belongs to the IL-6-type cytokine family, which shares the gp130 subunit in signaling cascade activation. Specifically, IL-11 signals via IL-11RA, leading to gp130 homodimerization and STAT3 activation. Beyond its known role in stimulating the maturation of megakaryocytes, it also exerts anti-inflammatory effects in T-cells and macrophages. Our preliminary observations indicated that both high-density lipoproteins (HDL) and sphingosine 1-phosphate (S1P) dose dependently induce IL-11 production in SMCs, via S1P2 stimulation. Since HDL-S1P showed antiatherogenic potential, we investigated the effects of exogenous administration of IL-11 to athero-prone mouse models and in different in vitro settings.

Materials and Methods. LDLR-/- mice on cholesterol-rich diet received human recombinant IL-11 (oprelvekin) at 50 µg/kg or vehicle i.p. thrice a week for 12 weeks. At sacrifice hearts and aortae were dissected for morphometric and immunostaining analysis of lesions. For in vitro analysis, cultured macrophages were exposed to IL-11, lipid charge or other stimuli and analyzed accordingly.

Results and Conclusions. IL-11 treatment significantly reduced lesion size and extension (-50%) either at the aortic root or at enface analysis, compared to control group. Moreover, necrotic core areas were also reduced (-30%). In aortic roots, apoptosis was lower in IL-11 treated group, which showed a significant increase in efferocytosis rate compared to controls. Macrophages exposed to IL-11 were resistant to AcLDL engulfment, a decrease in scavenger receptor (SR-A1, CD36) gene expression, while showing a significant increase in ABCA1 gene expression and cholesterol efflux to apolipoproten A-1.In conclusion, our data point to an anti-atherogenic role of IL-11, through the modulation of macrophage cholesterol handling and anti-inflammatory function.

IMPACT OF DIFFERENT OBESITY AND METABOLIC PHENOTYPES ON CARDIOVASCULAR OUTCOME IN PATIENTS AFTER CORONARY REVASCULARIZATION

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Methods. A sample of 674 consecutive acute coronary syndrome (ACS) patients were recruited from January 2008 to January 2010 during their hospitalization in our Department of Cardiology. Clinical blood pressure (BP) readings were obtained in the supine position, after 5 minutes of quiet rest, with an aneroid sphygmomanometerHeight and weight were measured with participants

wearing light clothes without shoes; height was measured to the nearest centimeter, and weight was measured to the nearest onehalf kilogram. BMI was calculated as body weight (kilograms) divided by the squared height (meters) and further sub-divided into 2 categories: <25 kg/m² (normal weight) and >30 kg/m² (obese). Examinations were performed by a single investigator who was unaware of the patients' BP and other clinical data.Metabolic status. Waist and hip circumferences were measured to the nearest centimeter with the patient standing and arms hanging relaxed. Then we categorized all participants in four group according to absence or presence of MetS and obesity as following: metabolically healthy and normal weight (MHNW), metabolically unhealthy but normal weight (MUNW), metabolically healthy but obese (MHO) and metabolically unhealthy and obese (MUO).

Study outcomes. The patients were followed from coronary revascularization until they experienced one of the primary endpoints. Clinical outcome was evaluated by monitoring major adverse cardiovascular events (death, fatal or non-fatal re-infarction with or without PCI and stroke), seen as cumulative cardiovascular (cCV) events for all statistical analyzes. Procedure-related AMI within 24 hours was not included in the end-point.Statistical Analysis. Data are presented as means, standard deviations, and frequency of occurrence (%). To summarize, all unhealthy phenotypes had higher CV risk of recurrences compared with the reference group (MHNW); this finding did not change after further adjustments for age, sex, smoking and cholesterol levels. Our findings showed that metabolically unhealthy phenotypes were associated with increased CV risk despite ongoing therapy. This data emphasizes the importance of metabolic health also in patients with coronary revascularization.

NOVEL VARIANTS OF SAR1B GENE IN CHILDREN WITH CHYLOMICRON RETENTION DISEASE

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Background. Chylomicron Retention Disease (CMRD) is an exceedingly rare recessive disorder characterized by severe intestinal lipid malabsorption, due to impaired chylomicron secretion, associated with reduced plasma cholesterol and low density lipoprotein cholesterol (hypobetalipoproteinemia). CMRD is caused by mutations in SAR1B gene encoding Sar1b protein, a guanosine triphosphatase, involved in chylomicron transport in enterocytes. Here we describe three variants of SAR1B gene found in three unrelated children with recessive hypobetalipoproteinemia born from consanguineous parents.

Methods. Full clinical investigation including intestinal biopsy and sequencing of recessive hypobetalipoproteinemia candidate genes (MTTP and SAR1B).

Results. Case 1 was a 4 months old male with steatorrhea, fat soluble vitamins deficiency, failure to thrive and severe hypobetalipoproteinemia. Intestinal biopsy revealed lipid accumulation in enterocytes. He resulted to be homozygous for a novel SAR1B nonsense variant (c.49 C>T, p.Gln17*) predicted to encode a short truncated Sar1b protein.

Case 2 was a 4 years old male with intestinal fat malabsorption, lipid accumulation in enterocytes and severe hypobetaliproteinemia. He resulted to be homozygous for a novel SAR1B missense variant (c.409 G>C, p.Asp137His) affecting the guanine recognition site of Sar1b protein. Case 3 was a 6 years old malnourished and dysmorphic male with steatorrhea, fat-soluble vitamin deficiency, lipid accumulation in enterocytes and liver steatosis. He resulted to be homozygous for a ~6 kb deletion in SAR1B gene eliminating exon 2. Exon 2 deletion eliminates the first ATG translation initiation codon, a condition predicted to generate a Sar1b protein devoid of the normal aminoterminal domain.

Conclusions. Intestinal fat malabsorption in a child with recessive hypobetalipoproteinemia should raise the suspicion of a monogenic disorder of chylomicron formation/secretion such as ABL or CMRD. The analysis of the corresponding candidate genes allows a rapid diagnosis and avoids delays in the diagnostic process and in the implementation of appropriate treatment.

L-ARGININE PREVENTS OSTEOGENIC DIFFERENTIATION OF AORTIC INTERSTITIAL VALVE CELLS

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Introduction. Oxidative stress and reduced bioavailability of nitric oxide (NO) are considered pathological mechanisms potentially involved in the calcific degeneration of aortic valve.

Aim. To study the effects of L-Arginine (L-Arg), the main precursor of NO, on the osteogenic differentiation of aortic interstitial valve cells (VIC).

Methods. We isolated a clonal population of bovine VIC that expresses markers of osteogenic differentiation (such as alkaline phosphatase, ALP) and induces the calcification of collagen matrix after stimulation with endotoxin (LPS 500 ng/ml). VICs were treated in vitro with different combinations of LPS±L-Arg (50 or 100 nM) and cell extracts were collected to perform proteomic analysis (iTRAQ) and gene expression experiments (RT-PCR).

Results. L-Arg treatment prevents the over-expression of ALP in the VIC treated with LPS (p <0.001) and reduces the matrix calcification (p<0.05). The proteomic analysis allowed to identify 49 proteins with an altered expression profile after stimulation with LPS and significantly modified by the treatment with L-Arg. These include proteins involved in the redox homeostasis of the cells (such as Xanthine Dehydrogenase, Catalase, Aldehyde Oxidase), remodeling of the extracellular matrix (ADAMTSIA, Basigin, COL3A1) and cellular signaling (Fibrillin-1, Legumain, S100A13). The RT-PCR analysis confirmed the modifications of Fibrillin-1, ADAMTSLA, and Basigin, whose expression levels increase after stimulation with LPS and are reduced by L-Arg (p <0.05). Furthermore, treatment with L-Arg (100 nM) significantly reduces the over-expression of inflammatory molecules induced by LPS (TNFalpha, p<0.001, IL-6, p<0.001 and IL-1b, p<0.001).

Conclusions. L-Arg treatment prevents osteogenic differentiation of VIC and reduces matrix calcification. This effect is achieved through the modulation of proteins involved in the cellular redox system, the remodeling of the extracellular matrix and the inflammatory activation of the VIC.

PYROPHOSPHATE IS A POTENT INHIBITOR OF VASCULAR CALCIFICATION AND CAN BE USED TO DEVELOP BIOLOGICAL MATRIX RESISTANT TO ECTOPIC MINERALIZATION

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Background. Previous studies showed that the pro-calcific differentiation of interstitial aortic valve cells (VIC) is accompanied by reduced production of pyrophosphate (PPi), a potent inhibitor of calcification.Aim: To define the anti-calcification properties of various polyphosphate compounds and to develop calcificationresistant vascular/valve matrices.

Methods. An in vitro model of calcification induced by high serum concentrations (FSB 50%) was developed. Type I collagen matrices were treated for 12 days with FBS 50% added with different concentrations of PPi, nucleotides (ATP, ADP, AMP), alendronate, mevalonic acid5-PPi and farnesyl-PPi. Calcification of the matrix was quantified by colorimetric assay and histological stainining (Von Kossa). The same approach was used to evaluate the anti-calcification properties of the different compounds on decellularized porcine aortic valves, decellularized bovine pericardium and commercial aortic valve bioprosthesis. A tissue engineering technique was also developed to covalently bind alendronate and mevalonic5-PPi acid to the collagen matrix.

Results. PPi is able to inhibit, in a dose-dependent manner, the serum-induced calcification of type I collagen matrices (p<0.001). The same inhibition capacity was observed for ATP, ADP, 5-PPi mevalonic acid, farnesyl-PPi and alendronate (p<0.001 for all compounds). No effect was observed for the AMP (PPi-free compound). The same inhibitory effects were obtained by using the previous compounds on decellularized porcine valvular leaflets, decellularized bovine pericardium and commercial bioprosthesis. Subsequently, Alendronate and acid-mevalonic5-PPi were covalently anchored to a collagen type I matrix. Both matrix treatments were effective in inhibiting calcium deposition (p<0.05).

Conclusions. The PPi isolated or contained within different polyphosphate compounds is able to inhibit the mineralization of the extracellular matrix. This property can be exploited for the development of biological matrices, both valvular and vascular able to withstand the calcification processes.

COMORBIDITIES PREDICT DISABILITY IN OBESE PATIENT. PRELIMINARY DATA BASED ON THE ICF-OB QUESTIONNAIRE

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Obesity is a chronic condition resulting in decreased physicalfunction and disability. Recently a questionnaire (Q) based on The International Classification of Function, Disability and Health was developed to address disability in obese-patients (ICF-OB). Aim. The aim of study was:

1) to assess disability in obese-patients by means of ICF-OB-Q;

- to compare disability-score with other phenotypic scores such as the Edmonton-Obesity-Staging-System (EOSS) and the comorbidities-index;
- 3) to identify predictors of disability.

Methods. Observational study on 48 adult patients, M/F 12/36, age 47.5 years (range 18-69) BMI 38.9 \pm 5.8 kg/m². In all patients demographic, anamnestic, anthropometric, and biochemical data were collected. The EOSS stage and the comorbidities index has been defined. The ICF-OB-Q, consisting of 4 parts: Medical-Area (QAM), psychological-area (QAP), physical-function-area (QAF), dietitians-area (QAD), was administered by a team of specialists from four disciplines (medicine, phychology, physical-therapy, diet and nutrition). According to disability-scores, we defined 4 different conditions: Negligible-disability (score <0.5), Mild-disability (score between \geq 0.5 and <1.0), Moderate-disability (score \geq 1.5).

Results. Most patients had a mild/moderate-disability. According to EOSS-system, 6.3% of subjects were within stage-0; 12.5% in Stage-1, 39.6% in Stage-2, 39.6% in Stage-3 and 1 patient only in stage-4. 75% of patients had multiple comorbidities. The disability-scores recorded by QAM and QAF correlated with EOSS-staging-scores and the comorbidities-index (p<0.05). Schooling was significantly correlated with QAM-score (p=0.004) while employment correlated with QAP-score (p=0.019), EOSS-stage (p=0.007) and comorbidities-index (p=0.016). Moreover, in a multivariate-analysis, comorbidities-index was an independent predictor of disability in medical-area (β stand. 0.37 p<0.01; IC 0.27-1.88) and the disability of physical-function-area (β stand. 0.56 p<0.05; IC 0.55-3.9).

Conclusions. The ICF-OB-Q is a validated tool for detecting disability as a first step for a multidisciplinary approach to taking care of obese-patients. Our preliminary data can be reinforced in the much broader case studies.

THE CALABRIAN NETWORK ATEROSTOP 2 FOR DIAGNOSIS AND CURE OF HYPERLIPIDEMIA IN ADULT AND CHILDREN

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Background and Aims. To date, a reliable estimate of the number of people with Familial Hypercholesterolemia (FH) is available only for 23 countries. Considering an estimated theoretical prevalence of heterozygous FH in the population of 1/500, less than 1% of FH cases are diagnosed in many countries including Italy, demonstrating the low priority attributed to FH worldwide (1). About 4000 subjects may be affected by this fearsome pathology in Calabria (2), but of these currently less than 200 have been identified. Thus, there is the urgent need to implement strategies to identify FH in Calabria.

Materials and Methods. In 1998, a network for the diagnosis and treatment of hyperlipidemia was conceived and approved by Calabria Region. It involved Internists working in the local hospitals which were coordinated by University. All participants in this Net-

work were well trained in lipidology. In 2017 a pediatric network was conceived for the diagnosis also in children. After training courses, now the General Practitioners and outpatients pediatricians collaborate to send those with hypercholesterolemia to the Lipid Center of the Network. Recently, the Network performed a screening to find new FH cases.

Results. In the last 2 year a total of 1300 subjects with hypercholesterolemia were screened from different cities. 132 blood samples were collected and sent for genetic testing (by Lipigen Project). To date, 18 were FH positive with the following genetic variants:8 patients were heterozygosis for the LDLR gene (c.418G>A p. G14L and c.1775G>A p.G592G); 2 patients heterozygosis for the APOB gene (c.2938G>A p.A98Tand c.5856T>C p.H1952), 1 patient was homozygous for a variant of the LDLR gene (c.1109 A/C p.N370T), 2 double heterozygous for the variants LDLR/APOB(c.119A>C p.A37T and c.268-4T>A) and finally 2 patients with triple heterozygosis of the LDLR gene (c.265T>C p.(C89A) , APOB gene (c.12614C>T p.P425L) and LDLRAP1 gene (c.672C>T p. S224). Conclusion. The hospital-based network is an operational refer-

Conclusion. The hospital-based network is an operational reference for all practitioners in the region and helps to integrate efforts aimed at bridging the gap between scientific evidence and clinical practice. This organisational model takes advantage of hospital units, which devote a fraction of their time to the prevention of cardiovascular disease (3).

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EFFICACY AND SAFETY OF PCSK9 INHIBITORS IN CLINICAL PRACTICE: A MONOCENTRIC EXPERIENCE

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The recent approval of novel anti-PCSK9 therapy opened a new scenario for the treatment of hypercholesterolemic patients. **Aim.** To evaluate the efficacy and safety of PCSK9-inhibitors (PC-SK9i) in clinical practice.Methods: observational study on 59 patients (31M/28F) that from December 2015 to August 2018 started PCSK9i therapy on the top of previous lipid-lowering-therapy (16 with alirocumab 75 mg, 14 with alirocumab 150 mg and 29 with evolocumab 140 mg). Lipid profile and glucose were evaluated at 3, 6 and 12 months.

Results. 22 patients had mutations of LDLR-gene. 50% were statinintolerant, 68% were in secondary prevention, 70% had peripheral vasculopathy, 15% were affected by diabetes and 14% by pre-diabetes. At baseline (N=59), LDL-c (median, interquartile range) was 140 [123-180] mg/dl; at 3 months (N=50) 55 [29.2-81.2] mg/dl, at 6 months (N=37) 56 [31-71.5] mg/dl, at 12 months (N=23) 49 [37-90] mg/dl. At 3 months 76% of the patients were on target for LDL-c, 70% at 6 months and 74% at 12 months. At 6 months the median reduction of LDL-c from basal was 86 mg and at 12 months 90 mg, indipendently from the PCSK9i used, from age and sex, from previous histo-

ry of CHD, from gene mutations and from diabetes. Statin-intolerant patients had a significative reduction in LDL-c values, but remained at higher LDL-c values if compared with normotolerant patients. Therapy with PCSK9i was well tolerated. One patient reported a reduction of cognitive function. One died for heart attack. One patient was a non-responder. We observed an increasing trend in glucose levels during the follow-up, mainly between 6 and 12 months.

Conclusion. Our experience confirmed efficacy and safety of PCSK9i. Further studies are needed to explore their long-term effect on glucose levels and to explore why rare patients are non-responder.

EFFICACY AND TOLERABILITY OF BUPROPION/NALTREXONE (MYSIMBA) IN OBESE PATIENTS: A MONOCENTRIC EXPERIENCE

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Obesity is a complex desease that requires chronic managment of weight and obesity-related comorbidities. The recent approval of fixed combination of bupropion/naltrexone widened the scenario of its pharmacological treatment.

Aim. To evaluate efficacy and tolerability of bupropion/naltrexone in obese patients.

Methods. Observational study on 18 patients (17F; median BMI 34.6 kg/m²) that from November 2017 to August 2018 started bupropion/naltrexone-therapy. Weight, blood-tests and arterial-blood-pressure (BP) were evaluated at 1, 3 and 6 months.

Results. 5 patients had a history of previous bariatric-surgery and 5 previous liraglutide-treatment. No patients were taking other weight-lowering-therapies.33% of patients did physical activity, 67% were no-smokers and all patients were under a Dietologist. 33% had concomitant controlled hypertension, 39% pre-diabetes, 33% hypercholesterolemia, 22% corrected hypothiroidism, 6% PCOS, 17% binge-eating-desorders. 2 patients interrupted the drug for inefficacy and 6 for side-effects, mainly gastrontestinal (nausea); one patient experienced insonnia, one had an increase of BP after 10 days and one an itchy erithema. Among patients who continued assumption, the majority reached the full daily dose, except one that continued with 2 tablets/day because of nausea.At baseline (N=18), weight (median, interquartile range) was 93.5 [88.4-99] kg; at 1 month (N=6) 94.5 [90.9-98.2] kg and at 3 months (N=8) 91.3 [76.1-99.6] kg.At 3 months, 37.5% of the patients had lost >5% of their baseline weight and 12.5% had lost >10%.BP slightly increased during follow-up.

Conclusion. Our preliminary data confirmed the efficacy of bupropion/naltrexone with weight lost > 5% in 50% of the population at 3 months, but almost half of patients interrupted the drug for side effects or inefficacy.

AN ALTERED LIPID OXIDATION PREDICTS METABOLIC SYNDROME AND TYPE 2 DIABETES

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Background. Metabolic Syndrome (MetS) and Type 2 diabetes (T2D) are clinical conditions involving the impaired uptake and utilization of glucose, altered lipid metabolism and the disruption of the metabolic signaling pathways that regulate insulin secretion from pancreas. Diabetes has been linked to an impaired ability to oxidize fatty acids. Fat oxidation can be assessed clinically by a respiratory quotient measurement during fasting. We hypothesised that a respiratory quotient might predict metabolic syndrome and type2 diabetes onset.

Methods. In this longitudinal study we used an existing database of 233 individuals who had complete nutritional and biochemical data at baseline and after 12-month follow-up. All participants underwent an indirect calorimetry to measure the respiratory quotient. We excluded participants with diabetes, chronic diseases and those who had changed food habits in the previous three months. Only 88 subjects met the inclusion criteria.

Results. Two individuals developed type 2 diabetes and 10 metabolic syndrome after one year. Mean basal respiratory quotient was 0.91 ± 0.09 in those who developed metabolic syndrome/diabetes. Participants in the high respiratory quotient group (>0.91) had a higher incidence of metabolic syndrome/diabetes than those in the low quotient group (25% vs 8% p=0.04). In the high respiratory quotient group, Kaplan-Meier curves showed a greater probability of having metabolic syndrome/diabetes than those in the low respiratoryquotient group (log Rank χ 2 test=8.44; p=0.004). A multivariable Cox proportional hazards model demonstrated that energy expenditure and weight increase did not predict metabolic syndrome/diabetes [HR (95% CI) = 1 (0.996-1.005), p=0.86 and 3.9 (0.407-38.061), p=0.23 respectively).

Conclusions. A greater probability of metabolic syndrome/diabetes was found in individuals with a basal respiratoryquotient of >0.91 than in those with a respiratoryquotient of ≤ 0.91 after 1 year. In the short term anthropometric measurements and their variation overtime were not correlated with metabolic syndrome/diabetes.

ASSESSMENT OF ARTERIAL STIFFNESS IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA BEFORE AND AFTER TREATMENT WITH IPCSK9

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Background. Familial Hypercholesterolemia (FH) is a genetic disease characterized by elevated levels of serum LDL cholesterol (LDL-C) and high cardiovascular risk (CVR). The prognosis of FH patients correlates with lifelong LDL-C levels, and therapy is aimed to achieve and maintain LDL-C target over years, for high (primary prevention, LDL-C <100 mg/dl) and highest (second-ary prevention, LDL-C <70 mg/dl) CVR patients. Endothelial and vascular dysfunction occur years before clinical manifestations of atherosclerosis (in the early stages of atherogenesis), even before the instrumental recognition of atherosclerotic lesions. PWV is considered a reliable and early marker of arterial stiffness and an independent prognostic predictor for cardiovascular mortality.

Aim. To evaluate the effectiveness of six months of treatment with anti-PCSK9 monoclonal antibody on lipid profile and vascular arterial function, in a setting of patients with heterozygous familial hypercholesterolemia (HeFH) in primary prevention, who did not achieve the expected LDL-C levels despite the best conventional therapy (at least 6 months) with high efficacy statin at the maximum tolerated dose in association with ezetimibe.

Materials and Methods. We enrolled 19 patients (12 male and 7 female, aged between 35 and 69 years) with HeFH in primary prevention, for which it was considered useful to start treatment with PCSK9 inhibitor drugs; 12 patients received treatment with Evolocumab 140 mg every 2 weeks; 7 patients were treated with Alirocumab at a dose of 75 (1) or 150 (6) mg every 2 weeks. At the time of enrollment (T0) and 6 months after the treatment with monoclonal antibodies (T1) we performed blood chemistry, arterial stiffness and anthropometric parameters. Complete lipid profile, fibrinogen, ApoB, Lp(a), GOT, GPT, CPK, fasting glucose, insulin and many other parameters were determined by routine methods. High sensitivity CRP was determined using the ELISA method. Both the Lipid Centre involved in the study performed PWV measurement by SphygmoCor. Carotid artery echo-Doppler scan was performed in accordance with the ESC/ESH guidelines.

Results. After 6 months of treatment (T1) with iPCSK9 compared to baseline values (T0) we found a significant reduction in levels of inflammation indices (CRP), lipid profile (LDL-C, TC, triglycerides, HDL-C, Lp(a), ApoB). Furthermore, PWV showed a small but significant reduction a T1 (9.33 ± 2.2 vs 10.19 ± 2.0 , $\Delta=8.9\%$, p<0.001). Due to the very small sample size, to verify whether arterial properties improvement, as measured by PWV, associate with Δ changes of other study variables is not statistically plausible.

Conclusions. After 6 months of treatment with monoclonal anti-PCSK9 antibodies, the levels of CRP, LDL-C, Lp(a), TC, TG, were significantly reduced in patients with heterozygous familial hypercholesterolemia; moreover, the artery properties significantly improved after therapy. This did not allow to speculate on a pathophysiological model in which LDL-C or CRP lowering allow PWV improvement in a direct and/or indirect way; therefore, larger observation should address this focus, also given the biological plausibility of the association.

PERSONALIZED REGIMEN FOR PCSK9 INHIBITORS: A THERAPEUTIC OPTION WHICH MAINTAINS EFFICACY AND REDUCES COSTS

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Background. PCSK9 inhibitor (PCSK9i) represent a breakthrough in the treatment of hypercholesterolemia, however PC-SK9i therapy have significant limitation in term of sustainability of economic public healthcare. The aim of the study was to verify the hypothesis that a longer interval between PCSK9i administration maintains LDL cholesterol-lowering efficacy.

Methods. We enrolled 14 FH patients (mean age 60 ± 9 years, 11 male) with known cardiovascular disease and LDL-C level below 70 mg/dl after at least 3 months of PCSK9i therapy. Therapy was administered every 2 weeks (11/14 evolocumab 140 mg, 3/14 alirocumab 150 mg) on tot maximally tolerated lipid lowering therapy (statins 5/14, ezetimibe 5/14, statins+rezetimibe 2/14, statins+fibrate 2/14). After 3 months, the interval between PCSK9i administration was extended to 3 weeks for at least 3 months.

Results. The PCSK9i, administered with a 3-week interval, maintained the desired LDL-C targe (40±18 at 2-week vs. 59±25 at 3-week intervals). Only 2/14 patients had to discontinue the 3-week interval regimen because LDL-C was above 70 mg/dl.

Conclusions. There are two ways to improve the cost-effectiveness of PCSK9 inhibitors: lower drug-cost and limit the use to high-risk patients. Inevitably, these two options are incompatible with the low negotiation margin of pharmaceutical companies and with the ethical issues related to the patient's right to receive the best available care. In our study, we sought a third alternative: longer dosing intervals. Certainly, we cannot demonstrate that prevention of cardiovascular events is maintained with the longer dosing interval by which the LDL cholesterol-lowering efficacy is preserved. These data deserved be confirmed in a larger population.

SPHINGOSINE-1-PHOSPHATE (S1P) RECEPTOR TYPE 1 EXERTS ATHEROPROTECTIVE EFFECTS IN MURINE MACROPHAGES

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Introduction. Atherosclerosis is a chronic inflammatory disease leading to the formation of vascular lesions caused by both an excessive accumulation of cholesterol and cell death in arterial walls. High Density Lipoproteins (HDLs) are thought to exert multiple protective effects against atherosclerosis. Indeed, HDLs mediate cholesterol transport from the atherosclerotic plaque through the interaction with cholesterol transporters expressed in macrophages. The protective effects of HDLs have been partially attributed to specific molecules, to which they serve as carriers, such as sphingosine1-phosphate (S1P) (1, 2). The aim of this study was to investigate molecular mechanisms underlying the putative atherosphate (S1P) /S1P-receptor 1 (S1PR1) axis in modulating macrophage functions relevant to the pathogenesis of atherosclerosis.

Methods. We used a transgenic mouse model overexpressing the S1PR1 in macrophages. The functional macrophage phenotype has been evaluated by examining surface markers of macrophage polarization and activation and differential gene expression by mean of flow cytometry and qPCR technique, respectively. To investigate the receptor-mediated lipoprotein uptake, we assessed the cholesterol accumulation in peritoneal macrophages by determining the uptake of fluorescently labelled acetylated and oxidized low density lipoproteins (LDL). The cholesterol transport from macrophages was evaluated with a radiotracer technique using apolipoprotein A-I (apoA-I) and HDLs as extracellular acceptors. The expression of proteins regulating cholesterol handling, inflammatory response and the expression of cell surface receptors involved in the foam cells formation were determined by qPCR. The propensity of S1P to prevent innate immunity signalling in macrophages and relative effect on M2 polarization, was examined in vitro by evaluating the liberation of anti-inflammatory cyto/chemokines such as IL-1RA, IL-10, IL-4 and CCL20 from peritoneal macrophages.

Results. Compared to WT cells, peritoneal macrophages overexpressing S1PR1 showed an increased expression of cholesterol transporters ABCA1 and ABCG1. Accordingly, an improved cholesterol transport to acceptors apoA-I and HDLs was observed in S1PR1-overexpressing macrophages. The favourable effects exerted by S1PR1 on cholesterol uptake and transport were likely related to the increased expression and activity of a transcription factor liver X receptor (LXR). Moreover, LXRs regulates the expression of several other important factors modulating macrophage biology in lesions undergoing regression, such as Arg-1, IRF8, Lgm1. In particular, Arg-1 expression is enhanced through binding of hematopoietic transcription factors IRF8 and PU.1 to its promoter (3). These results have been recapitulated in the present study. Our preliminary results suggest that S1PR1 overexpression induces M2a alternative macrophages phenotype and inhibits inflammatory response.

Conclusions. Current results suggest that S1P exerts beneficial atheroprotective effects in macrophages by modulating cellular cholesterol metabolism and inflammatory response mainly through interaction with the macrophage S1PR1. Hence, S1PR1 may be considered as a potential target for future therapies against atherosclerotic disease.

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ASSESSMENT OF S100A12 PLASMA LEVELS IN SUBJECTS WITH OR WITHOUT FAMILIAL HYPERCHOLESTEROLEMIA

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Background and Aims. Inflammation is a key regulatory process that links hypercholesterolemia and immune mechanisms promoting atherosclerosis. Inflammatory biomarkers may be helpful to better define the atherosclerotic burden in patients with high cholesterol levels such as familial hypercholesterolemia (FH). Our aim was to evaluate the concentration of S100A12 protein in FH patients and its association with pulse wave velocity (PWV) and carotid intima-media thickness (IMT).

Methods and Results. We measured lipid profile, S100A12, PWV and IMT in 94 hypercholesterolemic participants. FH diagnosis was attributed to patients with a Dutch score \geq 4 and confirmed with the presence of a genetic mutation. FH patients had a greater amount of low-density lipoprotein and apolipoprotein B and a lower amount of high-density lipoprotein and apolipoprotein AI than non-FH subjects (4.37±1.78 vs 3.8±0.55 mmol/L, p<0.05; 1.15±0.35 vs 1.04±0.15 g/L, p<0.05; 1.29±0.28 vs 1.44±0.34 mmol/L, p<0.05; 1.35±0.2 vs 1.49±0.28 g/L, p<0.01). FH patients had higher S100A12 levels than non-FH subjects (12.55±5.34 vs 7.89±4.68 ng/mL, p<0.001). PWV and IMT were higher in FH patients than non-FH subjects (8.63±2.02 vs 6.98±0.73 m/s, p<0.05; 0.77 [0.63-0.97] vs 0.66 [0.58–0.77] mm, p<0.01). S100A12 was independently correlated with genetic mutation (p<0.01), PWV and IMT (p for both <0.001).

Conclusion. FH patients exhibited higher S100A12 levels than non-FH subjects.

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DISEASE ACTIVITY SCORE PREDICTS CARDIAC AND VASCULAR IMPAIRMENT IN VERY EARLY PSORIATIC AND RHEUMATOID ARTHRITIS PATIENTS

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Background. Patients with inflammatory joint disease (IJD), including rheumatoid (RA) and psoriatic arthritis (PsA), exhibit increased rates of cardiovascular disease (CVD) morbidity and mortality. Chronic inflammation and immune dysregulation are thought to play a substantial role. Aim: To evaluate if disease activity score (DAS) could predict CV risk in terms of cardiac and vascular impairment, before clinical signs of atherosclerosis are manifested. The prevalence of subclinical atherosclerosis has been already assessed in RA and PsA, but how precociously also myocardial functionality is impaired remains unknown. In order to address this question, we assessed myocardial and arterial impairment, circulating CD34+ cell count, vitamin D levels, additionally to DA scores in IJD patients of recent onset.

Methods. DA scores were estimated in patients with RA (n=41) and PsA (n=35) without traditional CV risk factors, and 58 matched healthy controls (HC). Besides CRP-DAS28, BASDAI, PASI, VAS, HAQ were also calculated. Furthermore, global longitudinal and circumferential strain (GLS and GCS) were estimated to assess the myocardial function, Pulse Wave Velocity (PWV) and carotid intima-media thickness (cIMT) were measured to assess artery vascular involvement; circulating CD34+ counts were evaluated by flow cytometry, and vitamin D levels by HPLC. CRP, ESR, Fibrinogen were also determined by routine methods as inflammatory markers.

Results. We verified whether DA scores, and particularly CRP-DAS28, are able to predict cardiac and vascular involvement; consistently, we performed multivariate regression models considering GLS, GCS, PWV, cIMT and CD34+ as response variable(s), respectively. DAS28, CRP, ESR, Fibrinogen, age, gender, disease duration, BMI, lipid profile, systolic and diastolic blood pressure values, and Vitamin D levels. We found that DAS28 predict all the cardiovascular indices taken into account, and also CD34+ cell counts. As further analysis, we repeated the statistics in RA and Psa patients, separately, and also in two different subgroups according to DAS28 score, with a cut-off level of 2.9 (low activity diseased vs high activity diseased subjects). In RA we found a linear association between DAS28, and GLS, GCS, PWV, cIMT and CD34+ cells; these results were maintained also when patients were subdivided basing on disease activity grade. In PsA we found DAS28 as predictor of GLS, GCS, PWV, and CD34+ cells; however, this association was confirmed only for GLS in lower disease activity patients. In all patients, the predictive power of DAS28 was lacking in presence of an IMT >1.3.

Conclusions. DAS28 was found as a strong predictor of cardiac and vascular involvement in early staged IJD; if confirmed on larger scale, this observation could suggest the clinical evaluation by scores for patients routine CV risk stratification, at least when the early stage of IJD is confirmed by routine carotid US IMT examination (cIMT<1.3 mm), in absence of additional CV risk factors.

IDENTIFICATION OF P.LEU167DEL APOE GENE MUTATION BY NEXT GENERATION SEQUENCING IN A LARGE HYPERCHOLESTEROLEMIC FAMILY

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Introduction. Familial Hypercholesterolemia is a genetic disorder caused by mutations in LDLR, APOB or PCSK9 genes. Recently other genes and genetic loci (16q22.1 and 8q24.22) have been associated with hypercholesterolemia. APOE variants are well known determinants of lipid variability and a peculiar form of hyperlipidemia known as type III hyperlipoproteinemia.

Materials and Methods. We have studied a large family with definite clinical diagnosis of hypercholesterolemia. Sanger sequencing analysis did not revealed any pathogenic mutations in LDLR, APOB and PCSK9 genes.. More, we performed MLPA analysis to evaluate CNVs in LDLR gene and no mutations were identified. During the last years the NGS technology has been applied to analyze a large number of genes related to lipid metabolism. We performed NGS analysis in the proband by using a custom panel of 17 high-cholesterol-related genes by Ion Torrent PGM. This approach allowed the resequencing of candidate genes in addition to other recently discovered genes associated to hypercholesterolesterolemia.

Results and Conclusion. The data output of NGS analysis confirmed the absence of mutations in LDLR, APOB and PCSK9 genes and allowed to identify in the proband an APOE mutation in heterozygosity (p.Leu167del). The family cascade screening revealed the presence of the same mutation in the affected subjects while no carriers of mutation were found among the normolipidemic ones. As already known, p.Leu167del derange ApoE interaction with lipids and the affinity of apoE to its receptors and causes hypercholesterolemia.In conclusion we report the third kindred with FH due to an APOE gene mutation.

BIGLYCAN-INDUCED INFLAMMATORY RESPONSE IN HUMAN MONOCYTE: EX VIVO EXPERIMENTAL MODEL

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Background. Monocytes are critically involved in the pathogenesis of atherosclerosis, capable of secreting many factors such as chemokines, cytokines, growth factors, and reactive oxygen species, thus contributing to wall lesion development. Biglycan (BGN), a small leucine rich proteoglycan, plays a pivotal role in initiating lipid deposition in the arterial subintimal space, by its ability to bind and retain apoB- containing lipoprotein, including LDL, VLDL and IDL. Furthermore BGN, in its soluble form, acts as an endogenous ligand for Toll-Like receptors (TLR2, TLR4), mediating innate immunity and inflammation.

Methods. We studied the role of BGN in an experimental model of biglycan-induced inflammatory response in human monocytes isolated ex vivo from ten healthy subjects. Monocytes were cultured in six-well culture plates at a density of 1*106 cells/well and treated with BGN (1.25 µg/ml). A separate set of monocytes receiving BGN plus a TLR-2 and/or TLR-4 siRNAs were treated 48 h before BGN addiction in order to block their activity. TLR-2, TLR-4, IL-1β and IL-6 mRNA expression was assessed by qPCR and expressed by n-fold increase with respect to baseline and unexposed controls; TLR-2, TLR-4, IL-1β and IL-6 proteins were evaluated by a colorimetric commercial kit.

Results. The addition of BGN (1.25 μ g/ml) to monocytes induced a high mRNA expression and protein production of pro-inflammatory mediators such as IL-1 β and IL-6, as well as NF-kB, TLR-2 and TLR-4 activation. The involvement of TLR-2 and TLR-4 in the mediation of BGN action was confirmed by using the specific siRNAs. **Conclusion.** In light of these finding we can suggest further studies in order to evaluate the effects of soluble BGN also in other cells involved in the onset and progression of atherosclerosis, including endothelial cells and endothelial progenitor cells, to deepen to current knowledge of atherogenesis, and also to provide the rationale of novel therapeutic approaches.

A COMPLEX GENOTYPE IDENTIFIED IN A PROBAND AFFECTED BY SEVERE HYPERTRIGLYCERIDEMIA

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Primary forms of HTG include mild-to-moderate and severe forms; the rare severe hypertriglyceridemias are thought to be monogenic autosomal recessive and caused by homozygous or compound heterozygous loss of function mutations of few known genes pathophysiologically involved in the intravascular lipolysis of the TG-rich lipoproteins namely lipoprotein lipase (LPL), apolipoprotein CII (APOCII), apolipoprotein AV (APOAV), glycophosphatidylinositol (GPI)-anchored high-density lipoprotein-binding protein 1 (GPI-HBP1). lipase maturation factor 1 (LMF1) and GPD1. The proband. a 19 years old boy, showed a severe hypertriglyceridemia (up to 1400 mg/dL). No hepatosplenomegaly was observed and secondary forms of hypertriglyceridemia were excluded. Family clinical history was lacking because he was an adopted child, so we could not determine the mode of inheritance of the HTG trait. We decided to use a Next Generation Sequencing approach to study the coding exons and intron/exon boundaries of genes affecting the main pathways of triglyceride metabolism. The targeted resequencing of candidate genes led to the discovery of a complex genotype characterized by three different rare variants in three candidate genes: p.Asn318Ser in LPL gene, p.Ser107Ala in GPIHBP1 gene and p. Lys77Gln in APOC2 gene. All variants were confirmed by Sanger sequencing; LPL variant was found in homozygosity, while GPIH-BP1 and APOC2 variants were found in heterozygosity. The variant p.Asn318Ser in LPL gene has been already identified in heterozygosity in different subjects and classified as "non deleterious", "disease causing" or "tolerated" based on SNPs3D, Mutation Testing and SIFT. GPIHBP1 and APOC2 variants were classified as "probably damaging" and "possibly damaging" respectively by using bioinformatic tool Polyphen-2. More studies are needed to understand the impact of these variants in the development of the hypertriglyceridemia in this proband.We report a case of HTG likely caused by a high polygenic burden determined by complex heterozygosity for variants in LPL, APOC2 and GPIHBP1 genes.

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

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Aim. Heterozygous Familial Hypercholesterolemia (HeFH) is the most common genetic disorder characterized by high levels of LDL cholesterol (LDL-C) and associated with an increased risk of coronary heart disease (CHD). In addition to the monogenic variant, it has been reported that the HeFH phenotype may also have polygenic molecular bases (PFH). To date few studies have compared HeFH and PFH in terms of atherosclerotic burden. The aim of this study is the evaluation of atherosclerotic coronary artery disease by CT scan in subject with monogenic HeFH as compared to individuals with PFH.

Methods. Ninety one subjects, aging 18-75 years, clinically diagnosed as FH based on a DLCN score >3, were screened for mutation in the major FH-causing genes, LDLR, APOB, PCSK9 and LDLRAP1 by next generation sequencing. In those who were mutations negative, a 6-SNP genetic risk score (6-GRS) was calculated to determine the presence of a polygenic background, as previously reported. Seventy three patients were found to carry monogenic HeFH and 18 were classified as PFH based on an elevated 6-GRS. All patients were asked to undergo coronary CTA imaging. Coronary atherosclerosis burden was evaluated based upon the presence and severity of coronary stenosis and coronary calcium (Agaston score).

Results. In comparison with PFH patients, those with HeFH were younger (51±8yrs vs. 46±13 yrs, respectively, P=0.103), had higher levels of LDL-C (167±53 mg/dl vs. 185± 85mg/dl, respectively; P=0.709) and reported higher use of statins (38.9% vs. 76.4%, respectively; P<0.005). The prevalence of other cardiovascular risk factors was comparable between groups.We found that a coronary involvement was almost absent in patients under the age of 30 years. Among HeFH, 24 patients (32.9%) had non-significant coronary artery lesions (stenosis <50%) and 16 patients (21.9%) had coronary lesions of which at least one significant (stenosis \geq 50%). Among patients with PFH, 7 patients (38.9%) had one or more lesions of which at least one significant. Overall, 54.8% of HeFH and 55.6% of PHF showed any grade of coronary atherosclerosis and this difference was not statistically significance. Two patients

Conclusions. In this small pilot study coronary atherosclerotic burden is similar between HeFH and PH individuals. Nevertheless, larger studies are needed in order to better clarify this issue.

ROLE OF LRP1 LOCUS AS POSSIBLE GENETIC DETERMINANT OF BICUSPID AORTIC VALVE

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Background. Bicuspid aortic valve (BAV) represents the most common congenital heart defect, as it affects 0.5-2% of the general population. An increased risk of aortic valve stenosis, as well as aortopathy, has been documented in BAV patients. Moreover, presence and severity of calcification contributes to worsen the clinical outcome. Actually, disease progression may also involve processes sharing risk factors with the atherosclerotic process, among which lipid profile alteration. Genetic variants in LRP1 gene, encoding for the Low Density Lipoprotein Receptor-Related Protein 1, a receptor belonging to low density lipoprotein receptors family and involved in multiple cellular processes, have also been associated with the aneurysmal disease.

Aim. Of the present study was to investigate the role of LRP1 as possible genetic determinant of BAV.

Methods. Thirty-eight BAV patients admitted to the Referring Center for MFS and Related Disorders (Tuscany, Italy) have been analyzed by Next Generation Sequencing (NGS) approach (Illumina), based on a targeted sequencing panel of 97 genes suspected to be associated with BAV or BAV-associated conditions or known to be involved in aortic wall remodeling including LRP1.

Results. Data from NGS evidenced the presence of LRP1 rare genetic variants in 6 out of 38 BAV patients [c.527T>A (p.Val176Asp); c.1458G>A (p.Pro486=); c.7363G>A (p.Gly2546Ser); c.6874C>T (p.Arg2292Cys); c.2323G>C (p.Ala775Pro); c.7838G>A (p.Arg2613Gln)]. For one of these 6 patients, relatives were available for performing genetic analysis. In this family, p.Val176Asp LRP1 was observed to segregate with BAV phenotype. This variant was also classified as potentially pathogenetic by all in silico prediction tools used.

Conclusions. Data from the present study allow to support the role of LRP1 locus as potential candidate gene for BAV, thus contributing to evidence its possible involvement in modulating the pathophysiological processes underlying disease development.

CALCIUM INTAKE BEFORE DIETARY INTERVENTION IN A HYPERCHOLESTEROLEMIC CHILDREN POPULATION

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Introduction. Hypercholesterolemic children (HC) families often change nutritional habits before receiving specialistic advice, thus reducing total lipid intake. This may lead to deficiency of macro and/or micronutrients, like calcium.

The purpose of this study is to evaluate HC population calcium allowance before any dietary intervention.

Subjects and Methods. We evaluated the calcium, total lipids and saturated fats intake through a self-administered food survey (dietary recall) on 151 HC (cholesterol LDL>110mg/dl) with median age of 7.2 years (range 1-14), 71 males and 80 females. None reported previous dietary intervention. Nutritional habits of children within the 7-11 age group were compared with a control population with similar sex and age attributes. Results were confronted using student test.

Results. The chart shows intake of calcium (mg/day, avg±DS), total lipids and saturated fats (%Cal/day, avg±DS) of the sample and the age based advised levels of allowance.

Age	Number	Total Lipids	Saturated Fats	Calcium	Larn 2014 Ca ²⁺
<7	70	29.5±5.2	11.3±3.5	989±567	900
7-11	60	29.5±5.3	9.8±2.5	788±333	1100
>11	21	28.8±5.1	9.8±2.6	795±291	1300

HC with calcium assumption less than 70% of LARN's in three age groups were respectively: 11.4, 36.7 and 52.4%. The mean calcium intake of age group 7-11 was significantly lower in males than females (696 ± 275 vs 892 ± 375 mg/day, p=0.02). Calcium intake was similar between the sample and control population (7-11 years old) whilst total lipids intake (29.5 ± 5.3 vs $34.1\pm6.2\%$ Cal/day) and saturated fats (9.8 ± 2.5 vs $12.2\pm3.2\%$ Cal/day) were considerably lower in HC (p<0.001).

Conclusions. Although HC changed their nutritional habits before evaluation, the average daily calcium intake is not reduced, in age group 7-11 at least.

Allegedly HC replaced whole milk with skimmed milk and dairy products with less lipid content instead of removing dairy products which are their main dietary source of calcium.

Since calcium intake is below recommended levels, oral calcium supplementation should be considered regardless of lipidic framework especially in the age range 11-14 years.

ALIROCUMAB IN DYSLIPIDEMIC PATIENTS: THE SANTA CROCE E CARLE HOSPITAL OF CUNEO EXPERIENCE

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Introduction. Dyslipidemic patients are at increased cardiovascular risk and often do not achieve LDL goals with statins/ezetimibe. Moreover drug intolerance could challenge the clinician. Alirocumab is a monoclonal antibody targeted to PCKS-9 utilized to reduce LDL-Cholesterol levels that could be utilized also in patients with statin intolerance. Aim of the Study: was to retrospectively evaluate the effect of a 6 months treatment with alirocumab of 8 dyslipidemic patients according to Italian Medicines Agency (AIFA) prescription rules.

Subjects and Methods. 8 dyslipidemic patients were evaluated before and 6 months after alirocumab treatment (75 or 150 mg s.c. every two weeks). 4 patients fulfilled a diagnosis of Heterozygous Familial Hypercholesterolemia, the other 4 were diagnostic of Familial combined Dyslipidemia. Four patients were statin intolerant. Data after six month treatment are available of only 7 patients. Student's T-test was performed to compare means of the variables studied before and after the treatment.

Results. (means±S.D.) age 54.3 ± 14.4 years, Male/Female =7/1, Body Mass Index 26.04 ± 3.02 kg/m², Systolic Blood Pressure 130.2 ± 12.9 mmHg, Diastolic Blood Pressure 80.0 ± 9.6 mmHg, total cholesterol 312.7 ± 52.3 mg/dl, HDL-cholesterol 47.9 ± 12.1 mg/ dl, Triglycerides 197.3 ± 100.1 mg/dl, calculated LDL-cholesterol 225.4 ± 56.4 mg/dl, Non-HDL-Cholesterol 264.9 ± 56.6 mg/dl. After treatment lipid values were: total cholesterol 187.6 ± 39.5 mg/dl (p<0.0001 vs baseline), HDL-cholesterol 51.7 ± 13.1 mg/dl (p=0.09), Triglycerides 147.8 ± 65.6 mg/dl (p=0.064), calculated LDL-cholesterol 106.7 ± 42.5 mg/dl (p<0.0001 vs baseline), Non-HDLcholesterol 135.9 ± 40.6 mg/dl (p<0.0001 vs baseline). In particular absolute LDL-Cholesterol reduction was 118.7 ± 30.7 mg/dl corresponding to 53.6 ± 10.6 percent reduction. No adverse events were reported among patients (in particular no injection site reactions were reported).

Conclusions. Alirocumab 75 mg or 150 mg every two weeks is a very effective and safe treatment to reduce significantly LDL-C and Non-HDL-cholesterol levels in patients with familial dyslipidemias with or without statin intolerance. A trend toward an improvement also of HDL-cholesterol and Triglycerides was also observed although not significant.

EVALUATION OF SUBCLINICAL MYOCARDIAL DAMAGE IN PATIENTS WITH CHRONIC INTESTINAL INFLAMMATORY DISEASE BEFORE AND DURING TREATMENT WITH BIOLOGICAL DRUGS

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Background. The idiopathic inflammatory bowel diseases comprise two types of chronic intestinal disorders: Crohn's disease and ulcerative colitis. The prevalence of IBD in Western countries is estimated to be up to 0.5% of the general population, with growing incidence. The current treatment of IBD includes mesalazine (oral and rectal formulations), glucocorticoids (CCS, conventional and other forms like budesonide or beclomethasone), antibiotics (typically ciprofloxacine and metronidazole), immunosuppressants (mostly azathioprine/6mercaptopurine or methotrexate) and anti-TNF agents (infliximab, adalimumab, certolizumab pegol and golimumab). Recently, the antiintegrin monoclonal antibody vedolizumab and the antibody against IL-12/23 ustekinumab have been approved for IBD.

Aim. To evaluate the subclinical cardiac and vascular damage in patients with refractory (as considered after six months of conventional therapy with CCS plus mesalazine) chronic intestinal inflammatory disease before and after treatment with biological drugs (infliximab, adalimumab, vedolizumab). Furthermore, circulating CD34+ cell count was also assessed.

Methods. Pulse Wave Velocity (PWV), global longitudinal strain (GLS) and circulating CD34+ cells were evaluated to estimate subclinical cardiovascular involvement in 16 patients with IBD, before (T0) and after (T1) a six-months treatment with biological drugs. Carotid-femoral PWV was measured by routine methods. GLS was measured by speckle tracking echocardiography. Circulating CD34+ were counted by flow cytometry. In addition, inflammatory indices (ESR, GRP, fibrinogen) and EF% were also evaluated.

Results. At T1, no statistically significant differences were detected as regards ESR, PWV, EF with respect to T0; in contrast, some parameters appeared statistically improved as compared to baseline, including: CRP (p=0.013), GLS (p<0.001) and CD34+ (p<0.001). The interdependence analysis performed on the mean percent changes showed a significant correlation between Δ PWV and Δ GLS: as Δ PWV decreases Δ GLS increases, improving ventricular performance.

Conclusions. Patients with IBD have a greater risk of developing CV disease, especially when IBD is biological uncontrolled; in our study we have shown that in refractory IBD biological drugs may have a favorable effect on inflammatory status and symptoms/ biological compensation, but also as regards CV risk as suggested by favorable change in plasma levels of CRP, circulating levels of CD34+ and GLS values This study needs to be enhanced and reproduced on larger patients cohort to confirm this preliminary data and to address the question of whether therapy with these drugs may have a role also in favorably modulating CV risk.

THE ACTIVITY OF LYSOSOMAL ACID LIPASE IN DRIED BLOOD SPOT IN NON-ALCOHOLIC FATTY LIVER DISEASE AND IN CRYPTOGENIC CIRRHOSIS: 5 YEARS OF LABORATORY EXPERIENCE

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Introduction. Lysosomal acid lipase (LAL) plays a key role in lipid metabolism through the hydrolysis of cholesteryl esters and triglycerides in lysosome. In this study, lysosomal acid lipase activity was evaluated in dried blood spot (DBS) of adult patients with non-alcoholic fatty liver disease (NAFLD), cryptogenic cirrhosis (CC) or cirrhosis of different aetiology.

Methods. Dried blood spot (DBS) samples were prepared spotting 75 ul of blood. LAL activity is measured using the fluorimetric substrate 4-methylumbelliferyl palmitate in the presence of cardiolipin and Lalistat 2, respectively activator and inhibitor of LAL.

Results. In the first study, LAL activity was measured in 240 NAFLD patients. Median LAL activity was significantly reduced in NAFLD [0.78 vs 1.15 p<0.001]. Our findings suggest a strong association between impaired LAL activity and NAFLD. The second study was about a multicentre cohort including 274 patients with liver cirrhosis of different aetiology from 19 centres of Internal Medicine, Gastroenterology and Hepatology distributed throughout Italy. In the whole cohort, median LAL activity value was 0.58 nmol/spot/h. 0.49 and 0.65 in the groups of CC and known-aetiology cirrhosis, respectively (p=0.002). We found a marked reduction of LAL activity in patients with cryptogenic cirrhosis compared to the other known aetiologies. In another paper, we investigated the association between spleen dimensions and LAL activity in NAFLD patients. We included 425 consecutive patients who underwent abdominal ultrasound to evaluate hepatic steatosis and spleen dimensions. LAL activity was lower in 56 patients with splenomegaly, as compared to those without. At multivariable logistic regression analysis, age, LAL activity and platelets were significantly associated with splenomegaly. These results suggest that LAL may contribute to spleen enlargement in this setting. Further studies will clarify the role of LAL in chronic liver diseases.

PCSK9 MUTATIONS: EFFECTS BEYOND LDL CHOLESTEROL

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Purpose. PCSK9 loss of function (LOF) mutations are associated with alterations in circulating levels of triglyceride-rich lipoproteins and obesity. These observations call for better understanding about the specific effect of PCSK9 in determining these phenotypes and whether this is independent from the presence/absence of LDLR FH-causing mutations.

Methods. FH diagnosis was confirmed by genetic analysis in five subjects from the LIPIGEN Registry, who were heterozygous probands of the p.Leu22_Leu23dup PCSK9 Gain-Of-Function (GOF) mutation (group 1). Clinical, anthropometric and biochemical data of these subjects were compared with those from:

a) heterozygous FH probands (n=12) for mutations on both PCSK9 and LDLR (group 2);

b) carriers of the PCSK9 LOF R46L variant (n=41) (group 3) and non-carriers controls (group 4) (n=38).

Results. PCSK9 LOF (group 3) carriers showed a reduction in LDL-C (-8.89%), ApoB (-4.8%) but increased Body Mass Index (BMI) (+14.5%) versus controls. The same obese phenotype was observed in heterozygous FH for the mature protein secretion reducing PCSK9 mutation (group 1), despite expected elevation in ApoB and LDL-C. In fact, PCSK9 GOF carriers showed a similar phenotype of PCSK9 LOF subjects, in terms of triglycerides (120±66 vs 118±57 mg/dL, p=0.933) and BMI (30.45±3.9 vs. 28.15±3.95 kg/m², p=0.225). Finally, triglycerides and BMI of PCSK9 GOF patients were even higher compare to those from FH probands for mutation on both LDLR and PCSK9 (group 2), despite similar LDL-C and ApoB levels.

Conclusions. PCSK9 mutations causing alterations in mature protein secretion are associated with a metabolic pattern, that seems to be independent from the presence of LDLR FH-causing mutations. These data suggest the need of investigating the presence of further mechanisms involved in protein synthesis and maturation.

POST-PRANDIAL LIPEMIA AND CD36

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Aim. CD36 (Cluster of Differentiation CD36) is a membrane glycoprotein found at the surface of many cells types which binds with high affinity oxidized lipoproteins and free fatty acids (FFA). While the role of CD36 in the arterial wall during atherosclerosis has been extensively studied, on the other hand its expression at relatively high levels also in circulating cells such as leukocytes (PBMCs) is intriguing. We therefore asked a) whether CD36 expression in these cells type associates with increased fatty acids flux during post-prandial lipemia and b) if this is related to altered inflammatory pattern in these cells, modulating cellular responses.

Methods. Subjects underwent a postprandial phase study and were asked to undergo an oral lipid load. The concentration of FFA in plasma and PBMCs and changes in the expression of gene and protein expression in PBMCs during post-prandial lipemia (PP) were evaluated. In the attempt to study whether modulating CD36 expression in vivo associates with different postprandial response, the lipid load was given to carriers of an intronic variant of CD36 gene, the rs1761667, associated with reduced transcript and production of the protein.

Results. After three hours from the OFL, we confirmed significant increases in plasma triglycerides (TG), which was paralleled to the increase in TG content in triglyceride-rich lipoproteins (TGRLs) and to an increased IL-6, IL-1 β , MCP -1 and TNF- α genes expression, supporting a correlation between cellular inflammation and the PP phase. An increase of FFA in plasma and PBMCs was also observed, suggesting that the elevation of plasma lipemia is associated with an intracellular accumulation of FFA in circulating cells. Elevations in CD36 gene expression (+14±11 fold of induction) were observed in the same cells. Moreover cells isolated from the carriers of mutant allele during the PP phase, showed both a reduction in the transcript (0.44±0.29 fold of induction), in CD36 protein levels (-21%), and reduced concentration of FFA, compared to subjects with the common allele.

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

ALCOHOL PATTERN CONSUMPTION DIFFERENTLY AFFECTS THE EFFICIENCY OF MACROPHAGE REVERSE CHOLESTEROL TRANSPORT IN VIVO

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Moderate alcohol consumption is inversely correlated with cardiovascular morbidity and mortality, whereas binge consumption has the opposite effect. Cardiovascular disease can be prevented or retarded by the reverse cholesterol transport (RCT), in which excess cholesterol is removed from the macrophages of the artery wall. We aimed to evaluate whether moderate and binge consumption of alcohol differently impact RCT in vivo. RCT was measured with a standardized, radioisotope-based technique in apolipoprotein E knock out mice. Group I (n=10). received placebo, mimicking the abstainers; group II (n=10) received 0.8 g/kg alcohol/day for 28 days, mimicking a moderate intake; group III (n=10), received 0.8 g/kg alcohol/day for 5 days/week and 2.8 g/kg alcohol/day for 2 days/week, mimicking a moderate/binge intake. On day 26, mice were injected with 3H-cholesterol loaded macrophages and the radioactivity was quantified in plasma, liver and feces. Alcohol intake caused a dose-dependent increase in plasma total cholesterol (272 mg/ dl+59, 283 mg/dl+53, 374 mg/dl+86 in group I, II and III respectively). Binge consumption significantly increased plasma non-HDL cholesterol (126 mg/dl+40, 113 mg/dl+25, 165 mg/dl+65; in group I, II and III) and triglycerides (110 mg/dl+36, 88 mg/ dl+16, 136 mg/dl+30; in group I, II and III). Radioactivity in plasma (1.7%+0.6, 2.6%+1.9; 2.0%+0.5; in group I, II and III) and liver (2.4%+0.7, 4.2%+ 0.8, 3.4%+1.0 in group I, II and III) was higher in the moderate alcohol group. The elimination of radioactivity in the feces was similar in all groups: 5.3%+2.3, 4.9%+ 2.3, 4.1%+0.9; in group I, II and III. Overall, the removal of radioactivity from macrophages along the RCT pathway was higher in the moderate group: 12.2%+3.1, 15.1%+ 3.7; 13.3%+2.4; in group I, II and III respectively. Moderate alcohol consumption in mice was associated with limited impact on macrophage RCT, whereas binge consumption exerted pro-atherosclerotic effects, including the increase of total and non-HDL cholesterol and the impairment of cholesterol excretion from the body.

MALE HYPOGONADISM NEGATIVELY IMPACT ON SERUM LIPOPROTEIN FUNCTIONS RELATED TO CHOLESTEROL HOMEOSTASIS

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Background and Aim. Low testosterone levels are associated with an increased incidence of cardiovascular (CV) events, but the underlying biochemical mechanisms are not yet fully understood. The clinical condition of hypogonadism, characterised by low levels of testosterone, offers a unique model to unravel the possible role of lipoprotein-associated abnormalities in CV risk. In particular, the assessment of the functional capacities of high-density lipoproteins (HDL) may provide novel insights besides traditional risk factors. Thus, to determine whether reduced testosterone levels correlate with lipoprotein functionality, HDL cholesterol efflux capacity (CEC) and serum cholesterol loading capacity (CLC) were evaluated in a series of hypogonadal patients and control subjects. Methods. Hypogonadal men (n=19) and healthy aged matched men selected as controls (n=12) were enrolled for the study and characterized for plasma lipids with standard methods. Testosterone concentrations were measured by electrochemiluminescence immunoassay. HDL CEC was evaluated by cell-based radioisotopic techniques allowing the measurements of the single cholesterol efflux pathways. Serum CLC was assessed by fluorimetric techniques.

Results. Hypogonadism significantly reduced HDL ATP-binding cassette transporter A1 (ABCA1)- and ATP binding cassette transporter G1 (ABCG1)-mediated efflux (-21% and -45.6%, respectively) with a 14.3% decrement of total CEC. In the whole series, a positive correlation between testosterone levels and both total HDL CEC (r2=0.345, p=0.0008) and ABCG1 HDL CEC (r2=0.360, p=0.0006) was observed. Conversely, serum CLC, which was markedly raised (+44.5%) in hypogonadal patients, inversely correlated with testosterone levels (r2=0.233; p=0.008). HDL-C concentrations did not correlate with either testosterone levels or HDL CECs.

Conclusion. Altogether, these findings show that in hypogonadal patients pro-atherogenic lipoprotein-associated changes leads to reduced cholesterol efflux and increased influx. These results also offer for the first time a functional biochemical explanation for the increased CV risk in patients affected by low levels of testosterone.

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