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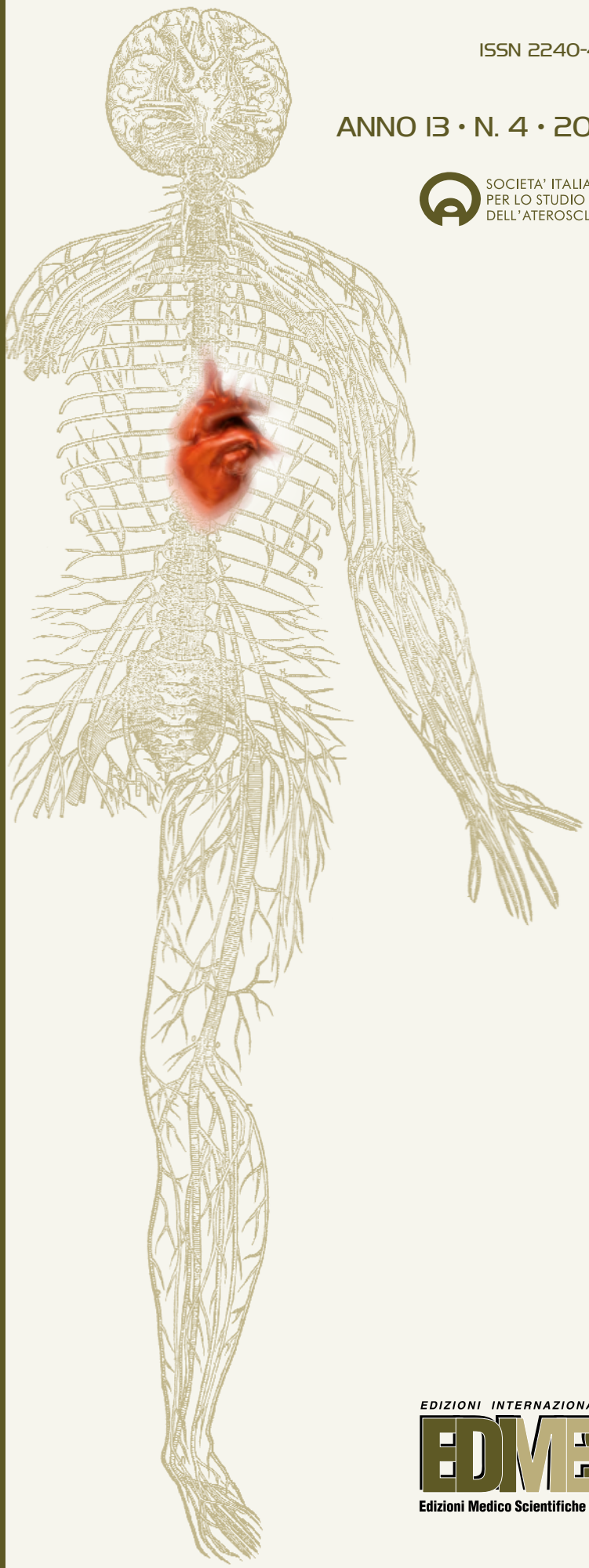

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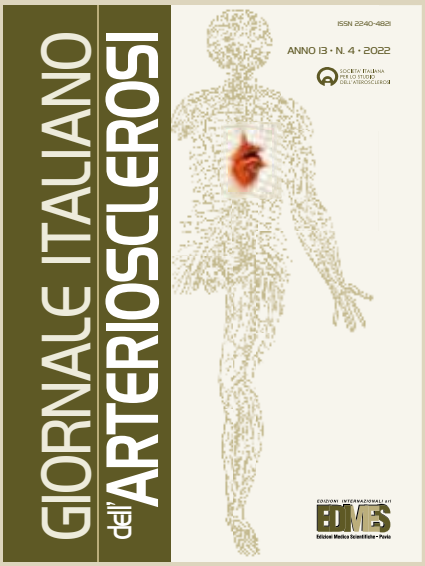

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per lo Studio dell'Aterosclerosi (SISA)

In copertina: De Humani Corporis Fabrica di Andreas Vesalius (Basilea, 1543)

Anno 13 • N. 4 • 2022

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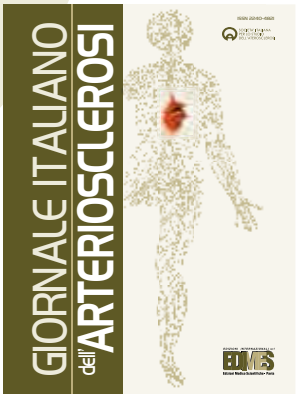

SOCIETA' ITALIANA PER LO STUDIO DELL'ATEROSCLEROSI

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Il "Giornale 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:
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b) quesiti relativi agli argomenti trattati per una verifica di auto apprendimento;
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Bibliografia
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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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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.

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PRESENTAZIONE DEL NUMERO

CLINICA

Stratificazione del rischio nei pazienti FH
Anche nei soggetti affetti da ipercolesterolemia familiare (FH) il rischio ischemico è modulato da altri fattori oltre ai livelli elevati di LDLc: la stratificazione del rischio consente di identificare tra i soggetti con fenotipo FH, quelli a rischio maggiore di eventi ischemici precoci.

Risk stratification in FH patients
Even in subjects suffering from familial hypercholesterolemia (FH) the ischemic risk is modulated by other factors in addition to the elevated LDLc levels: the risk stratification allows to identify among the subjects with the FH phenotype, those at higher risk of early ischemic events.

MECCANISMI DI MALATTIA

La PCSK9 oltre i lipidi: ruolo nello stress ossidativo e nella trombosi.
Vengono analizzate le funzioni pro-aterogene di PCSK9, indipendenti dalla regolazione dei livelli circolanti LDLc: queste suggeriscono che PCSK9 potrebbe rappresentare un nuovo biomarker di eventi cardiovascolari e i farmaci che ne inibiscono l'effetto (PCSK9-I) uno strumento idoneo a contrastare l'aterosclerosi agendo, non solo sui livelli circolanti di LDLc, ma anche su altri processi patogenetici come l'attivazione piastrinica e la formazione del trombo.

PCSK9 beyond lipids: role in oxidative stress and thrombosis
The pro-atherogenic functions of PCSK9 are analyzed, independent of the regulation of circulating LDLc: these suggest that PCSK9 could represent a new biomarker of cardiovascular events and the drugs that inhibit its effect (PCSK9-I) a suitable tool to counteract atherosclerosis by acting not only on the circulating levels of LDLc, but also on other pathogenetic processes such as platelet activation and thrombus formation.

FARMACI

L'effetto *drucebo* nei pazienti intolleranti alle statine
Nella rassegna sono riassunti i meccanismi fisiopatologici e le evidenze cliniche riguardanti la miopatia da statina al fine di fornire indicazioni pratiche sulla gestione dei pazienti con intolleranza alle statine.

The drucebo effect in statin intolerant patients
In this review the physiopathological mechanisms and clinical evidence regarding statin myopathy are summarized to provide practical information on the management of patients with statin intolerance.

Medicina, Scienza e Società

COVID-19: il virus che ama i trigliceridi e trova come nemico il fenofibrato
Con linguaggio giornalistico si analizza la relazione tra trigliceridi e mortalità da COVID-19, strizzando l'occhio al possibile effetto preventivo dei fibrati.

COVID-19: the virus that loves triglycerides and finds fenofibrate as its enemy
With journalistic writing, the relationship between triglycerides and COVID-19 mortality is analyzed, with a wink to the possible preventive effect of fibrates.

NOTIZIE DA CONGRESSI INTERNAZIONALI

Meeting annuale dell'European Society of Cardiology (ESC) 2022

Annual meeting of the European Society of Cardiology (ESC) 2022

Giornale Italiano dell'Arteriosclerosi 2022; 13 (4): 5-14

CLINICA

STRATIFICAZIONE DEL RISCHIO CARDIOVASCOLARE NEI PAZIENTI CON IPERCOLESTEROLEMIA FAMILIARE

Cardiovascular risk stratification in patients with familial hypercholesterolemia

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[3]Fondazione SISA, Milano

SUMMARY

People with familial hypercholesterolemia (FH) have an increased risk of developing cardiovascular disease (CVD), mainly due to the elevated levels of LDL cholesterol (LDL-C) since birth. However, this risk is not the same in all FH subjects and appears to be modulated by other risk factors besides LDL-C. Among these, traditional risk factors have a different predictive value in FH subjects compared to the general population, since they can underestimate the CVD risk in FH subjects, already with an inherently high baseline risk. The aim of this work was to present the current knowledge regarding parameters and methods that can help implement the stratification of CVD risk in subjects with the FH phenotype, to better identify those who need a more intensive approach early. An in-depth study of the genotype can provide important information, since it has been suggested that the monogenetic or polygenic basis of the disease may have a different impact on CVD risk, as well as the assessment of the levels of other lipid parameters such as HDL and Lp(a). Cardiovascular imaging also represents a promising tool for overcoming the obstacles represented by the stratification of cardiovascular risk in HF, including carotid intima-media thickness, coronary calcium score and coronary angiography with computed tomography. Some of these factors have already been integrated into some risk algorithms developed for FH subjects, showing interesting results even though they cannot replace clinical judgment.

Keyword: *Familial hypercholesterolemia, cardiovascular risk stratification, atherosclerosis, cardiovascular disease.*

Indirizzo per la corrispondenza
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Introduzione

L'ipercolesterolemia familiare (FH) è una malattia genetica di frequente riscontro nella pratica clinica (1, 2), caratterizzata da elevati livelli di colesterolo LDL

(LDL-C) sin dalla nascita, con conseguente insorgenza precoce dell'aterosclerosi e aumento del rischio di sviluppare malattie cardiovascolari (CVD) (3, 4).

I fattori di rischio tradizionali, quali età, sesso maschile, fumo di sigaretta, ipertensione arteriosa, livelli elevati di LDL-C e bassi di HDL-C, svolgono tutti un ruolo nei pazienti con FH, ma il loro valore predittivo è diverso da quello della popolazione generale. Sebbene il rischio cardiovascolare nei pazienti con FH sia principalmente determinato dal grado di innalzamento dei livelli di LDL-C, il rischio di malattia coronarica (*Coronary Heart Disease* – CHD) nella FH non è dovuto esclusivamente alla concentrazione plasmatica di LDL-C: la gravità e l'espressione clinica sono variabili persino all'interno della stessa famiglia, in cui tutti sono portatori dello stesso difetto genetico, che codifica per il recettore delle LDL (*LDLR*) (5). È stata riportata una significativa variabilità nell'incidenza di eventi cardiovascolari (CV) nei pazienti con FH, anche tra quelli portatori delle stesse mutazioni genetiche, con livelli comparabili di LDL-C (6, 7). Inoltre, alcuni pazienti con FH eterozigote (HeFH) possono andare incontro a eventi CV prematuri nonostante una terapia ipolipemizzante massimale, mentre altri non sviluppano CVD nonostante un marcato innalzamento di LDL-C (8). Le evidenze suggeriscono come una complessa interazione di molteplici modificatori di rischio CV, tra cui fattori ambientali e genetici, giochino un ruolo nello spiegare l'elevata eterogeneità del fenotipo clinico e la variabilità dell'incidenza di CVD nei pazienti FH, al di là dei livelli di LDL-C (9).

Gli eventi coronarici precoci sono un fenomeno accertato nei pazienti con FH, con un'età media di insorgenza dei sintomi coronarici pari a 45 anni negli uomini e 55 anni nelle donne (10). Poiché, ad eccezione dei casi più gravi, i segni fisici dell'FH non sono presenti fin dalle prime decadi di vita ma si sviluppano più tardi, definire una diagnosi in individui giovani è spesso difficile. L'età di insorgenza e la gravità della CVD nei pazienti con FH sono abbastanza variabili, perciò è difficile decidere quanto aggressivo debba essere il trattamento per prevenire la progressione dell'aterosclerosi e come monitorare tale progressione in questi pazienti. La stratificazione del rischio di aterosclerosi coronarica subclinica nei pazienti con FH consentirebbe di identificare i soggetti eleggibili per un trattamento intensivo in età più giovane. Attualmente non è disponibile un test di *screening* affidabile per prevedere l'entità e la progressione dell'aterosclerosi e degli eventi cardiovascolari maggiori nei soggetti FH asintomatici. Le attuali linee guida raccomandano di non utilizzare i classici algoritmi di rischio, come il Framingham o lo SCORE, nei pazienti FH, in quanto questi punteggi di rischio si basano su dati provenienti dalla popolazione generale e sottostimano significativamente il rischio cardiovascolare nell'arco della vita dei pazienti con FH: è stato proposto l'utilizzo di alcuni *marker* di aterosclerosi, così come la valutazione di alcuni fattori strettamente associati al rischio cardiovascolare nei soggetti FH (11).

Genotipo

In molti individui con un fenotipo clinico di FH non è possibile identificare una variante monogenica associata alla patologia (12, 13). Questi soggetti possono avere una causa poligenica, ambientale o monogenica sconosciuta di ipercolesterolemia. Rimane tuttora dibattuto il ruolo dell'indagine genetica in questi soggetti; al di là delle ripercussioni sullo *screening* familiare, è stato suggerito che la base monogenica o

poligenica abbia un effetto differente sul rischio cardiovascolare, a parità di livelli di LDL-C (14). Trinder e coll. hanno condotto uno studio di coorte utilizzando i dati dalla UK Biobank e analizzando l'associazione del genotipo con il rischio di rivascolarizzazione coronarica e carotidea, infarto miocardico, ictus ischemico e mortalità per tutte le cause tra soggetti con FH monogenica, ipercolesterolemia poligenica (>95° percentile del punteggio poligenico) o ipercolesterolemia non genetica a livelli comparabili di LDL-C. È stato riscontrato un *trend* significativo e graduale verso un maggior rischio di CVD tra i soggetti con ipercolesterolemia non genetica, ipercolesterolemia poligenica e FH monogenica (*hazard ratio* [HR] 1,26; IC95% 1,03-1,55; P=0,03). Questi risultati sono coerenti con quelli di una precedente indagine che ha rilevato l'associazione della FH monogenica con un rischio di CVD significativamente maggiore rispetto all'ipercolesterolemia poligenica, a livelli simili di LDL-C (15). Una possibile spiegazione dell'aumento del rischio è che l'ipercolesterolemia monogenica possa manifestarsi più precocemente nel corso della vita rispetto all'ipercolesterolemia poligenica, portando a una maggiore esposizione cumulativa a elevati livelli di LDL-C (16). È possibile che nel gruppo poligenico altri fattori di rischio per CHD siano meno prevalenti, per esempio livelli più elevati di lipoproteina(a) o livelli più bassi di colesterolo ad alta densità (HDL-C) (10). È anche possibile che l'ipercolesterolemia poligenica risponda meglio della FH monogenica ai farmaci ipocolesterolemizzanti (17).

Fattori lipidici

Il peso relativo dell'HDL-C nella predizione del rischio CV nei pazienti FH è piuttosto elevata rispetto ad altri punteggi predittivi del rischio (Framingham e SCORE) nella popolazione generale. Nella FH, l'LDL-C estremamente elevato porta a un'aterosclerosi subclinica precoce; è stato suggerito come la capacità di trasporto inverso del colesterolo, rappresentata dai livelli di HDL-C, diventi un fattore chiave per limitare la progressione dell'aterosclerosi e il verificarsi di eventi CVD (18). La funzionalità delle HDL definisce il grado di protezione dall'aterosclerosi: si ritiene dunque che influisca sui futuri eventi cardiovascolari. Diversi studi hanno riportato una diminuzione delle funzioni delle HDL nei pazienti con FH: in uno studio che ha analizzato 259 soggetti con FH e 208 soggetti senza FH, le dimensioni delle HDL erano significativamente più piccole nei soggetti con FH (19). Un altro studio ha analizzato i principali componenti del trasporto inverso del colesterolo in 12 soggetti con FH e 12 soggetti sani (20): le particelle HDL2 dei pazienti con FH mostravano una ridotta capacità di efflusso del colesterolo attraverso lo *scavenger receptor* SR-BI e ABCG1. Per quanto riguarda l'associazione della funzionalità delle HDL con l'aterosclerosi nei soggetti con HeFH, Ogura e coll. hanno riportato che una ridotta capacità di efflusso del colesterolo era associata a un maggior rischio di malattia cardiovascolare su base aterosclerotica (ASCVD) (21). Questi risultati suggeriscono la valutazione delle funzionalità delle HDL come potenziale misura per predire i rischi cardiovascolari aterosclerotici nei pazienti con HeFH.

La lipoproteina(a) [Lp(a)] è un fattore di rischio accertato per le malattie cardiovascolari (22) e, indipendentemente dai livelli di LDL-C, è stato costantemente riportato che il suo livello sierico è significativamente più alto nei pazienti con FH, soprattutto in quelli con un evento coronarico precoce (23). Nello studio SA-

FEHEART, che ha analizzato 1960 soggetti HeFH e 957 soggetti non FH, è emerso un aumento del livello di Lp(a) nei soggetti FH con ASCVD (24). Inoltre, il livello di Lp(a) era un predittore di ASCVD nei soggetti FH. L'aumento del rischio era indipendente dall'età, dal sesso, dallo stato di fumatore, dalle altre lipoproteine e dal tipo di mutazione nel gene *LDLR*. In particolare, livelli di Lp(a) >50 mg/dL con mutazione *LDLR* negativa (ovvero associata a una assenza quasi completa della funzionalità recettoriale) erano associati al maggior rischio cardiovascolare. Lo studio SAFEHEART ha riportato anche un'altra analisi di 2927 familiari di 755 soggetti con HeFH (25). Nel corso di un follow-up di oltre 5 anni, nei soggetti con HeFH e un livello elevato di Lp(a), il rischio cardiovascolare era maggiore, indipendentemente dai fattori di rischio convenzionali. Un recente studio ha fornito ulteriori evidenze sull'associazione della Lp(a) con la stenosi della valvola aortica nei soggetti con HeFH (26), e il ruolo predittivo di Lp(a) sugli eventi di ASCVD è stato confermato anche in una coorte giapponese di soggetti HeFH (27).

Tecniche di imaging

Sebbene esistano molti marcatori clinici e di laboratorio in grado di fornire informazioni prognostiche incrementali, il perfezionamento della stratificazione del rischio con misure di aterosclerosi coronarica subclinica in soggetti FH asintomatici sembra essere il più promettente. È importante chiarire che il ruolo della diagnostica per immagini nell'FH è molto diverso rispetto all'uso abituale nella popolazione generale. Nel contesto dell'FH, l'utilità dell'*imaging* è quella di identificare i soggetti che potrebbero essere idonei ad approcci terapeutici più aggressivi, oltre alle modifiche dello stile di vita e alla terapia con statine. Le modalità di *imaging* studiate nei pazienti con FH includono lo spessore dell'intima-media carotidea (cIMT), il punteggio del calcio coronarico (CAC) e l'angiografia con tomografia computerizzata (TC) coronarica.

Spessore dell'intima-media carotidea

Per quanto riguarda la cIMT, diversi studi clinici hanno dimostrato che le variazioni della cIMT rimangono sensibili alle variazioni dei livelli di LDL-C e che la cIMT può essere utilizzata nella valutazione della progressione dell'aterosclerosi carotidea (28). I pazienti con FH in età pediatrica presentano valori di cIMT più elevati rispetto ai controlli di pari età con livelli lipidici normali (29), il che suggerisce l'utilità di un marcatore non invasivo del rischio cardiovascolare su base aterosclerotica (ASCVD). In effetti, i bambini con FH avviati alla terapia con statine e seguiti per 20 anni hanno riportato una minore progressione della cIMT e un minor numero di eventi cardiovascolari rispetto ai loro genitori (30). Nonostante questa osservazione, non è stata dimostrata una correlazione tra la cIMT e la malattia vascolare aortica o coronarica nei pazienti con FH (31).

Punteggio del calcio coronarico

Il punteggio CAC è stato valutato nelle popolazioni FH per un'ulteriore stratificazione del rischio di ASCVD. Una metanalisi pubblicata nel 2020 (32) ha dimostrato che l'assenza di CAC, forte marcatore di basso rischio (33), è stata riscontrata comunemente tra i soggetti con FH (45% della popolazione in studio). Questa osservazione è importante se si considera che la malattia coronarica si manifesta molto più precocemente nelle persone con FH rispetto alla popolazione generale. Infatti, Miname e coll. (34) hanno seguito 206

persone con FH geneticamente determinata per 3,7 anni e hanno riscontrato che la CAC era l'unico marcatore indipendentemente associato a futuri eventi ASCVD: punteggi CAC elevati erano correlati a una maggiore incidenza di eventi cardiovascolari avversi maggiori, mentre in assenza di CAC non sono stati osservati eventi. Lo studio di Gallo e coll. (35) supporta il ruolo della CAC come strumento di stratificazione del rischio di ASCVD nella HeFH gli autori hanno seguito 1624 pazienti francesi e spagnoli con FH confermata dal test genetico per una mediana di 2,7 anni, sottoposti al test CAC: i partecipanti che hanno presentato eventi clinici hanno mostrato punteggi CAC più elevati (387 [range interquartile: 146-879] vs 8 [range interquartile: 0-109]) e una maggiore frequenza di CAC >100 (82,72% vs 26,18%); solo 3 partecipanti (3,7%) con eventi avevano un punteggio CAC pari a 0 rispetto ai 627 (40,64%) senza eventi.

Il punteggio CAC sembrerebbe dunque avere un ruolo importante nella discriminazione precoce delle forme più gravi di HeFH, nonostante l'assenza di fattori di rischio aggiuntivi tradizionali. I risultati di questa valutazione possono tradursi in un miglioramento e in un'intensificazione personalizzata della terapia ipolipemizzante. Sono necessari ulteriori studi per comprendere meglio il processo fisiopatologico alla base delle calcificazioni coronariche in questa malattia genetica e spiegarne l'elevata variabilità del fenotipo.

Angiografia con tomografia computerizzata (TC) coronarica

Infine, è stato dimostrato che la TC coronarica può contribuire a stratificare il rischio nei pazienti asintomatici con FH. La TC coronarica è una modalità di *imaging* non invasiva utile per valutare l'aterosclerosi coronarica in pazienti sintomatici e asintomatici ad alto rischio. L'esame diretto del lume vasale mediante TC ha dimostrato una capacità diagnostica paragonabile a quella dei metodi invasivi per la visualizzazione dei dettagli anatomici e del grado di stenosi del lume coronarico e per la valutazione del carico lipidico della placca (36). L'*imaging* cardiovascolare rappresenta uno strumento promettente per superare le principali sfide rappresentate dalla stratificazione del rischio cardiovascolare nella FH. Data l'espressione eterogenea del fenotipo FH, l'uso selettivo dell'*imaging* offre un metodo promettente di stratificazione, nonostante il rischio di base intrinsecamente elevato di questa popolazione.

Integrazione dei fattori in algoritmi di rischio

Gli studi pubblicati sulla FH eterozigote (HeFH) hanno suggerito diversi fattori di rischio clinici associati all'ASCVD. Tenendo conto di queste evidenze, l'*International Atherosclerosis Society* (IAS) ha recentemente proposto una definizione di FH grave (37), questa comprende tre diversi approcci basati sullo stato clinico dei pazienti: nei soggetti con HeFH senza storia di ASCVD, la diagnosi di FH grave è definita dai livelli di LDL-C senza trattamento e dal numero di fattori di rischio correlati (*Figura 1*), in quelli con aterosclerosi coronarica subclinica dalla presenza alla TC di calcificazione o di stenosi dell'arteria coronaria, mentre in quelli con storia clinica di ASCVD la FH è, per definizione, grave.

Pérez-Calahorra e coll. hanno valutato l'associazione tra FH grave e malattia cardiovascolare, trovando che la forma grave era associata alla presenza di malattie cardiovascolari (*odds ratio* [OR] 3,016; IC95% 3,136-4,257; P<0,001) (39). Uno studio

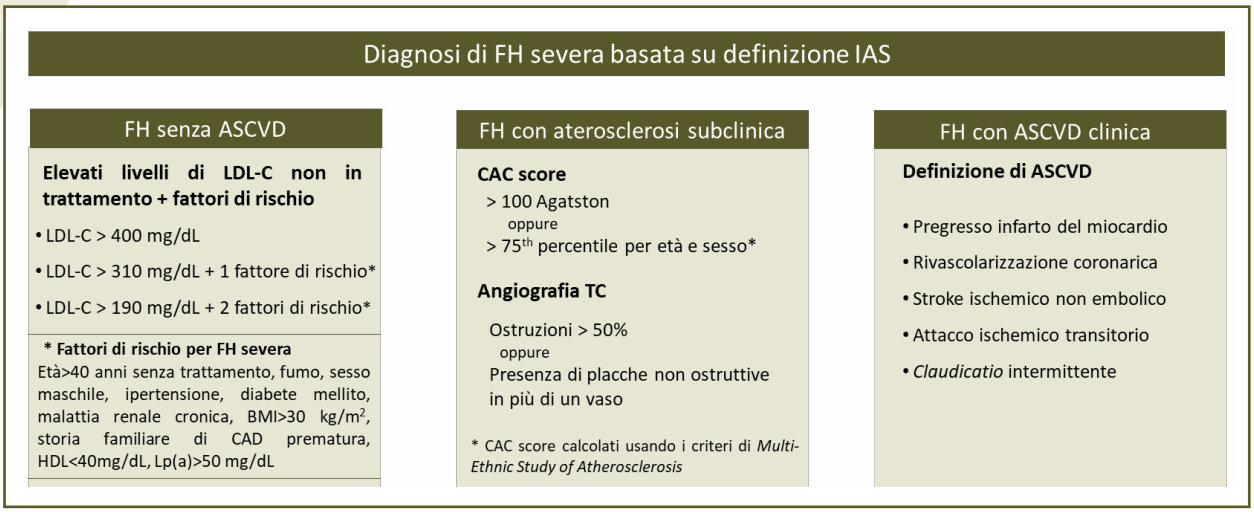

Figura 1 - Definizione di HeFH grave secondo la IAS (*modificata da* (38).

recente ha esaminato se la definizione di gravità in accordo con l'IAS potesse predire la mortalità cardiaca: i soggetti con FH grave presentavano il 64% in più di mortalità per malattia coronarica rispetto a quelli non gravi (P=0,007).

Nel 2017 è stato proposto il Montreal FH-SCORE, un calcolatore del rischio di ASCVD specifico per soggetti FH (40, 41). Lo *score* utilizza cinque fattori di rischio clinici: età, HDL-C, sesso maschile, ipertensione e fumo, arrivando a spiegare il 43,8% della variazione di frequenza delle CVD. Un valore maggiore di 20 è risultato associato a un rischio di 10,3 volte superiore di sviluppare eventi futuri rispetto valori inferiori.

Pérez de Isla e coll. hanno stabilito l'equazione di rischio SAFEHEART utilizzando il registro prospettico spagnolo (42). Questo studio ha incluso 2404 pazienti con HeFH con un *follow-up* medio di osservazione di 5,5 anni: età, sesso maschile, storia di precedenti ASCVD, ipertensione, aumento dell'indice di massa corporea, fumo, livelli di LDL-C e di Lp(a) sono risultati predittori indipendenti di futura insorgenza di ASCVD; più recentemente (35), l'aggiunta della CAC al modello ha mostrato di migliorare significativamente la predizione. Inoltre, l'aggiunta della CAC ha riclassificato il rischio in quasi la metà (45,4%) dei partecipanti allo studio: rispettivamente nel 40% e nel 50% di quelli considerati ad alto e basso rischio dal SAFEHEART. Una limitazione dell'equazione di rischio SAFEHEART è che questa coorte includeva pazienti con FH in contesti di prevenzione primaria e secondaria; un'altra limitazione è rappresentata dal fatto che il SAFEHEART includeva solo individui spagnoli e nessun'altra etnia.

L'FH-Risk SCORE (43) è stato sviluppato utilizzando un'ampia coorte prospettica multinazionale di pazienti con FH senza storia di ASCVD. Questo punteggio di rischio include sesso, età, HDL-C, LDL-C, ipertensione, fumo e livelli di Lp(a). Un FH-Risk-SCORE più alto è stato associato a una peggiore sopravvivenza libera da ASCVD a 10 anni (HR 5,52; IC95% 3,94-7,73; P<0,0001), sopravvivenza libera da eventi a 10 anni (HR 4,64; IC95% 2,66-8,11; P<0,0001) e sopravvivenza a 30 anni dovu-

ta a morte per cause cardiache (HR 10,73; IC95% 2,51-45,79; P=0,0014).

Il ruolo del diabete è stato indagato in diversi studi. Sebbene sia evidente come la sua presenza possa peggiorare il quadro di rischio CV di un paziente FH e si configuri come uno degli obiettivi del trattamento farmacologico, la sua capacità predittiva in aggiunta ad altri fattori di rischio è risultata minima, poichè la prevalenza di questa patologia è solitamente bassa nei soggetti FH (44, 45).

Conclusioni

I dati sinora raccolti suggeriscono come sia possibile dare priorità a fattori di rischio diversi dal solo valore di LDL-C nel trattamento dei pazienti con FH, poiché essi svolgono un ruolo cruciale nel perfezionamento della stratificazione del rischio cardiovascolare. Un'applicazione clinica di questo approccio potrebbe essere quella di modulare il trattamento in base al rischio nella prevenzione primaria. Naturalmente, il primo passo del trattamento farmacologico rimane l'ottimizzazione della terapia con statine in tutti i soggetti con FH, indipendentemente dal rischio di ASCVD previsto a 10 anni. Inoltre, il trattamento dovrebbe essere iniziato in giovane età, poiché l'esposizione cronica a LDL-C elevate è il fattore di rischio più importante per l'ASCVD nei pazienti con FH. Come secondo passo, i medici dovrebbero valutare e trattare altri fattori di rischio per l'ASCVD, come l'ipertensione, il diabete e il fumo, attraverso modifiche dello stile di vita o trattamenti farmacologici appropriati. Come terzo passo, i pazienti con un rischio moderato o elevato di eventi ASCVD potrebbero essere prioritariamente sottoposti a una terapia ipolipemizzante aggiuntiva. Sebbene queste stratificazioni del rischio possano essere applicate ai pazienti con FH nella pratica clinica, sono necessarie ulteriori indagini per determinare se questo approccio è in grado di migliorare gli esiti cardiovascolari della malattia. Inoltre, è importante sottolineare che l'u-

RIASSUNTO

I soggetti affetti da ipercolesterolemia familiare (FH) presentano un aumentato rischio di sviluppare malattia cardiovascolare (CVD), principalmente determinato dall'incremento dei valori di colesterolo LDL (LDL-C) fin dalla nascita. Tuttavia, questo rischio non è lo stesso in tutti i soggetti FH e pare essere modulato da altri fattori di rischio oltre al LDL-C. Tra questi, i fattori di rischio tradizionali hanno un valore predittivo differente nei soggetti FH rispetto alla popolazione generale, poiché possono sottostimare il rischio CVD in soggetti FH, già con un rischio di base intrinsecamente elevato. Scopo di questo lavoro è stato quello di presentare le conoscenze attuali riguardo a parametri e metodiche che possono contribuire ad implementare la stratificazione del rischio CVD in soggetti con fenotipo FH, per meglio identificare quelli che necessitano precocemente di un approccio più intensivo. L'approfondimento del genotipo può fornire informazioni importanti, poiché è stato suggerito come la base monogenetica o poligenica della patologia possa avere un impatto differente sul rischio CVD, così come la valutazione dei livelli di altri parametri lipidici quali HDL e Lp(a). Anche l'*imaging* cardiovascolare rappresenta uno strumento promettente per superare gli ostacoli rappresentati dalla stratificazione del rischio cardiovascolare nella FH, includendo lo spessore dell'intima-media carotidea, il punteggio del calcio coronarico e l'angiografia coronarica con tomografia computerizzata. Parte di questi fattori sono già stati integrati in alcuni algoritmi di rischio sviluppati per i soggetti FH, mostrando risultati interessanti pur non potendo sostituire il giudizio clinico.

Parole chiave: *Ipercolesterolemia familiare, stratificazione del rischio, aterosclerosi, malattia cardiovascolare.*

so degli *score* di rischio non deve sostituire il giudizio clinico, poiché molti altri fattori che non fanno parte degli algoritmi proposti possono modulare il quadro clinico del paziente, come il diabete, l'insufficienza renale, lo stile di vita, i fattori ambientali o genetici, nonché la presenza di malattie infiammatorie.

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MECCANISMI DI MALATTIA

LA PCSK9 OLTRE I LIPIDI: RUOLO NELLO STRESS OSSIDATIVO E NELLA TROMBOSI

PCSK9 beyond lipids: role in oxidative stress and thrombosis

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SUMMARY

Proprotein convertase subtilisin/kexin type 9 (PCSK9), mainly secreted in the liver, is a key regulator of cholesterol homeostasis inducing LDL receptors degradation. Beyond lipid metabolism, PCSK9 is involved in the development of atherosclerosis promoting plaque formation in mice and human, impairing the integrity of endothelial monolayer and promoting the events that induce atherosclerosis disease progression. In addition, the PCSK9 ancillary role on atherothrombosis process are widely debated. Indeed, recent evidence showed a regulatory effect of PCSK9 on redox system and platelet activation.

In particular, the role of PCSK9 in the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox2) system, of MAP-kinase cascades and of CD36 and LOX-1 downstream pathways suggests PCSK9 as a significant cofactor in the atherothrombosis development.

This evidence suggests that serum levels of PCSK9 could represent a new biomarker for the occurrence of cardiovascular events. Finally, other evidence showed that PCSK9 inhibitors, a novel pharmacological tool introduced in clinical practice in the last years, counteracted phenomena above described.

Key words: *PCSK9, platelets, thrombosis, anti-PCSK9, oxidative stress.*

Introduzione

L'Organizzazione Mondiale della Sanità (OMS) ha stimato che circa un terzo dei decessi a livello mondiale è attribuibile alle malattie cardiovascolari (CVD) e, dunque, la loro prevenzione rappresenta una delle sfide più importanti dei nostri tempi.

L'aterosclerosi è il processo anatomopatologico delle grandi arterie associato allo sviluppo di CVD. Tra i vari fattori predisponenti lo sviluppo dell'aterosclerosi, la lipoproteina a bassa densità (LDL) è l'u-

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nico a soddisfare i criteri di causalità (1). Tuttavia, il processo infiammatorio gioca un ruolo chiave nella promozione della transcitosi dell'LDL attraverso l'endotelio e della ritenzione delle stesse nella parete arteriosa (2). In questo sito, le particelle di LDL subiscono processi ossidativi enzimatici e non enzimatici che inducono la produzione di LDL ossidate (ox-LDL) (3, 4) le quali, a loro volta, esercitano un ruolo pro-ossidatante e pro-infiammatorio (2). Anche le piastrine giocano un ruolo nel processo di ossidazione delle LDL. Infatti, le piastrine, attivate dallo stimolo infiammatorio, aderiscono alle cellule dell'endotelio e amplificano il processo immuno-infiammatorio favorendo la chemiotassi delle cellule immunitarie innate (5) e il rilascio di mediatori pro-ossidativi e pro-infiammatori (6) che favoriscono la produzione di agenti pro-trombotici come le ox-LDL (7).

La proproteina convertasi subtilisina kexin tipo 9 (PCSK9), un enzima appartenente alla classe delle serin-proteasi e descritta per la prima volta nel 2003 (8), viene sintetizzata nella cellula come zimogeno solubile e convertito nella sua forma attiva dopo un processo autocatalitico nel reticolo endoplasmatico (9). La PCSK9 è un regolatore critico dell'omeostasi del colesterolo in quanto agisce come controregolatore dell'espressione dei recettori LDL (LDLR) sulla superficie cellulare (10). La PCSK9 è secreta principalmente dagli epatociti ma è espressa anche nella parete arteriosa, dove può influenzare l'emostasi locale e contribuire direttamente allo sviluppo dell'aterosclerosi (11). Data la forte correlazione tra dislipidemia e CVD, la riduzione dell'LDL-c, tramite l'inibizione dell'asse LDLR-PCSK9, contribuisce drasticamente a ridurre il rischio di CVD (12, 13).

Nell'ultimo decennio sono stati sviluppati diversi inibitori della PCSK9 (PCSK9-I) tra cui due anticorpi monoclonali umani, alirocumab ed evolocumab. I PCSK9-I legano la PCSK9 circolante, prevengono la produzione del complesso PCSK9-LDL-LDLR e il successivo turn-over dell'LDLR (14). La somministrazione di PCSK9-I, quando utilizzati in aggiunta alle statine, riduce l'LDL circolante di circa il 40-65% (15, 16). Studi interventistici di fase III hanno dimostrato che sia alirocumab che evolocumab riducono gli eventi cardiovascolari (17, 18). Negli ultimi anni sono state prodotte una serie di evidenze sui potenziali effetti ancillari dei PCSK9-I che potrebbero fornire ulteriori spiegazioni sui meccanismi alla base della protezione da CVD, in aggiunta a quanto attribuibile alla marcata riduzione delle LDL.

Ruolo dello stress ossidativo nel processo aterotrombotico

Numerose evidenze suggeriscono che lo stress ossidativo gioca un ruolo chiave nei processi di disfunzione endoteliale e nell'induzione di tutti quei meccanismi alla base della progressione delle CVD, inclusa l'attivazione piastrinica (19). Lo stress ossidativo è un fenomeno caratterizzato dallo squilibrio tra la produzione di specie reattive dell'ossigeno (ROS) e la capacità riducente dei sistemi biologici atti allo smaltimento di questi prodotti (20). Diversi sistemi enzimatici come la xantina ossidasi, le nicotinamide adenin dinucleotide fosfato (NADPH) ossidasi, le fonti mitocondriali di ROS e l'ossido nitrico sintasi (NOS) sembrano svolgere un ruolo nell'equilibrio di questo sistema (21). La NADPH ossidasi gioca un ruolo fondamentale nello stress ossidativo, come dimostrato dalla completa soppressione della produzione cellulare dei ROS nei pazienti affetti da malattia granulomatosa cronica legata al cromosoma

X (X-CGD), patologia caratterizzata dal deficit ereditario di Nox2, subunità catalitica della NADPH (22).

Nell'uomo, l'albumina e l'acido urico rappresentano le principali molecole antiossidanti circolanti (23, 24). Altri antiossidanti non enzimatici includono le vitamine E e C, il coenzima Q10 e i polifenoli che sono introdotti attraverso la dieta (25) e il glutatione (GSH), composto tiolico a basso peso molecolare sintetizzato di origine endogena (26).

L'infiammazione vascolare di basso grado e lo stress ossidativo sono fortemente implicate nella patogenesi delle CVD, intervenendo nelle varie fasi della progressione delle placche e nello sviluppo delle complicanze trombotiche (27, 28). Inoltre, anche le piastrine, oltre ad essere coinvolte nel rilascio di molecole infiammatorie e pro-trombotiche, sono in grado di produrre ROS (29). La produzione di ROS piastrinici, principalmente generati dalla NADPH ossidasi, favorisce un meccanismo di amplificazione dell'attivazione piastrinica, favorendo il rilascio di agonisti piastrinici come l'adenosina difosfato (ADP), inducendo la formazione di isoprostani e ox-LDL ed il rilascio di molecole pro-aterogene come il ligando CD40 (CD40L) (30).

Diversi studi hanno dimostrato una stretta connessione tra la produzione di ROS da parte della Nox2, un'isoforma NADPH ossidasi, e l'attivazione delle piastrine. In particolare, l'interazione tra Nox2 e l'attivazione piastrinica è stata inizialmente studiata in piastrine di pazienti con malattia X-CGD (31). In questi pazienti, affetti da un deficit genetico di NADPH ossidasi (31), è stata dimostrata una ridotta produzione e rilascio di CD40L in seguito a stimolazione piastrinica con il collagene e la trombina, oltre alla quasi completa soppressione della produzione del radicale anione superossido (O^{2-}) (32).

Le piastrine rappresentano una fonte di ROS e contribuiscono alla formazione delle ox-LDL che, a loro volta, stimolano una maggiore assorbimento di LDL da parte dei macrofagi.

Il CD36 e LOX-1 sono due recettori *scavanger* piastrinici che svolgono un ruolo importante nello sviluppo dell'aterosclerosi. CD36 è un ligando per le ox-LDL ed è in grado di innescare l'attivazione di cascate di segnali inducenti l'attivazione delle piastrine, in particolare attraverso l'espressione della P-selectina e l'attivazione dell'integrina $\alpha IIb\beta 3$ (un recettore del fibrinogeno) (33). Dunque, il legame delle ox-LDL con il CD36 è uno dei meccanismi di induzione dell'iperreattività piastrinica e gioca un ruolo cruciale nell'assunzione del fenotipo pro-trombotico da parte delle piastrine (33).

La produzione di ROS da parte del segnale delle ox-LDL mediante il CD36 richiede il coinvolgimento delle chinasi appartenenti alla famiglia Src e l'attivazione di Nox2. Non a caso tale attivazione può essere facilmente inibita in vitro con l'utilizzo degli inibitori del CD36 e Nox2 (gp91dstat) (34). Allo stesso modo, la produzione di ROS risulta inibita nei topi $Nox2^{(-/-)}$ (34), confermando il ruolo cruciale di questo enzima nel processo di attivazione (35). Il realizzarsi di tutti questi processi favorisce l'instaurarsi di un circolo vizioso di ossidazione delle LDL e l'attivazione piastrinica (36). Anche LOX1 è coinvolto nella regolazione dell'up-take di ox-LDL da parte sia delle cellule endoteliali che delle piastrine. Infine, l'espressione di LOX1 così come il CD36, e la sua interazione con le ox-LDL inducono l'aggregazione piastrinica, contribuendo alla formazione dei trombi (36).

PCSK9 e stress ossidativo

Dati recenti hanno evidenziato che PCSK9 ha funzioni pro-aterogene indi-

pendenti dal suo effetto sulla regolazione dei livelli lipidici circolanti. La PCSK9 è altamente espressa nelle cellule muscolari lisce dei vasi e nelle placche aterosclerotiche. Inoltre, la sua espressione può essere regolata da molti mediatori pro-aterogeni, inclusi i ROS prodotti dalla NADPH ossidasi, responsabili della formazione di ox-LDL (37). Le particelle di ox-LDL possono essere captate dai recettori LOX1 e CD36 e, una volta internalizzate, inducono la sovra-regolazione dell'espressione della PCSK9 perpetrando lo stimolo pro-aterogeno. Infatti, la PCSK9, tramite un meccanismo che coinvolge l'attivazione della Nox2, è in grado di stimolare la formazione di ox-LDL (34). La valutazione della relazione tra PCSK9, stress ossidativo e produzione di ox-LDL aterogene è stata esplorata tramite studi in vitro ed in vivo sia su modelli animali che sull'uomo, coinvolgendo pazienti ad alto rischio cardiovascolare.

In particolare, è stato dimostrato che le cellule endoteliali (EC) e le cellule muscolari lisce (SMC) trattate con differenti concentrazioni di proteina PCSK9 umana ricombinante (hPCSK9) mostrano un aumento della produzione di ROS in maniera dose-dipendente (38). Inoltre, la somministrazione della PCSK9 influenza l'attivazione della NADPH ossidasi migliorando l'espressione delle subunità citosoliche dell'enzima, come $p47^{phox}$ e $gp91^{phox}$ (38). Modelli murini geneticamente deficienti di $p47^{phox}$ e $gp91^{phox}$ hanno messo in luce la relazione tra PCSK9 e produzione di ROS (38, 39). In particolare, in questi modelli animali, un aumento dell'espressione del gene codificante per la PCSK9, indotto da una trasfezione plasmidica, induce un aumento della produzione di ROS nei macrofagi (39). Ancora, Ding et al. mostrano che i macrofagi dei topi $gp91^{phox-/-}$, $p47^{phox-/-}$ e $p22^{phox-/-}$ presentano livelli più bassi di ROS rispetto ai topi di tipo selvatico (WT) (38).

Recentemente, uno studio condotto su pazienti affetti da fibrillazione atriale (FA) ha evidenziato non solo un aumento dei livelli circolanti della PCSK9 e dell'attivazione della sua cascata di segnalazione tramite un meccanismo stress ossidativo dipendente, ma anche che i livelli della PCSK9 discriminano i pazienti ad aumentato rischio cardiovascolare (40) e correlano con l'incremento del tasso di generazione di ROS e di ox-LDL (41). Infine, un'analisi *post-hoc* di uno studio prospettico condotto su 907 pazienti con FA ha dimostrato che i livelli circolanti di PCSK9 e LPS si associano all'attivazione della Nox2 e ad un rischio più elevato di sviluppare eventi cardiovascolari (42).

Effetto antiossidante di PCSK9-I

Da studi recenti è emersa un'associazione tra la PCSK9 e lo stress ossidativo. Pertanto, l'inibizione della PCSK9 potrebbe rappresentare una nuova strategia per ridurre il rischio cardiovascolare associato allo stress ossidativo. Lankin et al. hanno riportato, in pazienti in prevenzione secondaria trattati con evolocumab, una riduzione della concentrazione plasmatica di ox-LDL ma nessuna influenza sull'attività degli enzimi antiossidanti, tra cui glutatione perossidasi, superossido dismutasi e catalasi (43).

Parallelamente, alirocumab, in ratti con danno epatico indotto da alcool, è stato in grado di modulare lo stress ossidativo diminuendo i prodotti di perossidazione lipidica nel fegato (43). Gli effetti antiossidanti di evolocumab durante la condizione di stress ossidativo sono stati studiati anche in vitro, in colture di cellule endoteliali di vena ombelicale umana (HUVEC), dimostrando di essere capaci di contrastare i danni causati dall'H_2O_2 in eccesso (44).

Infine, i dati emersi da uno studio multicentrico, condotto in 80 pazienti con ipercolesterolemia familiare eterozigote (HeFH), riportano che lo stress ossidativo risulta significativamente inibito da 6 mesi di trattamento con PCSK9-I (45). Infatti, sia i livelli di attivazione di Nox2 che di produzione di ox-LDL si riducevano significativamente rispetto ai valori registrati al basale (45).

Il ruolo della trombosi nella malattia cardiovascolare aterosclerotica

Quella della trombosi arteriosa è una patogenesi complessa e dinamica che origina a partire da una placca aterosclerotica lesa. La formazione di trombi consegue al rilascio di molecole pro-trombotiche, all'adesione delle piastrine alla parete vascolare e alla loro conseguente aggregazione. Questi processi, insieme all'attivazione della cascata coagulativa che è, nel sito danneggiato, responsabile della produzione di fibrina, porta alla formazione e alla crescita dei trombi.

Il ruolo delle piastrine in questo contesto fisiopatologico dipende dalla loro capacità di legare specifici recettori che vengono espressi sull'endotelio danneggiato (46), come ad esempio il legame del collagene espresso sulla superficie endoteliale lesa con lo specifico recettore piastrinico (47). Quindi, le piastrine, attraverso le loro glicoproteine di superficie, interagiscono con il collagene e il fattore di von Willebrand (vWF), cambiano forma ed aderiscono al sito della lesione. Questo evento porta alla secrezione di α-granuli che rilasciano ADP, serotonina e TxA2 portando al reclutamento di ulteriori piastrine (48). Infine, la generazione di trombina, attiva la cascata coagulativa che prende parte alla formazione di trombi (47). Una maggiore reattività piastrinica ed il conseguente aumentato rischio trombotico sono associati a condizioni patologiche, come l'aterosclerosi, ipercolesterolemia, diabete, ipertensione, fumo di sigaretta e obesità (49, 50), che possono contribuire alla crescita del trombo attraverso l'amplificazione e la propagazione dell'aggregazione piastrinica (51).

PCSK9 e attivazione piastrinica

Recentemente sono stati condotti diversi studi sperimentali, preclinici e clinici, volti a di verificare l'ipotesi che la PCSK9 circolante possa influenzare, con diversi meccanismi, l'attivazione delle piastrine. I risultati di questi studi chiariscono il ruolo della PCSK9 nel processo trombotico. In particolare, il modello animale *knock-out* per PCSK9 ($PCSK9^{-/-}$) mostra una riduzione della trombosi indotta da FeCl3 nell'arteria carotidea e della formazione di trombi non occlusivi (52). In linea con questi dati, si osserva, in un modello murino di trombosi simile al precedente, che PCSK9 induce la trombosi in vivo mentre, al contrario, il suo inibitore, l'evolocumab, inibisce tale effetto (53). Inoltre, le piastrine di topi $PCSK9^{-/-}$, rispetto alle piastrine provenienti da topi con fenotipo selvatico (Wt), mostrano una ridotta attivazione piastrinica, come testimoniato dai ridotti livelli di glicoproteina (Gp)IIb/IIIa, di sP-selectina e dalla ridotta formazione di aggregati piastrine-leucociti (52) (54).

È ben documentato che sP-selectina e CD40L sono due marcatori di attivazione piastrinica associati al rischio cardiovascolare (55). Esperimenti condotti su 30 conigli maschi affetti da dislipidemia, il trattamento con 10-deidrogingerdione, un nuovo inibitore della proteina di trasferimento dell'estere del colesterolo (CETP) in grado di sopprimere l'espressione di

PCSK9, mostrano una marcata diminuzione sia dei livelli di CD40L solubile che della sP-selectina (56). In aggiunta, la riduzione di questi marcatori correla positivamente con la soppressione dei livelli della PCSK9 (56). Queste osservazioni dimostrano che la PCSK9 potrebbe influenzare in maniera significativa l'attivazione piastrinica e gli eventi trombotici associati.

Navarese et al. hanno esaminato l'associazione tra PCSK9, aggregazione piastrinica e insorgenza di eventi cardiovascolari avversi maggiori (MACE) in pazienti con sindrome coronarica acuta (SCA), trattati con inibitori P2Y12 (prasugrel o ticagrelor) e sottoposti a intervento coronarico percutaneo (PCI) (57). I dati dello studio mostrano un aumento dei livelli della PCSK9 associato ad una maggiore reattività piastrinica ed una maggiore ricorrenza di CVD. Più in dettaglio, pazienti con livelli di PCSK9 più elevati mostrano un rischio di sviluppare eventi coronarici ricorrenti 2,62 volte maggiore rispetto a pazienti

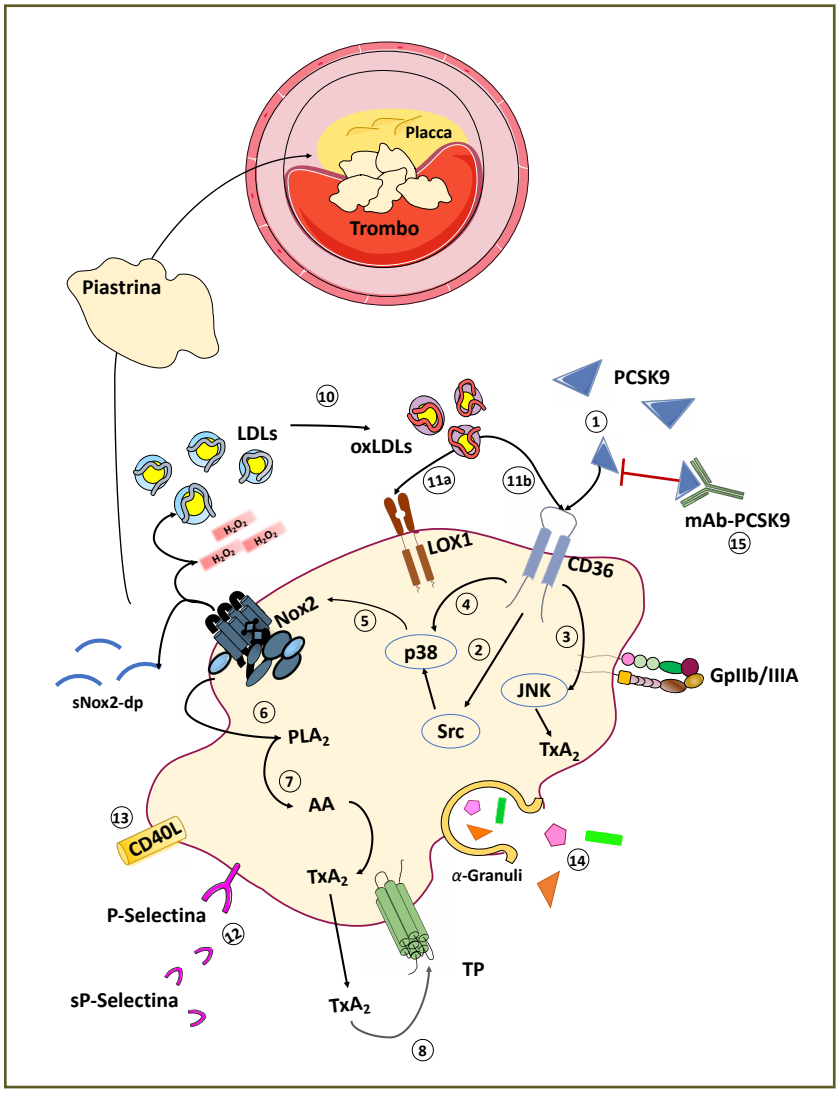

Figura 1 - Effetti di PCSK9 sull'attivazione delle piastrine.

(1) PCSK9 lega direttamente il recettore CD36 sulla superficie delle piastrine inducendo l'attivazione piastrinica e la segnalazione a valle tra cui l'attivazione delle chinasi (2) Src e (3) JNK. Inoltre, la PCSK9 aumenta la generazione di ROS mediante (4) fosforilazione di p38MAPK, (5) attivazione di Nox2, (6) fosforilazione di PLA2, (7) attivazione del metabolismo dell'AA e (8) segnalazione di TxA2. (9) La produzione di ROS mediata da Nox2 aumenta (10) la formazione di ox-LDL che amplificano l'attivazione piastrinica attraverso i recettori piastrinici (11a) LOX1 e (11b) CD36. Tutti questi eventi agiscono come segnale di amplificazione per l'attivazione piastrinica portando a (12) espressione di p-selectina, (13) espressione di CD40L e (14) rilascio del contenuto di granuli. (15) mAbs-PCSK9 inibiscono tutti questi meccanismi.

Abbreviazioni. AA: acido arachidonico; CD40L: ligando CD40; Gp: glicoproteina; H2O2: perossido di idrogeno; LDL: lipoproteine a bassa densità; ox-LDL: lipoproteine a bassa densità ossidate; PCSK9: proproteina convertasi subtilisina/kexina 9; PLA2: fosfolipasi A2; ROS: specie reattive dell'ossigeno; sNOX2-dp: peptide solubile derivato da NOX2; TP: recettore del trombossano; TxA2: trombossano A2; mAbs: anticorpi monoclonali.

con livelli plasmatici di PCSK9 più bassi (57). Questi risultati sono corroborati dagli esperimenti in cui hPCSK9, aggiunta al plasma ricco di piastrine (PRP) di soggetti sani, è in grado di aumentare significativamente l'aggregazione piastrinica e ridurre il tempo di latenza di aggregazione dopo la stimolazione delle piastrine con epinefrina (52). Allo stesso modo, l'incubazione delle piastrine con la PCSK9, a concentrazioni riscontrate nella circolazione dei pazienti con FA, aumenta l'aggregazione piastrinica, la biosintesi del TxB2 e il rilascio di sP-selectina (41), marcatori di attivazione piastrinica in vivo (58) (41). Complessivamente, questi dati rafforzano l'ipotesi per la quale l'aumento dei livelli della PCSK9 è correlato a un aumento della reattività piastrinica e di conseguenza ad un rischio aterotrombotico più elevato.

Nonostante tutte queste evidenze, l'effetto diretto della PCSK9 sulle piastrine, indipendentemente da quello generato dalla dislipidemia conseguente al suo legame con LDLR, è ancora dibattuto.

La dislipidemia induce la generazione di ox-LDL che, a loro volta, facilitano l'attivazione piastrinica legando i recettori *scavenger* quali il CD36 e LOX1 presenti sulla superficie delle piastrine (33, 59). Dunque, la PCSK9 non lega esclusivamente il LDLR ma ha, come ulteriori bersagli molecolari anche il recettore delle lipoproteine a densità molto bassa (VLDL), il CD36 e LOX1 (39). A conferma di tale dato, *in vitro,* la PCSK9 induce l'aumento dell'attivazione piastrinica, dello stress ossidativo e della fosforilazione delle proteine coinvolte nel *pathway* di attivazione della Nox2, come la p38, la p47phox e la fosfolipasi A2 (PLA2). Questi fenomeni sono amplificati dall'aggiunta di LDL esogene e attenuati dall'utilizzo dell'inibitore del CD36. Inoltre, un'analisi di co-immunoprecipitazione ha rivelato l'interazione diretta tra PCSK9 e CD36 (41). Successivamente, questi risultati sono stati confermati dal gruppo di Qi et al. che, attraverso esperimenti *in vitro* e *in vivo,* hanno dimostrato che la PCSK9 incrementa l'aggregazione piastrinica, il rilascio di ATP da parte dei granuli densi, l'attivazione dell'integrina $\alpha IIb\beta 3$ e il rilascio di P-selectina dai granuli α (53). Topi knock-out per CD36 confermano che gli effetti della PCSK9 sull'attivazione piastrinica dipendono dal CD36 (53). Quindi, sulla base di questi dati, si potrebbe affermare che la PCSK9 amplifica, tramite un legame diretto con il CD36, l'attivazione piastrinica e la trombosi, attivando le vie di segnalazione a valle (*Figura 1*)

Effetto anti-piastrinico di PCSK9-I

I PCSK9-I, dopo 6 mesi di trattamento, oltre a ridurre lo stress ossidativo, riducendo l'attivazione della Nox2 e bloccando la via dell'acido arachidonico (45), riducono l'attivazione piastrinica come dimostrato da Barale et al. in uno studio condotto su 23 pazienti con HeFH (60). Infatti, lo studio ha documentato la diminuzione dei livelli circolanti di alcuni marcatori di attivazione piastrinica, come il CD62P, il CD40L solubile, il fattore piastrinico-4 (PAF-4) e la sP-selectina (60). Questi risultati sono stati recentemente confermati da uno studio multicentrico condotto su una popolazione più ampia di pazienti con HeFH (n=88) in cui, anche in questo caso, 6 mesi di trattamento con PCSK9-I riducevano l'attivazione piastrinica e inoltre, la diminuzione del TxB2 sierico correlava positivamente con i livelli di PCSK9 circolanti (45) (*Figura 1*).

Conclusioni

Alla luce delle scoperte appena descritte è possibile affermare che la PCSK9 è una delle attrici principali nel processo aterotrombotico. PCSK9 è in grado di modu-

lare direttamente e indirettamente diversi percorsi coinvolti nella produzione di ROS e nell'attivazione delle piastrine, come rappresentato nella Figura 1.

L'introduzione di PCSK9-I potrebbe rappresentare un nuovo strumento per contrastare questo processo attraverso la riduzione sia del colesterolo LDL che dell'attivazione piastrinica e della formazione di trombi.

Tuttavia, la disponibilità di questa nuova classe di farmaci non deve far dimenticare che anche strategie di prevenzione cardiovascolare hanno mostrato effetti favorevoli sui livelli circolanti di PCSK9. Ad esempio, un'elevata aderenza alla Dieta Mediterranea è stata associata a livelli più bassi di PCSK9 (42). In particolare, l'olio extravergine di oliva, alimento ricco di composti antiossidanti, mostra un'associazione indipendente e inversa con i livelli di PCSK9 circolanti (42) suggerendo la Dieta Mediterranea, e/o la supplementazione con antiossidanti un potenziale strumento per regolare i livelli di PCSK9 e ridurre il rischio cardiovascolare.

RIASSUNTO

La proproteina convertasi subtilisina/kexina di tipo 9 (PCSK9), secreta principalmente nel fegato, è un regolatore chiave dell'omeostasi del colesterolo che induce la degradazione dei recettori bloccandone il riciclo sulla membrana del recettore per le LDL. Oltre al metabolismo lipidico, la PCSK9 è coinvolta nello sviluppo e nella progressione della malattia aterosclerotica infatti, promuove la formazione della placca sia in modelli murini che nell'uomo compromettendo l'integrità dell'endotelio.
Il ruolo della PCSK9 sul processo di aterotrombosi è ampiamente dibattuto. In effetti, prove recenti hanno mostrato un effetto regolatorio della PCSK9 sul sistema redox e sull'attivazione delle piastrine. In particolare, il ruolo della PCSK9 nell'attivazione del sistema della nicotinamide adenin dinucleotide fosfato (NADPH) ossidasi, delle MAP-chinasi e delle vie a valle di CD36 e LOX-1 suggerisce la PCSK9 come fattore importante nello sviluppo dell'aterotrombosi.
Queste evidenze indicano che il livello sierico della PCSK9 potrebbe rappresentare un nuovo predittore di eventi cardiovascolari. Infine, dati pre-clinici e clinici hanno mostrato che gli inibitori della PCSK9, introdotti nella pratica clinica negli ultimi anni, contrastano i fenomeni sopra descritti.

Parole chiave: *PCSK9, piastrine, trombosi, anti-PCSK9, stress ossidativo.*

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FARMACI

LA GESTIONE DEI PAZIENTI CON SINTOMI MUSCOLARI ASSOCIATI ALLE STATINE TRA REALE PREVALENZA ED EFFETTO "DRUCEBO"

The management of patients with muscle symptoms associated with statins between real prevalence and the "drucebo" effect

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SUMMARY

Muscle symptoms are the main adverse effects associated with statin therapy representing one of the reasons for poor adherence to treatment and failure to achieve optimal cholesterol-lowering treatment goals. The real prevalence of statin myopathy, as demonstrated by the results of randomized trials and meta-analyzes, is however much lower than perceived. In fact, statin therapies are often associated with a "drucebo" effect, i.e. an erroneous negative expectation regarding the frequency and severity of side effects. A correct diagnostic classification of patients with statin intolerance, which considers the impact of the "drucebo" effect, may allow for better therapeutic management and for reducing cardiovascular risk. This review summarizes the pathophysiological mechanisms and clinical evidence regarding statin myopathy in order to provide practical guidance on the management of patients with statin intolerance.

Key Words: *Statin, myopathy, SAMS, drucebo effect, cholesterol-lowering therapy.*

Introduzione

L'ipercolesterolemia è un fattore causale della progressione delle malattie cardiovascolari aterosclerotiche e l'utilizzo di farmaci ipocolesterolemizzanti è un elemento imprescindibile delle terapie volte alla riduzione del rischio cardiovascolare. Gli effetti avversi delle statine rappresentano uno dei principali motivi della mancata aderenza e del mancato raggiungimento degli obiettivi terapeutici. Gli effetti collaterali più frequenti, e motivo principale

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di sospensione della terapia con statina, sono rappresentati dai sintomi muscolari (Statin Associated Muscle Symptoms - SAMS) (1).

Gran parte dei sintomi muscolari riferiti dai pazienti in corso di terapia con statina può essere attribuita ad una sproporzionata aspettativa negativa da parte dei pazienti, in relazione alla frequenza e alla gravità di tali sintomi. Questa dispercezione è determinata dall'effetto "nocebo" delle statine o come è stato definito in termini semanticamente più corretti effetto "drucebo negativo", ovvero l'effetto avverso causato dall'aspettativa negativa correlata all'assunzione di un farmaco (2). L'intolleranza alle statine e la conseguente sospensione della terapia rappresentano un problema clinico globale di complessa gestione. I sintomi muscolari che si presentano in corso di terapia con statine possono infatti avere molte cause differenti. L'errata attribuzione di tali sintomi alla terapia con statine è molto frequente e si associa ad un aumento del rischio di eventi cardiovascolari e di mortalità (3). Una metanalisi recentemente pubblicata ha valutato per la prima volta la prevalenza globale dell'intolleranza alle statine sulla base dei dati di oltre 4 milioni di pazienti, dimostrando che essa è relativamente bassa (9.1%), quando determinata utilizzando le definizioni internazionali riconosciute, e risulta spesso sovrastimata (4).

L'obiettivo del presente lavoro è quello di riassumere i meccanismi fisiopatologici della miopatia da statina, passare in rassegna le evidenze cliniche riguardanti tale evento avverso e fornire indicazioni pratiche sulla gestione dei pazienti con intolleranza alle statine. Limitare l'inappropriata sospensione della terapia, cercando di identificare i soggetti con vera intolleranza alle statine può consentire la massima riduzione del rischio cardiovascolare e contribuire ad ottenere la migliore gestione del trattamento in termini di costo/efficacia.

Elenco degli argomenti trattati

- Meccanismi fisiopatologici e fattori di rischio della miopatia da statine
- Il concetto di effetto "drucebo" e il suo impatto clinico nella terapia con statine
- Strategie diagnostico-terapeutiche per i pazienti con sintomi muscolari associati alle statine

Miopatia da statina: definizione, fattori di rischio e meccanismi fisiopatologici

Definizione

I SAMS (Statin Associated Muscle Symptoms) comprendono una vasta gamma di effetti avversi che vanno dalla mialgia senza incremento della creatinkinasi (CK) fino alla rara rabdomiolisi. La mialgia è generalmente caratterizzata da una sensazione di debolezza o indolenzimento simmetrica a carico dei muscoli prossimali (5).

La rabdomiolisi da statine è un evento avverso raro, ma particolarmente grave, e si associa ad un marcato rialzo dei livelli di CK (i.e. spesso oltre 40 volte la norma) e al riscontro di insufficienza renale con mioglobinuria.

Fattori di rischio

Sono stati messi in evidenza numerosi fattori che possono predisporre all'insorgenza di SAMS. Alcuni di essi sono strettamente legati a caratteristiche proprie del paziente, quali sesso, età, etnia, fattori genetici e patologie concomitanti, quali l'ipotiroidismo non adeguatamente trattato, il diabete, l'insufficienza renale (6).

Tra i fattori di rischio esogeni, legati alla esposizione ad elementi esterni al pazien-

Tabella I - Fattori di rischio che predispongono all'insorgenza di SAMS (statin-associated muscle symptoms).

Fattori di Rischio Endogeni	Fattori di Rischio Esogeni
Età avanzata *Sesso femminile* *Etnia asiatica* *Massa corporea ridotta* *Anamnesi positiva per dolore muscolare e/o articolare* *Anamnesi positiva per incremento di CK* (soprattutto se CK>10 ULN) *Anamnesi familiare positiva per miopatia* *Miopatia indotta da statine o altri farmaci ipolipemizzanti* *Patologie neuromuscolari di natura infiammatoria o metabolica* Atrofia muscolare spinobulbare Deficit di alfa-1,4- glucosidasi acida o maltasi acida Deficit di carnitina palmitoil transferasi II Dermatomiosite Distrofia miotonica di tipo I e II Ipotermia maligna Malattia di McCardle Miastenia grave Mioglobinuria ricorrente Miopatia mitocondriale Miopatia necrotizzante Miosite da corpi di inclusione Neuropatia periferica Polimiosite Sclerosi laterale amiotrofica *Insufficienza renale grave* (III-V stadio KDOQI) *Epatopatia acuta o scompensata* *Ipertensione/scompenso cardiaco* (secondari a patologie renali) *Ipotiroidismo non trattato o sottotrattato* *Diabete mellito* *Infezione acuta* *Ostruzione vie biliari* *Traumi gravi con elevata richiesta metabolica* *Deficit vitamina D* *Polimorfismi genetici* Isoenzimi del citocromo P Mutazione Lipin-1 Polimorfismo del trasportatore ABC Variante del gene RYR Variante del gene SLCO1B1	*Esercizio fisico intenso e/o non abituale* *Elevata posologia della terapia ipolipemizzante con statine* *Abuso di alcool* *Abuso di sostanze stupefacenti (es. cocaina, anfetamina, eroina)* *Interazione farmacologica* Acido nicotinico Amiodarone Azoli Farmaci antipsicotici Farmaci immunosoppressori come la ciclosporina Fibrati tra cui soprattutto il gemfibrozil Inibitori delle proteasi Macrolidi Nefazodone Verapamil Warfarin *Consumo di succo di pompelmo o mirtillo (>1 litro/die circa)* *Integratori non regolamentati (es. lievito di riso rosso, funghi pleurotus, ecc.)* *Interventi chirurgici*

ABC, ATP-binding cassette; CK, creatine kinase; KDOQI, kidney disease outcomes quality initiative; RYR, recettore della rianodina; SLCO1B1, solute carrier organic anion transporter family member 1B1; ULN, upper limit of normal.

te, si annoverano il consumo di alcol o droghe, l'esercizio fisico e terapie concomitanti che possono esacerbare o scatenare i SAMS (7). Il dosaggio, le caratteristiche farmacocinetiche delle diverse statine e le interazioni farmacologiche sono elementi che condizionano fortemente il rischio di sviluppo di SAMS (8). I fattori di rischio più comunemente associati ai SAMS sono elencati in *Tabella 1.*

Meccanismi fisiopatologici

Sono stati ipotizzati numerosi meccanismi alla base del danno muscolare indotto da statine. L'inibizione dell'idrossimetilglutaril-CoA-reduttasi (HMGCoAR) interferisce con il metabolismo del mevalonato e di altre molecole tra cui il farnesil-pirofosfato. Quest'ultimo è implicato nella prenilazione di alcune proteine come il coenzima Q10 che riveste un ruolo importante del metabolismo energetico mitocondriale e nella stabilizzazione delle membrane cellulari. Molti studi hanno dimostrato che le statine, riducendo i livelli di coenzima Q10, causano un danno muscolare interferendo con la funzionalità mitocondriale e con il rilascio del calcio dal reticolo sarcoplasmatico, provocando così l'attivazione delle caspasi con l'induzione dell'apoptosi (9) (*Figura 1A*).

È stato descritto anche un danno muscolare immunomediato da statine. In individui immunologicamente suscettibili, le statine possono indurre la sintesi autoanticorpi diretti contro HMGCoAR, associandosi ad una miosite necrotizzante che non sempre migliora con la sospensione della statina, ma che necessita di terapia steroidea (10) (*Figura 1B*).

La riduzione della colesterolemia indotta dalle statine è stata associata ad alterazioni ultrastrutturali dei miociti e ad un aumento della quantità di steroli all'interno degli stessi, per l'iperespressione dei recettori per le LDL-C indotta dal trattamento ipocolesterolemizzante (11) (*Figura 1C*).

È stato osservato che alterazioni genetiche dei trasportatori ATP-binding cassette (ABC), coinvolti nell'efflusso di colesterolo e delle statine, possono alterare le proprietà farmacocinetiche e farmacodinamiche delle statine (12). Una mutazione con perdita di funzione del gene SLCO1B1, che codifica per un trasportatore epatico delle statine, implica un aumento della concentrazione plasmatica delle statine con conseguente aumento del rischio di SAMS (13).

Alcuni polimorfismi del citocromo P450 predispongono allo sviluppo di SAMS essendo associati a elevati livelli plasmatici delle statine. Le diverse statine differiscono tra loro riguardo alla via metabolica che subiscono: lovastatina, simvastatina e atorvastatina vengono metabolizzate soprattutto dal CYP3A4; fluvastatina è metabolizzata principalmente da CYP2C9 e, in minor misura, da CYP3A4; rosuvastatina viene eliminata per lo più per via biliare ed in minor misura da CYP2C9 e CYP2C19; pravastatina viene eliminata principalmente mediante solfatazione. Il CYP3A4 metabolizza anche molti farmaci tra cui antifungini azolici, macrolidi, inibitori delle proteasi, antidepressivi triciclici, amiodarone, warfarin e ciclosporina. L'inibizione del CYP3A4 indotta da questi farmaci favorisce un incremento del rischio di effetti avversi da statina (14) (*Figura 1D*).

È stato ipotizzato un ruolo nel danno muscolare indotto da statine da parte dell'atrogina 1, una proteina coinvolta nel catabolismo muscolare. Un suo incremento indotto dall'assunzione del farmaco si riflette in un maggior catabolismo muscolare (15) (*Figura 1 E*).

Inoltre, un'alterata omeostasi del calcio potrebbe contribuire al danno indotto da statine. Varianti genetiche del RyR (recet-

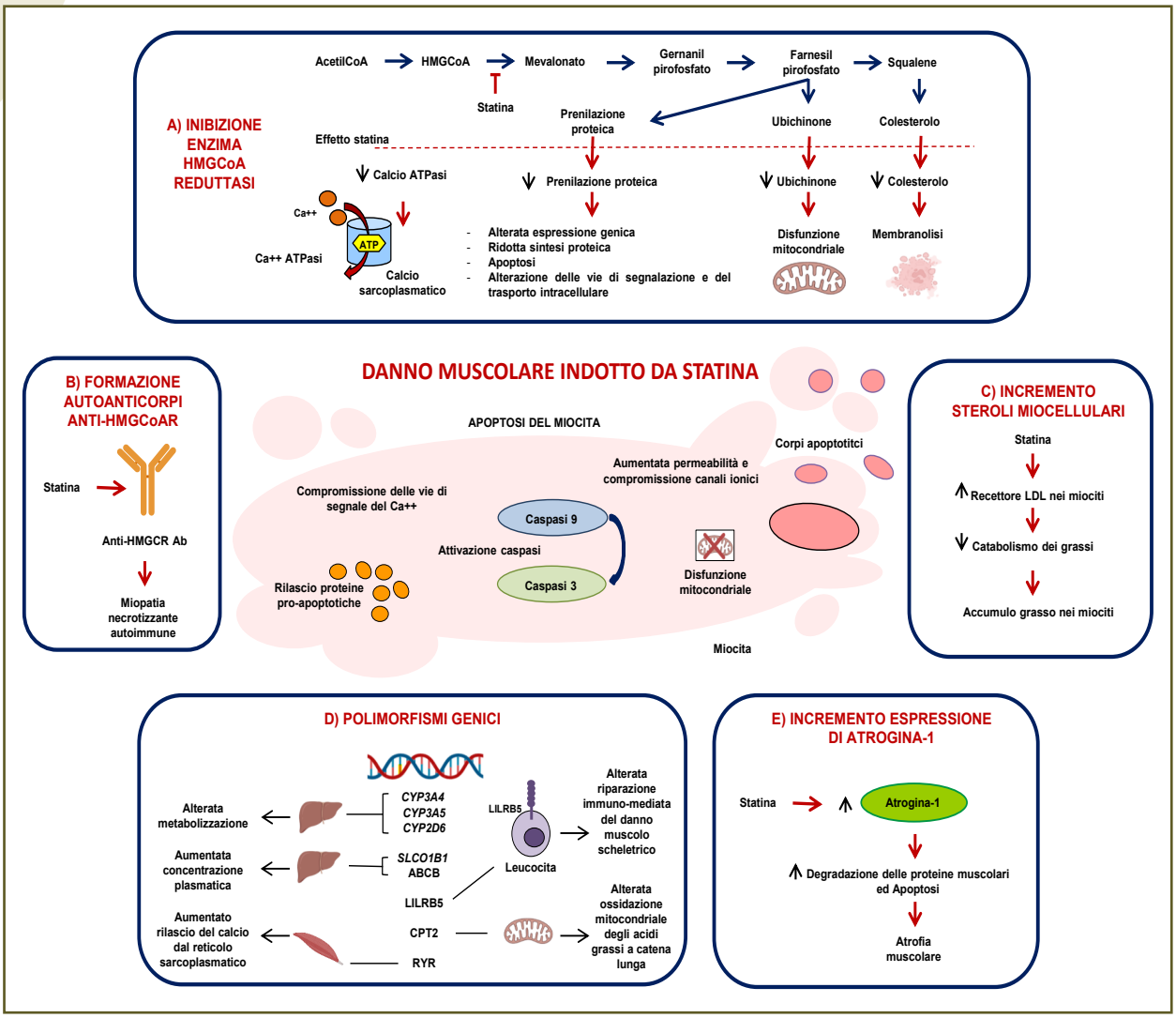

Figura 1 - Apoptosi del miocita e meccanismi fisiopatologici alla base del danno muscolare indotto dalle statine.

A) Inibizione enzima HMGCoA-R (idrossi-metil-glutaril-coenzimaA-reduttasi). L'inibizione statino-indotta dell'enzima HMGCoA-R determina un blocco del metabolismo del mevalonato con conseguente alterazione della funzione mitocondriale e della stabilizzazione delle membrane cellulari. *B) Formazione autoanticorpi anti-HMGCoA-R.* Il trattamento con statine può portare alla produzione di anticorpi anti-HMGCR, con possibile effetto patogeno diretto sul tessuto muscolare, soprattutto in soggetti geneticamente predisposti. *C) Incremento degli steroli miocellulari.* L'iperespressione dei recettori per le LDL (low density lipoprotein) indotta da statina determina un aumento della quantità di steroli all'interno dei miociti. *D) Polimorfismi genici.* Varianti dei geni che codificano per gli enzimi metabolici del citocromo P (CYP) 450; variante con perdita di funzione del gene SLCO1B1 (solute carrier organic anion transporter family member 1B1); varianti dei geni dei trasportatori ABC (ATP-binding cassette); mutazioni di LILRB5 (leukocyte immunoglobulin-like receptor subfamily B member 5); mutazione di CPT (carnitina-palmitoil transferasi); mutazione di RYR (recettore della rianodina). *E) Incrementata espressione di atrogina-1.* L'atrogina-1, proteina coinvolta nel catabolismo muscolare, viene stimolata positivamente dalle statine.

I meccanismi sopra riportati di danno muscolare mediati dalle statine, determinano apoptosi del miocita attraverso compromissione delle vie di segnale del Ca++, attivazione di pathway proapoptotici, aumentata permeabilità e compromissione di canali ionici, con disfunzione mitocondriale ed attivazione della caspasi 9 e caspasi 3.

tore della rianodina), coinvolto nel rilascio di calcio dal reticolo sarcoplasmatico, che ne inducono un'iperespressione, si associano ad un maggior rischio di danno muscolare (16) (*Figura 1 D*).

Ci sono altre evidenze che correlano il danno mitocondriale con la miopatia da statina. Ad esempio, varianti genetiche che provocano una ridotta espressione di CPT2 (carnitina-palmitoil transferasi 2), enzima coinvolto nell'ossidazione degli acidi grassi a lunga catena nei mitocondri, si associano ad un maggior rischio di sviluppo di SAMS (17).

L'effetto drucebo nella miopatia da statine

Il concetto di effetto "drucebo"

I sintomi muscolari che si presentano in corso di terapia con statine possono avere molteplici cause non necessariamente riconducibili all'effetto del farmaco. Oltre all'errata attribuzione alle statine di eventi avversi derivanti da altre cause, una considerevole percentuale di effetti collaterali può derivare dall'azione stessa di assumere il farmaco e dall'aspettativa che possa causare effetti collaterali. Nei *trial* con statine è stato infatti osservato spesso che viene riportato un maggior numero di eventi avversi negli studi in aperto rispetto a quelli in cieco.

L'effetto placebo descrive un'azione positiva che deriva dall'aspettativa che una sostanza inerte faccia bene, mentre l'effetto nocebo si riferisce al danno che deriva dall'aspettativa che una sostanza inerte arrechi danno. Benché tali termini (e.g placebo, nocebo) siano ancora comunemente applicati ai benefici e agli effetti collaterali dei farmaci (il dolore muscolare in caso di terapia con statine è spesso attribuito ad un effetto "nocebo"), poiché i farmaci per definizione non sono sostanze inerti, la terminologia effetto "placebo/nocebo" non è semanticamente corretta quando attribuita a farmaci. Di recente, l'International Lipid Expert Panel ha introdotto il concetto di effetto "drucebo" (DRUg + plaCEBO), che confronta l'intensità dei sintomi quando si utilizza un farmaco in condizioni di cieco e in condizioni *open-label*, fornendo una visione quantitativa della misura in cui i sintomi possono derivare dalla sola aspettativa. Gli effetti benefici causati dall'aspettativa piuttosto che dall'azione farmacologica del principio attivo (analoga a quella del placebo), sono definiti "effetto drucebo positivo", mentre gli effetti collaterali attesi (analoghi al nocebo) sono definiti "effetto drucebo negativo".

Per valutare il contributo dell'effetto "drucebo" all'interruzione delle statine e ai sintomi muscolari indotti da statine è stata effettuata una revisione sistematica di studi randomizzati e controllati sulla terapia con statine. Sono stati inclusi gli RCT che permettevano di quantificare l'effetto "drucebo" confrontando l'incidenza di sintomi muscolari rilevati tra le fasi in cieco e quelle "open-label" dei trial stessi. In questo modo sono stati selezionati cinque studi: da tutti è emerso un eccesso di effetti collaterali in condizioni di "open-label", con contributo dell'effetto "drucebo" al dolore muscolare associato alle statine tra il 38% e il 78% (18).

L'effetto "drucebo" delle statine nei trial clinici

Una recente metanalisi ha incluso gli RCT che hanno reclutato oltre 1.000 partecipanti, per una durata di trattamento prevista di almeno 2 anni e che hanno confrontato in doppio cieco terapia con statina *vs* placebo (19 studi, n=123.940) o regime statinico più intensivo *vs* meno intensivo (4 studi, n=30.724).

Nei 19 studi controllati con placebo, durante un *follow-up* medio ponderato di

4.3 anni, 16.835 (27.1%) dei pazienti assegnati alla statina rispetto a 16.446 (26.6%) di quelli assegnati al placebo hanno riferito dolore o debolezza muscolare (rate ratio [RR] 1.03; 95% CI 1.01-1.06). Durante il primo anno, la terapia con statine ha prodotto un aumento relativo del 7% di dolore o debolezza muscolare (1.07; 1.04-1.10), corrispondente a un eccesso assoluto di 11 eventi per 1000 anni-persona. Ne consegue che solo 1 su 15 di queste segnalazioni di sintomi muscolari da parte dei partecipanti assegnati alla terapia con statine era effettivamente dovuta al farmaco. Inoltre, dopo il primo anno non si è registrato un incremento significativo di prime segnalazioni di dolore o debolezza muscolare. Regimi di statine più intensivi (atorvastatina 40-80 mg o rosuvastatina 20-40 mg) hanno prodotto un RR di sintomi muscolari più elevato rispetto a regimi meno intensivi confrontati con placebo, con un modesto eccesso (1.05) nei regimi più intensivi dopo il primo anno. La terapia con statine ha prodotto un modesto aumento dei valori mediani di CK (0.02 x ULN), clinicamente non significativo. Il trattamento ha quindi causato un modesto eccesso di dolore muscolare, per lo più lieve, ma oltre il 90% di tutte le segnalazioni di sintomi muscolari da parte dei partecipanti in terapia con statine non era attribuibile ad un reale effetto avverso del farmaco (19).

Lo studio SAMSON (Self-Assessment Method for Statin Side-effects Or Nocebo) ha reclutato 60 pazienti che avevano da poco smesso di assumere statine a causa di effetti collaterali (prevalentemente, ma non esclusivamente SAMS). Nel corso di 12 mesi i partecipanti hanno alternato, in ordine casuale, periodi di 1 mese di statina, placebo o assenza di trattamento, riferendo ogni giorno l'intensità dei loro sintomi. L'innovativa inclusione di un periodo di assenza di trattamento ha permesso di stimare realmente l'effetto nocebo, ovvero la differenza nell'intensità dei sintomi tra l'assenza di trattamento e l'assunzione di una compressa di placebo inerte: la gravità dei sintomi era simile nei periodi di utilizzo di statine e placebo, ma significativamente minore nei periodi di assenza di trattamento. In altre parole, i sintomi derivavano dall'azione stessa di assumere delle compresse, non da effetti farmacologici della statina.

La maggior parte dei sintomi causati dalle compresse di statine erano dunque ascrivibili ad un effetto "drucebo" negativo. I risultati di tale studio suggeriscono inoltre che né l'intensità, né il *timing di onset/offset* dei sintomi rispetto all'assunzione delle compresse dovrebbe essere interpretato come indice di causalità farmacologica, poiché i sintomi tendono a presentarsi con lo stesso andamento temporale sia con il farmaco che con il placebo. È interessante sottolineare che sei mesi dopo la conclusione dello studio, oltre la metà dei partecipanti aveva ripreso la terapia con statine o era intenzionata a farlo (20).

Lo studio statinWISE ha arruolato 200 pazienti che avevano interrotto o stavano considerando di interrompere la terapia con statine, randomizzati ad assumere atorvastatina o placebo, per periodi alternati di due mesi, per un totale di un anno. Non è stata osservata alcuna differenza tra la gravità degli effetti collaterali durante i periodi di terapia con statine o con placebo. Due terzi dei partecipanti sono stati in grado di riprendere la terapia con statine (21).

La gestione terapeutica del paziente con intolleranza alle statine

Di fronte alla sfida terapeutica rappresentata dai pazienti con intolleranza alle statine bisogna avere la consapevolezza

che la maggior parte di loro potrebbe essere in grado di tollerare almeno alcuni regimi terapeutici che includono statine. Trovare un regime accettabile per il paziente può richiedere la sostituzione con una statina differente, la riduzione del dosaggio o, in casi particolari, l'uso di regimi posologici alternativi come l'assunzione a giorni alterni (*Figura 2*).

Tuttavia, per alcuni pazienti persisterà l'impossibilità di tollerare o la mancata volontà di assumere una qualsiasi statina. Altre terapie farmacologiche risultano pertanto spesso necessarie in aggiunta o in alternativa alla statina al fine di raggiungere gli obiettivi terapeutici. Resta fondamentale l'aderenza a modifiche dello stile di vita, quali l'adozione di un modello alimentare

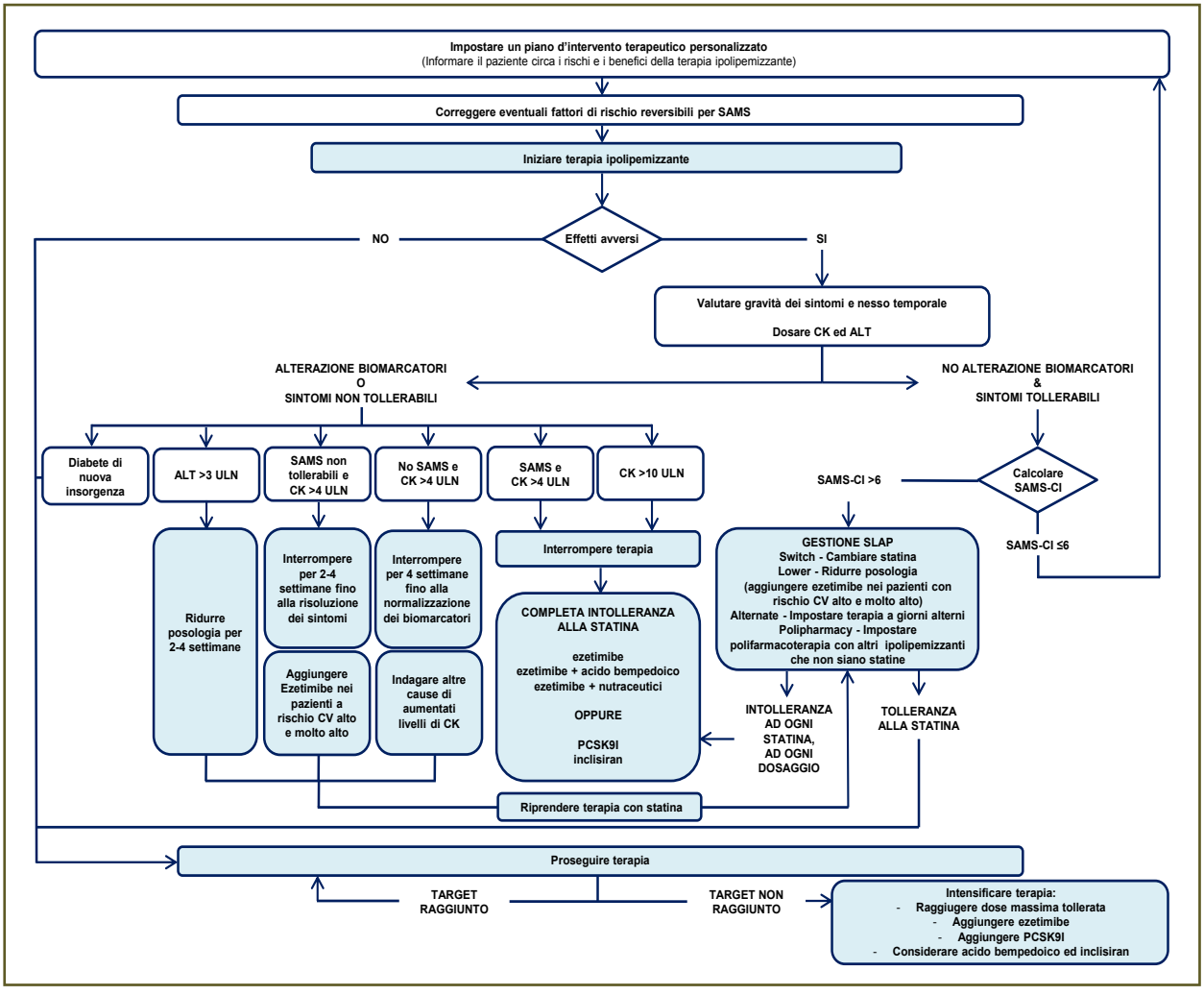

Figura 2 - Algoritmo per la gestione del paziente con intolleranza alla terapia ipolipemizzante con statina (modificato da Penson et al. (2))

ALT, alanine aminotransferase; CK, creatine kinase; CV, cardiovascular; PCSK9I, proprotein convertase subtilisin kexin type 9 inhibitors; SAMS-CI, statin-associated muscle symptoms-clinical index; SAMS, statin-associated muscle symptoms; SLAP, Switch statin, Lower dose, Alternate days, Polypharmacy with non-statin therapy; ULN, upper limit of normal.

sano, l'attività fisica regolare, perdita di peso in caso di sovrappeso o obesità e cessazione del fumo (2).

Le strategie per la gestione terapeutica dell'intolleranza parziale alle statine possono essere sintetizzate nell'acronimo SLAP (Switch, Lower dose, Alternate dosing, Polypharmacy; vedi paragrafo 4.4) (2).

La prevenzione dell'intolleranza alle statine

Sebbene la gestione degli eventi avversi associati a statine sia solitamente reattiva, i dati a disposizione sul loro effetto "drucebo" negativo suggeriscono di adottare un approccio proattivo mirato a prevenirne la comparsa e ridurre la probabilità di attribuire erroneamente alla terapia sintomi muscolari di altra origine.

I pazienti devono anzitutto essere informati sul razionale e sui benefici della terapia, così da poter prendere decisioni consapevoli sul rapporto rischio/beneficio delle eventuali alternative terapeutiche in caso di comparsa di eventi avversi. È necessario fornire al paziente una chiara spiegazione sulla reale probabilità di eventi avversi, nonché dettagli sul proprio obiettivo di LDL-C in relazione al rischio cardiovascolare stimato. Ciò risulta anche potenzialmente utile per migliorare la *compliance* terapeutica a lungo termine (2). Se esistono fattori reversibili noti che predispongono a SAMS, questi devono essere discussi con il paziente e possibilmente corretti prima di intraprendere una terapia con statine (22). L'esercizio fisico, comunemente prescritto tra le modifiche dello stile di vita, può essere associato a dolore muscolare e CK elevata. I pazienti devono essere informati del rischio di sviluppare disturbi muscolari associati all'esercizio e possibilmente svolgere programmi di esercizio personalizzati per minimizzare il rischio di lesioni dovute a sforzi inappropriati, specie per i pazienti in precedenza sedentari. L'ipotiroidismo predispone a SAMS e deve essere escluso o corretto prima di iniziare una statina (23, 24).

La polifarmacoterapia è molto comune nei pazienti trattati con statine e il rischio di interazioni è elevato (25). Nel caso si tratti di terapie a lungo termine potrebbe essere necessario preferire farmaci alternativi. Nel caso di brevi cicli terapeutici è consigliabile attenderne il termine prima di iniziare una statina (23).

Nei pazienti con un'anamnesi familiare di intolleranza alle statine e in quelli ad alto rischio di intolleranza alle statine (pazienti anziani, donne di aspetto minuto con superficie corporea ridotta, compromissione epatica/renale o polifarmacoterapia), si può prendere in considerazione l'inizio di una terapia combinata con una bassa dose di statina associata ad ezetimibe (2).

L'approccio iniziale al paziente con intolleranza alle statine

Quando i pazienti presentano sintomi che suggeriscono un'intolleranza alle statine, è necessario innanzitutto raccogliere un'anamnesi dettagliata dei sintomi e misurare i *biomarker* di danno muscolare (compresi ALT e CK). L'epoca di insorgenza e la remissione dei SAMS in relazione all'inizio ed alla sospensione della terapia, nonché le caratteristiche qualitative degli stessi, sono elementi spesso utilizzati per l'eventuale attribuzione dei sintomi all'effetto del farmaco. Si stima che oltre il 75% dei SAMS compaia entro le prime 12 settimane di trattamento e che il 90% si manifesti entro 6 mesi (23). È dunque improbabile che i sintomi che emergono dopo una durata maggiore della terapia siano causati dalle statine, a meno che non siano precipitati da un'interazione farmacologica o da qualche altro evento concomitante come un peggiora-

mento della funzionalità tiroidea, renale e/o epatica.

A causa della latenza dell'insorgenza e della cessazione degli effetti del farmaco, è inoltre improbabile che eventi avversi che si verificano immediatamente dopo l'inizio della terapia, o che si risolvono immediatamente dopo la sospensione, siano causati dalle statine (26).

Stimare la gravità dei sintomi che causano l'intolleranza alle statine permette al medico di adottare un approccio centrato sul paziente, bilanciando i potenziali benefici del trattamento con gli eventuali disagi associati. In caso di sintomi lievi e tollerabili, è utile tentare di motivare i pazienti a continuare la terapia ribadendone i comprovati benefici, poiché frequentemente la mialgia scompare dopo poche settimane (27).

Se non è possibile ottenere una riduzione ottimale dei lipidi a causa dell'intolleranza alle statine, le modifiche allo stile di vita e i nutraceutici possono essere utili per abbassare ulteriormente l'LDL-C. Una dieta ben bilanciata può ridurre l'LDL-C di >10%, l'esercizio fisico regolare del 5-7% e la perdita di peso dell'8-10% (28).

Un'ampia gamma di nutraceutici ha dimostrato effetti ipolipemizzanti ed è stata proposta per l'uso nell'intolleranza alle statine. Tra questi spicca per efficacia ipocolesterolemizzante il lievito di riso rosso, che può consentire una riduzione di LDL-C del 15-25%. Tuttavia, sono occasionalmente osservati effetti avversi muscolari associati all'assunzione di monacolina K. Va sottolineato che molti nutraceutici hanno effetti pleiotropici aggiuntivi antinfiammatori e antiossidanti, benefici sulla rigidità arteriosa e sulla funzione endoteliale, che potrebbero essere utili nella prevenzione delle malattie cardiovascolari (29).

Al fine di limitare nel tempo un'interruzione non necessaria della terapia con statine, una sospensione per 4-6 settimane (*dechallenge*), seguita dalla ripresa (*rechallenge*), può essere molto utile per determinare la causalità degli eventi avversi e limitare i danni al paziente. Tale strategia consente a 2/3 dei pazienti di riprendere la terapia con statine dopo SAMS (30). Tuttavia, quando possibile, la terapia con statine deve essere continuata (anche a dosi più basse o ricorrendo alla somministrazione a giorni alterni), per evitare interruzioni ingiustificate potenzialmente associate ad *outcome* sfavorevoli (31,32). Laddove la statina venga sospesa, una terapia farmacologica alternativa dovrebbe essere intrapresa immediatamente, soprattutto nei pazienti ad alto rischio cardiovascolare (33).

Tollerabilità dei sintomi muscolari e alterazione dei biomarker

In assenza di anomalie dei *biomarker* di danno muscolare, nella maggior parte dei casi è sicuro continuare la terapia con statine (23). Il *SAMS Clinical Index* (SAMS-CI) è uno *score* clinico utilizzato per la valutazione della plausibilità della relazione tra i sintomi muscolari e la terapia con statine (34). Esso considera la localizzazione, le caratteristiche qualitative ed il *timing* dei sintomi muscolari rispetto ad inizio e sospensione della terapia. In caso di SAMS-CI ≤6 un effetto causale delle statine è molto improbabile. Dopo aver escluso altre cause di dolore muscolare, è possibile attribuire i sintomi all'effetto "drucebo" negativo. In caso di SAMS-CI > 6 la probabilità di causalità aumenta. In caso di SAMS intollerabili o alterazione dei *biomarker* di danno muscolare, può essere necessario ridurre o sospendere la terapia con statina e intraprendere ulteriori indagini per garantire la sicurezza del paziente (2).

Un dolore muscolare intollerabile, anche in presenza di CK <4 xULN, richiede l'interruzione della terapia indipendentemente dalla conferma della causalità della

terapia con statine. È necessario tentare di ripristinare la terapia ipocolesterolemizzante il prima possibile e nei pazienti ad alto rischio andrebbe immediatamente iniziato il trattamento con ezetimibe (33).

Un'alterazione di CK >4 xULN, anche senza SAMS, richiede l'interruzione della terapia con statine per almeno quattro settimane ed un successivo controllo ematochimico. In caso di normalizzazione del CK, si dovrebbe ricominciare la terapia con statine a dosi più basse e si possono prendere in considerazione tutti gli elementi dell'algoritmo SLAP (vedi paragrafo 4.4 e *Figura 2*). È importante differenziare gli innalzamenti di CK come effetto della statina rispetto ad altre possibili cause, quali esercizio fisico intenso, farmaci, infezioni virali, alcolismo, danni muscolari, ipotiroidismo, malattie reumatologiche o sindromi coronariche acute.

In caso di SAMS associati a CK >4 xULN o in caso di CK > 10xULN anche in assenza di sintomi, ci troviamo probabilmente di fronte ad un'intolleranza completa alle statine, ovvero l'impossibilità di tollerare qualsiasi statina a qualsiasi dose. Questa situazione riguarda solo il 3-5% di tutti i pazienti con intolleranza alle statine. In tale scenario, l'algoritmo SLAP non è applicabile e si devono prendere immediatamente in considerazione farmaci ipocolesterolemizzanti alternativi (2).

La gestione del paziente che presenta effetti avversi di tipo non muscolare è meno controversa in quanto basata su elementi più oggettivi. In caso di diabete di nuova insorgenza non è indicato interrompere la terapia con statine (35). L'incidenza dell'innalzamento delle ALT (> 3 xULN) in corso di terapia con statine è bassa e l'alterazione, in genere transitoria, viene solitamente gestita riducendo la dose di statina. Dopo 2-4 settimane è spesso possibile tornare alla dose originaria (23).

La strategia SLAP

S - Switch: l'intolleranza alle statine può essere un effetto di classe, ma può anche manifestarsi come risposta ad un particolare farmaco. In tal caso il passaggio ad una differente statina può essere sufficiente a risolvere i sintomi dell'intolleranza. Il passaggio da un farmaco idrofilo (pravastatina e rosuvastatina) a uno lipofilo (atorvastatina, simvastatina, fluvastatina, lovastatina, pitavastatina) o viceversa, può essere utile nei pazienti affetti da SAMS, tuttavia, non può essere considerato una regola (36).

La sospensione delle statine e il *rechallenge* (anche con una statina diversa) nello studio PROSISA (Prevalence Of Statin-Associated Muscle Symptoms In Italy) ha permesso a 2/3 dei partecipanti di riprendere la terapia con statine dopo aver inizialmente riportato eventi avversi (30).

L - Lower dose: la riduzione della dose può fornire indicazioni preziose per capire se un disturbo è dose-dipendente dunque farmacologico oppure idiosincratico. È fondamentale ricordare che anche la dose più bassa di statina può essere importante nella prevenzione di eventi cardiovascolari. Se sono tollerate basse dosi di statine, la dose può poi essere aumentata lentamente (27).

A - Alternate dosing: la strategia di somministrazione a giorni alterni è stata esaminata da diversi RCT e i risultati sono stati riuniti in una metanalisi condotta dal Lipid and Blood Pressure Meta-analysis Collaboration (*LBPMC)*. Non sono emerse differenze statisticamente significative nell'abbassamento di LDL-C o trigliceridi tra somministrazione quotidiana e a giorni alterni di rosuvastina e atorvastatina (37, 38).

P – Polipharmacy: Quando i pazienti sono in grado di tollerare una dose ridot-

ta (o a giorni alterni) di statine, ma gli obiettivi terapeutici non sono raggiunti, può essere appropriata una terapia aggiuntiva con ipocolesterolemizzanti non statinici (23).

La terapia ipocolesterolemizzante non statinica

Ezetimibe

Ezetimibe riduce l'assorbimento intestinale del colesterolo bloccando la proteina Niemann-Pick C1-like 1 sugli enterociti.

Nei pazienti completamente intolleranti alle statine l'ezetimibe può essere utilizzato in monoterapia, potendo favorire una riduzione di LDL-C del 15-20% (39). Il trattamento può essere intrapreso immediatamente dopo la sospensione della statina, soprattutto nei pazienti ad alto rischio. Nei pazienti con intolleranza parziale, l'ezetimibe può essere associato efficacemente a basse dosi di statina, favorendo una riduzione di LDL-C fino al 27% (40).

Lo studio IMPROVE-IT ha dimostrato che ezetimibe, in associazione alle statine, determina un'ulteriore riduzione degli eventi cardiovascolari (41). Una recente analisi secondaria dei dati dello stesso studio (42) incentrata sui pazienti di età superiore a 75 anni, ha dimostrato che questo gruppo ha avuto il maggior beneficio assoluto dall'aggiunta di ezetimibe. Una combinazione di statine ed ezetimibe può essere particolarmente utile in questa popolazione, poiché gli anziani sono tra i pazienti più a rischio per eventi avversi da statine ad alte dosi (36).

Inibitori di PCSK9

La proproteina convertasi subtilisina/kexina di tipo 9 (PCSK9) è una proteina regolatrice che si lega ai recettori per le LDL sugli epatociti e ne promuove l'inattivazione mediante internalizzazione nel citoplasma. L'inibizione di PCSK9 aumenta i recettori per le LDL esposti sugli epatociti, con conseguente maggior rimozione delle particelle di LDL circolanti (43). *Alirocumab ed evolocumab* sono anticorpi monoclonali inibitori di PCSK9 iniettati per via sottocutanea ogni 2-4 settimane, riducono LDL-C di circa il 60% in monoterapia ed hanno mostrato risultati particolarmente incoraggianti in soggetti con intolleranza alle statine (44).

Lo studio GAUSS-3 ha reclutato partecipanti con intolleranza confermata alle statine, mostrando dopo 24 settimane di trattamento con evolocumab una riduzione del 53% del LDL-C con segnalazione di sintomi muscolari solo nel 21% dei pazienti trattati (45). Risultati analoghi sono emersi dallo studio ODYSSEY-ALTERNATIVE per alirocumab (LDL-C -45%) (46).

L'inibizione di PCSK9 ha dimostrato di ridurre significativamente gli eventi cardiovascolari in associazione alla terapia con statine negli studi FOURIER (evolocumab) (47) e ODYSSEY-Outcomes (alirocumab) (48).

La notevole efficacia nella riduzione dell'LDL-C, l'impressionante sicurezza e l'emergente profilo di riduzione degli eventi, rendono gli inibitori di PCSK9 uno strumento fondamentale nella gestione dell'intolleranza alle statine, sebbene l'elevato costo di tali farmaci rappresenti ancora un limite alla possibilità di un loro utilizzo su vasta scala.

Inclisiran

Inclisiran è uno *small interfering RNA* sintetico (siRNA) che si lega all'mRNA di PCSK9 e ne riduce la sintesi inibendone la traduzione.

Lo studio RCT ORION, condotto su pazienti con LDL-C elevato e alto rischio cardiovascolare, ha mostrato la riduzione di LDL-C dal 27,9% al 41,9% dopo la som-

ministrazione di una dose singola, e dal 35,5% al 52,6% dopo due dosi (49). Gli studi di follow-up hanno inoltre dimostrato che l'effetto ipocolesterolemizzante di due dosi di inclisiran persiste fino a 18 mesi, riducendo l'LDL-C fino al 55% (50).

Sebbene occorra attendere i risultati dello studio ORION-4 sugli *endpoint* cardiovascolari e non vi siano ancora studi specifici dedicati a pazienti con intolleranza alle statine, la modalità di somministrazione di inclisiran (2 dosi/anno) lo rende estremamente interessante nei pazienti con intolleranza o non aderenza alle statine (51).

Acido bempedoico

L'acido bempedoico è un inibitore dell'adenosina trifosfato-citrato liasi (ACLY), enzima coinvolto nella biosintesi del colesterolo, due tappe a monte rispetto a HMGCoAR. È un profarmaco convertito nel composto attivo (acido bempedoico-coenzima A) dall'acil-CoA sintetasi-1 a catena lunga (ASCV1L) nel fegato. A causa dell'elevato effetto di primo passaggio l'esposizione sistemica all'acido bempedoico è bassa, il che potrebbe spiegare la minor frequenza di osservazione di sintomi muscolari rispetto alle statine (52). Ha mostrato buona tollerabilità come monoterapia in pazienti con intolleranza alle statine e ha ridotto LDL-C del 28,7% con dosi fino a 240 mg/die (53).

In aggiunta alla terapia con statine a bassa o moderata intensità in corso, dosi di 120 e 180 mg/die di acido bempedoico hanno prodotto una riduzione di LDL-C rispettivamente del 17,3% e del 24,3% rispetto al placebo. È importante sottolineare che gli effetti collaterali, compresi i SAMS, non differivano tra i tre gruppi. Da notare inoltre che il 10% dei pazienti arruolati aveva una pregressa storia di interruzione della terapia con statina a causa di sintomi muscolari (54).

Nello studio di fase 3, CLEAR Tranquility, pazienti con storia di SAMS sono stati randomizzati ad acido bempedoico 180 mg o placebo per 12 settimane, dopo un *run-in* di 4 settimane con ezetimibe. L'acido bempedoico in aggiunta all'ezetimibe ha ridotto LDL-C del 28,5% rispetto al placebo. Le analisi per sottogruppi hanno suggerito una maggiore riduzione dell'LDL-C nei non utilizzatori di statine rispetto agli utilizzatori di statine a bassa dose (34,7% vs. 20,5%, rispettivamente), con bassa incidenza di sintomi muscolari in entrambi i gruppi (55).

L'efficacia dell'acido bempedoico nella riduzione degli eventi cardiovascolari è oggetto di indagine dello studio CLEAR Outcomes, attualmente in corso. Si tratta del primo studio su *endpoint* cardiovascolari, che ha incluso esclusivamente pazienti intolleranti alle statine (56).

Glossario

ALT: alanine aminotransferase
ABC: ATP-binding cassette
CYP: citocromo P
CK: creatine kinase
HMGCoA-R: idrossi-metil-glutaril-coenzimaA-reduttasi
ILEP: International Lipid Expert Panel
LBPMC: Lipid and Blood Pressure Meta-analysis Collaboration
LDL-C: low density lipoprotein-cholesterol
PCSK9I: proprotein convertase subtilisin kexin type 9 inhibitors
RCT: randomized controlled trial
RR: relative risk
SAMS: statin-associated muscle symptoms
SAMS-CI: statin-associated muscle symptoms-clinical index
SLAP: Switch statin, Lower dose, Alternate days, Polypharmacy with non-statin therapy
ULN: upper limit of normal

Conclusioni

L'intolleranza muscolare alle statine è un problema di grande rilevanza clinica, in quanto rappresenta uno dei principali determinanti della mancata aderenza alla terapia. Prima di interrompere definitivamente un trattamento ipocolesterolemizzante efficace, i medici dovrebbero essere consapevoli che molto spesso i sintomi muscolari possono essere attribuiti all'effetto "drucebo" negativo delle statine, legato ad una sproporzionata aspettativa negativa da parte del paziente riguardo gli effetti avversi del farmaco. È possibile che la grande attenzione dei media focalizzata sugli effetti negativi delle statine abbia portato a una prevalenza particolarmente elevata degli effetti "drucebo" negativi. Esistono criteri clinico-laboratoristici che possono essere utili nella definizione della "vera" intolleranza alle statine e sono stati formulati algoritmi per la gestione dei pazienti con SAMS, con l'obiettivo di evitare ingiustificate interruzioni del trattamento e garantire il raggiungimento degli obiettivi terapeutici.

Questionario di auto-apprendimento

1) Quale strategia bisogna mettere in atto in un paziente con incremento di CK>10 ULN in corso di terapia con statine?
a) Sostituire la terapia
b) Ridurre il dosaggio della terapia
c) Interrompere la terapia

2) Quali tra questi non è un fattore di rischio endogeno per l'insorgenza di SAMS:
a) Infezione acuta
b) Trauma grave con elevata richiesta metabolica
c) Esercizio fisico intenso e/o non abituale

3) Che cosa è il SAMS-CI:
a) Un indice clinico che considera localizzazione e timing dei sintomi muscolari rispetto ad inizio e sospensione della terapia statinica
b) Un indice clinico che considera localizzazione, caratteristiche qualitative e timing dei sintomi muscolari rispetto ad inizio e sospensione della terapia statinica
c) Un indice clinico che considera localizzazione, caratteristiche qualitative e timing dei sintomi muscolari rispetto all'inizio della terapia statinica

4) Quale tra le seguenti statine viene eliminata per lo più per via biliare?
a) Simvastatina
b) Rosuvastatina
c) Atorvastatina

5) Quale polimorfismo del citocromo P450 è coinvolto nelle interazioni farmacologiche delle statine:
a) CYP3A4
b) CYP3B12
c) CYP218

Risposte corrette: 1c, 2c, 3b, 4b, 5a.

RIASSUNTO

I sintomi muscolari sono uno dei principali effetti avversi associati alla terapia con statine e rappresentano uno dei motivi della mancata aderenza alla terapia e del mancato raggiungimento degli obiettivi ottimali del trattamento ipocolesterolemizzante. La reale prevalenza della miopatia da statine, come dimostrato dai risultati di trial randomizzati e metanalisi, è tuttavia nettamente inferiore rispetto a quanto percepito. È stato descritto infatti un effetto "drucebo" delle statine, ovvero un'errata aspettativa relativa alla frequenza ed alla gravità degli effetti collaterali ad esse associati. Un corretto inquadramento diagnostico dei pazienti con intolleranza alle statine, che tenga conto dell'impatto dell'effetto "drucebo", può consentire una migliore gestione terapeutica massimizzando la riduzione del rischio cardiovascolare. In questa rassegna sono riassunti i meccanismi fisiopatologici e le evidenze cliniche riguardanti la miopatia da statina al fine di fornire indicazioni pratiche sulla gestione dei pazienti con intolleranza alle statine.

Parole chiave: *Statina, miopatia, SAMS, effetto drucebo, terapia ipocolesterolemizzante.*

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MEDICINA, SCIENZA E SOCIETÀ

COVID-19: IL VIRUS CHE AMA I TRIGLICERIDI E TROVA COME NEMICO IL FENOFIBRATO

COVID-19: the virus that loves triglycerides and finds fenofibrate as its enemy

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È finita, si spera, la pandemia, e pesano i ricordi: 5 piani da fare senza ascensore; incontri con pazienti che (dopo i cinque piani) lamentavano la mancata assistenza, ristoranti chiusi ecc. Ma alcuni aspetti sono stati singolari e forse irripetibili. Pensiamo agli infettivologi. Orfani per vent'anni di nuovi antibiotici e quasi dimenticati, hanno trovato un nuovo vessillo da seguire: un virus RNA di oscura origine e tuttora senza terapie ben accertate. Le star televisive sono state loro, specialisti di alto livello, appassionati e che hanno anche la capacità di discutere, a volte di litigare. Visto però da un farmacologo, il Coronavirus è un argomento affascinante. Un virus ha come obbiettivo solo quello di sopravvivere, non importa se questa sua sopravvivenza possa impedire quella di un altro. E non far sopravvivere il virus è un compito complesso. In assenza di farmaci ma con ottimi vaccini, la situazione è stata quasi opposta a quella di 20 anni fa con il virus HIV, colpevole dell'AIDS. Qui, un enorme lavoro su un virus RNA, simile al COVID, non ha portato ad alcun vaccino, ma per fortuna i farmaci si sono dimostrati molto efficaci. Si sono aperte invece per il COVID idee creative. Una di queste ha portato ad un argomento per noi congeniale, i trigliceridi.

Il Coronavirus presenta una parte centrale, appunto di RNA: è ben nota e ben studiata ed apparenta il virus a quelli di altre specie, dal pipistrello al pangolino, che non hanno però portato a identificare molecole che impediscano efficacemente la moltiplicazione dell'RNA virale. Ma il virus ha anche un cappotto: il "capside", di materiale grasso, di "trigliceridi", che da un lato lo difende, dall'altro gli consente di interagire con la membrana delle cellule umane permettendogli di entrare.

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I trigliceridi sono costituiti da acidi grassi. Se si tratta di grassi saturi, la molecola è rigida, dura; con altri grassi, dai monoinsaturi agli omega-3, le molecole divengono via via più molli. I trigliceridi del capside COVID sono di grassi saturi, palmitico e miristico. È quindi una copertura molto solida che permette al virus una buona sopravvivenza a varie temperature ed anche di resistere sulle superfici esterne. Si tratta però di un punto debole: quando il virus si moltiplica, oltre che l'RNA deve anche moltiplicare i trigliceridi del capside. E qui entrano in gioco ricercatori dell'Università di Gerusalemme: i professori Nahmias ed Ehrlich hanno avuto la curiosità di andare a vedere come il virus si alimenti e come mai si dirige subito ai polmoni. La sorpresa è stata grande. Il virus, non essendo in grado da solo di provvedere al suo nutrimento, deve avvalersi di un organo umano. Non si tratta però del fegato, come avviene per la maggior parte dei virus, bensì dei polmoni. Quando vi arriva per contagio aereo mette in atto una serie di stimoli, che obbligano i polmoni a produrre trigliceridi per il suo capside. Come se non bastasse, blocca anche la capacità di quest'organo di demolire i trigliceridi neo-prodotti.

Purtroppo, tutta questa produzione di grasso è di grave danno. Il polmone ha una struttura ad alveare, a tralci sottili, che consentono un ottimale passaggio dell'aria. Se vi arrivano dei grassi si avvia subito un processo infiammatorio con evoluzione temibile. Non è una novità: ogni anno centinaia di bambini vanno incontro a polmonite "lipoidea": sono piccoli che assumono uno sciroppo (spesso di materiale oleoso) e lo mandano di traverso facendolo arrivare alle vie aeree. Fortunatamente riescono ad eliminare gran parte di questo materiale in un tempo relativamente breve. Il caso è ben diverso per COVID-19: il virus stimola il polmone di continuo e il grasso prodotto non viene eliminato. L'infiammazione persiste e, in un certo numero di casi sfortunati, può portare a morte.

Esistono sostanze farmacologiche che possano da un lato ridurre la sintesi dei trigliceridi polmonari, dall'altro aumentarne la demolizione? Dopo un'attenta indagine gli israeliani hanno trovato il farmaco giusto. Si tratta del fenofibrato, farmaco a noi ben noto per il trattamento delle ipertrigliceridemie, a livelli di 300-500 mg/dl e più. Il fenofibrato è molto efficace, non sempre, ma spesso, normalizza i dati. È poco noto che questo farmaco ha una distribuzione prevalente al fegato, ma seguono il rene e appunto i polmoni (1).

Gli israeliani hanno dimostrato che le cellule del polmone umano, esposte al virus COVID, accumulano trigliceridi. Se si aggiunge fenofibrato questi grassi si riducono fino a scomparire e non si verifica morte cellulare (2). È importante nell'uomo? Quando ho sentito questi dati, mi è subito venuto in mente un paziente AIDS in terapia. Non di rado, i trattamenti anti-AIDS fanno salire i trigliceridi, a valori anche molto elevati. Questo paziente, un operatore sanitario presso il Pio Albergo Trivulzio, aveva valori oltre 800 mg/dl e prendeva una dose di fenofibrato doppia di quella solita (290 invece di 145 mg al giorno). Come è ben noto, nella sua RSA a un certo punto è arrivata un'epidemia grave. Tutti con febbre e, purtroppo, parecchi decessi. Il nostro una sera va a letto e misura la febbre: 39°2! Terrore e programma di indagini la mattina successiva. Ma la mattina dopo la febbre non c'era più, era "guarito". Restava il dubbio sulla diagnosi, ma l'esame degli anticorpi dimostrava che l'infezione c'era stata.

L'esperienza nostra si è poi arricchita. Pazienti in trattamento da anni con il fenofibrato hanno avuto o infezioni non gravi,

di breve durata, oppure sono apparsi "immuni", pur vivendo in un ambiente con altissima percentuale di contagi. Ricordo una signora in trattamento con fenofibrato, contagiata assieme al marito. Ricoverati entrambi, lei è tornata a casa dopo tre giorni, il marito è purtroppo deceduto.

L'attività di ricerca sperimentale e clinica in questo ambito non è intensa come quella con antivirali o vaccini. Un problema è forse che il fenofibrato costa pochissimo: il trattamento per i dieci giorni di solito previsti può superare di poco i 3 euro. Comunque, sono in corso o si sono conclusi diversi studi: uno epidemiologico in Israele, Milano e a Bologna, ha esaminato tutti gli iperlipidemici in terapia farmacologica, diverse migliaia. Il dato più certo è che i trattati con fenofibrato hanno una minima incidenza di malattia (p.es. rispetto alle statine) e la mortalità è vicina allo zero (2).

Lo studio FERMIN, tuttora in corso, è un classico studio clinico in doppia cecità. I pazienti COVID di nuova diagnosi ricevono o una compressa al giorno di fenofibrato per dieci giorni o un placebo. È in corso in dieci paesi. Personalmente lo considero poco attendibile: il fenofibrato sembra attivo contro il virus se il trattamento è già in corso, non come nuova terapia. Infine, a Gerusalemme si è concluso lo studio FENOC, un nuovo studio controllato su un piccolo numero di pazienti, valutando numerosissime variabili, con risultati che sembrano convincenti.

È poi emersa l'idea che un nutraceutico, di minimo costo, possa essere altrettanto utile del fenofibrato, con il vantaggio dell'assoluta sicurezza. Il "conjugated linoleic acid" noto come CLA, è una molecola costituita da due isomeri dell'acido linoleico legati assieme. Al CLA sono state attribuite molte virtù: fa dimagrire, cura il diabete, il cancro, ecc. Ha molti estimatori e discrete vendite nelle parafarmacie ed erboristerie, anche se i dati scientifici non sono tanto convincenti. L'idea di provarlo in alternativa al fenofibrato sembra abbia successo, almeno in modelli animali. In commercio il CLA è disponibile in capsulone da 3 grammi. Ne occorrerebbero 2-3 al giorno, il prezzo è ragionevole. Qualcuno vuol provare?

Ma l'interesse per i lipidi del COVID non si è fermata ai trigliceridi. Di recente un gruppo inglese ha dimostrato la presenza di *aminofosfolipidi* nel capside virale, che nel sangue possono esercitare un'attività pro-coagulante (3), una vecchia idea sulla patogenesi delle pneumopatie da COVID. L'aspetto più interessante è che questi aminofosfolipidi superficiali sono molto sensibili ai componenti alcoolici dei prodotti usati come collutori per gargarismi, suggerendo che nella prevenzione da virus questa possa essere un'area non priva di interesse.

Tutte queste indagini sul quadro lipidico nell'infezione COVID non potevano lasciare da parte un'indagine aggiornata sui rapporti fra infezione e rischio cardiovascolare. L'ipertrigliceridemia sembra un fattore di rischio importante nell'ospedalizzato da COVID, essendo associata in modo indipendente con la mortalità (OR=2.3) (4). Ci viene poi in aiuto un recentissimo Editoriale su NATURE (5). L'infezione da COVID si associa ad effetti negativi a lungo termine: il "long COVID". Fra questi la salita di eventi vascolari è tra i più significativi: un'indagine su 150.000 veterani USA guariti dal COVID, a confronto con un ugual numero di soggetti pre-pandemia, seguiti per un anno, ha dimostrato un aumento di 3 volte di eventi cardiovascolari, di 2,8 di malattia renale cronica e 15 di malattia epatica cronica (6). Che efficacia potrebbe avere il fenofibrato come preventivo?

Nel frattempo, tengo con me una confezione di compresse di fenofibrato. Se mi viene la febbre od ho qualche sospetto, non si sa mai.

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NOTIZIE DA CONGRESSI INTERNAZIONALI

ESC 2022

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Nel mese di agosto 2022, si è tenuto il meeting annuale dell'European Society of Cardiology (ESC), in modalità mista.

Le nuove linee guida ESC sull'ipertensione polmonare sollecitano una diagnosi più precoce

Le nuove linee guida della Società Europea di Cardiologia (ESC)/Società Europea di Respirazione (ERS) per la diagnosi e il trattamento dell'ipertensione polmonare hanno abbassato il *cutoff* per definire l'ipertensione polmonare e hanno modificato l'algoritmo diagnostico in modo che la diagnosi venga fatta precocemente.

L'ipertensione polmonare non è una condizione rara, dal momento che colpisce circa l'1% della popolazione globale e circa il 10% di quella anziana.

Il valore di *cutoff* per definire l'ipertensione polmonare è stato abbassato da 25 mm Hg a 20 mm Hg perché c'erano evidenze che i valori anomali erano più bassi di quanto si pensasse inizialmente. Ogni aumento della pressione arteriosa polmonare rappresenta un aumento del postcarico per il cuore destro ed è associato a una mortalità più precoce. Tuttavia, questo *cutoff* più basso non si traduce ancora in nuove raccomandazioni terapeutiche, poiché l'efficacia della terapia dell'ipertensione arteriosa polmonare nei pazienti con pressione arteriosa polmonare media (mPAP) di 21-24 mm Hg è sconosciuta.

Il nuovo algoritmo diagnostico per i pazienti con sospetta ipertensione polmonare o dispnea inspiegabile è stato completamente rivisto.

La diagnosi viene effettuata nelle tre fasi seguenti:

- Fase 1: contatto con il medico di base. Il medico di base deve essere consapevole che esistono alcune forme rare di respiro affannoso e può pensare già all'ipertensione polmonare per un paziente che presenta segni clinici negativi in presenza di insufficienza cardiaca e che potrebbe essere inviato direttamente a un centro per l'ipertensione polmonare.
- Fase 2: rilevamento mediante ecocardiografia. Il medico di base invia il paziente con respiro affannoso a un cardiologo o a uno pneumologo, dove riceve una valutazione polmonare o cardiaca più completa.
- Fase 3: conferma con cateterismo car-

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diaco destro in un centro per l'ipertensione polmonare.

L'ESC aggiorna le linee guida per l'aritmia ventricolare e la morte improvvisa

Le nuove linee guida ESC per la gestione dei pazienti con aritmie ventricolari e la prevenzione della morte cardiaca improvvisa contengono una serie di aggiornamenti rispetto all'edizione del 2015.

Le linee guida contengono tre raccomandazioni di classe I per migliorare la sopravvivenza in caso di arresto cardiaco extraospedaliero. I defibrillatori automatici esterni (DAE) dovrebbero essere collocati in aree pubbliche, come le stazioni ferroviarie, dove possono verificarsi arresti cardiaci; la rianimazione cardiopolmonare (RCP) dovrebbe essere avviata dai presenti e si dovrebbe promuovere la formazione nella comunità per aumentare la RCP e l'uso dei DAE da parte degli astanti. Le linee guida indicano anche che dovrebbe essere presa in considerazione una App per chiamare i volontari nelle vicinanze a eseguire la rianimazione prima dell'arrivo dei paramedici (Classe IIa), un'applicazione già utilizzata con successo in Danimarca.

Le linee guida forniscono approfondimenti sui test genetici, includendo esempi per diverse malattie, come la cardiomiopatia dilatativa (DCM). Se un paziente con DCM si presenta in giovane età (< 50 anni) con blocco atrioventricolare o se il paziente ha una storia familiare di DCM o di morte cardiaca improvvisa in giovane età, il test genetico è una raccomandazione di Classe I. Se la frazione di eiezione ventricolare sinistra è pari o inferiore al 35% dopo almeno 3 mesi di terapia medica ottimale, si dovrebbe prendere in considerazione l'impianto di un defibrillatore cardioverter impiantabile (ICD) nei pazienti con DCM e insufficienza cardiaca sintomatica (classe NYHA II-III). Si tratta ora di una raccomandazione di classe IIa, declassata rispetto alla raccomandazione di classe I delle linee guida del 2015 a causa dello studio DANISH e del fatto che i farmaci stanno riducendo il tasso di morte cardiaca improvvisa in questa popolazione.

I beta-bloccanti, idealmente quelli non selettivi (nadololo o propranololo), sono raccomandati nei pazienti con sindrome del QT lungo (LQTS) con documentato prolungamento dell'intervallo QT, per ridurre il rischio di eventi aritmici (Classe I) e in tutti i pazienti con diagnosi clinica di tachicardia ventricolare polimorfa catecolaminergica (CPVT) (Classe I). Nei pazienti con LQTS e CPVT clinicamente diagnosticate, si raccomandano test genetici e consulenza genetica.

Nuove linee guida ESC per ridurre il rischio CV nella chirurgia non cardiaca

Le linee guida ESC sulla valutazione e la gestione cardiovascolare dei pazienti sottoposti a chirurgia non cardiaca sono state oggetto di un'ampia revisione rispetto alla versione del 2014.

Il loro obiettivo è sempre lo stesso: prevenire le complicanze emorragiche legate all'intervento, l'infarto/lesione miocardica perioperatoria, la trombosi dello stent, l'insufficienza cardiaca acuta, le aritmie, l'embolia polmonare, l'ictus ischemico e la morte cardiovascolare (CV).

Il documento classifica gli interventi chirurgici non cardiaci in tre livelli di rischio a 30 giorni di morte CV, infarto acuto del miocardio (IMA) o ictus. Il rischio basso (<1%) comprende la chirurgia dell'occhio o della tiroide; il rischio intermedio (1%-5%) comprende la sostituzione del gi-

nocchio o dell'anca o il trapianto renale; e il rischio alto (> 5%) comprende l'aneurisma dell'aorta, il trapianto di polmone o la chirurgia del cancro del pancreas o della vescica.

Le linee guida riportano che prima di un intervento chirurgico non cardiaco a rischio alto o intermedio nei pazienti con CVD nota, fattori di rischio CV (compresa l'età di 65 anni o più) o sintomi suggestivi di CVD:

- Si raccomanda di ottenere un ECG preoperatorio a 12 derivazioni (Classe I).
- Si raccomanda di misurare la troponina cardiaca T ad alta sensibilità (hs-cTn T) o la troponina cardiaca I ad alta sensibilità (hs-cTn I). Si raccomanda inoltre di misurare questi biomarcatori a 24 ore e 48 ore dall'intervento (Classe I).
- Si dovrebbe prendere in considerazione la misurazione del peptide natriuretico di tipo B (BNP) o del N-terminale-proBNP (NT-proBNP).

Tuttavia, per i pazienti a basso rischio sottoposti a chirurgia non cardiaca a rischio basso e intermedio non è raccomandato eseguire di routine un ECG preoperatorio, hs-cTn T/I o la concentrazione di BNP/NT-proBNP (Classe III).

FANS associati al rischio di insufficienza cardiaca nei soggetti diabetici

Le persone con diabete che assumono farmaci antinfiammatori non steroidei anche a breve termine possono avere un rischio maggiore del 50% di sviluppare un'insufficienza cardiaca, secondo i risultati di uno studio di registro nazionale su oltre 330.000 pazienti. Inoltre, sembra che siano particolarmente suscettibili i pazienti di età superiore a 79 anni o con livelli elevati di emoglobina A1c. Si tratta di un'osservazione rilevante, dato che i FANS continuano a essere prescritti con una certa facilità alle persone con diabete e che l'uso di questi agenti presenta dei rischi.

Lo studio ha identificato 331.189 pazienti con diabete di tipo 2 in registri nazionali danesi dal 1998 al 2018. L'età media era di 62 anni, 23.308 (7%) sono stati ricoverati in ospedale con insufficienza cardiaca durante il *follow-up*. Di questi, il 16% ha dichiarato di aver avuto almeno una prescrizione di FANS nell'arco di 2 anni e il 3% ha dichiarato di averne avute almeno tre. Il *follow-up* dello studio è iniziato 120 giorni dopo la prima diagnosi di diabete di tipo 2 e si è concentrato su pazienti che non avevano precedenti diagnosi di insufficienza cardiaca o malattia reumatologica. Lo studio ha utilizzato un disegno *case-crossover*, in cui ogni individuo funge da proprio controllo, così da minimizzare il confondimento ed essere adatto a studiare l'effetto dell'esposizione a breve termine su eventi immediati.

L'uso a breve termine (fino a 28 giorni) di FANS è stato collegato a un aumento del rischio di ospedalizzazione per insufficienza cardiaca (odds ratio [OR] 1,43; IC 95% 1,27-1,63). I ricercatori hanno identificato rischi ancora maggiori in tre sottogruppi: età di almeno 80 anni (OR 1,78; IC 95% 1,39-2,28), livelli elevati di A1c trattati con uno o meno farmaci antidiabetici (OR 1,68; IC 95% 1-2,88) e pazienti senza precedente uso di FANS (OR 2,71; IC 95% 1,78-4,23).

Nella coorte, celecoxib e naprossene erano usati raramente (0,4 e 0,9%, rispettivamente), mentre il 3,3% dei pazienti aveva assunto diclofenac e il 12,2% ibuprofene. Questi ultimi due FANS avevano OR di 1,48 e 1,46, rispettivamente, per l'ospedalizzazione per insufficienza cardiaca di nuova insorgenza (IC 95% 1,1-2,o e 1,26-1,69, rispettivamente). Non è emerso alcun aumento del rischio per celecoxib o naprossene.

Il beneficio della prevenzione CV secondaria della polipillola promette benefici per la salute globale: il trial SECURE

Secondo i risultati di uno studio multinazionale in pazienti con un precedente infarto miocardico (MI), una singola pillola contenente aspirina, un farmaco ipolipemizzante e un ACE-inibitore ha fornito una protezione progressivamente maggiore da un secondo evento cardiovascolare (CV) nel corso di diversi anni di follow-up.

Le curve hanno iniziato a separarsi già all'inizio dello studio, con una riduzione del 24% dell'*hazard ratio* di eventi CV avversi maggiori (MACE), e continuano a separarsi, per cui si può prevedere che i risultati sarebbero ancora più sorprendenti per un *follow-up* ancora più lungo.

Anche studi precedenti sulle polipillole hanno dato risultati positivi, ma l'ultimo studio, chiamato SECURE, è il più grande studio prospettico randomizzato ad aver valutato una singola pillola che combina più terapie per la prevenzione secondaria. Lo studio ha randomizzato 2.499 pazienti di età superiore ai 65 anni che avevano avuto un MI nei 6 mesi precedenti e almeno un altro fattore di rischio, come diabete mellito, disfunzione renale o una precedente rivascolarizzazione coronarica. I pazienti sono stati arruolati in 113 centri di studio in sette Paesi europei.

La polipillola consisteva in aspirina a dose fissa di 100 mg, nell'inibitore della HMG CoA reduttasi atorvastatina e nell'ACE inibitore ramipril. Per atorvastatina e ramipril, le dosi erano rispettivamente 40 mg e 10 mg, ma erano disponibili diverse versioni della polipillola per consentire la titolazione a una dose tollerata. L'età media degli arruolati era di 76 anni. Quasi un terzo (31%) erano donne. Al basale, la maggior parte dei pazienti aveva l'ipertensione (77,9%) e la maggior parte aveva il diabete (57,4%). Quando gli eventi dell'*endpoint* primario sono stati valutati singolarmente, la polipillola era associata a una riduzione relativa del 33% del rischio di morte CV (*hazard ratio* [HR] 0,67; P=0,03). Le riduzioni del rischio di MI non fatale (HR 0,71) e di ictus (HR 0,70) erano della stessa entità generale, anche se non hanno raggiunto la significatività statistica. Non c'è stata una riduzione significativa della rivascolarizzazione urgente (HR 0,96). Inoltre, la riduzione della mortalità per tutte le cause (HR 0,97) non era significativa. L'aderenza, monitorata a 6 e 24 mesi con la *Morisky Medication Adherence Scale*, è stata caratterizzata come bassa, media o alta. Un numero maggiore di pazienti nel gruppo in polipillola ha raggiunto un'aderenza elevata a 6 mesi (70,6% contro 62,7%) e a 24 mesi (74,1% contro 63,2%).

Lo studio DANCAVAS non raggiunge l'*endpoint* primario, ma indica i benefici di uno *screening* CV completo

In un nuovo studio danese, lo *screening* cardiovascolare completo basato sull'*imaging* negli uomini di età compresa tra 65 e 74 anni non ha ridotto in modo significativo la mortalità per tutte le cause, sebbene vi siano forti indicazioni di un beneficio in alcuni *endpoint* cardiovascolari nell'intero gruppo e anche nella mortalità dei soggetti di età inferiore a 70 anni.

Lo studio DANCAVAS ha assegnato in modo casuale 46.611 uomini danesi di età compresa tra 65 e 74 anni, in un rapporto 1:2, a sottoporsi allo *screening* (gruppo invitato) o a non sottoporsi allo *screening* (gruppo di controllo) per le malattie cardiovascolari subcliniche. Lo *screening* comprendeva una TAC con elettrocardiografia senza contrasto per determinare il punteggio del cal-

cio coronarico e rilevare aneurismi e fibrillazione atriale; misurazioni della pressione arteriosa alla caviglia per rilevare malattie delle arterie, periferiche e ipertensione e prelievo di un campione di sangue per rilevare diabete e ipercolesterolemia. Dei 16.736 uomini invitati, 10.471 (62,6%) hanno effettivamente partecipato allo *screening*.

Nelle analisi per *intention-to-treat*, dopo un *follow-up* mediano di 5,6 anni, l'*endpoint* primario (morte per tutte le cause) si è verificato in 2106 uomini (12,6%) nel gruppo invitato e in 3915 uomini (13,1%) nel gruppo di controllo (HR 0,95; IC 95% 0,90-1,00). L'*hazard ratio* per l'ictus nel gruppo invitato, rispetto al gruppo di controllo, è stato di 0,93 (IC 95% 0,86-0,99); per l'IMA 0,91 (IC 95% 0,81-1,03); per la dissezione aortica 0,95 (IC 95% 0,61-1,49) e per la rottura aortica 0,81 (IC 95% 0,49-1,35). L'*endpoint* composito post-hoc di mortalità per tutte le cause/ictus/MI era ridotto del 7%, con un hazard ratio di 0,93 (IC 95% 0,89-0,97).

L'analisi di sottogruppo ha mostrato che l'esito primario della mortalità per tutte le cause era significativamente ridotto negli uomini invitati allo *screening* di età compresa tra 65 e 69 anni (HR 0,89; IC 95% 0,83-0,96), senza alcun effetto negli uomini di età compresa tra 70 e 74 anni. In termini di costo-efficacia, i costi sanitari aggiuntivi totali sono stati di 207 euro per persona nel gruppo invitato, che comprendeva lo *screening*, i farmaci e tutte le visite mediche e ospedaliere. L'anno di vita aggiustato per la qualità (QALY) guadagnato per persona è stato di 0,023, con un rapporto incrementale di costo-efficacia di 9075 euro per QALY nell'intera coorte e di 3860 euro negli uomini di età compresa tra 65 e 69 anni, cifre che si confrontano favorevolmente con lo *screening* del cancro, caratterizzato da un rapporto costo-efficacia di 22.000 euro per QALY.

I dati potrebbero supportare questo tipo di *screening* cardiovascolare negli uomini di età inferiore ai 70 anni. In Danimarca, dove lo studio è stato condotto, la popolazione gode di un buon livello di assistenza sanitaria. In altri Paesi dove può essere più difficile accedere alle cure o dove la salute cardiovascolare non è così buona, un programma di *screening* di questo tipo avrebbe probabilmente un effetto maggiore.

I benefici di Dapagliflozin nell'HFpEF gettano nuove basi per il trattamento dell'insufficienza cardiaca: il trial DELIVER

L'inibitore SGLT2 dapagliflozin è diventato il terzo agente della classe a mostrare prove di efficacia nei pazienti con insufficienza cardiaca con frazione di eiezione conservata (HFpEF) grazie ai risultati di oltre 6.200 pazienti randomizzati nello studio DELIVER.

Questi risultati hanno dimostrato che il trattamento con dapagliflozin apporta benefici ai pazienti con insufficienza cardiaca indipendentemente dalla loro funzione ventricolare sinistra, se considerati insieme ai risultati precedentemente riportati nello studio DAPA-HF, che ha testato lo stesso farmaco in pazienti con insufficienza cardiaca con frazione di eiezione ridotta (HFrEF). L'analisi combinata prespecificata che includeva un totale di 11.007 pazienti con insufficienza cardiaca in tutto lo spettro dei valori di frazione di eiezione (con singoli pazienti che avevano valori inferiori al 20% o superiori al 70%) ha mostrato un beneficio consistente del trattamento con dapagliflozin nel ridurre significativamente l'*endpoint* combinato di morte cardiovascolare o ospedalizzazione per insufficienza cardiaca di circa il 22%, rispetto al placebo, in tutto l'intervallo di questa frazione di eiezione.

I risultati di DELIVER per dapagliflozin hanno anche evidenziato un apparen-

te effetto di classe per l'insufficienza cardiaca da parte degli SGLT2, a causa di risultati simili precedenti per altri due farmaci della classe: empagliflozin e sotagliflozin.

Una seconda analisi combinata prespecificata ha associato i risultati di DELIVER a quelli di un precedente studio che ha valutato empagliflozin in pazienti con HFpEF, EMPEROR-Preserved, su un totale di 12.251 pazienti, e ha mostrato risultati simili ma con un'apparente diminuzione dell'efficacia nei pazienti che si trovavano nella fascia più alta di funzione ventricolare sinistra conservata, con frazioni di eiezione superiori a circa il 65%. Nel solo EMPEROR-Preserved, i pazienti con frazioni di eiezione pari o superiori al 60% non hanno mostrato un beneficio significativo dal trattamento con empagliflozin, sebbene i dati abbiano mostrato una tendenza numerica alla riduzione degli eventi avversi.

Una terza analisi combinata, ha aggiunto a questi 12.000 pazienti i dati del DAPA-HF, dello studio con empagliflozin in pazienti con HFrEF (EMPEROR-Reduced) e di uno studio su un terzo inibitore SGLT2, sotagliflozin (SOLOIST-WHF). Anche in questo caso, i risultati hanno mostrato una coerenza trasversale tra gli studi e una riduzione significativa e complessiva del 23%, rispetto al placebo, del tasso di morte cardiovascolare o di ospedalizzazione per insufficienza cardiaca.

Secondo gli esperti, i risultati di DELIVER hanno ulteriormente consolidato un nuovo paradigma per il trattamento dei pazienti con insufficienza cardiaca, che sottolinea la necessità di iniziare rapidamente il trattamento con inibitori SGLT2 nei pazienti non appena ricevono una diagnosi di insufficienza cardiaca, senza la necessità di misurare e considerare prima la frazione di eiezione ventricolare sinistra del paziente.

ALL-HEART: nessun beneficio dell'allopurinolo nella cardiopatia ischemica

L'allopurinolo, un farmaco comunemente utilizzato per il trattamento della gotta, è un inibitore della xantina ossidasi e agisce riducendo i livelli di acido urico nel siero e lo stress ossidativo. Gli studi osservazionali hanno mostrato risultati variabili, mentre gli studi di intervento, la maggior parte dei quali con meno di 100 partecipanti, hanno suggerito potenziali miglioramenti in fattori quali la pressione sanguigna, la funzione endoteliale, l'ipertrofia ventricolare sinistra o lo spessore dell'intima media carotidea.

I risultati di un nuovo studio randomizzato dimostrano però che l'allopurinolo non ha fornito alcun beneficio in termini di riduzione degli eventi cardiovascolari (CV) nei pazienti con cardiopatia ischemica. Il trattamento di questi pazienti senza gotta con 600 mg di allopurinolo al giorno non ha prodotto alcun effetto sugli *endpoint* primari compositi, tra cui l'infarto miocardico non fatale (MI), l'ictus non fatale o la morte CV.

ALL-HEART è stato uno studio prospettico, randomizzato, in aperto, multicentrico. I pazienti con cardiopatia ischemica ma senza storia di gotta sono stati reclutati in 424 ambulatori generali del Regno Unito, a partire dal febbraio 2014 e con un *follow-up* che si è concluso nel settembre 2021. I partecipanti sono stati assegnati in modo casuale 1:1 a ricevere 600 mg di allopurinolo al giorno o l'assistenza abituale. Si trattava di uno studio decentralizzato, quindi il *follow-up* era in gran parte a distanza dopo le prime 6 settimane, e comprendeva l'utilizzo di dati di *record linkage* raccolti da *database* centralizzati dell'NHS *(National Health Service)* per i ricoveri e i decessi in Scozia e in Inghilterra. Il *follow-up* medio è stato di 4,8 anni.

In totale, nell'analisi finale per *intention-to-treat*, i pazienti sono stati 5721, e 639 hanno avuto un primo evento primario. Per quanto riguarda l'esito primario di MI non fatale, ictus non fatale e morte cardiovascolare, i ricercatori non hanno riscontrato differenze tra i gruppi, con un *hazard ratio* di 1,04 (IC 95% 0,89-1,21).

I risultati suggeriscono che allopurinolo non dovrebbe essere raccomandato per la prevenzione secondaria degli eventi in questo gruppo, sebbene rimanga un trattamento importante per la gotta.

Gli studi sugli antiossidanti finora condotti sono stati deludenti. Esistono prove chiare e convincenti che lo stress ossidativo è coinvolto nella patogenesi dell'aterosclerosi, eppure gli studi hanno dato risultati negativi. Per il successo futuro saranno necessari tre fattori: selezionare il paziente giusto, individuare un biomarcatore affidabile per la misura dello stress ossidativo e per la valutazione della terapia, e disporre di terapie mirate che agiscano sui principali fattori scatenanti dello stress ossidativo.

Il vaccino COVID-19 è sicuro nei pazienti con insufficienza cardiaca

I pazienti con insufficienza cardiaca HF sono a maggior rischio di ospedalizzazione, necessità di ventilazione meccanica e morte a causa del COVID-19 e la vaccinazione riduce il rischio di malattie gravi dovute al COVID-19.

In uno studio caso-controllo condotto in Danimarca, i pazienti affetti da insufficienza cardiaca che hanno ricevuto due dosi di vaccino COVID mRNA non hanno avuto maggiori probabilità di peggioramento della malattia, tromboembolismo venoso o miocardite entro 90 giorni rispetto a pazienti simili non vaccinati. Inoltre, nei 90 giorni successivi alla seconda iniezione, i pazienti vaccinati avevano meno probabilità di morire per qualsiasi causa rispetto ai pazienti non vaccinati.

I ricercatori hanno identificato 50.893 pazienti con HF sottoposti a doppio vaccino nel 2021 e li hanno appaiati a 50.893 pazienti non vaccinati con HF nel 2019 (prepandemia), della stessa età, sesso, durata dell'HF, uso di farmaci per HF, cardiopatia ischemica, cancro, diabete, fibrillazione atriale e ricovero con HF entro 90 giorni. Quasi tutti i pazienti del gruppo vaccinato hanno ricevuto il vaccino a mRNA di Pfizer/BioNTech (92%) e il resto ha ricevuto il vaccino a mRNA di Moderna (8%) nel 2021. I pazienti avevano un'età media di 74 anni e il 64% erano uomini. Erano affetti da HF da una media di 4,1 anni.

Durante i 90 giorni di *follow-up*, il rischio di morte per tutte le cause è stato significativamente inferiore nella coorte vaccinata rispetto a quella non vaccinata (-0,33 punti percentuali; IC 95% da -0,52 a -0,15 punti percentuali). Il rischio di peggioramento dell'insufficienza cardiaca era dell'1,1% in ciascun gruppo; la miocardite e il tromboembolismo venoso erano estremamente rari e i rischi per queste condizioni non erano significativamente diversi nei due gruppi.

L'albuminuria è legata a un maggior rischio di CVD nei pazienti diabetici

Meno della metà degli adulti danesi con diabete di tipo 2 nel 2015 è stata sottoposta a una recente valutazione dell'albuminuria: in coloro che si sono sottoposti al test, l'albuminuria si associava a un tasso maggiore del 50% di insufficienza cardiaca, infarto, ictus o morte per tutte le cause durante il *follow-up* di 4 anni, in uno studio condotto su oltre 74.000 residenti in Danimarca.

Anche i soggetti di questo studio con diabete di tipo 2 ma senza albuminuria presentavano un tasso del 19% di questi esiti

avversi, evidenziando il rischio "sostanziale" di malattie cardiovascolari a cui vanno incontro le persone con diabete di tipo 2, anche senza una chiara indicazione di nefropatia. Questo suggerisce che la soglia di rapporto albumina-creatinina urinaria (UACR) di almeno 30 mg/g per definire l'albuminuria potrebbe essere troppo alta.

Il profilo dell'albuminuria come marcatore di rischio per le persone con diabete di tipo 2 ha riscosso un crescente interesse dopo l'approvazione negli Stati Uniti nel 2021, poi in Europa nel 2022, del finerenone come agente specifico per gli adulti con diabete di tipo 2 e albuminuria.

Anche i pazienti con un rapporto di 10-29 mg/g presentano un rischio e dovrebbero essere presi in considerazione per il trattamento con finerenone. Anche in questi soggetti si osserva una progressione della malattia renale, ma più lenta, rispetto a coloro che soddisfano l'attuale soglia standard di albuminuria.

Nei due trial su finerenone, FIDELIO-DKD e FIGARO-DKD, sebbene il disegno di entrambi gli studi prevedesse l'arruolamento di persone con diabete di tipo 2 e un UACR di almeno 30 mg/g, alcune centinaia degli oltre 13.000 pazienti arruolati avevano valori di UACR inferiori a questo livello e l'analisi di questo sottogruppo potrebbe fornire importanti indicazioni sul valore del finerenone per le persone con albuminuria nei *range* attualmente ritenuti normali.

L'intelligenza artificiale è pronta a cambiare il paradigma della prevenzione del rischio CV

In genere, l'intelligenza artificiale (AI) viene applicata per analizzare un insieme complesso di variabili e stabilire correlazioni che non si possono ottenere facilmente con l'osservazione non assistita. Tuttavia, una derivazione dell'AI, talvolta definita AI causale, incorpora la causalità e non solo l'associazione, e sembra in grado di cambiare il paradigma della prevenzione degli eventi cardiovascolari (CV).

In uno studio innovativo, chiamato CAUSAL AI, questo approccio è stato esplorato con due fattori di rischio principali: l'aumento del colesterolo LDL (LDLc) e l'aumento della pressione sistolica (SBP). Sulla base di un algoritmo di *deep learning*, sono stati valutati gli effetti causali di questi fattori di rischio, che sono stati poi incorporati nella stima del rischio. Gli algoritmi di *deep learning* sono stati basati su studi di randomizzazione mendeliana che hanno valutato 140 varianti geniche associate all'LDLc e 202 varianti associate alla SBP.

Lo studio ha dimostrato che l'accuratezza della previsione del rischio può essere notevolmente migliorata con l'AI causale, ma soprattutto suggerisce che l'AI causale può prevedere l'impatto di azioni specifiche per ridurre questo rischio nel contesto della traiettoria del paziente verso gli eventi CV. L'integrazione degli effetti causali negli algoritmi di stima del rischio consente di stimare con precisione il rischio cardiovascolare basale causato dalle LDL e dalla SBP e il beneficio derivante dalla riduzione delle LDL, della SBP o di entrambe a partire da qualsiasi età e per qualsiasi durata.

In un test dell'impatto predittivo dell'AI causale, la previsione del rischio è stata condotta su 445.771 partecipanti alla UK Biobank. Rispetto agli eventi reali in questa popolazione, lo *score* di rischio CV ha costantemente sottostimato l'aumento del rischio causato dall'aumento delle LDL, della pressione o di entrambi nel corso della vita del paziente. Inoltre, ha sistematicamente sovrastimato il rischio di eventi cardiovascolari tra i partecipanti con LDLc, SBP o entrambi più bassi. Tuttavia, dopo aver incorporato l'effetto causale delle LDL e della pressione arteriosa, lo stesso *score*

è stato in grado di prevedere con precisione il rischio di eventi cardiovascolari.

I benefici di Evolocumab aumentano con un *follow up* più lungo: il trial FOURIER OLE

L'abbassamento dei lipidi a lungo termine con evolocumab riduce ulteriormente gli eventi cardiovascolari, compresa la morte per cause CV, senza segnali preoccupanti di sicurezza, secondo i risultati dello studio FOURIER con estensione in aperto (OLE).

Nello studio principale FOURIER, il trattamento con l'inibitore di PCSK9 per una mediana di 2,2 anni ha ridotto l'*endpoint* primario di efficacia del 15%, ma non ha mostrato alcun segnale sulla mortalità CV rispetto al placebo in pazienti con malattia aterosclerotica in terapia con statine.

Ora, con un *follow-up* di 8,4 anni - il più lungo fino ad oggi in qualsiasi studio su PCSK9 - la mortalità cardiovascolare è stata ridotta del 23% nei pazienti che sono rimasti in terapia con evolocumab rispetto a quelli originariamente assegnati al placebo (3,32% vs 4,45%; HR 0,77; IC 95% 0,60-0,99). Le curve di Kaplan-Meier durante FOURIER erano essenzialmente sovrapposte e solo dopo l'inizio del periodo di estensione in aperto con un *follow-up* a più lungo termine il beneficio in termini di riduzione della mortalità cardiovascolare è diventato evidente.

FOURIER-OLE ha arruolato 6635 pazienti (3355 randomizzati a evolocumab e 3280 a placebo), che hanno completato lo studio principale e hanno assunto evolocumab per via sottocutanea scegliendo tra 140 mg ogni 2 settimane o 420 mg mensili. Le visite previste dallo studio sono state effettuate alla settimana 12 e poi ogni 24 settimane. Il *follow-up* mediano è stato di 5 anni. Alla settimana 12, l'LDLc mediano era di 30 mg/dL e questo valore è stato mantenuto per tutto il *follow-up*. La maggior parte dei pazienti ha raggiunto livelli molto bassi di LDLc, con il 63,2% che ha raggiunto livelli inferiori a 40 mg/dL e il 26,6% inferiori a 20 mg/dL.

I pazienti assegnati in modo casuale a evolocumab rispetto al placebo nello studio principale presentavano un rischio inferiore del 15% per l'esito primario di morte CV, MI, ictus, ospedalizzazione per angina instabile o rivascolarizzazione coronarica. Il rischio di morte CV, MI o ictus era inferiore del 20% e, come già detto, del 23% per la morte CV.

Quando i dati sugli eventi cardiovascolari avversi maggiori sono stati analizzati per anno, la maggiore riduzione delle LDL si è verificata negli anni 1 e 2 dello studio principale (Δ 62 mg/dL tra i bracci di trattamento), evidenziando il ritardo del beneficio che ha continuato ad accumularsi nel tempo. C'è stato poi un effetto di trascinamento nel periodo di estensione: il beneficio è stato più evidente all'inizio durante l'estensione in aperto e poi, come ci si potrebbe aspettare quando tutti i pazienti sono stati trattati con la stessa terapia, ha cominciato ad attenuarsi con il tempo.

I tassi di incidenza annualizzati per i pazienti inizialmente randomizzati a evolocumab non hanno superato quelli dei pazienti trattati con placebo per nessuno degli eventi di interesse analizzati (eventi gravi di sicurezza, ictus emorragico, diabete di nuova insorgenza, eventi muscolari, reazioni al sito di iniezione e reazioni allergiche correlate al farmaco)

Due vecchi farmaci e un DOAC hanno mostrato risultati fallimentari in tutta la gamma di gravità del COVID-19: gli studi ACT

La scoperta che alcuni dei migliori farmaci per il trattamento dei pazienti affetti

da COVID-19 sono anche poco costosi, abbastanza sicuri e facilmente disponibili sarebbe una fortuna notevole. Ma se tali farmaci esistono, è improbabile che includano la colchicina o l'aspirina, come suggeriscono un paio di studi randomizzati che hanno arruolato pazienti con COVID ricoverati o trattati ambulatorialmente.

La colchicina non ha mostrato alcuna differenza in termini di ricovero, ventilazione meccanica o morte nell'arco di 6 settimane, né negli studi ospedalieri né in quelli ambulatoriali che costituiscono il programma di sperimentazione della terapia anticoronavirus (ACT). L'effetto dell'aspirina sulla trombosi maggiore, sull'ospedalizzazione o sulla mortalità nello studio ambulatoriale è stato analogamente neutro.

Nei pazienti COVID ricoverati, inoltre, l'aspirina associata a rivaroxaban, un anticoagulante orale diretto (DOAC) a basso dosaggio, non ha migliorato il rischio composito di eventi gravi come tromboembolismo venoso (TEV), necessità di supporto ventilatorio o morte. Il trattamento combinato sembrava aumentare il rischio di emorragie complessive, ma non aveva alcun effetto sulle emorragie clinicamente gravi.

Il risultato di questi due studi, soprattutto alla luce di studi precedenti sugli stessi temi, è che l'aspirina con rivaroxaban a basso dosaggio non dovrebbe essere utilizzata nei pazienti ricoverati con COVID, l'aspirina giornaliera da sola non apporta benefici ai pazienti ambulatoriali con COVID e la colchicina non ha alcun effetto sugli esiti clinicamente importanti in entrambi i contesti.

PANTHER: il clopidogrel dovrebbe diventare la "nuova aspirina" nella malattia coronarica?

I risultati di una nuova meta-analisi suggeriscono che un inibitore P2Y12, come clopidogrel o ticagrelor, potrebbe essere una scelta migliore dell'aspirina per la monoterapia antiaggregante nei pazienti con malattia coronarica. La meta-analisi PANTHER ha dimostrato che il trattamento con inibitori P2Y12 riduce il rischio di eventi ischemici successivi, in particolare di infarto miocardico (MI), rispetto all'aspirina, senza aumentare il rischio di sanguinamento.

Il rischio relativo per l'*endpoint* primario composito – morte cardiovascolare, IMA e ictus – è stato ridotto del 12% nei pazienti che hanno ricevuto un inibitore P2Y12, rispetto all'aspirina, soprattutto grazie a una riduzione relativa del 23% dell'IMA. Anche l'ictus è risultato numericamente ma non significativamente inferiore con la terapia con inibitori P2Y12.

Il rischio complessivo di emorragie maggiori non è risultato significativamente diverso, mentre le emorragie gastrointestinali e l'ictus emorragico si sono verificati meno frequentemente nei pazienti che hanno ricevuto un inibitore P2Y12 piuttosto che la monoterapia con aspirina.

L'attuale metanalisi ha coinvolto studi randomizzati che hanno confrontato la monoterapia con un inibitore orale di P2Y12 o con l'aspirina per la prevenzione secondaria di eventi ischemici in pazienti con cardiopatia conclamata, ma senza indicazione all'anticoagulazione orale. I sette studi inclusi sono stati CAPRIE, HOST-EXAM, GLASSY, TICAB, DACAB, ASCET e CADET. La popolazione dello studio era costituita da 24.325 pazienti provenienti da questi sette studi, di cui 12.178 assegnati alla monoterapia con inibitori P2Y12 (clopidogrel 62,0%, ticagrelor 38,0%) e 12.147 alla monoterapia con aspirina. La durata mediana del trattamento è stata di 557 giorni.

Il rischio per l'esito primario di efficacia - un composito di morte cardiovascolare, MI e ictus - è risultato inferiore con la mo-

noterapia con inibitori P2Y12 rispetto alla monoterapia con aspirina (5,5% vs 6,3%; HR 0,88; IC 95% 0,79-0,97). Il rischio di emorragia maggiore era numericamente ma non significativamente più basso con l'inibizione del P2Y12 rispetto all'aspirina (1,2% vs 1,4%; HR 0,87; IC 95% 0,70-1,09), ma vi era una chiara e significativa riduzione dell'ictus emorragico (HR 0,32) e dell'emorragia gastrointestinale (HR 0,75). Il rischio di eventi clinici avversi netti - definito come il composito dell'*endpoint* primario di efficacia e del sanguinamento maggiore - è risultato inferiore con la monoterapia con inibitori P2Y12 rispetto alla monoterapia con aspirina (6,4% vs 7,2%; HR 0,89; IC 95% 0,81-0,98).

Non sono state osservate differenze nell'effetto in nessuno dei sottogruppi esaminati, tra cui età, sesso, stato di fumatore, diabete, malattia arteriosa periferica (PAD), precedenti ictus o MI, storia di malattia renale, presentazione clinica e tipo di inibitore P2Y12 utilizzato. Tuttavia, i pazienti sottoposti a PCI hanno mostrato una riduzione del 30% dell'*endpoint* composito con un inibitore P2Y12. Questo è in linea con l'osservazione di una riduzione notevolmente elevata della trombosi dello stent con gli inibitori P2Y12 rispetto all'aspirina.

L'analisi ha rivisto un dogma presente in molte linee guida, che raccomandano la monoterapia con aspirina come prima linea per la prevenzione secondaria nei pazienti con malattia coronarica stabile, mentre un inibitore P2Y12 viene preso in considerazione solo nei pazienti con PAD o malattia cerebrovascolare. Questi risultati sono molto importanti e influenzeranno la pratica clinica, ma l'aspirina resta uno standard valido, in quanto è associata a una migliore compliance e a minori effetti avversi *off-target* (rispetto a ticagrelor), a una minore variazione nella risposta al trattamento (rispetto a clopidogrel) e molto probabilmente è più costo-efficace. Tuttavia PANTHER, e in particolare lo studio HOST-EXAM, fornisce buoni argomenti per utilizzare gli inibitori P2Y12 invece della monoterapia con aspirina, in particolare nei pazienti più giovani con una storia di rivascolarizzazione.

Segnale precoce di beneficio per Empagliflozin nel MI acuto: il trial EMMY

L'uso precoce dell'inibitore SGLT2 empagliflozin dopo un infarto miocardico acuto (MI) migliora i livelli di peptidi natriuretici e i marcatori della funzione e della struttura cardiaca, secondo i risultati di un nuovo studio randomizzato.

Gli inibitori SGLT2 hanno mostrato benefici nei pazienti con insufficienza cardiaca (HF) in tutto lo spettro della funzione ventricolare sinistra, riducendo il rischio di ospedalizzazione per HF e di morte cardiovascolare in caso di bassa frazione di eiezione (EF) nello studio EMPEROR-Preserved dello scorso anno e nello studio DELIVER recentemente riportato. Gli inibitori SGLT2 riducono anche il rischio di HF incidente in gruppi ad alto rischio come quelli con diabete di tipo 2 o malattia renale cronica.

Tenendo presente che i benefici emergono nel giro di poche settimane, indipendentemente dallo studio, e che l'infarto miocardico è una delle principali cause di insufficienza cardiaca, viene da chiedersi se il trattamento con un inibitore SLGT2 possa avere effetti benefici se iniziato subito dopo un infarto miocardico acuto.

A questa domanda ha voluto rispondere lo studio in doppio cieco EMMY, condotto in 11 centri austriaci da maggio 2017 a maggio 2022, che ha randomizzato 476 pazienti, entro 72 ore dall'intervento coronarico percutaneo per un infarto acuto, a empagliflozin 10 mg una volta al giorno o un

placebo, in aggiunta alla terapia post-MI indicata dalle linee guida, che comprendeva farmaci antiaggreganti (100%), statine (97%) e inibitori dell'enzima di conversione dell'angiotensina o bloccanti del recettore dell'angiotensina (96%).

L'età media era di 57 anni, il 13% aveva il diabete di tipo 2, l'11% aveva una storia di malattia coronarica e il 4,8% di MI. Il peptide natriuretico N-terminale pro b-tipo (NT-proBNP) mediano era di 1294 pg/mL. I livelli di NT-proBNP sono diminuiti rispetto al basale in entrambi i gruppi, ma l'esito primario di variazione media alla settimana 26 è stato inferiore del 15% nel gruppo empagliflozin, dopo l'aggiustamento per la concentrazione di NT-proBNP al basale, il sesso e lo stato del diabete (P=0,026). L'EF del ventricolo sinistro è migliorata in entrambi i gruppi, mentre i marcatori strutturali del volume sistolico finale del ventricolo sinistro e del volume diastolico finale del ventricolo sinistro sono migliorati e si sono ridotti nel gruppo empagliflozin.

L'antitrombotico a dose piena aiuta i pazienti selezionati dell'unità di terapia intensiva COVID-19

I pazienti ospedalizzati in terapia intensiva a causa di un'infezione acuta da COVID-19 hanno avuto un numero significativamente inferiore di eventi trombotici e di complicanze quando sono stati trattati con anticoagulanti a dose piena rispetto ai pazienti che hanno ricevuto una profilassi anticoagulante a dose standard, ma l'anticoagulazione a dose piena ha anche innescato un eccesso di eventi emorragici moderati e gravi, secondo i risultati di uno studio randomizzato.

I nuovi risultati dello studio COVID-PACT, condotto su una coorte esclusivamente statunitense di 382 pazienti in terapia intensiva con infezione da COVID-19, potrebbero portare a una modifica delle linee guida esistenti, che attualmente raccomandano la profilassi a dose standard sulla base dei risultati di studi precedenti. I nuovi risultati suggeriscono che l'anticoagulazione a dosi piene dovrebbe essere presa in considerazione per prevenire le complicanze trombotiche in pazienti critici selezionati con COVID-19, dopo aver valutato il rischio di eventi trombotici ed emorragie del singolo paziente. Tuttavia, sono stati evidenziati limiti metodologici dello studio, e queste evidenze devono essere ancora confermate.

Infarto miocardico nelle donne sotto i 50 anni

Le giovani donne (sotto i 50 anni) sono sempre più spesso colpite da infarto senza che i medici ne conoscano il motivo.

Le malattie cardiovascolari sono la principale causa di morte nelle donne, con un numero di decessi sette volte superiore a quello del cancro al seno. Il tasso di mortalità ospedaliera è significativamente più alto e, nonostante sia in calo, è nettamente superiore a quello degli uomini (più del doppio), in particolare nelle donne sotto i 50 anni. Inoltre, oltre ai fattori di rischio tipici, le donne presentano fattori di rischio specifici legati a cambiamenti ormonali, profili infiammatori ad alto rischio e trombofilia.

Lo studio Young Women Presenting Acute Myocardial Infarction in France (WAMIF) è stato progettato per determinare le caratteristiche cliniche, biologiche e morfologiche legate alla mortalità ospedaliera a 12 mesi da un infarto miocardico nelle donne sotto i 50 anni. Lo studio prospettico e osservazionale ha incluso tutte le donne di questa fascia d'età provenienti da 30 siti in Francia tra maggio 2017 e giugno 2019. L'età media delle 314 donne arruolate era di 44,9 anni. Quasi due terzi

presentavano un infarto miocardico con sopraslivellamento del segmento ST.

Il profilo di rischio ha rivelato che il 75,5% era fumatore, il 35% aveva una storia familiare di malattie cardiache, il 33% aveva avuto complicazioni in gravidanza e il 55% aveva recentemente vissuto una situazione di stress. L'analisi ha anche mostrato che l'uso di cannabis e la contraccezione orale erano fattori di rischio primari nelle donne con meno di 35 anni. Infatti, nonostante gli autori si aspettassero che molte di queste giovani donne avessero condizioni autoimmuni ampiamente atipiche, con alti livelli di infiammazione, hanno riscontrato che molte donne presentavano fattori di rischio classici, per lo più modificabili. Contrariamente a quanto atteso, inoltre, la maggior parte presentava un ateroma, spesso lesioni ostruttive, o addirittura una malattia a tre vasi, in quasi un terzo della coorte.

Dopo 1 anno dall'evento, il 90,4% non aveva sperimentato alcun tipo di evento CV e il 72% non aveva nemmeno un sintomo, diversamente da quanto suggerito da studi precedenti che avevano mostrato un eccesso di ospedalizzazione nelle donne post-infartuate.

36° CONGRESSO NAZIONALE SISA

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SOCIETA' ITALIANA
PER LO STUDIO
DELL'ATEROSCLEROSI

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STEATOEPATITE NON ALCOLICA E FIBROSI EPATICA IN UNA CASISTICA DI PAZIENTI OBESI SOTTOPOSTI A CHIRURGIA BARIATRICA

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Obiettivi dello studio. Valutare la prevalenza di steatoepatite non alcolica (NASH) e fibrosi epatica in pazienti con obesità grave candidati ad intervento di chirurgia bariatrica. È stato inoltre valutato l'andamento delle transaminasi plasmatiche, adiponectina e di alcuni scores non invasivi di fibrosi epatica avanzata a distanza di 6 e 12 mesi dall'intervento chirurgico.
Popolazione e Metodi. Abbiamo studiato un campione di 28 pazienti affetti da obesità grave (75% donne, età mediana 41 anni, BMI mediano 45 kg/m^2), che sono stati sottoposti ad intervento in elezione di sleeve gastrectomy (19 pazienti) o bypass gastrico (9 pazienti). In tutti i pazienti sono stati eseguiti accertamenti metabolici (incluso 2-h clamp euglicemico iperinsulinemico) al baseline ed è stata eseguita una biopsia epatica durante l'intervento chirurgico.
Risultati. Dei 28 pazienti inclusi nello studio, 16 (57%) hanno soddisfatto i criteri istologici per una diagnosi di NASH, mentre i restanti 12 (43%) pazienti non avevano NASH al baseline. Di questi 12 pazienti privi di NASH alla biopsia, 8 pazienti avevano steatosi macrovescicolare di grado lieve o severo (NAFL), mentre solo 4 pazienti (pari al 14.3% del campione totale) erano esenti da NAFLD alla biopsia epatica. Per quanto riguarda il grado di fibrosi epatica, 4 pazienti (14.3%) non avevano fibrosi (stadio F0), 14 (50%) pazienti avevano fibrosi moderata (F2) e 10 (35.7%) avevano "bridging fibrosis" (F3). Nessuno dei pazienti aveva cirrosi epatica (F4). Quando i pazienti venivano suddivisi sulla base della presenza/assenza di NASH e/o della severità di fibrosi epatica (F3 vs. F0-2), i due gruppi di pazienti erano comparabili per età, sesso e le principali variabili biochimiche esaminate, incluso transaminasi, APRI index, FIB-4 score e sensibilità insulinica (M-clamp). L'intervento chirurgico induceva, sia dopo 6 che 12 mesi, un marcato calo ponderale ed una significativa riduzione dei livelli circolanti di adiponectina in entrambi i gruppi. Al contrario, i valori di transaminasi e gli scores non invasivi di fibrosi epatica avanzata non hanno mostrato alcuna significativa variazione dopo 6 e 12 mesi dall'intervento chirurgico in nessuno dei gruppi di pazienti considerati (NASH vs. no-NASH e F3 vs. F0-2).
Conclusioni. Nei pazienti con obesità grave candidati a chururgia bariatrica la NAFLD è una patologia assai comune (essendo presente in circa 85% del campione) ed è già presente anche nelle sue forme istologiche più severe (NASH nel 57% dei casi e fibrosi avanzata nel 35.7% dei casi), pur rimanendo queste forme spesso clinicamente silenti (o paucisintomatiche) e senza accompagnarsi a significative alterazioni delle transaminasi circolanti. Ciò suggerisce la necessità di una diagnosi precoce e tempestiva delle forme più severe della NAFLD (che sono quelle associate ad elevato rischio di progressione verso la cirrosi ed HCC) in tutti i soggetti obesi che vengono sottoposti a chirurgia bariatrica (da eseguirsi almeno in fase intra-operatoria).

NEED TO BRIDGE THE GAP BETWEEN RESEARCH AND CLINICAL PRACTICE: THE UNMEASURED ADDED VALUE OF LP(A)

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Elevated plasma levels of lipoprotein(a) [Lp (a)] (≥50 mg/dL, observed in approximately 20% of the general population) represent an independent risk factor for cardiovascular (CV) disease. Lp(a) levels are mostly genetically determined, therefore they are relatively stable throughout life. The most recent European guidelines for the management of dyslipidaemias recommend assessing Lp (a) levels at least once in life, to identify individuals who, being exposed to high levels of this atherogenic lipoprotein since birth, suffer from a CV risk higher than that determined by elevated LDL-C levels alone. The SANTORINI study is an observational study that aims to evaluate how, in clinical practice, patients with high and very high CV risk are treated, collecting data at enrolment and after 12 months. Italy participates in this study with 1531 patients at very high risk and 446 at high risk. At enrolment, in the overall population, mean LDL-C and Lp(a) levels were 98.4 mg/dL and 52.5 mg/dL. When stratified by risk, very high-risk patients had mean LDL-C and Lp (a) values of 94.6 mg/dL and 48.7 mg/dL, respectively, while high-risk patients had values of 111.4 mg/dL and 77.9 mg/dL, respectively. In addition to the low percentage of subjects receiving an appropriate lipid-lowering therapy, which is reflected in the observed LDL-C values, it is also known that the levels of Lp (a), especially in the high-risk group, confer an increase individual risk, regardless of LDL-C levels. The assessment of Lp (a) levels, therefore, allows the identification of subjects at greater risk of CV events and avoids an underestimation of the risk, inappropriate therapies, and reduced goal achievement.

LIVER STIFFNESS RELATES TO AN INCREASED RISK FOR ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Non alcoholic fatty liver disease (NAFLD) is a common and emerging liver disease in adults and represents a large and growing public health problem. NAFLD is closely associated with obesity, type 2 diabetes mellitus, metabolic syndrome, and cardiovascular events. The diagnosis of NAFLD is based on the following three criteria: non- alcoholic, detection of steatosis either by imaging or by histology, and appropriate exclusion of other liver diseases. The prevalence is estimated around 25% worldwide, it is around 50% in type 2 diabetes and around 50% in patients with dyslipidemia. NAFLD patients are usually asymptomatic until the condition progresses to liver cirrhosis. Patients affected by NAFLD show a lower-than-expected survival because of increases in the risk of cardiac arrhythmias such as atrial fibrillation and ventricular arrhythmia. NAFLD is often detected based on the presence of hepatic steatosis on abdominal ultrasound (US) during routine health checkups. US is gold standard examination for NAFLD diagnosis, as it is a non- invasive method, relatively cheap and widely available. Liver Stiffness Measurement (LSM) through FibroScanTM (transitory elastography) is a reliable index to assess liver librosis. Similar diagnostic accuracy is shown by Shear Wave Elastography (SWE), a technique US devices have been improved with. Purpose of the study is to evaluate ASCVD risk in NAFLD where the liver fibrosis could represent, alone, a non-lipid marker of cardiovascular risk.

Methods. We evaluate 41 patients (15 F, aged 58.71±13.56) who presented NAFLD according to the recent international guidelines. We excluded from the study patients in steroid therapy. Using the liver ultrasound we evaluate the liver stiffness as fibrosis marker as Stiffness measured by share wave technology. As controls, we compared 88 patients who were NAFLD negative (33F, aged 57.39±9.91). To evaluate the cardiovascular risk we calculate for each patient Atherosclerosis Cardiovascular disease score (ASCVD-10-yr).

Results. In NAFLD group, arterial essential hypertension was in 58.53% (24) and diabetes in 34.15% (14). Similar percentages of comorbidities were found in Controls. Patients with NAFLD has an increased liver fibrosis (median fibrosis class 1 [1QR 1-3]), compared to controls (F 0) ($p<0.05$) and an increased cardiovascular risk compared to controls (ASCVD-10-yr 17.21±17.99% vs 7.93±7.68; $p<0.05$). Shear wave kPa range (Spearman r 0.37, $p<0.01$) Fib4 and NFS score were directly correlated to ASCVD-10-yr. The same direct correlation was found between ASCVD-10-yr and absolute liver stiffness values evaluated in kPa (Pearson r 0.39, $p<0.05$). In particular higher fibrosis (F2-F3) was related to a nearly 50% increased ASCVD in 10 years.

Conclusion. Our data suggest that NAFLD may relate to a reduced life-expectancy mostly due to cardiovascular disease. Dyslipidemia and dysregulation of glucose homeostasis are underlying risk factors in NAFLD that contribute to the increased ASCVD risk, but the predilection for ectopic fat deposition in the liver and other tissues, in particular epicardial fat, seems to be associated with heightened risk of ASCVD beyond the risk attributable to traditional risk factors.These data may be useful in evaluation of both cardiovascular disease and liver steatosis due the connection we found between the two parameters. Thus, a comprehensive evaluation may be useful in order to optimize the tailored therapy for these patients. It would also be interesting to follow the decrease in visceral fat storage in patients with new antidiabetic medication like GLP-1 receptor antagonists. Further studies are surely needed.

A NEW METHOD OF EXTRACELLULAR VESICLES SEPARATION AND CHARACTERIZATION HIGHLIGHTS THEIR IN VITRO HETEROGENEITY-RELATED BIOLOGICAL PROPERTIES

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Extracellular vesicles (EVs) partecipate, by transferring their cargo from cell to cell, in pathophysiological processes. Unfortunately, unproper separation and characterization methods impair the full comprehension of their functions. To overcome this problem, we set up an ultracentrifugation method to size-separate different EV populations derived from a melanoma cell line, following an algorithm developed by Livshits. We characterized EVs by transmission electron microscopy (TEM), atomic force microscopy and dynamic light scattering (Zetasizer). Fatty acid (FA) profile and protein content were evaluated by gas-chromatography and mass spectrometry. Purity from external proteins was assayed by CONAN method. Size analysis not only confirmed the existence of different EV populations, but also the theoretical sizes calculated by the algorithm. Gas-chromatography analysis revealed a continuous percentage increase in saturated FA ranging from parental cells to smaller EVs (33.61%-64.79%), suggesting different membrane properties among populations. Mass spectrometry analysis identified 2003 proteins with qualitative and quantitative differential distribution among the separated populations. As expected, the MetaCore pathway analysis performed on individual cargos evidenced, besides common signaling pathways, molecular properties that specifically characterize each fraction. This suggests distinctive behaviors and biological functions for different EVs. Finally, the interactomic analysis, performed on identified proteins from vesicles with smallest diameter, evidenced a complex and highly integrated network of involved in a fine regulation of target-cell and environmental plasticity. Our separation method may be applied to any cell line, being helpful in defining the role of specific EV populations in cardiovascular diseases and in finding new pharmacological treatments able to modulate EV functions.Supported by EXTRALIPO Bando SEED 2019.

EFFECT OF SEMAGLUTIDE ON GLOBAL LONGITUDINAL STRAIN AND GLOBAL MYOCARDIAL WORK EFFICIENCY IN PATIENTS WITH UNCONTROLLED TYPE 2 DIABETES MELLITUS AND OBESITY

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Background. Glucagon-like peptide-1 receptor agonists (GLP1-RAs) are peptide molecules that exert their action through potentiation of insulin secretion, suppression of glucagon release, delayed gastric emptying, and weight loss. Cardiovascular outcome trials (CVOTs) demonstrated that GLP1-RAs have effectively reduced cardiovascular (CV) risk in type 2 diabetes mellitus (T2DM) patients, so their use is recommended by guidelines. The objective of our work was to evaluate the effect, in a cohort of uncontrolled diabetic patients, of Semaglutide, on oxidative stress markers (8-isoprostane and NOX-2), platelet activation indicator (Sp-Selectin) and subclinical myocardial damage evaluated by measurement of deformation and efficiency parameters, obtained by speckle tracking echocardiography (STE).

Materials and Methods. We performed a retrospective analysis enrolling 70 Caucasian patients (mean age 65.5±8.2, 54 men and 16 women) who met the following inclusion criteria: diagnosis of T2DM within 5 years, uncontrolled T2DM, obesity. Exclusion criteria were previous CV events, atrial fibrillation, heart failure. All clinical evaluation and laboratory tests were performed at baseline and after six months of treatment. The serum values of oxidative stress markers (8-isoprostane, NOX-2) and platelets activation (Sp-selectin) were assessed with ELISA sandwich. Echocardiographic recordings were performed by a single blind operator. Continuous variables were expressed as mean±standard deviation. For all continuous variables, comparisons between baseline (T0) and post-treatment values (T6) were performed using paired Student's t test. A linear regression analysis was performed to assess the relationship between variation in Global Longitudinal Strain (GLS), GLS endo-epi ratio, and global myocardial work efficiency (GWE), expressed as Δ of variation between baseline and follow-up (ΔT0–6) and the variation of metabolic, inflammatory, oxidative stress and platelets activation covariates that significantly improved after the treatment (expressed as ΔT0-6).

Results. Among enrolled patients, 85.7% presented hypertension, 18.5% had chronic kidney disease and 41.4% had dyslipidaemia. All patients were in treatment with statins and metformin, 42.8% with insulin, 24.2% with diuretics, 37.1% wit ACE inhibitors, and 48.5% with angiotensin receptor antagonists. The mean dose of Semaglutide was 0.59±0.29 mg/week without serious adverse events. At six months, data showed significant improvement in hemodynamic and clinical parameters such as systolic and diastolic blood pressure (SBP, DBP), heart rate ($p<0.004$), NT-ProBNP ($p<0.0001$); and metabolic parameters: fasting plasma glucose, insulinemia, HOMA, IGF-1, HbA1c and BMI ($p<0.0001$). Lipid profile and renal function also showed an improvement ($p<0.0001$). In addition, there was a significant reduction in biomarkers of oxidative stress such as 8-isoprostane, Nox-2 ($p<0.0001$), biomarkers of platelet activity such as Sp-Selectin ($p<0.0001$) and high-sensitivity C-reactive protein (hs-CRP) ($p<0.0001$). In addition, we observed a significant improvement in left ventricular myocardial deformation parameters such as GLS ($p<0.0001$), GLS endo/epi ($p<0.0001$) and GWE ($p<0.0001$). The linear correlation analysis showed that ΔGLS endo-epi was inversely correlated with ΔHOMA ($p=0.001$), Δuric acid ($p=0.012$), ΔNox-2 ($p=0.016$), ΔSp-selectin ($p=0.010$); ΔGLS was inversely correlated with ΔHOMA ($p=0.011$), Δuric acid ($p=0.025$) and ΔSp-selectin ($p<0.0001$); ΔGWE was inversely correlated with ΔHOMA ($p=0.011$), Δuric acid ($p=0.025$) and ΔSp-selectin ($p<0.0001$). From stepwise multivariate linear regression model, ΔSp-selectin, ΔHOMA, Δuric acid and Δ NOX-2 justifying respectively 18.1%, 7.2%, 5.6% and 5.3% of ΔGLS endo-epi; ΔSp-selectin, Δuric acid and ΔHOMA justifying 27.8%, 4.4% and 5.6% of ΔGLS respectively. Instead ΔNOX-2, Δhs-CRP and ΔBMI justifying 22.3%, 15.6% and 4.0 % of ΔGWE respectively. Results of our study demonstrated that six months treatment with Semaglutide, in patients with uncontrolled T2DM and obesity, improved GLS, GLS endo-epi ratio and GWE. It's plausible that this improvement may be justified by the reduction of inflammatory, oxidative stress and platelet activation parameters together with favourable metabolic changes; thus promoting the protection of the cardiac microcirculation with improving in myocardial contractility.

GENETIC CHARACTERIZATION OF LIPOPROTEIN(A) KRINGLE IV TYPE 2 REPEAT POLYMORPHISM: COMPARISON BETWEEN DIGITAL DROPLET AND REAL-TIME PCR

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Background. Evidence to support the role of lipoprotein(a) [Lp(a)] as a risk factor for atherosclerosis and thrombosis continue to increase. Lp(a) levels strongly differ among individuals, and many studies have shown the relationship between variants in the LPA gene, encoding apolipoprotein(a), and the increase in Lp(a) levels, closely related to the increase in cardiovascular risk. The major genetic determinant of these levels is a copy number variation (CNV) polymorphism, consisting of a variable number of repeats of a 5 kb region that includes exons 4 and 5 of the gene, encoding the protein domain Kringle IV type 2 (KIV-2). A lot of studies were able to support causality between Lp(a) and clinical outcomes through the analysis of KIV-2 repeat polymorphism. However, the peculiar structural characteristics of this variant constitute a significant challenge to the development of accurate methods for its detection. In this study, we compared quantitative real-time PCR (qPCR) and digital droplet PCR (ddPCR) in KIV-2 repeats determination.
Methods. 100 patients with possible/probable/certain diagnosis of Familial Hypercholesterolemia according to the Dutch Lipid Clinic Network score were analysed. Demographic and laboratory/clinical characteristics of the study population were collected. CNV values were obtained with qPCR using the 7900HT Sequence Detection System and with ddPCR using the QX200 Droplet Generator and reader system. Telomerase reverse transcriptase gene (TERT) was used as a single-copy reference gene in both techniques. To make CNV values comparable within and between different plates, three internal controls (CNV: C1=41-47, C2=50, C3=63-68) were used.
Results. In the whole cohort, qPCR analysis showed median values of repeats of 29.45 [IQR: 20.89-41.49], while ddPCR of 10.24 [IQR: 8.92-12.26]. Correlation analysis between the two methods was slightly significant, because of the greater dispersion of data obtained by qPCR compared with ddPCR. In fact, we found a very huge discrepancy in results using qPCR; really, C1, C2, C3 internal controls measurement throughout different experimental sessions reported lower data dispersion and greater stability in ddPCR with respect to qPCR: control sample C2, used as a reference sample for qPCR and estimated to have around 50 repeats, was confirmed to have a mean value of 54.85±1.11 with ddPCR analysis; control C1 and C3 showed a mean±SD of 37.59±9.05 in qPCR vs 44.28±2.74 in ddPCR and 101.18±45.12 in qPCR vs 65.02±3.53 in ddPCR, respectively. Spearman's rho test showed an inverse proportional correlation between Lp(a) levels of each patient and the CNV polymorphism, as expected, but higher and significant when evaluated with ddPCR despite qPCR (R=-0.393, p<0.001 vs R=-0,220, p=0,028, respectively). Dividing patients in two groups based on Lp(a) concentration (300 mg/L as cut off), a significant lower number of repeats of the KIV2 domain emerged among patients with greater levels of Lp(a) compared with the other group in both methods but with strongly evidence with ddPCR than in qPCR (P<0.001 and P=0.003, respectively).
Conclusion. Data obtained support the contribution of this molecular characterization approach in CNVs measurement, so strengthening the need of confirming results in larger cohorts in the effort of identifying a suitable method for the evaluation of a complex polymorphic variant representing the main genetic determinant of Lp(a) levels. The achievement of this goal might pave the way to improve genetic characterization of Lp(a) trait, in particular in the dyslipidaemic population, in order to better frame the risk profile of these patients.

TRIGLYCERIDES-GLYCAEMIA INDEX PREDICTS CARDIOVASCULAR EVENTS IN NON-ALCOHOLIC FATTY LIVER DISEASE

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Introduction. Non-alcoholic fatty liver disease (NAFLD) prevalence is steadily growing. The NAFLD epidemic is strongly associated with that of obesity and type 2 diabetes. NAFLD is considered the hepatic manifestation of metabolic syndrome and most patients affected by NAFLD develop cardiometabolic complications rather than liver ones. Aim of the study was to investigate which of Triglycerides and glucose index (TyG) and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) indexes better predicts cardiovascular events in NAFLD patients.
Methods. This is a post-hoc analysis performed in 830 patients enrolled in the Plinio Study (Progression of LIver Damage and Cardiometabolic Disorders in Non-alcoholic Fatty Liver Disease: an Observational Cohort Study. ClinicalTrials.gov Identifier: NCT04036357). The study includes dysmetabolic patients investigated for the presence of NAFLD. TyG index (ln[Fasting triglycerides (mg/dl) x Fasting glucose (mg/dl)] / 2) and HOMA-IR (Fasting insulin (mg/dl) x Fasting glucose (mg/dl)/405) were calculated as insulin resistance scores. Data on incident Cardiovascular Events (CVEs) were collected during the follow-up.
Results. NAFLD was present in 82.8% of patients. Both TyG index (4.8±0.3 vs. 4.6±0.2; p<0.001) and HOMA-IR (3.4 [2.4-5.6] vs. 1.7 [0.9-2.5], p<0.001) were higher in patients with NAFLD. Higher tertile of both scores independently associated with NAFLD diagnosis (TyG index III tertile aOR: 4.02, p<0.001; Homa-IR III tertile aOR: 7.31, p<0.001) after correction for age, sex, obesity, diabetes, arterial hypertension, prior CVEs and low-Fib4 score. Median follow-up duration was 47.6 [23.9-75.7] corresponding to 3629.6 person-years in NAFLD subgroup. The incidence rate of CVEs in NAFLD patients was 1.5%/year. Multivariable regression analyses showed that TyG Index III tertile (aHR: 1.86; p<0.01) but not Homa-IR III tertile predicted CVEs incidence after correction for age, sex, obesity, diabetes, arterial hypertension, prior CVEs, low Fib-4 score.
Conclusion. Unlike the HOMA-IR, TyG index predicts CVEs in NAFLD patients, and its use could help identify patients in need of more careful cardiovascular prevention.

IN VITRO AND EX VIVO STUDIES TO EVALUATE THE EFFECTS OF EDOXABAN ON PLATELET FUNCTION

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Background. All anticoagulants are expected to have an indirect effect on platelet function since they interfere with the generation or activity of thrombin, but the impact of Direct Oral Anticoagulants (DOACs) is largely unknown. Previous studies conducted with Dabigatran, Apixaban and Rivaroxaban showed a reduction in endogenous thrombin potential (ETP) and in platelet aggregation induced by thrombin and tissue-factor (TF) in a dose dependent manner. Aim of this study was to evaluate the effects of Edoxaban on platelet function by in vitro and ex vivo studies.
Methods. We evaluated platelet aggregation (PA), thrombin generation (TG) and thromboxane B2 (TXB2) levels in 20 healthy donors: samples were incubated in vitro with increasing concentrations of Edoxaban [E50, E150, E250 (ng/mL)] or vehicle as control. We also investigated the same parameters in 12 patients with Atrial fibrillation (AF) treated with Edoxaban (ex vivo study). A PAP-8 aggregometer was used to assess PA on PRP samples, induced by ADP (5 µM), TRAP-6 (10 µM), Human Thrombin (THR, 0.182 mU/µL) and TF. TG was measured using the Calibrated Automated Thrombogram System (CAT). Serum TXB2 was measured by using the TXB2 EIA kit, according to the manufacturer's instructions.
Results. The incubation of PRP with different Edoxaban concentrations significantly reduced TF-induced PA with respect to vehicle [E50 by 21% ($p=0.033$), E150 by 33% ($p=0.004$), and E250 by 52% ($p<0.001$)]. TF-induced PA was significantly lower in patients treated with Edoxaban than in controls ($p<0.001$). ADP and TRAP-6-induced PA was not inhibited by any Edoxaban concentrations in the in vitro study, and also ex vivo experiments failed to demonstrate any difference between ADP and TRAP-6-induced PA from AF patients treated with Edoxaban and controls. THR-induced aggregation in E150 group showed a trend towards a reduction, though did not reach the statistical significance. Among the parameters related to TG, Lag Time was significantly ($p<0.001$) and positively related to Edoxaban concentrations. Patients showed more prolonged Lag Time values ($p=0.031$) with respect to those observed in controls. ETP and Peak were significantly reduced in vitro ($p<0.001$) by the incubation of Edoxaban in a dose dependent manner. In in vitro study, ETP ratio values were significantly reduced according to increasing Edoxaban concentrations. AF patients showed reduced levels of ETP ratio with respect to controls ($p<0.001$). We found a 24% decrease in serum TXB2 concentration in the E250 group vs control ($p<0.01$), while the reduction is not significant in the other Edoxaban concentrations.
Conclusions. Our data show that Edoxaban is able to interfere with platelet function. In particular, it significantly reduces TF-induced platelet aggregation in a dose-dependent manner and also TG, although thrombin-induced aggregation is not affected by the drug, supporting Edoxaban's indirect effect on thrombin by inhibiting FXa. In addition, the reduction of TXB2 levels by Edoxaban suggests that the drug is endowed with an antiplatelet effect, which may, in turn, lead to the delayed/reduced formation of coagulation complexes reinforcing its antithrombotic potential.

ROLE OF DENDRITIC CELL IMMUNORECEPTOR 2 (DCIR2) IN EXPERIMENTAL ATHEROSCLEROSIS

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Background and Aim. DCIR2 (Dendritic cell immunoreceptor 2) is a C-type lectin receptor mainly expressed by dendritic cells, responsible for modulating the adaptive response towards pathogens. The peculiarity of this receptor is the inhibitory intracellular domain able to dampen the response driven by other immune cell receptors. Thus, given the strong impact of the immune system in atherogenesis, the aim of this project was to study the contribution of DCIR2 in atherosclerosis.
Materials and Methods. Male Dcir2-/- Ldlr-/- (DKO) and Ldlr-/- mice were fed a cholesterol-enriched diet for 12 weeks to encourage atherogenesis. Subsequently, paralleled to the analysis of circulating lipid and immune cells, the characterization of atheromatous plaque within ascending aorta was performed.
Results. Compared with the control counterpart, DKO mice showed decreased plasma cholesterol and triglycerides levels (-42%, $p<0.05$; -25%, $p<0.01$ respectively), which resulted in a better phenotype of the atherosclerotic plaque - characterized by the reduced plaque formation both at aortic sinus level (-58%, $p<0.01$) and along the first tract of the aorta (-64%, $p<0.05$) for 300µm -. Although atheromatous plaques were less necrotic (-91%, $p<0.05$), they did not differ either in the fibrotic component or in the content of smooth muscle cells and macrophages. Of note, while a significant decrease in circulating neutrophils and monocytes (-47%, $p<0.05$; -38%, $p<0.01$) was observed in the DKO mice compared to Ldlr-/-, the number of bone marrow myeloid precursors increased (+42% $p<0.05$).
Conclusions. DCIR2 is a receptor mainly expressed by dendritic cells and is involved in the modulation of the immune response; our data suggest that it is also implicated in the development of atherosclerosis. We are currently investigating whether this is related to a different regulation of the immune-inflammatory response.

THE DUAL ACTIVITY OF FUROXANS MAY AMELIORATE DIFFERENT ASPECTS OF ATHEROSCLEROSIS: AN IN VITRO STUDY ON SMOOTH MUSCLE CELLS

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Atherosclerosis is a multifactorial disease in which, beyond lipid accumulation and inflammation, nitric oxide (NO) unbalance and smooth muscle cell (SMC) proliferation play pivotal roles. To find a new pharmacological approach, we synthesized furoxans, which demonstrated to release NO in a controlled fashion and we tested their ability in inhibiting SMC proliferation, together with the comprehension of their mechanism of action. We measured SMC proliferation by cell counting (Coulter Counter) after 72 hours of incubation or by thymidine incorporation (20 hours). Proteomics was assessed by SILAC followed by MetaCore analysis or by western blot techniques.We demonstrated that all the tested furoxans inhibit SMC growth and cause rat aorta rings vasodilation, albeit with different potency. To comprehend their antiproliferative mechanism, after blocking the position 4 of their ring by a phenyl group, we found that their inhibitory potency paralleled with the electron-attractor capacity of the group in 3. Extending the study to related furoxans (in which groups in 3 and 4 are interchanged) and furazans (analogues without ring-opening capacity and therefore unable to release NO), we found that 4-Ph-3-R furoxans were the most potent inhibitors of SMC proliferation, followed by 3-Ph-4-R furoxans. Furazans were not effective, documenting that the opening of the ring is essential for growth inhibition. To understand the molecular basis of this effect, we demonstrated that the mechanism is neither cGMP- nor polyamine-dependent, the two main NO-mediated pathways involved in SMC proliferation. Finally, proteomic experiments assessed that specific proteins (12) and specific networks involved in cell homeostasis (e.g. SUMO1, BANF1) are modulated by furoxans, probably by interaction with adducts generated by their ring opening, rather than NO release. Altogether, thanks to their pharmacological flexibility compared with classical NO donors, furoxans may be tested in animal models of atherosclerosis to assess their efficacy as antiatherosclerotic molecules.

PCSK9 AND LEPTIN PLASMA LEVELS IN ANOREXIA NERVOSA

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Aim. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a regulator of low-density-lipoprotein cholesterol (LDL-C), a major risk factor of cardiovascular (CV) disease. Since the hormone leptin has been suggested to have a role in CV risk regulation, possibly by modulating LDL receptor expression through the PCSK9 pathway, nutritional status may represent a potential regulator. Thus, evaluation of PCSK9 levels in human eating disorders appears of interest. In this report we evaluated the lipoprotein profile, PCSK9 and leptin levels in subjects affected by Anorexia Nervosa (AN), to improve the understanding of the metabolic alterations in this disease.
Methods and Results. We have set up a case-control observational study, enrolling 20 anorexic adolescent females and 20 normal adolescent females as control group, age and sex matched. AN subjects showed a lower BMI, total cholesterol and LDL-C respect to control group, with lipoprotein levels in the normal range. Further, adolescent AN girls show significantly higher PCSK9 (+24%, $p<0.005$) and lower leptin levels (-43%, $p<0.01$), compared to the control group.
Conclusions. Raised levels of PCSK9 and reduced leptin, stimulate further research unravelling the role of liver and adipose tissue in the handling of PCSK9/LDL metabolism in adolescent anorexia nervosa.

ANGPTL3 AND PCSK9 DIRECTLY INTERACT AND COORDINATE THE REGULATION OF CELLULAR METABOLISM IN VITRO

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Background and Aims. ANGPTL3 and PCSK9 are known regulators of lipoprotein metabolism. Patients harboring homozygous loss of function mutations in the ANGPTL3 gene, show reduced levels of circulating PCSK9, indicating a possible coordinate regulation of these two proteins. This study aimed to establish whether the two proteins can cross-regulate in different conditions of nutritional availability.
Material and Methods. ANGPTL3, PCSK9, or both genes were overexpressed in HepG2 cells grown in glucose-rich (Feeding) and glucose-poor (Fasting) conditions. We performed Real-time PCR to study ANGPTL3 and PCSK9 mRNA levels, Co-immunoprecipitations (Co-Ip) to verify protein-protein interaction, and western-blotting to quantify the produced proteins and apoB secretion both intracellularly and extracellularly in the culture medium.
Results. Glucose determines a 5-fold increase in the ANGPTL3 mRNA levels and a 1.5-fold increase in the PCSK9 mRNA levels in HepG2 cells. The Co-immunoprecipitation in baseline growth conditions highlighted a direct protein-protein interaction of PCSK9 and ANGPTL3 intracellularly. The western blot analysis showed that the two proteins have a similar secretion pattern dependent on glucose availability in the culture medium. ANGPTL3 overexpression determines PCSK9 intracellular accumulation in fasting conditions, the opposite is observed in the overexpression of PCSK9. The double overexpression determines a consensual secretion increase of both proteins, more evident in feeding conditions. In addition, also apoB secretion appears to be tightly dependent on glucose levels showing a substantial increase in the case of ANGPTL3 and PCSK9 overexpression (Fold-change x2,8).
Conclusion. ANGPTL3 and PCSK9 are transcriptionally cross-regulated, they respond to changes in glucose availability and show co-secretion patterns in vitro. The two proteins are in close intracellular interaction, they are finely regulated in the same direction in response to metabolic stimuli and both promote apoB secretion and accumulation in culture media.

THE LOW-DENSITY LIPOPROTEIN RECEPTOR-MTORC1 AXIS COORDINATES CD8 T CELL ACTIVATION

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Background. Activation of T lymphocytes combines functional to metabolic rewiring of cell machinery, including cholesterol homeostasis. Here we evaluated the role of LDLR, as a key regulator of cholesterol cellular uptake, on T cell biology.
Methods. Immunophenotypic characterization of T cells from WT and LDLR KO mice was performed in vitro (anti-CD3/CD28) and in vivo (ovalbumin vaccination) coupled to proteomics and WB analysis on isolated T cells. T cells from FH (familial hypercholesterolemia) patients, carrying mutations in the LDLR gene, were tested.
Results. LDLR mRNA expression increased after in vitro activation of CD8, but not CD4 T cells, suggesting a different regulation of cholesterol homeostasis between T cell subsets. Functionally, deficiency of LDLR mainly dampened CD8 vs CD4 activation as demonstrated by in vitro proliferation (-35%, $p<0.01$) and INFγ production (-39.6%,$p<0.01$), and in vivo proliferation and cytokine production (↓IFNγ $p<0.001$,↓IL13 $p<0.01$,↓perforin $p<0.05$) after ovalbumin vaccination. Addition of LDL to serum free media increased by roughly 15%($p<0.01$) CD8 proliferation in WT but not in KO and in CD4 cells. By proteomic and WB analysis we associated this phenotype to a reduced activation of mTORC1 (pmTOR -40%,$p<0.01$) and impaired lysosomal organization (reduced lysotracker and LAMP-1 expression). CD8 T cells from FH patients proliferated less (-36%,$p>0.05$) compared to sex- and age-matched controls; in addition, CD8 from FH vaccinated for seasonal influenza were tested in vitro with virus-derived peptides, showing a decreased granzyme production (-60.3%,$p<0.01$) compared to CD8 from vaccinated controls.
Conclusions. LDLR plays a critical role in regulating the immunometabolic responses in CD8 Tcells by fuelling the cholesterol-lysosome-mTORC1 axis.

EFFECT OF PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 INHIBITORS ON PULSE WAVE VELOCITY AND MONOCYTE-TO-HIGH-DENSITY-LIPOPROTEIN-CHOLESTEROL RATIO IN FAMILIAL HYPERCHOLESTEROLEMIA SUBJECTS: RESULTS FROM A SINGLE-LIPID-UNIT REAL-LIFE SETTING

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Introduction. Subjects with familial hypercholesterolemia (FH) are characterized by an increased amount of low-density lipoprotein cholesterol (LDL-C) that promotes a continuous inflammatory stimulus. Our aim was to evaluate the effect of proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9-i) on inflammatory biomarkers, neutrophil-to-lymphocyte ratio (NLR), monocyte-to-high-density lipoprotein ratio (MHR), and on early atherosclerosis damage analyzed by pulse wave velocity (PWV) in a cohort of FH subjects.
Methods. In this prospective observational study, we evaluated 56 FH subjects on high-intensity statins plus ezetimibe and with an off-target LDL-C. All subjects were placed on PCSK9-i therapy and obtained biochemical analysis as well as PWV evaluation at baseline and after six months of PCSK9-i therapy.
Results. After six months of add-on PCSK9-i therapy, only 42.9% of FH subjects attained LDL-C targets. As expected, a significant reduction of LDL-C (-49.61%, $p<0.001$) was observed after PCSK9-i therapy. Neutrophil count (NC) and MHR were reduced by PCSK9-i (-13.82% and -10.47%, respectively, p value for both<0.05) and PWV significantly decreased after PCSK9-i therapy (-20.4%, $p<0.05$). Finally, simple regression analyses showed that Δ PWV was significantly associated with Δ LDL-C ($p<0.01$), Δ NC and Δ MHR (p value for both <0.05).
Conclusions. In conclusion, PCSK9-i therapy significantly improved lipid and inflammatory profiles and PWV values in FH subjects; our results support the positive effect of PCSK9-i in clinical practice.

EFFICACY AND SAFETY OF LOMITAPIDE IN PEDIATRIC PATIENTS WITH HOMOZYGOUS FAMILIAL HYPERCOLESTEROLEMIA: IS DISCONTINUATION OF LIPOPROTEIN AFHERESIS POSSIBLE?

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Introduction. Lomitapide is a microsomal transfer protein inhibitor approved for the treatment of adults with homozygous familial hypercholesterolemia (HoFH). The use of lomitapide in HoFH pediatric subjects is described only by few case-reports.AIM OF THE STUDY: In this study, as part of the first multicenter trial in the word, we evaluated the efficacy and the safety of lomitapide on top of conventional therapy with statin, ezetimibe and lipoprotein apheresis (LA) in pediatric subjects with HoFH.
Subjects and Methods. 2 males and 2 females, aged between 6 and 10 years, with HoFH (3 out of 4 with genetic diagnosis) were included in the study. Lomitapide was initiated at a starting dose of 2 mg/day and progressively escalated up to 20 mg/day. The efficacy of lomitapide was defined by the LDL cholesterol change, hepatic function and liver ultrasound were assessed.RESULTS: Untreated LDL cholesterol (LDL-C) was 794±53 mg/dl (mean±SD). With rosuvastatin 5-20 mg plus ezetimibe 10 mg plus weekly LA, LDL-C was 324±27 mg/dl. The addition of lomitapide 20 mg/day, resulted in a robust LDL-C decrease (221±52 mg/dl after 28-40 weeks of treatment), allowing the reduction of LA frequency in 3 children (every two weeks) and the LA suspension in 1. Furthermore, lomitapide induced reduction and/or resolution of cutaneous xanthomas present at the beginning of the study. Three out of 4 patients showed a transient mild elevation of AST and ALT (<2xULN), and the mild hepatic steatosis evaluated by liver ultrasound at the beginning of the study remained stable during the therapy. At present, none of the children has discontinued lomitapide treatment.
Conclusions. Our findings suggest that lomitapide is effective and safe in children with HoFH, leading to a significant reduction in LDL-C and allowing a reduction/suspension of LA with a considerable impact on the quality of life of children and their families.

IDENTIFICATION OF A NOVEL NONSENSE MUTATION IN THE APOB GENE BY NEXT GENERATION SEQUENCING

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Introduction. Familial hypobetalipoproteinemia (FHBL) is an autosomal codominant disorder of lipoprotein metabolism characterized by low plasma levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and apolipoprotein B (apoB) below the 5th percentile of the distribution in the population. It may be due to loss-of-function mutations in APOB or, less frequently, in PCSK9 genes. The most frequent dominant monogenic form of HBL is the Familial Hypobetalipoproteinemia type-1 (FHBL-1, OMIM # 615558). The 50% of FHBL-1 cases is caused by mutations in APOB gene which result in assembly defects and secretion of lipoproteins containing apoB. Most of the FHBL-1 subjects are heterozygous carriers of nonsense pathogenetic variants and frameshift of the APOB gene which interfere with the complete translation of the mRNA coding the apoB protein, determining the formation of truncated forms of apoB. FHBL heterozygotes are generally asymptomatic but often develop fatty liver.
Materials and Methods. We designed a custom panel for Next Generation Sequencing (NGS) in order to analyze known genes involved in FHBL by Ion Torrent GeneStudio S5 Plus. We sequenced the FHBL candidate genes in 10 patients presenting LDL-C and ApoB levels below the 5th percentile.
Results and Conclusion. In the majority of subjects no functionally relevant mutations in candidate genes were detected. Two unrelated patients was found to be carrier of a novel heterozygous nonsense mutation in the exon 26 of the APOB gene (c.10324C>T, p.Gln3442Ter). The mutation lead to the formation of a premature stop codon and an apoB truncated protein of an expected size of 75.8% of wild type apoB (apoB-75.8).In this work we describe a novel nonsense mutation of the APOB gene responsible for FHBL identified by a Next generation sequencing approach.

SUBOPTIMAL ADHERENCE TO STATIN THERAPY IN CHILDREN AND ADOLESCENTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA DESPITE A HIGH THERAPEUTIC EFFICACY: IS THE CARDIOVASCULAR RISK UNDERESTIMATED?

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Background. European guidelines currently support the initiation of statin by age 8-10 years in patients with heterozygous familial hypercholesterolemia (HeFH) to slow the progression of endothelial disfunction and to reduce the risk of cardiovascular disease in adulthood. However, to date, there is lack of data on adherence to statins in the paediatric population. Therefore, we describe our real-life paediatric experience about efficacy and adherence of statin therapy.
Methods. This is a monocentric, observational study recruiting children and adolescents with genetically confirmed HeFH. Anthropometric measures, fasting lipid profile and therapeutic data were collected at diagnosis of HeFH [T0], on lipid-lowering diet [T1], four weeks after starting statin therapy [T2] and yearly during the first two years on statin [T3 and T4].
Results. 24 HeFH children and adolescents (17/24 female) were started on statin at a mean age of 13.77±3.09 years (12 on atorvastatin, 10 on pravastatin and 2 on simvastatin). On lipid-lowering diet, lipid metabolism did not change significantly [LDL-C 237.61±47.18 vs. 218.22±50.15 mg/dl, p 0.11], while on statin the improvement was quick and persistent [LDL-C T1 218.22±50.15, T2 163.85±27.64, T3 153.12±34.90, T4 156.37±34.11 mg/dl, $p<0.05$]. The mean reduction of LDL-C in comparison to baseline levels was: -34.68±12.99% at T2 and -30.42±20.78% at T4. Despite this efficacy and excluding one case of statin-intolerance, 9/23 patients (about 39%) dropped out after one year of statin therapy with a higher prevalence among families without an history of precocious cardio-vascular events ($p<0.05$).
Conclusions. We report an overall scarce adherence to statins in our paediatric HeFH population despite an efficacy in line with international data. GP involvement, a more effective communication with patients and their families to emphasize the high HeFH-related cardiovascular risk, and a periodic follow-up including telemedicine may be tools to achieve a better adherence.

"IN MEDIO STAT VIRTUS": EFFICACY AND SAFETY OF STATINS ON ALTERNATE DAYS

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Background. Statins are the main treatment for hypercholesterolemia but patients taking statins sometimes refer muscle-related symptoms, a frequent cause of treatment discontinuation.As an alternative to high-intensity statin monotherapy, moderate-intensity statin with ezetimibe combination therapy can lower LDL cholesterol concentrations effectively and reduce adverse effects.AIM The aim of the study was to evaluate the efficacy of therapy with statin/ezetimibe combination on alternate days and only ezetimibe every other days, both in terms of safety of this treatment and in terms of overall reduction of the lipid profile, in a population of patients, predominantly in primary prevention, with myalgia statin-related, previously treated with statin/ezetimibe combination every day.
Methods. In this study were involved 49 subjects (19 male; 30 female. Median age: 61 years) in primary (46 subjects) and secondary (3 subjects) prevention with statin/ezetimibe combination treatment every day. The lipid profile (cholesterol tot; HDL; triglycerides) and CPK values were analyzed at baseline and also 3 months after the introduction of therapy with rosuvastatin/ezetimibe (5/10 mg) combination on alternate days and only ezetimibe (10 mg) every other days. LDL was calculated using Friedewald formula.
Results. In the analyzed population median baseline values were: total cholesterol 250 mg/dL, LDL-C 172,3 mg/dL, HDL-C 52,3 mg/dL; triglycerides 163 mg/dL and CPK 261 U/l.3 months after the introduction of alternative treatment median values were: total cholesterol 185.4 mg/dL, LDL-C 100 mg/dL, HDL-C 53 mg/dL; triglycerides 118 mg/dL and CPK 194 U/l.It has been showed a reduction of nearly 40 % LDL values and 33 % CPK values, with improvement of safety and compliance to therapy.
Conclusion. Our data suggest good efficacy of treatment with statin/ezetimibe combination on alternate days and only ezetimibe every other days thanks to the improvement of compliance. Moreover it could represent an advantageous therapy in terms of pharmacoeconomy.

CAROTID AND AORTIC INTIMA-MEDIA THICKNESS IN PEDIATRIC AGE: COMPARISON BETWEEN FAMILIAL HYPERCHOLESTEROLEMIA AND POLIGENIC DISLIPIDEMIAS

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Objectives. Carotid and aortic intima-media thickness (cIMT and aIMT) are surrogate markers of subclinical atherosclerosis. Patients with primary hyperlipidemias have higher cIMT and aIMT values than controls, even in pediatric age. AIM: The aim of the study was to evaluate cIMT and aIMT in pediatric subjects with monogenic (familiar hypercholesterolemia- FH) and polygenic dyslipidemias (PD) and to investigate any lipid profile or clinical data correlations.
Patients and Methods. The study included 390 hyperlipidemic subjects (135 FH, 255 PD; age 5-18 years). Clinical data (age, sex, weight, BMI) and pre-therapy lipid profile were analyzed by standard methods; cIMT and aIMT were tested by B-mode ultrasound with a 7.5-10 MHz linear array transducer.
Results. Significantly higher TC, LDL-C, non-HDL-C levels and lower triglycerides were observed in the FH group compared to the PD group. Mean cIMT values were higher in FH vs PC (0.462±0.079 vs 0.445±0.058 mm, p=0.014) as were left cIMT values (0.462±0.080 vs 0.443±0.059 mm, p=0.006). Stratifying by age, these differences were just confirmed since 10 years of age. No significant results were obtained for right cIMT or aIMT. In the FH group, mean and left cIMT values positively correlated with TC, non-HDL-C, aIMT, age, weight, BMI and male sex. In the PD group correlations were confirmed with aIMT, age, weight, BMI and male sex, but not with TC and non-HDL-C.
Conclusion. Present results confirm the early onset of atherosclerosis, particularly in FH subjects. The increased cardiovascular risk, even compared to other forms of primary dyslipidemia, stresses the importance of an early diagnosis and treatment.

TELEMEDICINE FOR THE CARE OF PEDIATRIC PATIENTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA IN THE COVID-19 PANDEMIC ERA AND BEYOND

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Telemedicine is the use of electronic methods to deliver health care and/or health education from a distance. It is a useful tool in providing a modality for continued care as expected during pandemic era.
Methods. We conducted a single-centre prospective pilot study to evaluate the feasibility and applicability of telemedicine services in the management of children with Familial Hypercholesterolemia. The objective was to provide consultation for drugs and select children who needed inpatient care. The study was conducted during the period between March 2020 and June 2020. The first 30 parents of children affected by FH and routinely followed in our center and asked for consultation were informed about the availability of teleconsultation services. A digital platform for a teleconference (Zoom or Jetsi) was used to connect pediatricians and families. The medical data received was entered in the internal electronic medical records system (OBG clinico) and a summary was e-mailed to the family. At the end of the e-consult, the parents/caregivers were asked to complete a brief survey and rate the teleconsultation experience on a scale of 0 (not satisfied) to 5 (fully satisfied).
Results. During the study period, a total of 22 e-consults were done; 8/30 parents refused teleconsultation because of little confidence in the technology. At least 4 fathers and 18 mothers personally completed the survey. The study group comprised 22 patients - 8 boys and 14 girls. The mean age of the children in this study was 10,7 years (range 5-17 years). The patient/family satisfaction score for e-consults was "5" in 54,5% and "4" in 45,4% of the parents. Based on our experience, we conclude that telemedicine may be an effective modality in triaging children with Familial Hypercholesterolemia for follow-up and in providing individualized tailored advice. This results in enhanced satisfaction due to a stronger doctor-patient-family relationship and may improve clinical outcomes also outside the pandemic period.
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PROPROTEIN CONVERTASE SUBTILISIN KEXIN TYPE 9 INHIBITORS REDUCE PLATELET NETS RELEASE THAT DRIVE THROMBOSIS IN FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction. Elevated low-density lipoprotein-cholesterol levels (LDL-C) contribute to chronic systemic inflammation in heterozygous familial hypercholesterolemia (HeFH). it is known that the treatment of HeFH patients with PCSK9 inhibitors (PCSK9i) significantly reduces LDL-C levels. Moreover, neutrophil extracellular traps (NETs) release has been shown to induce activation of endothelial cells and platelets, resulting in a proinflammatory response. Thus, NETs may play a role in triggering atherosclerotic plaque formation and thrombosis. This study aims to describe the inflammatory profile of HeFH patients and explore the effect of PCSK9 inhibitor (PCSK9i) on NETs release, and thrombus formation, and finally, investigate the molecular mechanisms governing its occurrence.
Materials and Methods. We studied 40 patients with heterozygous familial hypercholesterolemia (HeFH) on treatment with the maximum tolerated statin dose ± ezetimibe before and after six months of PCSK9i therapy. We analyzed NETs release and thrombus formation by measuring in plasma citrullination of histone H3 (CitH3) and thrombus-formation analysis system (T-TAS), respectively. Furthermore, we investigated by in vitro study if plasma post-PCSK9i reduced NETs release and thrombus formation.
Results. In vivo studies showed decreased circulating levels of CitH3 and under laminar flow platelet-dependent thrombus growth in HeFH patients after PCSK9i treatment compared to patients before treatment. In vitro study showed that plasma from HeFH patients after PCSK9i treatment decreased NETs release by neutrophils and thrombus formation.
Conclusion. This study provides evidence that HeFH patients showed an increased NETs release and thrombus growth that were inhibited by the treatment with PCSK9i which may represent a novel approach to counteract inflammation in these patients.

GENETIC SUSCEPTIBILITY IN VACCINE INDUCED THROMBOTIC THROMBOCYTOPENIA (VITT)

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Background. In late February 2021, a prothrombotic syndrome was observed in a small number of individuals who received the adenoviral vector-based vaccine Vaxzevria (AstraZeneca). Similar pictures were observed in some individuals who received the Janssen (Johnson & Johnson) vaccine. This syndrome has been named vaccine-induced thrombotic thrombocytopenia (VITT) and is characterized by multiple venous/arterial thrombosis, with atypical venous thrombosis of cerebral and splanchnic districts, associated with thrombocytopenia. Hemorrhagic manifestations and sometimes disseminated intravascular coagulation (DIC) are present. Evidences suggest that this rare syndrome is caused by platelet-activating antibodies directed against platelet factor 4 (PF4), a chemokine stored in the platelets' alpha granules necessary for platelet aggregation, also known to be involved in the atherosclerotic plaque formation. Aim of this work is to apply a Whole Exome Sequencing (WES) analysis approach, for the identification of possible genetic predisposition profiles underlying VITT.
Methods. Due to the referral role of the Center for Atherothrombotic Diseases (University of Florence/AOU Careggi) for the management of COVID-19 patients and diagnosis of VITT, fifty patients were examined. Ten out of fifty patients were diagnosed with VITT. In our analysis we used Next Generation Sequencing techniques, by a WES approach, with Illumina NextSeq500 platform and SureSelect XT HS enrichment kit (Agilent Technologies), to analyse six out ten VITT patients.
Results. VITT patients analyzed were 6 females (mean age 64,2±13,8), who received an adenoviral vector-based vaccine. WES analysis revealed a total of 140,563 variants. Rare variants (MAF <1%) identified range from 1,619 to 1,774 and their distribution by type (frameshift, missense, splicing, nonsense, UTR, samesense, intronic) is similar in the six patients.In this work we decided to focus on rare variants involved in different biological processes underlying VITT.We found a total of 89 rare variants in genes involved in integrin signalling pathways (ITGA2B, ITGAD, ITGB4, GP6, FGA, FGB), in thrombocytopenia (MASTL, PDIA6, FYB, MYH9), and other genes inducing/inhibiting platelet aggregation/activation processes. Interestingly, the two patients (VITT05 and VITT18), with most severe clinical complications, showed a higher number of rare variants identified in such pathways (21 and 27 variants, respectively). Among the abovementioned pathways, 15 variants with putative functional effect have been identified in genes encoding for molecules of the integrin pathway, which display an additional role in the atherosclerotic mechanism/process. Interestingly, both VITT05 and VITT18, patients with a more severe phenotype, carried variants in GP6 gene, encoding a collagen receptor involved in collagen-induced platelet adhesion and activation.
Conclusions. WES analysis exhibits a considerable number of variants in molecular pathways involved in integrin signalling, thrombocytopenia, platelet aggregation/activation and atherosclerotic processes. The two patients with the worst clinical outcome presented a significantly higher number of suggestive rare variants with respect to other patients investigated; consequently, it is not possible to exclude the potential contribution of a greater number of rare suggestive variants in the modulation of the phenotype of patients with worse clinical course. Further investigation on other mechanisms (inflammation, immunity, viral response) and functional assays are needed for more clarity with respect to the impact of genetic background on VITT susceptibility.

A SMALL LIPOPROTEIN THAT CAN MAKE THE DIFFERENCE: THE ROLE OF LIPOPROTEIN(A) IN CARDIOVASCULAR RISK STRATIFICATION AND REVERSE SCREENING IN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLAEMIA

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Background and Aims. Familial hypercholesterolemia (FH) is a genetic disease involving 1:250 subjects in the general population. FH detection and treatment, starting from childhood, helps gaining decades of life. Cardiovascular-disease (CVD) risk stratification is fundamental in FH children. Lipoprotein(a) [Lp(a)] is universally recognized as an independent CVD risk factor. Lp(a) values above 30 mg/dl are considered a threshold for increased CVD. Moreover, Lp(a) determination in FH children can improve CVD family risk stratification.
Matherials and Methods. In a 30 months period, 70 patients were referred for the first time to the Centre for Paediatric Dyslipidaemias of Guglielmo da Saliceto Hospital in Piacenza for hypercholesterolemia; 33/70 (20 male, age mean±sd 11.2±4.3 years) were assessed for suspect FH. Pediatric evaluation, anthropometric parameters, complete lipid profile including Lp(a) and genetic analysis for FH were performed. Nutritional intervention and follow up were started. If patient's Lp(a) level was ≥30 mg/dl, Lp(a) assessment in first degree family members was recommended.
Results. Lipid profile of the study population was (mean±sd, mg/dl): total cholesterol 266.3±46.7, LDL-cholesterol 187.8±36.8, HDL-cholesterol 56.3±12.3, triglycerides 108.6±62.4. Mean Lp(a) level was 27.9 mg/dl. 7/33 patients had Lp(a) ≥30 mg/dl, so Lp(a) assessment was recommended in their parents and 4 out of 7 patients had at least one parent with Lp(a)≥30 mg/dl. 4 out of 7 patients with elevated Lp(a) and 12/26 patients with normal Lp(a), after a 6 month nutritional treatment, were put on lipid lowering pharmacological therapy.
Conclusions. Lp(a) evaluation in FH pediatric patients can improve CVD risk stratification, so as to start early drug therapy after nutritional and lifestyle intervention. Elevated Lp(a) in children is an alarm sign that should lead clinicians to perform reverse screening in adult components of the child's family. Pediatric Lipidologists have a fundamental role in CVD prevention in the whole family.

TRANSCRIPTOMICS: AN ENTICING APPROACH TO UNDERSTANDING THE PATHOPHYSIOLOGY AND CLINICAL OUTCOMES OF ACUTE ISCHEMIC STROKE

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Background. Acute ischemic stroke (AIS) represents one of the principal causes of neurological morbidity and mortality worldwide and is characterized by a multifactorial etiology, caused by interactions among blood vessel and environmental and genetic factors. For a prompt and efficient cerebral blood restoration, intravenous thrombolysis with rt-PA is often combined with mechanical thrombectomy (MT). MT represents a golden ticket from a research perspective, providing cerebral thrombi (CT) as new study material, enabling in-depth studies concerning their cellular composition and etiology correlation. In addition, it represents a key pillar for the creation of virtual predictive models of different gene expression based on annotated histopathological evidence. The trend towards a medicine based on personalized and individualized prevention and treatment strategies has led to the need to investigate the genetic aspect of the disease, due to its significant contribution to the genesis of the ischemic event. For that, focus of the research is the highlighting and exploration of profiles in which peripheral blood (PB) mirrors CT through the analysis of global gene expression profiles, and the identification of promising markers that can serve as sentinels for different pathophysiological mechanisms and/or determinants of clinical outcomes, such as haemorrhagic transformation, 24h edema, modified 3 months Rankin scale-mRS, death. This approach could allow to gain deeper insights into the pathogenesis of the disease through investigation of the relationship between gene expression and phenotypic differences.
Methods. We performed gene expression profiles of RNA samples obtained from 40 CT and 37 PB of 52 patients. The CT obtained during MT were stored in RNA later, while PB, collected before and 24 hours after MT, in tubes containing a reagent that protects RNA from degradation and minimizes ex vivo changes in gene expression. RNA was extracted by PAX gene blood miRNA kit; the global gene expression profile was assessed by Affymetrix technology using GeneChip Human Transcriptome Array 2.0, allowing the analysis of 44,699 genes, with more than 285,000 full-length transcripts coverage. Data analysis was performed in R environment with dedicated pipelines.
Results. Data processing and the application of appropriate filtering criteria showed an average of analyzable probe sets of 440,085 in CT and 602,874 in PB. In the two different type of specimens 20,341 were found to be common features, whereas 3 and 562 symbols were unique in CT and PB, respectively. The Gene Ontology (GO) enrichment analysis allowed the identification of the biological processes, common and peculiar, in CT and PB, indicating that peripheral and local mechanisms of damage and response to damage are present in both. The significance analysis of microarrays, according to different outcomes and GO analysis, brought into focus 221 significant biological processes associated with poor outcome according to mRS in CT, and 27 terms associated with 24h edema in PB. Among significant terms in CT, those associated with regulation of neutrophil mediated immunity and activation play a crucial role. Concerning PB, particularly significant enriched terms were associated with regulation and activation of transcriptomes of cells.
Conclusions. Our results provided interesting insights into the mechanisms underlying the AIS and the response to treatments. In particular, the analysis of CT and PB gene expression profiles, differentially expressed probe sets and their biological processes alterations according to stroke outcomes, has not only confirmed and extended several known pathophysiological mechanisms, but also suggested novel pathways to be explored that may provide an important starting point for expanding knowledge on this cryptic disease.

SPHINGOSINE-1-PHOSPHATE (S1P) RECEPTORS AND CHOLESTEROL TRANSPORTERS EXPRESSION IN MACROPHAGES

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Background and Goals. Atherosclerosis is a chronic multifactorial disease characterized by an accumulation of cholesterol in arterial macrophages. The bioactive lipid sphingosine-1-phosphate (S1P) acts as an extracellular and intracellular signaling molecule regulating the immune, cardiovascular and nervous system. Several lines of evidence point to a crucial role for the S1P/S1P-receptor (S1PR) axis in cancer and in chronic inflammatory diseases, such as atherosclerosis. In vivo studies have shown that pharmacological stimulation of S1PR can exert atheroprotective effects. Fingolimod (FTY720, Gilenya®), an unselective S1PR modulator, was the first oral therapy approved for relapsing–remitting multiple sclerosis. In animal models, fingolimod can reduce the progression of atherosclerosis. We explored the effects of S1P/S1PR stimulation on the expression of cholesterol transporters in macrophages in vitro.
Materials and Methods. Murine macrophages were cultivated under cholesterol normal or loading (acetylated LDL, AcLDL) conditions and exposed or not to different concentrations of S1PR modulators. The expression of target genes and proteins, such as ABCA1, ABCG1 and SR-BI, in macrophages, was evaluated by real-time qPCR and Western blot.
Results. Treatment with S1PR modulators, particularly fingolimod, affected cholesterol transporters expression, regardless of AcLDL stimulation. Treatment specifically increased the expression of SR-BI, which is normally downregulated under cholesterol loading conditions.
Conclusions. Our preliminary observations suggest that the modulation of S1PRs may affect the expression of ABCA1, ABCG1 and SR-BI in macrophages. This effect may partially account for the atheroprotective role attributed to S1P/S1PR axis stimulation in the context of atherosclerosis.

FOUR VARIANTS IN CREB3L3 GENE ARE ASSOCIATED WITH DIFFERENT PHENOTYPES

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Aim. Dyslipidemia are characterized by an heterogeneous genetic background with several genes influencing different lipid fractions. The CREB3L3 gene was recently suggested as a non-canonical gene causative of hypertriglyceridemia (HTG). We aim to report our variants in CREB3L3 identified in patients with suspect of different diseases.
Patients and Methods. Four patients with different clinical suspects (2 with HTG) were screened by NGS using a large panel of 94 genes associated with dyslipidaemia, cholestasis and/or liver diseases. Variant presence was confirmed by Sanger sequencing.
Patient 1: a 9 years old girl with TG 8280 mg/dL, total cholesterol 834 mg/dL, HDL-cholesterol 26 mg/dL, acute lymphoblastic leukemia treated with PEG-arginase.
Patient 2: a 25 years old woman hospitalized urgently for acute pancreatitis with TG> 1500mg/dL.
Patient 3: a 5 years old girl analyzed to confirm the status of carrier for a variant causing Wilson's disease.
Patient 4: a 58 years old man with clinical suspect of FH.
Results. All patients resulted heterozygotes for rare variants in CREB3L3 gene: patient 1 for the nonsense variant c.724C>T - p.(Arg242*), previously reported as causative of HTG; patient 2 for the new missense variant c.742C>T - p.(Arg248Cys); patient 3 for the new splicing variant c.577-1G>A predicted to cause the loss of only 2 aminoacids p.(Gln193_Gln194del); Patient 4 for the missense variant c.700C>G - p.(Leu234Val) and LDLR gene (c.1135T>C - p.(Cys379Arg). Only the first variant was classified as likely pathogenic, whereas the last 3 variants were classified as USV. The last 2 variants were identified in patients without HTG.
Conclusions. Among 4 patients carrying different variants in CREB3L3 gene, only 2 patients showed HTG. Only one of these patients carried a variant classified as likely pathogenic. This report highlights that variants in CREB3L3 gene should be carefully evaluated for the association with HTG.

THE IMPACT OF LIPOPROTEIN(A) GENOTYPE ON THE PHENOTYPE OF INDIVIDUALS WITH CLINICAL FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction. Evidence suggests that LPA genotypes, associated with elevated lipoprotein(a) [Lp(a)] levels, can result in a phenotype suggestive of clinical familial hypercholesterolemia (FH). This study aimed at determining the prevalence of LPA risk variants in FH individuals enrolled in the Italian LIPIGEN study, with (FH/M+) or without (FH/M-) a causative variant.
Methods. We selected adults (aged ≥18 years) with a clinical diagnosis of FH and with the genetic test performed in a centralized laboratory searching for possible causative variants in candidate genes and evaluating the two common Lp(a)-raising single nucleotide polymorphisms rs3798220 and rs10455872. A Lp(a) genetic score was calculated for each participant by summing the number risk-increasing alleles. We compared baseline lipid levels and clinical variables by presence of FH causative mutations and LPA genotype.
Results. A total of 930 FH/M+ and 765 FH/M– patients were identified. Among them, 10.2% and 21.0% were characterized by one or two copies of either rs10455872 or rs3798220, respectively. FH/M- subjects had higher levels of Lp(a) than FH/M+ patients (median values 41 mg/dL [9-103] vs 19 mg/dL [8-41], p<.0001), with increasing Lp(a) concentrations among subjects with the same FH genetic background based on increasing value of Lp(a) genetic score. The adjustment of LDL-C levels based on lipoprotein(a) concentration reduced from 68% to 42% the proportion of subjects with LDL-C level ≥190 mg/dL. Overall, in the 4.6% of clinically diagnosed FH patients, the phenotype was not explained by a monogenic or polygenic aetiology, but genotype associated with high Lp(a) levels emerged as the sole culprit.
Conclusion. Our study supports the importance of measuring lipoprotein(a) in patients with familial hypercholesterolemia to improve the diagnosis and the prediction of cardiovascular risk. Subjects in whom hypercholesterolaemia is driven by high Lp(a) values, would benefit more from therapies targeting this protein.

THE SPECTRUM OF FATTY LIVER DISEASE AND THE ROLE OF GENETIC, METABOLIC AND LIFESTYLE FACTORS ASSOCIATED WITH ITS SEVERITY IN HETEROZYGOUS APOLIPOPROTEIN B-RELATED FAMILIAL HYPOBETALIPOPROTEINEMIA

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Introduction and Aims. Heterozygous apolipoprotein B (APOB)-related familial hypobetalipoproteinemia (FHBL) is a genetic disorder characterized by low levels of LDL cholesterol and apoB owing to disrupted secretion of apoB-containing lipoproteins. Patients with heterozygous APOB-related FHBL are prone to develop fatty liver disease (FLD) that has been anecdotally associated with the risk of progression toward cirrhosis. However, the actual prevalence of and the factors associated with severe FLD in APOB-related FHBL patients still remain to be elucidated. Our study aims to characterize the spectrum of FLD and to determine the association of genetic, metabolic and lifestyle factors with the severity of FLD in a single cohort of heterozygous APOB-related FHBL patients.
Methods. 21 adults with genetically-proven heterozygous APOB-related FHBL (men 57.1%, age 51[23-74] years) were consecutively enrolled in the Lipid Clinic in Modena. Genetic and clinical data were retrieved. The presence and severity of liver steatosis was evaluated by liver ultrasonography; measurement of liver stiffness with ultrasound elastography techniques (Fibroscan® and 2-dimensional shear wave elastography (2D-SWE)) was performed. A subgroup of patients underwent liver biopsy; histological presence of nonalcoholic steatohepatitis (NASH) and staging of fibrosis were defined according to Kleiner's criteria. Patients were classified as carriers of significant fibrosis if Fibroscan® and/or 2D-SWE yielded liver stiffness results ≥8Kpa and/or histological fibrosis stage was ≥F2. Lifestyle habits were evaluated by self-administration of three-day food diary, Sofi's Mediterranean diet adherence score and international physical activity questionnaires.
Results. 4 patients had APOB missense or intronic splice-site variants and 17 patients were carriers of APOB truncating mutations, of whom 5 determine the synthesis of protein longer than apoB-48 and 12 shorter than apoB-48. The prevalence of abdominal obesity, arterial hypertension, type 2 diabetes mellitus (T2DM) and metabolic syndrome was 57.1%, 28.6%, 14.3% and 14.3%, respectively. 16 patients (76.2%) had liver steatosis at ultrasound, whose grade was mild-to-moderate in 10 patients and severe in 6 patients. The presence of any grade of liver steatosis was significantly associated with apoB length: only 1 out of 5 patients (20%) carrying truncated apoB longer than apoB-48 vs. 11 out of 12 subjects (91.7%) carrying truncated apoB shorter than apoB-48 showed liver steatosis (p=0.010). However, the severity of liver steatosis was not associated with apoB length. The main factors significantly associated with the presence of severe steatosis were indices of adiposity (BMI, p=0.036; waist circumference, p=0.006), glucose homeostasis (fasting glucose, p=0.008; HbA1c, p=0.045) and insulin resistance (HOMA-IR, p=0.018), and markers of liver injury (GOT, p=0.036; GPT, p=0.011). Of note, the presence of severe steatosis was significantly associated with significant fibrosis (p=0.031).4 patients had liver stiffness ≥8Kpa, 1 of which with overt cirrhosis. 5 out of 6 patients submitted to liver biopsy showed NASH with significant liver fibrosis (4 with F2 and 1 with F3 stage). In total, significant fibrosis according to liver stiffness and/or histology was present in 6 patients (28.6%). Significant fibrosis was not associated with APOB length, but was significantly associated with metabolic comorbidities (abdominal obesity, p=0.019; insulin resistance, p=0.004; T2DM, p=0.015), platelet count (p=0.003) and markers of liver injury (GPT, p=0.029; GGT, p=0.045).With regards to lifestyle, few patients were adherent to Mediterranean diet (16.7%) and most of them reported unhealthy dietary habits with excessive fat intake (80%). Moreover, 23.8% of patients were inactive.
Conclusions. FLD affect the majority of patients with heterozygous APOB-related FHBL, especially when apoB length is shorter than apoB-48. However, the severity of liver steatosis and fibrosis seems to be influenced by metabolic comorbidities, rather than by APOB mutations per se. Efforts to promote healthy lifestyles and prevent obesity and diabetes should be made in order to avoid FLD progression.

HDAC INHIBITION PROMOTES OSTEOGENIC DIFFERENTIATION IN VALVE INTERSTITIAL CELLS

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Background. Calcific aortic valve disease (CAVD) is the most common valvulopathy in the Western world, but the pathophysiological processes involved in the disease are still poorly understood. The progression of CAVD depends mainly on the differentiation of valve interstitial cells (VICs) towards an osteogenic phenotype. Valproic acid (VPA), an inhibitor of histone deacetylases (HDAC), has been shown to promote the osteogenic differentiation of different cell types, but evidence is lacking on its potential involvement in CAVD.
Aim. This research project aims to determine the potential effects of VPA and HDAC inhibition in isolated VICs.
Methods. Isolated human VICs were treated with 5 mM VPA alone or in an osteogenic medium (OM) for 12 days. At the end of the treatment, proteins and RNA were extracted for western blotting analysis and gene expression studies (RT-PCR). The activity of alkaline phosphatase (ALP) and calcium deposition were quantified through colorimetric assays.
Results. Isolated VICs treated with VPA showed an increase in ALP activity compared to the control group, which became statistically significant ($p<0.05$) when the cells were in OM. A significant overexpression of osteogenic markers (such as ALP and BMP2) and IL-6 was detected when VICs were treated with OM and/or VPA. The supplementation of VPA to the OM also increased calcium deposition in isolated VICs. Moreover, the protein expression of acetylated histone H3 was significantly increased in an OM supplemented with VPA, due to VPA-mediated HDAC inhibition.
Conclusions. These preliminary data suggests that VPA induces the osteogenic differentiation of VICs, probably through HDAC inhibition. Further studies are needed to confirm these findings and understand whether the pharmacological modulation of HDAC may represent a therapeutic strategy for CAVD.

METABOLIC AND CARDIAC MORPHO-FUNCTIONAL IMPROVEMENTS AFTER PCSK9 INHIBITORS ADMINISTRATION

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Background. Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) play a key role in cholesterol's metabolism regulation through the degradation of low-density lipoprotein receptor (LDL-R). Recent studies suggest its possible role in cardiovascular (CV) diseases, promoting vascular inflammation, reactive oxygen species generation and atherosclerotic plaque formation. Furthermore, higher TG/HDL ratio has recently emerged as a marker of increased atherosclerotic extension and it can identify subjects with higher CV risk profile. Global longitudinal strain (GLS), global myocardial work efficiency (GWE), reservoir (PALS) and pump (PACS) atrial function evaluated by speckle-tracking echocardiography are able to identify early subclinical ventricular and atrial cardiomyopathy. In the last years, PCSK9 inhibitors have been introduced as innovative therapies for LDL plasma levels' reduction, showing strong CV protection. The aim of our study was to investigate, at baseline and after 6-months follow-up, PCSK9 inhibitors effects in patients with established coronary disease who were statin-intolerant and/or not reaching the target of LDL-C <55 mg/dl using the maximum tolerated drug dosage.
Materials and Methods. We enrolled 30 patients (24 males and 6 females, mean age 66±8 years), 97% showed arterial hypertension, 70% chronic kidney disease, 67% polidistrectual atherosclerosis, 50% type 2 diabetes mellitus (T2DM) and 20% chronic heart failure NYHA class II-III. All patients underwent main anthropometric and hemodynamic parameters evaluation, biochemical analysis, oxidative stress markers assessment, and advanced echocardiogram at baseline and after six months of therapy. The serum values of oxidative stress marker (NOX-2) and platelets activation (Sp-selectin) were assessed with ELISA sandwich. Echocardiographic recordings were performed using an E-95 Pro ultrasound system (GE Technologies, Milwaukee, Wisconsin, USA) using a 2.5 MHz transducer.
Results. There were no significant differences regarding systolic blood pressure (SBP), heart rate and glycaemia after six month of therapy. As expected, lipid profile was greatly improved in all the subjects, reaching the target of LDL-C <55 mg/dl. We obtained a statistically significant reduction of total-cholesterol (Δ=-32%, p<0.0001), LDL-C (Δ= -60%, p<0.0001), TG (Δ=-26%, p<0.0001), TG/HDL ratio (Δ= -27%, p<0.0001); an increase of HDL-C (Δ=+9%, p=0.001) and an improvement of glomerular filtrate evaluated by CKD-EPI (Δ=+6%, p=0.007). We observed a statistically significant reduction of NOX-2 (Δ=-27%, p<0.0001) and Sp-selectin (Δ=-36%, p<0.0001). Concerning echocardiographic parameters, we obtained a statistically significant increase of PALS (Δ= +16.9%, p<0.0001), PACS (Δ=+21%, p<0.0001), GWE (Δ=+9.7%, p<0.0001), GLS (Δ=+24%, p<0.0001) and a statistically significant reduction of global wasted work (GWW) (Δ=-15%, p<0.0001), left atrial volume index (LAVI) (Δ= - 8%, p<0.0001) and E/e' ratio (Δ= -19%, p<0.0001) respectively. The linear correlation analysis showed that ΔPACS was significantly and inversely correlated with ΔTG/HDL (r=-0.406, p=0.013) and ΔNOX-2 (r=-0.416, p=0.011); ΔPALS was significantly and inversely correlated with TG/HDL (r=-0.473, p=0.004) and ΔNOX-2 (r= 0.435, p=0.008); ΔE/e' was significantly and directly correlated with ΔTG/HDL (r=0.654, p<0.0001) and ΔNOX-2 (r=0.438, p=0.008); ΔGWE was inversely correlated with ΔNOX-2 (r=-0.422, p=0.01).Our study demonstrated for the first time that PCSK9 inhibitors are able to reduce left ventricular filling pressure, to increase atrial function (reservoir and pump) and global cardiac performance. Furthermore, PCSK9 inhibitors are able to increase CKD-EPI after six months of treatment in high CV risk population. Our results could be partially explained with a reduction of oxidative stress markers, inflammation and cardio-lipotoxicity, probably linked to a modulation of PCSK9's heart expression and its toxic effect on CV and renal function. In addition, we observed a TG/HDL ratio's reduction related to cardio metabolic and lipid profile improvement. Further studies are necessary to better investigate systemic benefit in a larger population with longer follow-up.

BLACK GARLIC AND POMEGRANATE STANDARDIZED EXTRACTS FOR BLOOD PRESSURE IMPROVEMENT: A NON-RANDOMIZED DIET-CONTROLLED STUDY

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Recently released position papers by the European Society of Hypertension (ESH) and the Italian Society of Hypertension (SIIA) provide therapeutic recommendations for the use of nutraceuticals in the management of high blood pressure (BP) and hypertension, opening up new perspectives in the field. This not-randomized diet-controlled clinical study aimed to evaluate if daily dietary supplementation with back garlic and pomegranate (namely SelectSIEVE® SlowBeat) could advantageously affect BP in individuals with high-normal BP or stage I hypertension. Enrolled subjects were adhering to a Mediterranean DASH (Dietary Approaches to Stop Hypertension) diet for two weeks before deciding whether to continue following Mediterranean DASH diet alone or in association with SelectSIEVE® SlowBeat. At the end of the study, dietary supplementation with SelectSIEVE® SlowBeat was associated with significant improvement in systolic blood pressure (SBP) and diastolic blood pressure DBP compared to baseline. SBP improved also in comparison with control. In conclusion, the study shows that dietary supplementation with extracts from black garlic and pomegranate safely exert significant improvements in BP in healthy individuals adhering to a Mediterranean DASH diet.

EFFICACY AND SAFETY OF LOMITAPIDE IN FAMILIAL CHYLOMICRONAEMIA SYNDROME

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Background and aims. Familial chylomicronaemia syndrome (FCS) is a rare autosomal recessive disorder, resulting in elevated triglycerides (TGs), abdominal pain and pancreatitis. Treatment options are limited. Lomitapide, a microsomal triglyceride transfer protein inhibitor, is approved for the treatment of homozygous familial hypercholesterolaemia. Whether its therapeutic use may be extended to FCS remains unknown. The aim of this study was to evaluate the efficacy and safety of lomitapide in adult patients with FCS.
Methods. The open-label, single-arm 'LOCHNES' study of lomitapide in FCS enrolled patients >18 years with genetically confirmed FCS, elevated fasting TG ≥750 mg/dL and history of pancreatitis. Patients were administered lomitapide to maximum tolerated dose for 26 weeks. The primary endpoint was the percent change in TGs from baseline to Week 26.
Results. Eighteen patients enrolled with median baseline TG levels 1803.5mg/dL (97.5% CI, 1452-2391 mg/dL). At Week 26, median fasting TGs reduced to 305mg/dL (97.5% CI 219-801mg/dL; 70.5% reduction); median lomitapide dose was 35 mg/day; 13 patients achieved TGs ≤750 mg/dL. Adverse events were mild-to-moderate and mainly related to gastrointestinal tolerability. Liver imaging at baseline and Week 26 revealed hepatic fat increases from median 12.0% to 32.5 %, while median hepatic stiffness remained normal. No patient experienced acute pancreatitis or severe abdominal pain during lomitapide treatment.
Conclusions. Lomitapide is effective and well tolerated in reducing TGs in FCS patients with a history of pancreatitis. Larger studies are warranted to determine lomitapide effectiveness in FCS.

DIETARY CHOLINE SUPPLEMENTATION WORSENS ATHEROSCLEROSIS DEVELOPMENT AND MODULATES MULTIPLE METABOLIC PATHWAYS IN EKO MICE

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Background & Aim. Scientific evidence revealed that there is a positive correlation between increased risk of cardiovascular events and plasma levels of TMAO, a molecule originating from the degradation of dietary choline by the gut microbiota. This study was aimed at investigating whether dietary choline affects additional metabolic pathways besides that leading to TMAO production.
Methods. Ten-week-old EKO female mice were fed for 16 weeks two standard rodent diets differing for a low (0.09%) or high (1.2%) choline content. Atherosclerosis development was quantified at the aortic sinus and targeted plasma metabolomic was performed. Furthermore, hepatic gene expression of selected target genes was analyzed by qPCR.RESULTS. Confirming previous observations, high choline intake was associated with greater atherosclerosis development and increased plasma levels of TMAO. In contrast, despite the different dietary content, plasma choline concentration did not differ between the two groups. Likewise, cholesterolemia and triglyceridemia were not significantly affected by choline intake. Interestingly, high choline feeding was associated with lower plasma levels of the thiol group-containing amino acid homocysteine and a concomitant increase of its related metabolites, methionine, sarcosine and carnitine. In agreement with the observed metabolomic changes, EKO mice fed high-choline diet displayed a significant increase in the expression of Aldh7a1, Slc44a1, Sardh and Gnmt in liver, together with a trend towards a higher expression of Chdh, Bhmt and Dmgdh.
Conclusions. Taken together, our data confirm that an increased dietary intake of choline worsens atherosclerosis burden and leads to increased plasma levels of TMAO. Interestingly, choline intake not only influences the choline-TMAO pathway, but also modulates metabolic processes affecting methionine, sarcosine, glycine levels, as well as homocysteine plasma concentrations. These observations shed a new light on the scenarios through which dietary choline might influence the development of atherosclerosis and modify cardiovascular risk.

IMPACT OF OPA1 AND MITOCHONDRIAL DYNAMICS ON SYSTEMIC LIPID METABOLISM AND ATHEROSCLEROSIS

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Aims. Mitochondria, in eukaryotic cells, are one of the principal organelles involved in cellular metabolism, energy generation, calcium homeostasis, hormones, sterol synthesis and bile acids (BAs) production. Mitochondria continuously undergo biogenesis, fusion, fission and mitophagy, maintaining a continuous balance between all forms. On these premises, we test the impact of OPA1, an essential protein of the inner mitochondria membrane fusion, on mitochondrial interaction with other organelles and the following impact on lipid metabolism and the development of metabolic dysfunction and atherosclerosis.
Methods. OPA1 liver KO (Opa1LKO) on HFD and Opa1LKO, OPA1 He male mice, OPA1 Tg on LDLR Ko background were fed with Western type diet (WTD) respectively for 12 weeks and 20 weeks. Inverse calorimetry, ITT, GTT, Lipid Tolerance Test (LTT) were performed, and paraffin-embedded tissues were used for histological analysis. Frozen tissues were further used for integrated OMICs analysis.
Results. Opa1LKO mice display altered systemic metabolism and reduced body weight due to altered lipoprotein circulation without alteration on muscle functionality. Opa1LKO mice altered bile acid production, as showed by RNAseq and proteomics data, leading to the manifestation of cholestasis and the consequent hepatic fibrosis. Opa1LKO mice show an improved glycemic profile, reduced liver steatosis and a reduction in circulating triglycerides levels following LTT. In line on LDLR KO background, Opa1 hepatocyte deficiency is associated with reduced lipoprotein amount and OPa1 overexpression is therefore associated with increased lipoprotein metabolism. Despite this increased lipoprotein metabolism, no differences were observed in atherosclerotic plaque development.
Conclusion. Hepatic Opa1 deficiency protects mice from HFD-induced metabolic dysfunction resulting in a reduction of lipid metabolism as a consequence of an alteration in bile acids production. OPA1 systemic modulation despite major alteration on lipoprotein metabolism is not strongly affecting atherosclerotic plaque development.

EFFICACY OF LONG TERM TREATMENT WITH EVINACUMAB IN FAMILIAL HOMOZYGOUS HYPERCHOLESTEROLEMIA (HOFH): FROM TRIAL TO REAL WORLD EXPERIENCE

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Background. Homozygous Familial Hypercholesterolemia (HoFH) is a genetic disorder, characterized by absent (null-null) or impaired (non-null) LDL-receptor activity, resulting in a rimarkable increase of low-density lipoprotein cholesterol (LDL-C), early onset coronary atherosclerosis, and premature death; thus requires aggressive LDL-C-lowering to prevent complications. Traditional therapeutic strategies, such as lifestyle modifications, invasive treatment, as apheresis and drugs to reduce LDL cholesterol, are insufficient to achieve the objectives of ESC 2019 guidelines in these patients. Evinacumab, a human monoclonal antibody inhibitor of angiopoietin-like protein 3 (ANGPTL3), is the latest lipid-lowering drug, which received FDA approval in February 2021(exclusively in patients with HoFH) in addition to other lipid-lowering therapies, after a phase III trial, ELIPSE HoFH.
Material and Methods. Seven of our patients (3 males and 4 women) with HoFH, who participated in the ELIPSE trial, continued intravenous infusion of evinacumab (with compassionate use) at 15 mg / kg of body weight over at 60 minutes once monthly from October 2021 to date.
Results. In line with the data of the trial, after 12 months of treatment, our real word experience confirms a stable reduction of LDL-C (from 277 mg/dl to 92 mg/dl , p 0.002), triglycerides((from 80 mg/dl to 34 mg/dl , p 0.18) and HDL (from 43.2 mg/dl to 34.1 mg/dl , p 0.001) and at the same time the absence of adverse events.
Conclusions. The significant reduction of LDL-C and the need for new treatment options for HoFH, in the absence of side effects, make evinacumab an important step in the therapy of HoFH, although high costs and intravenous administration still limit its use and approval by AIFA.

INVESTIGATING THE EFFECT OF DIABETIC CONDITIONS ON THE PROGRESSION OF CALCIFIC AORTIC VALVE DISEASE

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Background. Calcific aortic valve disease (CAVD) is the most common heart valve disease. To date, no effective pharmacological therapy has proven to halt or delay its progression. Diabetic subjects are at higher risk of developing cardiovascular complications, including CAVD. Moreover, diabetes contributes to the progression of CAVD, but the pathophysiological mechanisms are still not completely understood.
Aim. This research project aims to assess the molecular mechanisms leading to diabetic CAVD. In particular, we investigated the effects of a high glucose treatment in isolated VICs and of hyperglycemic conditions on animal models of CAVD.
Methods. LDLr-/- and LDLr-/-:ApoB100/100 mice were fed with either a diabetogenic or control diet for 6, 12 and 26 weeks. The aortic valves were collected for RNA sequencing followed by gene expression analysis. For the in vitro system, non-human primate VICs were treated with low- or high-glucose culture media (5.5 mM or 25 mM, respectively) and inorganic phosphate (Pi; final concentration 2.6 mM) to induce their osteogenic differentiation. After 5 days of treatment, the total RNA was extracted for gene expression analysis (RT-qPCR) and calcium deposition was determined through a colorimetric assay.
Results. Based on our findings, hyperglycemic conditions downregulate cardiogenic pathways and cardioprotective genes (including GATA4, TBX5, NPPA, and NKX2-5) in the aortic valves of LDLr-/- and LDLr-/-: ApoB100/100 mice. Moreover, the diabetogenic diet promotes the expression of genes involved in inflammatory and immune processes, accelerating the progression of CAVD.In our in vitro model, a high glucose treatment alone does not directly affect calcium deposition or the osteogenic differentiation of isolated VICs. In addition, elevated glucose levels dysregulate cardiogenic genes (such as NKX2-5 and TBX5) in our in vitro model. Taken together, these findings suggest that further mechanistic studies are warranted and may lead to the discovery of novel potential targets for diabetic CAVD.

AN UNTARGETED LIPIDOMIC ANALYSIS REVEALS DEPLETION OF SEVERAL PHOSPHOLIPID CLASSES IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA ON TREATMENT WITH EVOLOCUMAB

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Background. Familial hypercholesterolemia (FH) is caused by mutations in genes involved in low-density lipoprotein cholesterol (LDL-C) metabolism, including those for pro-protein convertase subtilisin/kexin type 9 (PCSK-9). The effect of PCSK-9 inhibition on the plasma lipidome has been poorly explored.
Methods. Using an ultra-high-performance liquid chromatography-electrospray ionization-quadrupole-time of flight-mass spectrometry method, the plasma lipidome of FH subjects before and at different time intervals during treatment with the PCSK-9 inhibitor Evolocumab was explored.
Results. In 25 FH subjects, heterozygotes or compound heterozygotes for different LDL receptor mutations, untargeted lipidomic revealed significant reductions in 26 lipid classes belonging to phosphatidylcholine (PC), sphingomyelin (SM), ceramide (CER), cholesteryl ester (CE), triacylglycerol (TG) and phosphatidylinositol (PI). Lipid changes were graded between baseline and 4- and 12-week treatment. At 12-week treatment, five polyunsaturated diacyl PC, accounting for 38.6 to 49.2% of total PC at baseline; two ether/vinyl ether forms; seven SM; five CER and glucosyl/galactosyl-ceramide (HEX-CER) were reduced, as was the unsaturation index of HEX-CER and lactosyl-CER (LAC-CER). Although non quantitative modifications were observed in phosphatidylethanolamine (PE) during treatment with Evolocumab, shorter and more saturated fatty acyl chains were documented.
Conclusions. Depletion of several phospholipid classes occurs in plasma of FH patients during treatment with the PCSK-9 inhibitor Evolocumab. The mechanism underlying these changes likely involves the de novo synthesis of SM and CER through the activation of the key enzyme sphingomyelin synthase by oxidized LDL and argues for a multifaceted system leading to vascular improvement in users of PCSK-9 inhibitors.

A CASE OF SEVERE MULTIFACTORIAL CHYLOMICRONEMIA SYNDROME TREATED WITH VOLANESORSEN, ARE THESE PECULIAR CASES NEGLECTED AND UNDERTREATED?

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Background. Chylomicronemia can be either monogenic (Familial Chylomicronemia Syndrome or FCS) or multifactorial (Multifactorial Chylomicronemia Syndrome or MCS). Unlike FCS, MCS is usually asymptomatic and more responsive to pharmacological treatment, although there are cases that are in a grey zone between the two conditions. Our goal was to detect new cases of FCS and patients with MCS with a clinical phenotype similar to FCS among a wide cohort of patients.
Materials and Methods. For our single centre retrospective study based in Turin (Italy) we extracted data from a wide laboratory database, ranging from 2016 to 2020. Data from patients with triglycerides>885 mg/dl in more than one detection were collected in order to rule out secondary non-metabolic conditions causing hypertriglyceridemia, such as renal failure and neoplasms. Remaining subjects were subjected to a phone interview and a visit to fill out Moulin and Lipigen scores. Willing patients were then sent to a salivary genetic test for known genes for FCS.
Results. Starting from a database of 563,765 blood tests for triglycerides, 10 patients were selected for the phone interview, clinical evaluation and score assessment. 4 of them were subjected to the genetic test. One patient resulted double heterozygous for variants of LPL and APOA5 genes. This subject had a high Moulin and Lipigen score and a history of hardly manageable hypertriglyceridemia and recurrent pancreatitis. Since the subsequent start of a treatment with volanesorsen in February 2022, triglycerides have been in range and pancreatitis events have not occurred yet.
Conclusions. Under the definition of MCS a broad spectrum of conditions are included, and some are "clinically FCS" but not "genetically FCS". Probably It is now time for a stratification of clinically significant MCS patients and for gathering data in order to possibly expand the indications for volanesorsen to these subjects.

ROLE OF OPTIC ATROPHY 1-MEDIATED MITOCHONDRIAL DYNAMICS IN KUPFFER CELLS ON SYSTEMIC METABOLISM

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Aim. Kupffer cells are liver tissue-resident macrophages essential to liver pathophysiology as they play a critical role in the innate immune response. They are able to influence immune activity but also systemic metabolism through the synthesis of proteins with metabolic activity (i.e., CETP). OPA1 (Optic Atrophy 1) is a dynamin-related protein located in the inner mitochondrial membrane, which has a pro-fusion activity and thus is one of the main leaders involved in mitochondrial dynamics, since it regulates cristae morphology and consequently also oxidative phosphorylation. This project aims to study the role of mitochondrial dynamics within Kupffer cells and its possible effects on systemic metabolism by exploiting mice selectively lacking OPA1 in Kupffer Cells.
Methods. OPA1 flox/flox Clec4F-Cre+ and control mice were fed a standard diet for 22 weeks. The metabolic phenotype was assessed by indirect calorimetry through metabolic cages. Blood and liver were collected for immunophenotyping by flow cytometry analysis. Plasma total cholesterol and triglycerides dosages were performed. Liver histology was assessed with specific tissue stainings.
Results. OPA1 flox/flox Clec4F-Cre+ showed less energy expenditure (-9.44%; $p<0.05$), less oxygen consumption (-9.44%; $p<0.05$) and less carbon dioxide production (-9,48%; $p<0.01$), despite an increase in movement (+26.6%) flox/flox Clec4F-Cre- control mice. Systemic immune profile was similar, while the percentage of Kupffer Cells in the liver was reduced in OPA1 flox/flox Clec4F-Cre + compared to OPA1 flox/flox Clec4F- Cre - (-25% $p<0.05$). No significant differences in cholesterol and triglyceride levels were observed between the two groups as well as in liver histology.
Conclusions. Our data suggest that OPA1-mediated alteration of mitochondrial dynamics affects Kupffer cells and impacts systemic energy phenotype. To unveil whether this pathway plays a role in liver diseases, ongoing studies are investigating the role of OPA1 deficiency in KC in models of diet-induced obesity.

DIAGNOSIS AND MANAGEMENT OF FAMILIAL CHYLOMICRONEMIA SYNDROME IN A NEWBORN GIRL

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Introduction. Familial chylomicronaemia syndrome (FCS) is a rare, severe, monogenic, recessive disorder caused by loss-of-function mutations in both alleles of one or more of the genes that control the intravascular lipolytic cascade of triglyceride (TG)-rich lipoproteins. FCS is characterized by severe hypertriglyceridemia (TGs >10 mmol/L - 886 mg/dL) due to the accumulation of chylomicrons during fasting. FCS patients also develop eruptive xanthomas, lipemia retinalis, recurrent abdominal pain, acute and/or recurrent pancreatitis, hepato-splenomegaly and memory loss. The standard of care of FCS is based on a strict dietary regimen with <10% of energy from fat and supplementation with medium-chain TGs. Long-term adherence to this diet is poor.
Material and Methods. Here we describe a female neonate, born at term of uneventful pregnancy from healthy non-consanguineous parents. At 6th day of life a capillary blood micro-sample showed lactescent serum. TG levels were 3632 mg/dl and the clinical suspicion of FCS was made. A Next Generation Sequencing (NGS) custom panel was used to analyze candidate genes involved in the pathways of triglyceride synthesis and metabolism. Implementation of a nutritional management plan was started.
Results and Conclusions. NGS analysis allowed to identify a previously described homozygous pathogenetic mutation in LPL gene (c.829G>A p.Asp277Asn). Genetic molecular cascade screening allowed to identify the mutation in heterozygosity in both parents. Milk formula supplemented with medium chain triglycerides (MCT) oil, vitamins and oligoelements ensured an adequate intake of nutrients and TGs were stably <500 mg/dl over the weeks. At 6 months complementary feeding was introduced with a specific low-fat diet. Feeding has been well tolerated and TG levels have been as low as 339 mg/dl. In conclusion, early diagnosis and nutritional management of FCS in newborn are crucial to guarantee adequate growth and neuro-psycho-motor development and prevention severe complication.

PATHOGENICITY EVALUATION OF VARIANTS ASSOCIATED WITH FAMILIAL HYPERCHOLESTEROLEMIA: COMPARISON BETWEEN GUIDELINES

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Aim. Familial Hypercholesterolemia (FH) is the most frequent genetic disease caused by pathogenic variants in three main causative genes (LDLR, APOB and PCSK9) and characterized by high level of LDL-cholesterol and higher cardiovascular risk. There are two forms of this disease: the heterozygous (HeFH) and the homozygous one (HoFH), characterized by the presence of one and two pathogenic variants, respectively. We aim to perform the pathogenicity evaluation of a large number of variants identified in our laboratory according to different guidelines.
Materials and Methods. The pathogenicity evaluation of 193 variants, identified in the laboratory from 2008 to 2022, was made comparing the ACMG's guidelines (Richards et al. 2015) with the most recent FH-specific suggestions (Chora et al. 2018 for APOB and PCSK9 and ClinGen - Chora et al. 2022 - for LDLR).
Results. Using the ACMG's guidelines for the classification of the 137 variants identified in LDLR, 18 are USV and 113 are pathogenic/likely pathogenic, while following the most recent suggestion 38 are USV and 92 are pathogenic/likely pathogenic. The different weight given to several functional assays, widely used to test the protein function, is the major determinant of classification changes for 13 variants. Some variants identified in patients with a clear HoFH phenotype and just in a single HeFH relative were reclassified as USV. No differences were observed between the different guidelines in the evaluation of the 45 variants identified in APOB and the 11 ones identified in PCSK9.
Conclusions. Despite new guidelines suggested criteria specific for the FH genetic features that are very useful for a standardization of pathogenicity evaluation, their application resulted in many variants classified as USV. These guidelines should be improved allowing to consider with more strength the available data, very few in case of the rarest variants.

THE IMPACT OF STATIN THERAPY ON IN-HOSPITAL PROGNOSIS OF PATIENTS AT HIGH-TO-VERY HIGH CARDIOVASCULAR RISK ADMITTED WITH COVID-19

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Background. Compelling evidence suggests that statins may reduce the risk of COVID-19-related complications through their multiple pleiotropic effects. This study aimed to assess the impact of preadmission statin therapy and its continuation upon hospitalization on clinical outcomes of patients at high-to-very high cardiovascular (CV) risk with COVID-19, as well as to assess the possible influence of preadmission statin therapy on endothelial function at hospitalization.
Methods. A cohort of hospitalized COVID-19 patients at high-to-very high CV risk was retrospectively enrolled. The association between statin therapy and either endothelial function, as assessed by brachial artery flow-mediated dilation (bFMD) at hospital admission, or the composite endpoint of intensive care unit (ICU) admission/in-hospital death was assessed through univariable and multivariable analyses.
Results. Among 342 enrolled patients (mean age 79 ± 11 years, males 60%), 119 (35%) were treated with statins prior to hospital admission whereas 223 (65%) were not. Upon hospitalization, 91 patients continued statin therapy, 28 patients discontinued it, and 3 patients introduced it de novo. Also, 25 (7%) patients were admitted to ICU, 75 (22%) patients died, and 92 patients (27%) met the composite endpoint of ICU admission/in-hospital death. At multi-adjusted Cox regression preadmission statin therapy was associated with up to a 70% reduced risk of ICU admission/in-hospital death (HR 0.296, 95% CI 0.161-0.541, p<0.001). Also, at different multi-adjusted linear regressions it was positively associated with bFMD at hospital admission. However, no longer freedom from ICU admission/in-hospital death emerged according to statin continuation versus discontinuation upon hospitalization (p= 0.674).
Conclusions. Statins may positively affect the prognosis of hospitalized COVID-19 patients at high-to-very high CV risk, possibly due to their endothelium-protective effects, at least in the early phases of infection. In the COVID-19 era, statins may have an adjunctive role against COVID-19 adverse outcomes in patients at high-to-very high CV risk.

DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMIZED CLINICAL TRIAL COMPARING THE EFFECT OF A COMBINED NUTRACEUTICAL CONTAINING RED YEAST RICE ASSOCIATED TO ARTICHOKE EXTRACT OR BERBERINE ON LIPID PROFILE AND LIVER PARAMETERS IN PATIENTS AFFECTED BY POLYGENIC HYPERCHOLESTEROLEMIA

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Background. Increasing evidence suggests that the combination of low-dose lipid-lowering nutraceutical compounds is an effective and safe tool to improve total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) in subjects affected by mild-to-moderate hypercholesterolemia.
Aim. To test comparatively and versus placebo the lipid-lowering efficacy of berberine and artichoke extract in combination with low dose of monacolins from red yeast rice.
Methods. A 3-arms, randomized, double-blind, placebo-controlled, parallel-groups clinical trial was carried out on 60 healthy adult volunteers with a diagnosis of polygenic hypercholesterolemia. Enrolled subjects were randomized to be treated with monacolins 2.8 mg + artichoke 200 mg [ATC group], monacolins 2.8 mg + berberis aristata 588 mg [BBR group] or placebo.
Results. After 8 weeks of treatment, all patients experienced a significant improvement in baseline TC, LDL-C, apolipoprotein B (Apo-B) (P<0.01 always) (ATC group: TC= -18.9%, LDL-C= -26.7%, Apo-B = -19.6%; BBR group: TC= -18.4%, LDL-C= -25.8%, apoB= -23.2%; placebo: TC= -6.2%, LDL-C= -8%, Apo-B= -8.4%). Observed LDL-C variations in actively treated subjects were statistically significant not only compared to baseline but also compared to placebo. Subjects in ATC and BBR group respectively reached significantly lower body mass index and improved baseline high-density lipoprotein cholesterol (HDL-C) and triglycerides levels. Finally, baseline waist circumference and the hepatic steatosis index significantly decreased in both ATC and BBR.
Conclusion. In our short-term trial, subjects affected by polygenic hypercholesterolemia experienced a significant improvement in several cardiovascular risk factors in both monacolin-berberine and monacolin-artichoke treated patients.

EFFECT OF DIETARY SUPPLEMENTATION WITH DIURIPRES® ON BLOOD PRESSURE, VASCULAR HEALTH AND METABOLIC PARAMETERS IN INDIVIDUALS WITH HIGH-NORMAL BLOOD PRESSURE OR STAGE I HYPERTENSION: THE CONDOR RANDOMIZED STUDY

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Our aim was to evaluate if a nutritional intervention with a dietary supplement (Diuripres®) containing magnesium, standardized extract of orthosiphon, hawthorn and hibiscus could positively affect blood pressure (BP), vascular health and metabolic parameters in 60 individuals with high-normal BP or stage I hypertension. Participants followed a low-fat low-sodium Medi-terranean diet for 4 weeks before being randomly allocated to 8-week treatment with 2 pills each day of either Diuripres® or placebo. Diuripres® significantly decreased systolic BP compared to placebo after 4 weeks (3.1±0.8 mmHg; p< 0.05) and more consistently after 8 weeks (3.4±0.9 mmHg; p<0.05). At 8-week follow-up, after correction for multiple testing, dietary supplementation with Diuripres® was associated with significant improvements in diastolic BP (-3.1±0.6 mmHg; p<0.05), aortic BP (-4.3±0.4 mmHg; p<0.05), and high-sensitivity C-reactive protein (hs-CRP; 0.04±0.01 mg/dL; p<0.05) in comparison with baseline. The reductions in diastolic BP (–3.8±0.7 mmHg; p< 0.05), aortic BP (-5.2±1.0 mmHg; p<0.05) and hs-CRP (-0.03±0.01 mg/dL; p< 0.05) were also significant compared to placebo. Therefore, our study show that dietary supplementation with Diuripres® may be useful in individuals with high-normal BP or stage I hypertension.

IMPACT OF ANTI-OESTROGEN THERAPY ON SERUM LEVELS OF LIPOPROTEIN(A) IN WOMEN: A SYSTEMATIC REVIEW AND META-ANALYSIS OF DOUBLE-BLIND PLACEBO-CONTROLLED CLINICAL STUDIES

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Endocrine-therapy for breast cancer is associated with an increased risk of developing atherothrombotic events, of which high serum levels of lipoprotein(a) (Lp(a)) are a well-recognized risk factor. The aim of our study was to systematically evaluate the effect on Lp(a) of different endocrine therapies for breast cancer in high-risk women. A systematic literature search was conducted in multiple electronic databases to identify the randomized, double-blind, placebo-controlled clinical studies on this topic. Effect size for changes in Lp(a) was expressed as mean difference (MD) and 95% confidence intervals (CI). Data were pooled from 10 clinical trials comprising 24 treatment arms, which included 2049 women (1128 women in the active-treated arms and 921 women in the control arms). Unexpectedly, meta-analysis of data suggested that anti-oestrogen therapy in women significantly reduced Lp(a) [MD= -5.92% (95%CI: -9.05%,-2.8%)]. Then, the increased risk of thromboembolic events in women undergoing endocrine-therapy for breast cancer does not appear to be mediated by an increase in Lp(a) serum levels.

A CASE OF SITOSTEROLEMIA: HEMATOLOGICAL DIAGNOSIS WITHOUT ABNORMAL LIPID PROFILE

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Background. Sitosterolemia is a rare genetic disorder, characterized by phytosterol accumulation in the blood due to mutations of ATP-binding cassette (ABC) transporters ABCG5 and ABCG8. The latter promote the secretion of phytosterol into the bile and the intestinal lumen. Subjects affected by sitosterolemia are almost asymptomatic, while elevated plasma low-density lipoprotein cholesterol (LDL-C) and accelerated atherosclerosis are often found. Other manifestations include hematologic problems such as hemolytic anemia with stomatocytosis and splenomegaly. The aim of this study is to describe a case of a 30-year-old Italian male with a diagnosis of sitosterolemia by hematological phenotype.
Material and Methods. The patient came to the attention of the Hematologists for microcytic anemia and splenomegaly. Hematologists, excluding the main causes of microcytic anemia, and performed tests that revealed the possible diagnosis of sitosterolemia. Then, he came to the Lipid Clinic of San Martino Hospital in Genoa without atherosclerotic process, as documented by carotid ultrasound, and with the following lipid profile: total cholesterol 140 mg/dL, high-density lipoprotein cholesterol 46 mg/dL, calculated LDL-C 73 mg/dL, triglicerides 104 mg/dL. Subsequently, genetic analysis and the sitosterols dosage were performed.
Results. Genetic tests of the patient found the following mutations: c.293 C>G, p.Ala98Gly and c.80 G>C, p.Gly27Ala of ABCG5. He also presented high plasma levels of Colestanol, Campesterol and β-sitosterol (1,71 mg/dL, 0,45 mg/dL and 0,40 mg/dL respectively). Therefore, patient was diagnosed with sitosterolemia, and Ezetimibe 10 mg therapy and low phytosterol diet was administered.After 4 months of therapy, we did not observe changes in the lipid profile while a reduction in plasma phytosterols was reported (Colestanol 1 mg/dL, Campesterol 0.34 mg/dL and β-sitosterol 0.35 mg/dL).
Conclusion. This case report confirms that sitosterolemia may not show elevated plasma LDL-C. The identified mutations, although described as benign in the literature, may be responsible for the proband's haematological findings.

EFFICACY OF DIFFERENT NUTRACEUTICAL COMBINATIONS CONTAINING MONACOLIN K AND BERBERINE ON LIPID PROFILE: THE EXPERIENCE OF THE OUTPATIENT LIPID CLINIC OF GENOA

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Background. The 2019 ESC/EAS guidelines for the management of dyslipidemia suggest nutraceutical treatment as adjuvant of lifestyle intervention to reach the LDL cholesterol (LDL-C) target in low cardiovascular risk. On June 2022, the European Union established a daily limit for all monacolins in supplements of less than 3 mg. The main focus of this study was to evaluate the efficacy in improving lipid profile of the new formulation of Normolip 5 Forte® ESI (NUT 1), composed of 2,2 mg of monacolin K and 500 mg of berberine; secondary, we aim to compare the efficacy of NUT1 with two other nutraceuticals with different composition produced by the same pharmaceutical company.
Material and Methods. This single-center open-label clinical trial included 90 patients with polygenic hypercholesterolemia not controlled by diet alone, referring to the Lipid Clinic of San Martino Hospital in Genoa. Patients were equally divided to receive NUT1 or a nutraceutical containing 2.2 mg of monacolin K without berberine (NUT2) or a supplement composed of 10 mg of monacolin K without berberine (NUT3). At baseline, anthropometric characteristics, lipid profile and risk score were evaluated. After 8-weeks intervention, all considered parameters were revaluated.
Results. After 8 weeks of treatment with NUT1, a significant reduction in total cholesterol (-13%, p<0.0001) and LDL-C (-18%, p<0.0001) was reported. Comparing the three different nutraceuticals, we observed that NUT1 effect on lipid profile was similar to the NUT2, with a LDL-C reduction of -17% (p<0,0001); instead, patients administered with NUT3 reported a greater reduction of LDL-C (-24%, p<0,0001) compared to NUT1.
Conclusion. This study confirms that the association of monacolin and berberine is effective in improve lipid profile; however, patients who received NUT2 achieved a similar LDL-C reduction compared to patients who received NUT1. Furthermore, a greater reduction of LDL-C was observed in subjects who received NUT3.

HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA IN CHILDREN: CASE REPORT

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B.L is a young boy born in January 2008, who underwent a dermatological examination in 2016 after the appearance of skin lesions in the popliteal fossa with no other symptoms associated. The dermatologist diagnosed cutaneous xanthomas and indicated the execution of a lipid profile. Baseline exams (October 2016) showed TC 539 mg/dl and LDL-C 452 mg/dl (HDL-C and TG in range), confirmed in a second lipid profile a few days later (LDL-C 511 mg/dl). He was therefore sent for a specialist consultation. The family history revealed a hypercholesterolemia in both the parents (mother, LDL 152 mg/dl, treated with atorvastatin, father, LDL 136 mg/dl, not treated) and in maternal grandfather and grandmother. No history of early cardiovascular disease. Remote physiological and pathological anamnesis was negative. Clinical examination revealed 3 little skin xanthomas (2-3 mm diameter) in the popliteal fossa, cardiovascular and other system examination was normal; his weight was 33,4 kg (75° pct), height 131 cm (50-75° pct) and BMI 18,5 kg/m^2 (75° pct, overweight). Other laboratory exams (thyroid profile, renal profile and fasting plasma glucose) were normal, as well as baseline AST, ALT and CK. Genetic examination (NGS) was performed and two different mutations were found (c.1048C>T: premature stop codon in exon 7 of LDL-R, c.1775G>A encoding for a defective LDL-R). The family segregation study revealed that the first one was inherited from the father and second one from the mother. A first step of therapy was started with physical activity and dietary recommendations (CHILD-2 and school menu changes), and statin therapy (starting with Simvastatin 20 mg/day). The other in-depth investigations were negative (echo Doppler and carotid intima-media-thickness, echocardiogram and ECG, stress test and computed tomography of coronary arteries). The therapy was gradually increased in the next months until the dosage of 40 mg of Rosuvastatin and 20 mg of Ezetimibe, with the achievement of an LDL-C of 210 mg/dl. In June 2020 Evolocumab 140 mg x2/month was started, later increased to 420 mg once a month and then 420 mg twice a month, reaching a LDL-C of 120 mg/dl. Drugs were always well tolerated, with normal follow-up exams. Familial hypercholesterolemia (FH) is often underdiagnosed and undertreated, particularly in children, since physical signs are rarely observed. Lipid profile screening would guarantee the early identification of these patients and genetic analysis should be performed on childhood patients with family history or clinical data very suggestive for FH. Risk factors can be identified in children and adolescents and early treatment can reduce LDL-C burden, improve endothelial function and attenuate the development of atherosclerosis, adding decades of healthy living.

THE GENETIC MANIPULATION OF HDL/APOA-I LEVELS IN MICE AFFECTS THE LIPID DEPOSITION IN PERIPHERAL ORGANS

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The reverse cholesterol transport is a multistep process whereby excess cholesterol is conveyed by HDL from the peripheral tissues to the liver for excretion. In this study, the impact of the genetic manipulation of HDL/apoA-I levels on lipid deposition in intestine, liver, kidney and adrenals was investigated. Mice with extremely low plasma HDL levels, deficient for both murine apoA-I and apoE (DKO), were compared with mice characterized by elevated HDL, deficient for both apoA-I/apoE, but overexpressing human apoA-I (DKO/hA-I). Mice, both female and male, were fed a standard rodent diet until one year of age. Plasma lipids were quantified by enzymatic methods. Intestine, liver, kidney and adrenal morphology was evaluated by light microscopy on frozen sections. Plasma total cholesterol concentration in DKO mice was comparable with that of wild-type mice and 3-fold lower than that observed in DKO/hA-I mice. Plasma HDL-C was almost absent in DKO mice and strongly elevated in DKO/hA-I mice. The H&E-stained sections did not reveal the presence of morphological alterations in the tissues analyzed: intestinal villi and crypts were regular, steatosis in liver parenchyma, as well as foam cells in renal glomeruli were absent and adrenal size was comparable in both genotypes. The neutral lipid-specific staining with Oil Red O showed instead interesting differences. The intestine did not exhibit HDL-mediated effects on lipid deposition. On the contrary, in the hepatic parenchyma, an increased accumulation of lipids around the centrilobular vein was observed in DKO/hA-I mice only. In addition, within the glomeruli and the adrenal cortex of DKO/hA-I mice, lipid accumulation was significantly higher than in DKO. On summary, although DKO mice are almost completely devoid of HDL and prone to atherosclerosis development, they do not exhibit signs of abnormal lipid accumulation in liver, kidney and adrenals, as in DKO/hA-I mice, characterized by elevated HDL levels.

STATIN-INDUCED AUTOIMMUNE MYOSITIS: A PROPOSAL OF AN "EXPERIENCE-BASED" DIAGNOSTIC ALGORITHM FROM THE ANALYSIS OF 69 PATIENTS

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Background. Statin-induced autoimmune Myositis (SIAM) represents a rare clinical entity that can be triggered by a prolonged statin treatment. Its pathogenetic substrate consists of an autoimmune-mediated mechanism, evidenced by the detection of antibodies directed against the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (anti-HMGCR Ab), target enzyme of statin therapies.
Objective. To evaluate the different features of SIAM cases described in literature and to extrapolate a diagnostic algorithm. Methods: We have analyzed the clinical data of 69 patients diagnosed with SIAM. Sixty-seven patients have been collected from the fifty-five available and complete case records regarding SIAM in the literature; the other 2 patients represent our direct clinical experience and they are described in detail as case records. From the analysis of the clinical features of 69 patients, we have constructed a proposal of an "experience-based" diagnostic algorithm for SIAM, in order to facilitate the diagnosis of nuanced SIAM clinical cases. The algorithm involves Anti-HMGCR antibody testing, musculoskeletal MR, EMG/EEG of upper-lower limbs and, where possible, the muscle biopsy.
Conclusion. We advise a close monitoring of symptoms during statin treatment and the employment of this algorithm to early recognize and treat adverse events such as SIAM, avoiding no longer reversible and sometimes fatal complications.

THE LIFETIME RISK OF ATHEROSCLEROTIC CARDIOVASCULAR DISEASE DOES NOT VARY BASED ON GENETICALLY DETERMINED OR CLINICALLY MEASURED LIPOPROTEIN(A) LEVELS

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Lipoprotein(a) (Lp(a)) concentration has been causally associated with increased risk of atherosclerotic cardiovascular disease (ASCVD). Whether ASCVD risk varies according to genotype determined or measured Lp(a) levels is still unknown.A total of 445,744 participants (mean age: 57.3 years; female sex: 54.3%) enrolled in the UK Biobank were included in this Mendelian randomization analysis. For each participant, we calculated the LPA genetic risk score by summing the number of risk-increasing alleles inherited at rs3798220 and rs10455872 variants. The primary outcome was the incidence of major coronary events (MCE), a composite of fatal or non-fatal myocardial infarction, or coronary revascularization. Using adjusted Cox proportional hazards models and Kaplan-Meier curves, we compared the cumulative lifetime risk of MCE among subjects with different LPA genotype and measured Lp(a) levels, expressed as hazard ratio (HR) and 95% confidence interval (95%CI).Participants with one copy of either rs10455872 or rs3798220 had a HR for MCE of 1.47 (95%CI, 1.42-1.51) compared with wild-type subjects (median Lp(a): 146.3 nmol/L and 13.6 nmol/L, respectively). Stratifying the population according to measured Lp(a) concentrations comparable to those observed for the genetic score, we found similar increased MCE risk for the same Lp(a) change (HR 1.47; 95%CI, 1.41-1.53). Moreover, even among subjects with the same LPA genotype, increasing quintiles of measured Lp(a) concentration were associated with a step-wise increase in MCE risk. Conversely, among subjects with different LPA genotype, but similar median Lp(a) concentrations, the lifetime risk of incident MCE was comparable.Our findings demonstrated that LPA genetic risk score and measured Lp(a) concentration provide comparable risk for incident MCE. However, since measured Lp(a) showed a wide range of values among individuals with the same genotype, with the risk changing accordingly, we emphasize the importance of measuring Lp(a) level in clinical practice to better identify patients at risk, regardless of genotype.

EVIDENCE FOR THE REFINEMENT OF THE DIAGNOSTIC APPROACH IN CHILDREN AND ADOLESCENTS AFFECTED BY FAMILIAL HYPERCHOLESTEROLEMIA: DATA FROM THE LIPIGEN STUDY

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Background. The high cardiovascular risk associated with familial hypercholesterolemia (FH) makes early identification crucial. However, the detection of FH subjects in childhood poses several issues. We aimed to highlight and quantify limits of a diagnosis of FH in childhood based on the presence of the typical features of FH, included in one of the most applied diagnostic tool, the Dutch Lipid Clinic Network (DLCN) score, and to suggest additional parameters to refine the FH diagnosis at young age.
Methods. From the LIPIGEN study, we selected 1188 (≥18years) and 708 (<18years) FH patients with a positive genetic test for heterozygous FH, with no missing DLCN parameters about physical examination and personal clinical history, and untreated LDL-C value available. The prevalence of the main FH features was compared between the two groups, and data about premature CHD also in second-degree family members were integrated in a paediatric sub-group (N=374).
Results. The lower prevalence of typical FH characteristics in children/adolescents vs adults was confirmed: tendon xanthoma 2.1% vs 13.1, arcus cornealis 1.6% vs 11.2%, respectively. No children presented clinical history of premature CHD or cerebral/peripheral vascular disease (in adults 8.8% and 5.6%, respectively). The presence of tendon xanthoma and/or corneal arcus as well as hypercholesterolemia in first-degree relatives were comparable among adults and children (18.7% vs 20.0% and 92.9% vs 93.5%, respectively). The prevalence of premature CHD in first degree relatives was significantly higher in adults compared to subjects under 18 years (38.9% vs 19.7%). Within the paediatric sub-group with data about second degree relatives (representative of the whole paediatric cohort), a premature CHD events in parents was reported in 63 of 374 subjects (16.8%), but the percentage increased to 54.0% extending the evaluation also in second degree relatives.
Conclusion. In children, the DLCN parameters are clearly less informative than in adults. A refinement of the diagnostic approach with a tailored data collection is needed for the diagnosis of FH at young age.

CORONARY ARTERY CALCIUM IS STRONGLY ASSOCIATED WITH PULSE WAVE VELOCITY AND LDL-CHOLESTEROL BURDEN IN SUBJECTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Background and Aims. Familial hypercholesterolemia (FH) is a genetic disorder characterized by high plasma levels of low-density lipoprotein cholesterol (LDL-C) and premature cardiovascular (CV) diseases. Coronary artery calcification (CAC) assessment and arterial stiffness measured as pulse wave velocity (PWV) are accurate in CV risk assessment, but so far data on HeFH are missing. Evaluation of aortic stenosis by Doppler echocardiography with markers of severity, aortic valve area (AVA) and mean gradient (MG) are still unresolved. In this study we evaluated CAC, PWV and the relationship between aortic valve calcium/stenosis severity in a HeFH cohort in order to improve risk stratification and therapy timing.
Methods. One-hundred genetically characterized HeFH subjects were recruited at our lipid clinic and CAC, PWV measurement and LDL-C burden calculation were assessed. Physiologic/structural determinants of aortic valve area (AVA)/mean gradient (MG) relationship associated with aortic stenosis were also analyzed.
Results. Mean age was 45±16 years. 25% of patients had hypertension;15% were in secondary prevention. On univariate analysis, we found strong positive correlations between CAC and both PWV ($r=0.52$ $p>0.0001$) and total LDL-C burden ($r=0.52$ $p<0.0001$). No other associations with lipid parameters were found. Multivariate analysis showed that CAC was independently associated with PWV adjusted for sex, total LDL-C burden, systolic blood pressure, smoking, LDL-C, HDL-C and statin treatment.
Conclusions. Arterial stiffness is independently correlated with CAC in HeFH subjects with similar total LDL-C burden and CV risk profile. The assessment of PWV in HeFH patients could represent a valuable tool to refine the CV risk and therapeutic management.

HE DIES YOUNG WHO IS DEAR TO THE GODS

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Since the discovery and commercialisation of antibiotics, cardiovascular diseases have become the most significant cause of death in western economies. The identification of risk factors (family history, LDL-hypercholesterolaemia, hypertension, diabetes, smoking, sedentariness...) prompted basic research to look for drugs reducing these factors. It is to the credit of the Padua school that it intuited the multiplicative nature of the risk of the association of some of these factors, later named the Metabolic Syndrome: includes, in addition to diabetes and conditions preceding it (IFG; IGT), hypertension, hypertriglyceridemia, low LDL-cholesterol values, and racially determined values of abdominal circumference. This datum, apparently purely anthropometric, is strongly pathogenetically connected with the elements of the syndrome, explaining various alterations, due to the flow of NEFAs from the abdominal district to the liver: hypertriglyceridemia due to the 'mass effect' of the NEFAs themselves; alteration of the post-synthetic metabolism of lipoproteins, with the formation of small, dense HDL. Much has been debated about the existence of this morbid form ('Requiescat in pace' wrote one of its early detractors). A controlled study demonstrated an increased CV risk in the presence of 3, 4 or 5 elements of this syndrome. Another metabolically deleterious effect of NEFAs, exerted in this case in the systemic circulation, is the one described by Randle, christened by his name, or 'glucose-fatty-acid cycle': describes the competition for cellular use of glucose by fatty acids, resulting in the problematic cellular internalisation of sugar, with persistence of sugar in the circulation. At the level of endothelial cells, it exerts an atherogenic effect summative to that due to the increased synthesis of triglyceride-rich lipoproteins (TRLP). In gynoid obesity, the centripetal flow of NEFAs occurs through the inferior vena cava: this pathogenetic aspect, explains the lower impact of this condition on CV disease, as it lacks the increased synthesis of TRLP. In 'pear' obesity, CV morbidity is still higher than in subjects with normal BMI. A Caucasian male (P.P.) with no history of family history of CV disease, with a personal history of significant sporting activity in his youth, who was a personal friend during his summer stay at our ASL, was entrusted to us as a patient in July 2002 by his wife, who was concerned about his persistent weight gain, also in relation to his strong diabetic family history. The patient reported nocturia in recent times. The patient's anthropometric data were: height cm 181; weight kg 107.000; BMI 32; abdomen circumference cm 104. Objective examination documented: pure MV over all range; cardiac action rhythmic, normo frequent, distal arterial pulses pulsating, symmetrical, BP 145/95 (he had not checked blood pressure habitually for years); abdomen globular, treatable, organs within limits. The patient was a smoker of 30 cigarettes a day; drank one bottle of wine a day, occasionally super alcohol. . Informed of the definite diagnosis of diabetes, and of the high cardiovascular risk due to the concomitant metabolic syndrome and cigarette smoking, he was prescribed Metformin in graduated doses up to 2,550 mg, Aspirin 100 mg, Atorvastatin 20 mg, as well as an invitation to take aerobic physical activity, to assiduously monitor his blood pressure, to stop smoking, and to lose weight. He also received a prescription to undergo ECG and funduscopy. The patient was puzzled by our instructions, objecting that he was in good health and 'felt great'; he would discuss our prescription with his doctor. He claimed to be able to normalise all altered parameters with his lifestyle. Back at home, he discussed our prescriptions with the doctor: both considered them 'excessive'. He informed us that he would follow up with behavioural therapy alone. The following spring, we were informed by his wife that the patient, seized one evening, first by thoracic discomfort, then by frank precordial pain radiating to the left arm, was quickly transported to the local hospital, where he was diagnosed with an extensive anterior infarction and taken to the haemodynamic room, where he unfortunately did not arrive alive. The case is emblematic in several respects: death occurs in most cases within 90 minutes of the onset of symptoms; the high morbidity and mortality from IMA in the diabetic, a patient whose mortality is as high as the second heart attack in the non-diabetic; the patients' guilty nihilism, sometimes shared by the treating physicians, in not aggressively treating diabetes and all the other factors of the syndrome, when present. The fatal event in the case described, could have benefited from the pleiotropic effects of Metformin on cardiovascular disease, at a dosage of 2,550 mg; of Aspirin in primary prevention; of the reduction of cholesterolaemia, also due to the pleiotropic effect of these molecules, on plaque stabilisation and nitric oxide production. The therapeutic inertia of physicians in applying the guidelines of the Scientific Societies is known as a cause of morbidity and mortality. Similarly, non-acceptance of treatment, or discontinuation of treatment by patients, is another cause of treatment failure. P.P. (29/02/1956-22/03/2003) joking about his own date of birth, predicted that he would die young anyway, claiming that he would turn one year every four, having been born on the last day of February of a leap year. His prediction was unfortunately true: in addition to unchangeable constitutional factors, there were environmental factors that were not adequately dealt with.

SCREENING OF POLYMORPHISMS F2 RS1799963, F5 RS6025 AND IDENTIFICATION OF RARE COAGULATION VARIANTS IN COVID-19 PATIENTS

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Background and Aims. Severe SARS-CoV-2 infections are characterized by perturbation of physiological coagulation mechanisms. COVID-19 is associated with a high incidence of thrombotic complications, such as venous thromboembolism or arterial thrombotic events (myocardial infarction and cerebral events). An association between thrombophilia and the most severe clinical course of COVID-19 has been suggested. Thrombophilia is a condition of altered haemostasis, characterized by increased blood clotting, which predisposes to adverse thrombotic events. This condition may be due to hereditary factors, acquired changes or, in the prevalence of cases, an association of genetic and acquired factors. Among the hereditary thrombophilia factors, the most frequent causes are factor V Leiden polymorphism (rs6025) in F5 gene, and the G20210A polymorphism (rs1799963) in F2 gene. Aim of the study was to identify genetic variants and/or genetic profiles associated with severity of the disease and thrombotic events susceptibility.
Methods. Starting from these considerations, an NGS analysis was conducted on a cohort of n=40 patients with COVID-19; genetic analysis included a sequencing panel of 11 genes (PROC, PROS1, FGA, FGB, FGG, SERPINC1, F2, F5, F10, PLAT, PLG) known to be involved in the coagulation process. Moreover, a genotyping analysis of rs6025 and rs1799963 polymorphisms has been conducted through Real Time PCR on the whole cohort of n=994 patients hospitalized at the AOU Careggi with COVID-19.
Results. As regards NGS analysis, 29 rare variants (MAF≤1%) have been identified at the heterozygous state in 24 of the 40 patients studied: 7 missense variants (on the F10, F2, PLAT, SERPINC1, F5 and FGB genes), 13 synonymous variants (on FGB, F2, PLAT, PLG, PROC and F5 genes), 4 variants concerning zone 3'-5' UTR/downstream (on F10, PLAT, PROC and F5 genes) and 5 non-deep intronic variants (on F5, PROC, F10 and PLG genes). In particular, there are five rare variants which were identified in two different patients each. A higher prevalence of rare missense variants with potential pathogenic prediction in ICU or death patients (26.7%) was observed than in ordinary ward patients (8%). Concerning common genetic thrombophilia, in the whole n=994 patients cohort, n=45 were heterozygous for the rs1799963 polymorphism and n=31 were heterozygous for rs6025 polymorphism. MAF for F2 G20210A was 0.023, higher than that reported in the literature for the population of Tuscany (0.016), while for the FV Leiden was 0.016, comparable to that observed in the tuscan population (0.020). Among a subgroup of n=324 patients, for which information concerning the clinical outcome was available, emerged that in patients who developed a thromboembolic event (5.9%) there was a higher allelic frequency, but not statistically significant, of FVL polymorphism, compared with patients who did not develop such an event (0.026 vs. 0.018, p=0.519); no differences were observed for F2 G20210A polymorphism.
Conclusions. The presence of common genetic factors of hereditary thrombophilia does not seem to indicate a significant contribution in modulating the risk of developing thromboembolic complications in SARS-CoV-2 patients; on the other hand, NGS results show that genetic variability, due to rare variants, might modulate clinical severity of COVID-19 disease in patients.

THE OPTIMIZED INTAKE OF PROTEIN-RICH FOODS TO REDUCE CARDIOVASCULAR DISEASE RISK CAN HELP MITIGATE CLIMATE CHANGE

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Ischemic heart disease is the leading cause of mortality in Europe, contributing to half of total cardiovascular disease (CVD) deaths. Food choices account for 50% of all CVD deaths and ~37% of total greenhouse gas (GHG) emissions, with red meat at the top of the list of harmful foods for planetary health.To identify the most suitable intakes of different protein-rich foods to reduce CVD risk, we performed a systematic evaluation of the relationship between their habitual consumption and CVD incidence or mortality in meta-analyses of prospective studies. Thereafter, we calculated the impact on climate of the transition from the current intake of protein-rich foods in Europe towards the optimal intake for CVD prevention, using the Carbon Footprint indicator. We identified 100g and 25g weekly of red and processed meat as the most appropriate intakes for the optimization of CVD prevention, corresponding to a reduction of 87.6% and 91.7% respectively from current consumption. Among dairy products, cheese and full-fat milk should be reduced (-52.9% and -69% respectively) while yogurt consumption should markedly increase (up to 200g/day, +528.8%). A much higher intake would be required for legumes (+716.6%), while fish and eggs should be increased to a lesser extent. Overall, these variations would avoid the emission of 18.7 kgCO2eq./capita/week (-56.6%). The increase in GHG emissions linked to higher consumption of protein-rich foods like legumes, yogurt, fish and nuts, would not counterbalance the reduction due to lower consumption of meat and full-fat dairies (+5.4 vs. -23.9 kgCO2eq.). These results indicate that in the attempt to optimize CVD prevention by appropriate food choices, reducing the intake of red and processed meat as well as full-fat dairies and replacing them with vegetable protein sources but also with yogurt and moderate amounts of fish - particularly fatty fish - and eggs, could also reduce by almost 60% GHG emissions linked to protein-rich foods consumption in Europe.

GENETIC HETEROGENEITY OF SEVERE HYPERTRIGLYCERIDEMIA: LOW PREVALENCE OF THE FAMILIAL CHYLOMICRONAEMIA SYNDROME

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Aim. Severe Hypertriglyceridemia (HTG) is an inherited disease characterized by very high plasma levels of triglycerides (TG) and a considerably increased risk of acute pancreatitis.The monogenic autosomal recessive form (Familial Chylomicronaemia Syndrome - FCS) is due to 2 pathogenic variants in 5 known genes involved in metabolism of the TG-rich lipoproteins (LPL, APOA5, APOC2, GPIHBP1 and LMF1) and associated to very severe phenotype. High levels of TG are often due to multifactorial chylomicronaemia syndrome (MCS) caused by heterozygous rare variants in canonical FCS genes and/or in non-canonical genes recently associated with TG metabolism. We aim to compare genetic status in a cohort of patients with severe hypertriglyceridemia.
Patients and Methods. Thirty seven unrelated patients were recruited based on plasma levels TG>10mmol/L and screened for rare variants in candidate FCS genes. Multiplex ligation-dependent probe amplification was used to detect large rearrangements in the LPL gene. Recently, the genetic analysis was performed by NGS with a large panel of genes involved in lipid metabolism.
Results. Among studied patients, 9 carried two pathogenic variants in canonical genes (4 were true homozygotes, 4 were compound heterozygotes and 1 was double heterozygote). In 11 patients we detected only one variant classified as pathogenic: 9 patients with variants in canonical genes (6 LPL - 3 APOA5); 2 patients with variants in non-canonical genes (GCKR and CREB3L3). No pathogenic variants were identified in 17/37 patients (45.9%) but 5 of them were carriers of additional rare variants of uncertain significance (USV) in canonical and non-canonical genes.
Conclusions. Genetic screening revealed that only a small number of patients suffer from FCS since they presented 2 pathogenic variants. These results highlight the genetic heterogeneity of the disease and the great usefulness of NGS to define complexes genotypes including of potential pathogenic variants, also in non-canonical genes.

BODY MASS INDEX MODULATES THE IMPACT OF SHORT-TERM EXPOSURE TO AIR PARTICULATE MATTER ON HIGH DENSITY LIPOPROTEIN FUNCTION

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Background. The exposure to environmental pollution has been associated with an increased risk of ischemic heart disease and stroke, with increased susceptibility in obesity. Air pollutants trigger inflammation and oxidative stress, which may impact the atheroprotective functions of HDL. Aim of the present study was to assess the relationship between short-term exposure to air particulate matter (PM) and HDL function, and the modifying effect of BMI.
Methods. Daily exposures to PM10 and PM2.5 of 50 subjects with overweight/obesity and 41 healthy volunteers with BMI<30kg/m2 were obtained from fixed monitoring stations. HDL function was assessed as their ability to promote nitric oxide (NO) release by endothelial cells and to reduce cholesterol mass in macrophages.
Results. HDL ability to induce NO production progressively declined with the increase in BMI. No association was found between HDL-mediated NO production and PM10 or PM2.5 exposures. Nevertheless, a significant modifying effect of BMI on PM10 was observed on the day before the recruitment. In subjects with a normal BMI, a positive association between day-1 PM10 exposure and HDL-mediated NO production was found, but this compensatory response was lost in participants with higher BMI values. Similar results were obtained with HDL ability to reduce macrophage cholesterol mass, although this functional parameter was independent from BMI and the modifying effect of BMI on PM10 exposure measured the day-1 was less evident.
Conclusions. The compensatory response of HDL function after exposure to PM was progressively lost at increasing BMI levels. The impaired ability of HDL to promote NO release could contribute to the endothelial dysfunction induced by PM and could help to explain the susceptibility of subjects with obesity to the detrimental effects of pollution.

TREATMENT WITH NATRACEUTICAL IS SAFE AND EFFECTIVE IN REDUCING LDL AND REACHING THERAPEUTICAL GOAL IN PATIENTS TREATED WITH PCSK9-I AND INTOLERANT TO STATIN AND EZETIMIBE

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Background. Anti-proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9-i) are usually added to statins (S)±ezetimibe (E), but a percentage of patients cannot use S and/or E due to intolerance. Furthermore, a percentage does not reach the LDL target with PCSK9-i alone. In these subjects, the addition of a natraceutical could be helpful.
Patients and Methods. Aim of our study was to evaluate, in a series of patients treated with PCSK9-i intolerant to S and E and not in therapeutic target, the reduction of LDL, the achievement of therapeutic target on LDL and the safety by adding a natraceutical. Eighteen patients already on treatment with full dose of PCSK9-i (14 evolocumab, 4 alirocumab) unable to reach the therapeutic target and intolerant to S and E, were treated for six weeks with a natraceutical (monacolin K 10 mg plus octacosanol 12 mg). The mean age was 61.94±7.01 years, BMI 26.5±0.71 (kg/m^2). All were assessed for total cholesterol, HDL, LDL, triglycerides (mg/dl), ALT and CPK before treatment and at six weeks with natraceuticals.
Results. Initial mean values were: total cholesterol 184.11±30.07, HDL 60.94±13.20, triglycerides 132.79±46.97, LDL 98.83±30.61. After six weeks mean values were: BMI 26.6±0.20, total cholesterol 148.56±31.31 (-19.31%), HDL 64.56±13.34 (+5.94%), triglycerides 121±51.76 (-8.86%), LDL 64.33±29.06 (-34.91%); twelve (66.67%) of patients reached therapeutic goal for LDL. No adverse events or significant changes in ALT and CPK were reported.
Discussion. In our study, the addition of a natraceutical to PCSK9-i therapy was effective in reducing LDL values and achieving the target according to the 2019 guidelines. Of particular interest is the intensity of the LDL reduction (-35%), which appears to be greater than expected (24%).
Conclusions. This reduction of LDL and the absence of adverse events could justify the use of natraceuticals in this patient group, although further randomised studies are needed.

SUCCESSFUL TREATMENT WITH LOMITAPIDE IN A PATIENT WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA AND SEVERE FATTY LIVER DISEASE

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Introduction and Aims. Homozygous familial hypercholesterolemia (Ho-FH) is a rare condition due to biallelic mutations in low-density lipoprotein-receptor (LDL-R) pathway genes characterized by very high level of LDL cholesterol (LDL-c) from birth and an extremely high risk of premature atherosclerotic cardiovascular disease (ASCVD), determining low quality of life and life expectancy. Lomitapide, an oral microsomal triglycerides transfer protein (MTTP) inhibitor, is an effective therapeutic option for Ho-FH, but caution should be observed when used in patients with fatty liver disease (FLD) and increased liver enzymes since it is associated with onset/worsening of liver steatosis. Here we present a case in which lomitapide was safely used in an adult Ho-FH patient with pre-existing severe FLD.
Case presentation. A 39-year-old man with severe hypercholesterolemia since young age (LDL-c up to 405 mg/dl) and a history of premature coronary heart disease with residual stable angina, was referred to the Lipid Clinic in Modena for the suspicion of FH. He also presented an overt metabolic syndrome (visceral obesity, arterial hypertension, impaired fasting glucose) and FLD with elevated liver enzymes and elastosonographic evidence of moderate liver fibrosis. His lipid-lowering therapy (LLT) included rosuvastatin 20 mg, ezetimibe and evolocumab 140 mg twice a month without reaching the LDL-c goal. Both parents were affected by hypercholesterolemia and a history of ASCVD. Genetic analysis revealed homozygous pathogenic mutation in LDL-R gene. LLT was further enhanced by increasing evolocumab up to 420 mg twice a month and starting LDL-apheresis with a negative impact on quality of life. For this reason, lomitapide 5 mg daily together with a personalized low-fat diet were started, obtaining a significant weight loss and improvement of lipid profile without any gastrointestinal adverse event. However, liver enzymes elevation higher than 5-fold the baseline values was observed, leading to lomitapide discontinuation until exclusion of secondary causes of hypertransaminasemia and baseline liver enzymes values restoration. After one month wash-out, lomitapide was gradually reintroduced up to 5 mg daily without recurrence of hypertransaminasemia, leading to long-term LDL-c target achievement and LDL-apheresis discontinuation. The optimal adherence to low-fat diet and the weight loss resulted in improvement of FLD and fibrosis despite lomitapide therapy.
Conclusion. Ho-FH requires a complex and combined treatment. Co-existence of metabolic comorbidities makes Ho-FH management even more difficult. Lomitapide can be safely used also in Ho-FH patients with FLD and hypertransaminasemia, but its use requires strict follow-up of liver disease and a multidisciplinary approach with tailored lifestyle advices. When considering lomitapide therapy in this setting, low-fat diet should ideally be started in advance and weight stabilization should be obtained before treatment introduction.

A DIFFERENTIAL PROTEOMIC ANALYSIS ON HUH7 HEPATOCARCINOMA CELL LINE NATURALLY OVEREXPRESSING OR HYPOEXPRESSING LOW DENSITY LIPOPROTEIN RECEPTOR

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Background. LDL receptor (LDLR), chiefly expressed in liver, is the main regulator of LDL plasma levels, and carriers of LOF mutations on LDLR gene are affected by a rare disease that causes early atherosclerosis and fatal and non-fatal cardiovascular events. LDLR expression is highly regulated both at transcriptional/post-transcriptional (es. hsa-miR-140-5p) and post-translational levels (es. PCSK9, IDOL). The aim of this study was to identify new modulators of LDLR and cholesterol/triglycerides (chol/TG) metabolism.
Methods. A polyclonal HuH7 human hepatocarcinoma cell line was incubated with fluorescently labeled LDLs and sorted in two subpopulations naturally expressing, respectively, high and low LDLR expression in order to identify differentially expressed proteins (DEPs) by the means of mass spectrometry technique (cut-off: ±1.95 fold-change). DEPs involved in LDLR modulation and/or in chol/TG metabolism have been selected and Gene Ontology database has been used for the annotation analysis. Ingenuity pathway analysis by Qiagen has been used to elaborate the upregulated vs downregulated pathways in the two subpopulations of cells.
Results. The proteomic analysis on HuH7 High and Low pointed out an enrichment in 7 downregulated DEPs (CPS1, CES1, ABAT, MT1X, GLRX) and in 5 upregulated DEPs (AGR2, ACOT7, AKR1C1, FLNA, ANXA3). Among the downregulated DEPs, CES1 and CPS1 are of greatest interest. CES1 (carboxylestherase 1) is highly expressed in liver and involved in TG metabolism and in the protection against hepatic steatosis. CPS1 (carbamoil-phosphate synthase), is a mitochondrial protein involved in urea cycle and it has been recently associated with HDLc levels in a genome wide association study (GWAS). Among upregulated proteins, ACOT7 (acyl-CoA thioesterase 7), who hydrolyses acyl-CoA thioesters into free-fatty acids (FFA). Moreover, ACOT7 resulted overexpressed in biopsies from patients with HCC (where an overproduction of FFA has been observed). Interestingly, the analysis on up- and down-regulated pathways unveiled the modulation of the FXR/RXR axis, thus leading to an important modulation in cholesterol metabolism (CYP7A1), biosynthesis (CYP51A1, FDFT1), transport (ABCA1, ABCG1-5-8), efflux (APOA4, CD36), lipoprotein synthesis (LPL, CETP, PLTP) and lipogenesis (SREBP-1c, FASN, SCD1, ACACA, MLXIPL).
Conclusions. The proteomic analysis shed light on several up- and down-regulated DEPs between HuH7 with High and Low LDLR expression. The Gene Ontology functional analysis revealed that CPS1, CES1, and ACOT7 are annotated as involved in "lipid metabolism" and "fatty acid metabolism", and the Ingenuity pathway analysis confirmed the modulation of pathways involved in these processes. Even if the activity of some of these proteins is mostly studied in tissues or cell populations different from the liver or hepatocytes (es. CES1 has been widely studied in macrophages, while ACOT7 is known for it role in FFA formation in the brain), it would be of great interest to further investigate their potential contribution into the hepatic tissue in relation to life-threatening pathologies such as familial hypercholesterolemia.

ULTRASOUND AND ELASTOSONOGRAPHY: THE "EYES" OF THE DOCTOR IN PATIENTS WITH METABOLIC SYNDROME AND NAFLD UNDERWENT TO DIET INTERVENTION

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NAFLD is the most widespread liver disease, characterized by fatty acids liver accumulation and subsequent fibrosis with a prevalence that is estimated to be around 50% in patients with metabolic syndrome. There are several imaging techniques that can accurately diagnose fatty liver. Recently, ultrasound has acquired a leading role in the diagnosis and follow-up of fatty liver disease. Furthermore, elastosonography and in particular shear wave represent a valid alternative to liver biopsy. We evaluated the effects of lifestyle and nutritional interventions on hepatic steatosis through ultrasonographic and elastosonographic techniques. Thirty-two female subjects with metabolic syndrome were subjected to clinical, anthropometric, and laboratory assessments, as well as abdominal ultrasonographic/elastosonographic measurements taken from enrollment time (T0) and after 3 months (T1) of lifestyle modifications. After 3 months of lifestyle changes, significant weight loss was observed, with a marked improvement in all adiposity indices. The laboratory parameters at T1 showed significant decreases in total and LDL cholesterol, triglycerides, basal blood glucose, 120 min glycaemia, basal insulin and HOMA Index ($p<0.001$). A similar improvement was observed at T1 for steatosis degree ($p<0.01$) and elastosonographic measurements (Kpa $p<0.001$). After 3 months, the liver size showed improvement with positive correlations to all previous variables. Hepatic stiffness (Kpa) positively correlated with neck circumference, visceral fat, and ALT, with basal insulin, gamma-GT, and AST, and with waist circumference, WhtR, and fat mass. The degree of steatosis was positively correlated with more variables and with greater statistical significance at T1 with respect to T0. Particularly, the positive correlations between the degree of steatosis and neck circumference ($p<0.001$), HOMA Index, and triglycerides ($p<0.001$) appeared to be very significant. In conclusion NAFLD management may include liver ultrasonographic and elastosonographic techniques to better manage and follow-up patients.

PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 GIVES ATHEROGENIC PROPERTIES TO EXTRACELLULAR VESICLES RELEASED BY VASCULAR SMOOTH MUSCLE CELLS

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Extracellular vesicles (EVs) mediate intercellular and inter-system communication. They play a role in pathological conditions, e.g. atherosclerotic cardiovascular diseases (ASCVD), participating in atherosclerosis onset and progression. Besides endothelial, monocytes and macrophages, vascular smooth muscle cells (VSMCs) are involved in atheroma formation. They can influence neighboring cells through bioactive molecules, some of which are packed into EVs. VSMCs express and secrete proprotein convertase subtilisin/kexin type 9 (PCSK9), crucial in the pathophysiology of ASCVD and influences VSMCs differentiation, migration and proliferation.
Aim. To unveil whether the overexpression of PCSK9 in VSMCs affects the composition of EVs favoring a pro-atherogenic phenotype.
Methods. EVs were isolated from VSMCs wild-type (VSMCs-WT-EVs) or overexpressing PCSK9 (VSMCs-PCSK9-EVs) and tested on EA.hy926 endothelial cells, THP-1 monocytes, THP-1-derived-macrophages and in embryos of zebrafish. The following approaches were used: flow cytometry, WB, mass spectrometry, qPCR, nanoparticle tracking analysis, transmission electron microscopy, mitochondrial-bioenergetics analysis.
Results. VSMCs-WT-EVs and VSMCs-PCSK9-EVs expressed specific markers of EVs (tetraspanins, Alix, and β1-Integrin) with no differences in their concentrations (625.17±235.23/mL/cell count and 926.17±815.26/mL/cell count, respectively), size (235.78±29.78 nm and 233.16±16.3 nm, respectively) and morphology. VSMCs-PCSK9-EVs, expressing higher levels of PCSK9, carried a different pattern of proteins and miRNAs linked to inflammation. VSMCs-PCSK9-EVs led to a rise in the expression of adhesion molecules and pro-inflammatory cytokines in endothelial cells. In monocytes and macrophages, exposure to VSMCs-PCSK9-EVs raised the expression of MCP-1, IL-1α, IL-1β, IL-6 and IL-8 as well as the phosphorylation of the inflammatory protein STAT3. VSMCs-PCSK9-EVs enhanced the migratory capacity of THP-1, decreased that of macrophages and reduced THP-1 basal and maximal mitochondrial respiration. VSMCs-PCSK9-EVs increased the uptake of oxidized LDL in macrophages. Injection of VSMCs-PCSK9-EVs in the hindbrain ventricle of zebrafish embryos favored a local recruitment of macrophages.
Conclusion. In atheroma formation, PCSK9 seems to play an atherogenic role by means of EVs derived from VSMCs.

HOW DIFFERENT DIETS SHAPE PLASMA AND AORTA LIPIDOME: A STUDY IN THE APOE KNOCKOUT MOUSE MODEL

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Introduction. Specific lipid molecules circulating in plasma at low concentrations have emerged as biomarkers of atherosclerotic risk. The aim of the present study was that of evaluating, in an athero-prone mouse model, how different diets could affect plasma and aorta lipidome.
Materials and Methods. Thirty-six apoE knockout mice were divided in 3 groups and fed for 12 weeks with diets (standard, Western-type and Paigen) differing for cholesterol and fatty acid (FA) content. Atherosclerosis was measured at the aortic sinus and aorta. Lipids were quantified in plasma and aorta with a hybrid triple quadrupole/linear ion trap mass spectrometer equipped with an ultra-high pressure liquid chromatography system.
Results. Taking into consideration the entirety of the dataset, the variation of the bulk lipid content of each sample in the plasma was overwhelmingly large with respect to any variation in the aorta. Conversely, the diversity of the relative amount of lipids that could be found in the aorta was much larger than in plasma.The cholesterol content of the diets was the main driver of lipid accumulation in plasma and aorta. The different composition of the diets resulted in sizeable differences in plasma of essential (linoleic acid) and nonessential (myristic and arachidonic acid) FA, even though the distribution pattern of single FA moieties followed a comparable trend for all diets. We found a comparable distribution, in plasma and aorta, of the main lipid components of oxidized LDL, including cholesteryl esters and lysophosphatidylcholines. Interestingly, LacCer, Glc/GalCer and individual ceramide species known in the clinic as markers of cardiovascular disease were found to accumulate in diseased aortic segments and increase with plaque development.
Conclusions. Both the cholesterol and FA content of the diets profoundly affected plasma lipidome. Aorta lipidome was likewise affected with the accumulation of specific lipids known as markers of atherosclerosis.

MEDITERRANEAN DIET IN HYPERLIPIDEMIC CHILDREN: EFFICACY AND KIDMED SCORE APPLICATION

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Introduction. Mediterranean Diet (MD) is worldwide known for its health benefits on cardiovascular diseases (CVD) but scanty data are available in dyslipidemic pediatric patients showing increased CV risk. .The aim of the study is to evaluate MD efficacy on lipid profile and adherence by the Mediterranean Diet Quality Index for children and adolescents (KIDMED score) to verify its application in children with primary hyperlipidemia.
Methods. The study included 157 dyslipidemic children (10.01±3.54 years; M/F 73/84). Dietary intake was evaluated twice, at baseline and three months after dietary advices (MD pattern), by food week diaries and KIDMED score application. On the basis of the score, patients were classified in the three levels of adherence: optimal; needed improvement; very low diet quality. Plasma lipid levels were evaluated by standard methods at baseline and after three months since the diet counselling.
Results. After dietary advices, KIDMED score improved in 65%, was unchanged in 18.5% and worsened in 16.6%. Stratifying patients on the basis of adherence level , 33.8% improved their class, 57.3% maintained the same category and 8.9% worsened their class. In particular, out of 80 people with very low diet quality at baseline, 53.8% improved their class. Out of 90 subjects with unchanged class, 54.4% increased the score. Finally, statistical analyses highlighted that the increase of adhesion scores lead to a decrease in non-HDL and LDL-C levels ($p<0.0001$), in particular, in patients who improved their class, LDL-C levels decrease by 5.8%.
Conclusions. Present results demonstrate the MD efficacy and the applicability of the KIDMED score as tool to verify the adherence to the diet in pediatric dyslipidemic patients and it could be proposed as a valid model in order to prevent cardiovascular diseases. However, it is a qualitative evaluation method which does not take into account quantitative target of CHILD I and II dietary protocols, standard of care for dyslipidemic children.

CORRELATION BETWEEN SLCO1B1 AND ABCB1 POLYMORPHISMS AND RISK OF ADVERSE STATIN REACTIONS

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Aims. tatins are the gold standard in reducing cardiovascular risk through lowering LDL cholesterols. However, 7 to 29% of statin-treated patients experience myopathy. The SLCO1B1 and ABCB1 gene are responsible for hepatic reabsorption and biliary and renal elimination of statins, respectively. The aim of the study was to evaluate the presence of a correlation between heterozygous rs 4149056 SLCO1B1 or homozygous rs 2032582 ABCB1 mutations and adverse events in patients treated with (rosuva, prava, atorva/simva) statins.
Methods. We monitored through clinical and laboratory parameters two statin metabolism's regulatory genes (SLCO1B1,ABCB1) in patients under statin therapy.
Results. SLCO1B1 gene: in patients treated with atorvastatin/simvastatin, 7 out of 8 patients experienced adverse events such as to discontinue therapy; in those treated with rosuvastatin, 50% in a group of 10 patients required a change of therapy while for pravastatin, only 1 out of 5 patients experienced side effects.ABCB1 gene: there was a marked intolerance towards atorvastatin/simvastatin (6 out of 6 patients discontinued therapy), while for pravastatin 2 out of 3 patients discontinued therapy and, about rosuvastatin, only 1 out of 6 had to discontinue therapy.
Conclusions. Despite the small sample size, the SLCO1B1 mutation appears to be strongly associated with an increased probability of adverse reactions from statins in drugs metabolized by cytochrome CYP450 3A4 (simvastatin and atorvastatin). Instead, rosuvastatin minimizes the risk of adverse effects in patients with the ABCB1 polymorphism. Although metabolism of pravastatin is not influenced by cytochromes, a greater number of adverse events have been observed in patients carrying the ABCB1 mutation. The genotyping of the polymorphisms rs 4149056 SLCO1B1 and rs 2032582 ABCB1, could help to improve the therapeutic management of the statins in future increasing the safety of the administration, and allowing to implement more and more a "tailored therapy".

THE INFLAMMATORY POTENTIAL OF DIET PREDICTS THE DEVELOPMENT OF PRE-CLINICAL CAROTID ATHEROSCLEROSIS

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Background. An inflammatory/atherogenic effect of some nutrients has been postulated, but not confirmed by epidemiological studies which provide conflicting results how this relationship matters for Cardiovascular Disease (CVD) risk. Assessing nutrient intake is complex and few inflammatory biomarkers were analyzed so far. Hence, to overcome this gap, we harnessed a panel of 368 inflammatory biomarkers, previously related to CVDs, to unveil these relations.
Methods. During the basal visit ('99-'01), records on dietary habits of 417 subjects without pre-clinical carotid atherosclerosis (determined by carotid ultrasonography; "SCA") were collected. Dietitians analyzed the dietary records to:
1) derive the caloric intake from total, saturated, monounsaturated, and polyunsaturated fats (as percentage of total energy intake (En%)) and
2) estimate the pro-/anti-inflammatory effect of diet via the Dietary Inflammatory Index (DII).

We measured plasma expression of 368 proteins (OlinkTM) to validate the estimated pro-/anti-inflammatory effect of diet (DII below or above the cohort median). The same subjects were re-evaluated after 11 years on average (10-11, 25th-75th percentiles) for carotid ultrasound to assess the development of SCA.
Results. At the basal visit, pro-inflammatory effects of diet were associated with increased En%SFA (rho:0.25, fdr<0.0001), decreased En%PUFA (rho:-0.18, fdr<0.0001) but neither with En%Fats (rho:0.07, fdr=0.633) nor En%MUFA (rho:0.00, fdr=0.929), implying that specific dietary lipids explain the higher values of DII. DII correlated with: increased expression of interleukin-6 (rho:0.25, fdr<0.0001), leptin (rho:0.18, fdr<0.05), T-cell surface glycoprotein (rho:0.18, fdr<0.05), and decreased expression of interleukin-27 (rho:-0.14, fdr<0.1). Of note, higher DII values at the first basal vascular examination predicted the development of SCA at follow-up, as subjects that developed SCA (n=177) presented with higher basal DII (1.86[0.85-2.50]) as compared to subjects that did not develop SCA (1.46[0.71-2.20]), p=0.015).
Conclusions. We support that an inflammatory effect of diet predicts the evolution of pre-clinical atherosclerosis. Larger studies are warranted to confirm this possibility.

A NUTRACEUTICAL CONTAINING BERGAMOT CITRUS AND ARTICHOKE IMPROVES LIVER STEATOSIS IN OBESE ADULTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Introduction. Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disorder associated with obesity, which leads to an increased risk of developing cardiovascular diseases. Currently, there is no approved medication for NAFLD management. The nutraceutical containing a polyphenolic fraction of bergamot and artichoke extract (BC) reduces intrahepatic fat in diabetic and non-diabetic subjects. We aim to evaluate the effects of this nutraceutical on the intrahepatic fat content in obese with NAFLD. In addition, we investigated the molecular mechanism of the BPF extract in hepatocyte cultures.
Methods. We analyzed the data of 31 non-diabetic obese with NAFLD enrolled in a previous clinical trial (ID ISRCTN12833814). Subjects received BC (1cp/die), or placebo for 12 weeks. Liver fat content (CAP) was assessed by transient elastography. For the in vitro study, we evaluated the effect of Bergamot extract in 2D and 3D human hepatoma cells and human primary hepatocytes.
Results. At baseline, there were no significant differences between groups. After 12 weeks, we found a greater CAP reduction in obese taking BC rather than placebo (-18±11 % vs -6±17 %, p=0.02) and a greater prevalence of improvement of NAFLD degree after taking the nutraceutical compared to placebo (80% vs 37%, p=0.03). Multivariate regression analysis shows that CAP change was significantly associated with baseline serum GGT levels (B=1.07, SE=0.25 p<0.001) and BC treatment (B=-23.8, SE=10 p=0.026). The 2D in vitro study showed that incubation with BPF decreases intracellular lipid content and is associated with an increase in expression levels of β-oxidation genes (Acox1, Pparα, and Ucp2). This result was confirmed by 3D spheroids and organoids.
Conclusions. BC is a promising non-pharmacological treatment to counteract the onset and progression of NAFLD, even in obese subjects. The underlying effect of liver fat reduction could be due to an increase in β-oxidation.

EFFECTS OF THYROID HORMONES THERAPY ON AN IN VITRO MODEL OF FATTY LIVER DISEASE

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Background. Thyroid hormone has a direct effect on cholesterol levels. Hypothyroid patients have increased cholesterol levels compared to individuals with normal thyroid function and have a greater risk of non-alcoholic liver disease (NAFLD). A recent study suggested that levothyroxine treatment increased the prevalence of liver fibrosis in obese individuals, but the effect on hepatic steatosis has been poorly investigated. Our aim was to evaluate the molecular mechanism of triiodothyronine (T3) and thyroxine (T4) on an in vitro model of hepatic steatosis.
Material and Methods. We evaluated the effect of 20 and 30 nM T3 or with 20 and 60 nM T4 for 96 hours in MCA-Rh7777 hepatic cells. We evaluated the intracellular lipids and triglycerides content. In addition, to understand the mechanism underlying this increase, we evaluated the genes involved in the metabolism of triglycerides, cholesterol and β-oxidation, as well as the pathways involved.
Results. In MCA-Rh7777, the incubation with T3 and T4 hormone increased intracellular lipid (p=0.01 and 0.002, respectively) and triglycerides (p=0.03 and p=0.001, respectively) content. In addition, we shown that T3 increased genes involved in triglycerides (Srebp1c: p=0.014; Acc: 20nM T3 vs CTRL, p=0.002), cholesterol (Srebp-2: p=0.006; HmgCs1: 20nM T3 vs CTRL, p=0.04) and β-oxidation (Cpt1α: 20nM T3 vs CTRL, p=0.006) metabolism. Furthermore, T3 incubation increased p-mTor pathways (p=0.03). Incubation with T4 hormone also increased gene involved in triglycerides (Srebp-1c: 20nM T4 vs CTRL, p=0.01; Scd1: p=0.005), cholesterol (HmgCr: 20nM T4 vs CTRL, p=0.008; HmgCs1: 0.02) and β-oxidation (Pparα: p=0.001) metabolism. In addition, T4 reduced p-Erk1/2 (p=0.002) and increased p-Ampk (p=0.003) pathways.
Conclusion. We found, for the first time, that replacement therapy with thyroid hormones induced liver fat accumulation thought the alteration of the expression levels of genes and pathways involved in hepatic lipid metabolism.

A NEW NUTRACEUTICAL (LIVOGEN PLUS®) IMPROVES LIVER STEATOSIS IN ADULTS WITH HYPERTRANSAMINASEMIA AND NON-ALCOHOLIC FATTY LIVER DISEASE

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Background. Non-alcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver-test results among adults with a prevalence of 13-23%. Currently, there is no approved medication for NAFLD treatment. Pre-clinical and clinical studies showed that several bioactive molecules in plants or foods (i.e., curcumin complex, bergamot polyphenol fraction, artichoke leaf extract, black seed oil, concentrate fish oil, picroliv root, glutathione, S-adenosyl-L-methionine and other natural ingredients) have been associated with improved fatty liver disease. Starting from these evidences, our purpose was to evaluate the effects of a novel combination of abovementioned nutraceuticals as a treatment for adults with fatty liver disease.
Methods. We analyzed the data of 53 participants with NAFLD and liver enzyme alteration enrolled in a previous clinical trial (ID ISRCTN70887063). The intervention group received six softgel capsules daily of a nutraceutical containing a combination of natural bioactive components for 12 weeks. The control group received six softgel capsules daily of a placebo containing maltodextrin for 12 weeks. The primary outcome measure was the change in liver fat content (CAP score). CAP score, by transient elastography, serum glucose, lipids, transaminases, and cytokines were measured at baseline and after intervention.
Results. The CAP score reduction (%) was greater (nutraceutical: -15±3 % vs placebo: -3±3 %, p=0.006) in subjects with AST reduction after adjustment for confounding variables. In addition, we found a greater prevalence of improvement of NAFLD degree after taking the nutraceutical in the participants with AST reduction (58% vs 33%, p=0.04).
Conclusions. Our results showed that a new combination of bioactive molecules as nutraceutical was safe and effective in reducing liver fat content over 12 weeks in individuals with hepatic steatosis.

ANTI-HYPERTENSIVE DRUGS AND MORTALITY RISK IN ATRIAL FIBRILLATION PATIENTS ON ORAL ANTICOAGULANTS. THE NATIONWIDE START REGISTRY

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Background. Arterial hypertension is the most common cardiovascular comorbidity in patients with atrial fibrillation (AF). Data on the use of antihypertensive drugs are scarce as their association with mortality risk. Objective is to investigate the relation between antihypertensive drugs and mortality risk.
Methods. We analysed the use of single antihypertensive molecules in 5769 AF patients included in the nationwide Italian START registry. We also investigated the association of antihypertensive drugs with mortality risk.
Results. Mean age was 80.8 years, 46.1% were women; 80.3% of patients were hypertensive. Furosemide (30.1%) was the most frequent diuretic followed by hydrochlorothiazide (15.4%) and potassium canrenoate (7.9%). 61.1% received β-blockers: 34.2% bisoprolol, 6.2% atenolol. Additionally, 36.9% were on ACE-I: ramipril (20.9%), enalapril (5.3%) and perindopril (2.8%); 31.7% were on ARBs: valsartan (7.6) and irbesartan (6.4%). Amlodipine and lercanidipine were prescribed in 14.0% and 2.3%, respectively. ACE-I ($p<0.001$), α-blockers ($p=0.020$) and D-CCB ($p=0.004$) were more common in men, while ARBs ($p=0.008$), thiazide diuretics ($p<0.001$) and β-blockers ($p<0.001$) in women. During 22.61±17.1 months, 512 patients died. Multivariable Cox regression analysis showed that ACE-I (Hazard ratio [HR] 0.677 95% Confidence Interval [95%CI] 0.545-0.841, $p<0.001$) and ARBs (HR 0.572, 95%CI 0.447-0.732, $p<0.001$), were inversely associated with mortality. ACE-I/ARBs prevented mortality in patients with diabetes, ACE-I also in previous cardiovascular disease, and ARBs also in HF. ACE-I/ARBs prevented death both in women and men.
Conclusions. ACE-I/ARBs are inversely associated with mortality in AF. Our data suggest that ACE-I/ARBs should be considered to optimise clinical management of AF patients.

PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 (PCSK9) SERUM LEVELS AND ABNORMALLY HIGH ANKLE-BRACHIAL INDEX IN PATIENTS WITH ATRIAL FIBRILLATION

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Background. High ankle-brachial index (ABI) has been associated with increased risk of worse outcomes in the general population. Few data on atrial fibrillation (AF) do exist. Experimental data suggest that proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) contribute to vascular calcification but clinical data on this association are lacking. We want to investigate the relationship between circulating PCSK9 levels and abnormally high ABI in patients suffering from AF.
Methods. We analysed data from 579 patients included in the prospective ATHERO-AF study. An ABI ≥ 1.4 was considered as high. PCSK9 levels were measured coincidentally with ABI measurement. We used an optimized cut-off of PCSK9 >1150 pg/ml obtained from ROC curve analysis. All-cause mortality according to the ABI value was also analysed.
Results. 115 (19.9%) had an ABI ≥1.4. The mean age was 72.1 years and 42.1% of patients were women. Patients with ABI ≥1.4 were older, more frequently male and diabetic. Multivariable logistic regression analysis showed an association between ABI ≥1.4 and serum levels of PCSK9 >1150 pg/ml (Odds Ratio 1.835, 95%CI 1.133-2.970, $p=0.014$). During a median follow up of 41 months, 112 deaths occurred. At multivariable Cox regression analysis, ABI ≥1.4 was associated with an increased risk of mortality (Hazard Ratio 1.676, 95%CI 1.073-2.617, $p=0.023$).
Conclusions. In AF patients, PCSK9 levels relate to an abnormally high ABI, which is in turn associated with an increased mortality risk. This is the first clinical evidence of an association between PCSK9 and vascular calcification in AF patients.

THE GENETIC LACK OF ANGTPL3 DOES NOT ALTER HDL FUNCTIONALITY

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Aim. Individuals with loss-of-function mutations in ANGPTL3 gene express a rare lipid phenotype called as Familial Combined Hypolipidemia (FHBL2). FHBL2 individuals show reduced plasma concentration of total cholesterol and triglycerides as well as ApoB and Apo-AI -containing lipoproteins particles, including HDL.This feature is particularly remarkable in homozygotes in whom ANGPTL3 in the blood is completely absent. ANGPTL3 is a circulating inhibitor of LPL and EL and it is thought that EL hyperactivity causes plasma HDL reduction in FHBL2. Nevertheless, the consequences of ANGTPL3 deficiency on HDL functionality has been poorly explored. In this work, HDL particles isolated from homozygous and heterozygous FHBL2 carriers of Italian cohort of Campodimele (LT) were evaluated for their ability to preserve endothelial homeostasis compared with controls.
Methods. Six homozygous and 26 heterozygous carriers of LOF mutation S17* in ANGPTL3 gene, and 22 non-affected family members (controls), all belonging to the Italian families, volunteered for the study. HDLs were purified by sequential ultracentrifugation from serum and analyzed for subclasses composition. Furthermore, the ability of isolated HDL to modulate the release of nitric oxide (NO) and the expression of adhesion molecules was evaluated in cultured endothelial cells.
Results. ANGPTL3 deficiency alters HDL subclass distribution. As homozygous, but not heterozygous FHBL2 subjects have reduced content of large and increased content of small HDL with no alteration in HDL2 and HDL3 size. The plasma content of preβ-HDL was reduced in homozygotes (P=0.028 one way ANOVA) and showed a positive correlation with plasma levels of ANGPTL3 (R2=0.128 and P=0.04) with an estimated cut-point of Angptl3 level value below 138 ng/dL. However, changes in composition did not alter the functionality of FHBL2 HDL, as lipoprotein particles isolated from carriers retain their capacity to promote NO production and to inhibit VCAM-1 expression in endothelial cells. Consistently, no significant changes in circulating levels of soluble ICAM-1 and E-selectin were detected in carriers.
Conclusion. These results indicated that the reduction of HDL-C level and the alteration of HDL subclass distribution observed in subjects with genetically determined ANGPTL3 deficiency doesn't hamper the vaso-protective and the anti-inflammatory properties of this lipoprotein fraction.

ALTERATIONS OF LIPID OBSERVED IN COVID-19 PATIENTS

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Introductioni. It seems that during SARS-CoV-2 infection, total cholesterol, LDL-C, and HDL-C values decrease and lipids could play a fundamental role in viral replication.
Methods and Aim. We performed a retrospective analysis of 118 hospitalized patients with COVID-19, comparing pre-infection lipid profile (53 patients) to those measured on admission. Our aim was to evaluate whether SARS-CoV-2 infection could be involved in lipid profile alterations and study possible correlations with disease severity and clinical outcome.
Results. Median baseline values at the admission time were: total cholesterol at 136.89±42.73 mg/dL, LDL-C 81.53±30.35 mg/dL, and HDL-C 32.36±15.13 mg/dL; and triglycerides at 115.00±40.45 mg/dL, non-HDL-C 104.53±32.63 md/dL. Median values of pre-infection total cholesterol and HDL-C were significantly higher than those measured at the admission time (p value < 0.05). The C-reactive protein (CRP) negatively correlated with LDL-C (p=0.013) and HDL-C (p=0.05).
Conclusion. Our data underline suggest a possible relation between COVID-19 and lipid profile with a negative correlation between CRP, LDL-C, and HDL-C values, proposing the hypothesis that lipid lowering could follow the rising of the COVID-19 inflammatory state.

IMPACT OF Mrc1 DEFICIENCY IN BONE MARROW HOMEOSTASIS IN DYSMETABOLIC CONDITION

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Obesity and type 2 diabetes are associated with increased myelopoiesis, which involves the sinusoidal endothelium, that regulates leucocytes egression as a consequence of the presence of inflammatory molecules including the mannose receptor (Mrc1). Mrc1 is a C-type lectin receptor with high affinity for mannose, fucose and N-acetylglucosamine, which is known to regulate leukocytes trafficking on lymphatic sinusoids by direct interaction with the highly glycosylated CD44. Aim of this project was to investigate whether the Mrc1 deficiency affects obesity development in experimental models.
Methods. Mrc1 KO mice and wt littermates were feed with a high fat diet (HFD, 45% Kcal/die) for 20 weeks. Weight gain was monitored during the diet regimen and glucose and insulin tolerance were assessed. Extensive flow cytometry profiling and histological analyses were performed.
Results. After HFD feeding, Mrc1 KO mice presented a different hematopoiesis profile with a reduced number of neutrophils progenitors ($p<0.05$), mature neutrophils ($p<0.05$) and macrophages ($p<0.05$) in the bone marrow(BM) compared to WT mice. BM adipocytes, known to maintain HSC quiescence by supporting CXCL12 expression, increased in Mrc1 KO mice ($p<0.01$), as was the number of adipocytes expressing CXCL12 ($p<0.05$). The reduced innate branch hematopoiesis in KO mice was reflected in the reduction of circulating neutrophils and pro-inflammatory monocytes ($p<0.05$) and it is paralleled by a decreased infiltration of macrophages in the liver and visceral adipose tissue. The deficiency of Mrc1 results in a less marked liver steatosis compared to WT (3.370%±2.610 vs 7.970%±1.891, average±SEM; $p<0.05$) and with better insulinic and glucidic response. Furthermore, plasma proteomics analysis confirmed reduced transendothelial migration of leucocytes (z-score= -1.414, $p<0.05$). Overall our data show that Mrc1 deficiency, in an experimental model of obesity, promotes a less inflammatory BM phenotype, associated with reduced systemic inflammation and protection from metabolic dysregulation.

FIBRATE USE IS ASSOCIATED WITH LOWER INCIDENCE OF HEART FAILURE AMONG PEOPLE WITH TYPE 2 DIABETES IN THE REAL WORLD

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Background. Fenofibrate, a PPAR-alpha agonist mainly known for its triglycerides lowering effect, has been recently shown to reduce the incidence of heart failure (HF) in a post-hoc analysis of the ACCORD trial. The effect on HF was independent from fenofibrate's action on lipids and was mainly seen among subjects on standard glycemic control. We aimed to validate such findings in a real-world clinical setting.
Methods. This observational study was conducted on patients with type 2 diabetes evaluated at the University Hospital of Padua between 2008 and 2018. Detailed clinical information were linked to administrative databases with death certificates and hospital discharge codes. The association of fibrate prescription with the composite primary outcome of hospitalization for HF and cardiovascular mortality was tested with Cox proportional hazard models with time-dependent co-variates or Cox marginal structural models (MSM, with inverse-probability of treatment weighing evaluated at each visit) that allows to adjust for multiple confounding factors and biases. The interactions of baseline and follow-up HbA1c with fibrate treatment were tested in Cox models. A similar "falsification" analysis was run for omega-3 fatty acids, which have a similar indication as fibrates.
Results. We included 5419 patients, 41% women, with an average age of 66 and a diabetes duration of 7.6 years. Only 10% had pre-existing cardiovascular disease and 5.6% had prior HF. During a median follow-up of 7.3 years, patients were seen 12 times at the clinic, and we recorded 1710 events in 1136 patients. During the study, ≈5% of the population (n=265) was treated with fibrates. Fibrate use was associated with younger age, male sex, higher BMI, worst glycemic and lipid profile, greater use of antidiabetic medications, higher prevalence of NAFLD, but a lower prevalence of macrovascular disease and HF at index date. After accounting for these confounding factors, fibrate use was associated with a 39% lower risk of the primary endpoint (HR 0.61; 95% C.I. 0.41-0.92, p=0.02). The finding was confirmed in a more extensively adjusted model (HR 0.65; 95% CI 0.43-0.98; p=0.04) and similar results were obtained with Cox MSM models. We found no interactions between fibrate treatment and glycemic control. The effect of omega-3 fatty acids on the primary outcome was neutral (HR 1.03; 95% CI 0.85-1.23; p=0.8).
Conclusions. Our findings support the possible beneficial effect of fibrates on HF in patients with type 2 diabetes. Further studies are warranted to identify the mechanism of action and confirm whether fibrates might be considered a treatment option against the HF burden in diabetes.

FIXED ASSOCIATION OF MONACOLINE K AND OCTACOSANOL IS EFFECTIVE, AT ONE YEAR, IN MAINTAINING THE REDUCTION OF LDL IN PATIENTS AT MODERATE CARDIOVASCULAR RISK AND UNWILLING TO STATINS

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Background. Natraceuticals are bioactive elements that are used to reduce LDL values. After one and half months, treatment with monacolin K 10 mg combined with octacosanol 12 mg reduced LDL values by approximately 24% without side effects (B. Napolitano, SISA 2021). This may support use of natraceuticals to reduce LDL. In literature, however, there are insufficient data on the maintenance of effects on LDL and the safety of long-term natraceutical use.
Patients and Methods. We wanted to evaluate the efficacy and safety in reducing LDL values in 46 patients with pure hypercholesterolaemia, with moderate cardiovascular risk (SCORE >0.9% and <5% at 10 years), unwilling to take statin therapy, with a 54-week treatment with monacolin K at 10 mg and octacosanol at 12 mg once daily. All patients were assessed for body mass index (BMI in kg/m2), total cholesterol, HDL, LDL, triglycerides (in mg/dl), ALT and CPK before and at the end of treatment. At baseline: mean age 58±11.57 years, BMI 25±3.1, cholesterol 211.78±36.53, HDL 59.52±14.84, triglycerides 122.17±57.40, LDL 131.51±30.36 mg/dl.
Results. Six weeks after the start of therapy: BMI 25±1.2, cholesterol 178.14±40.38, HDL 57.09±16.54, triglycerides 116.62±39.19, LDL 95.29±32.01 (-27.48%). After fifty-four weeks: BMI 24±1.4 (-4.17%), cholesterol 170.17±33.93, HDL 58.04±15.38, triglycerides 96.7±35.21, LDL 91.13±27.69 (-30.53% compared to the start of therapy and -4.5% compared to six weeks after the start of therapy). No adverse events or significant changes in ALT and CPK were reported.
Discussion. In our study, a 54-week natraceutical therapy successfully reduced LDL levels without side effects. Six weeks after the start of therapy, the average reduction was 27%, 30.53% after 54 weeks and 4.5% compared to the first six weeks.
Conclusions. Treatment with a combination therapy of monacolin K and octacosanol usefully reduces LDL values and keeps them reduced for one year.

DISTINCTIVE PROATHEROGENIC LIPOPROTEIN PROFILE IN CHILDREN AND ADOLESCENTS WITH HIGH TRIGLYCERIDE-TO-HIGH DENSITY LIPOPROTEIN CHOLESTEROL RATIO: A MAGNETIC RESONANCE STUDY ON LIPOPROTEIN SUBCLASSES SIZE AND DISTRIBUTION

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Background and Aims. A high triglyceride-to-high density lipoprotein cholesterol (TG/HDL) ratio is used to refine cardiovascular risk estimation with high prognostic power even in healthy youths; nonetheless, the underlying mechanisms are debated. We hypothesized that these might reside in unfavorable combinations of lipoprotein subclasses, since the traditional density-based classification of lipoproteins overlooks the heterogeneity of particle subclasses differing in dimensions and atherogenic potential.
Methods. We examined data from the Yale Pathogenesis of Youth Onset Type 2 Diabetes study cohort including 630 children and adolescents of different ethnicity with overweight/obesity and devoid of other cardiovascular risk factors, undergoing a thorough metabolic characterization comprising 3-hour oral glucose tolerance test and abdominal magnetic resonance imaging. Lipoproteins were analyzed with a 400-MHz proton nuclear magnetic resonance (NMR) analyzer.
Results. Analysis was performed on 592 individuals aged 13±3 years, 58% females, BMI z-score 2.1±0.8, divided in quartiles of TG/HDL ratio. Independently from sex, age, ethnicity, BMI, fasting glucose, and insulin sensitivity, the highest TG/HDL quartile showed increased visceral adiposity, intrahepatic fat, worse metabolic profile, and increased absolute concentration of very-low-density (VLDL; +178%, $p<0.0001$), intermediate-density (IDL; +338%, $p<0.0001$), and low-density (LDL; +42%, $p<0.0001$) lipoprotein particles. TG/HDL ratio positively correlated with the average particle size of VLDL ($r=0.37$, $p<0.0001$), and negatively with size of both LDL ($r= -0.51$, $p<0.0001$) and HDL ($r= -0.69$, $p<0.0001$). The relative and absolute frequency of the proatherogenic large VLDL, very small LDL, and small HDL progressively increased across TG/HDL quartiles.
Conclusions. Children and adolescents with a high TG/HDL ratio display a distinctive, unfavorable lipoprotein profile characterized by high levels of ApoB-containing lipoproteins mainly driven by a relative and absolute increase in proatherogenic subclasses. This novel may partly explain the increased cardiovascular risk associated with high TG/HDL ratio, justifying its use to stratify atherosclerotic cardiovascular risk beyond traditional risk factors.

LIPOPROTEIN(A) DOES NOT HAVE A CLINICAL ARTERIAL OR VENOUS PROTHROMBOTIC EFFECT

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Background and Aim. Over the past decade, lipoprotein(a) [Lp(a)] has been the subject of controversy and debate about its physiological functions and roles in atherogenesis, thrombogenesis, and development of cardiovascular diseases. Lp(a) is an apoB-containing lipoprotein covalently bound to an apolipoprotein(a) [apo(a)], and is causally associated with the risk of cardiovascular disease. Because the apo(a) moiety has sequence homology with plasminogen, it has been suggested that Lp(a) may exert a prothrombotic effect. However, evidence on its role as a risk factor for venous thromboembolic events remains controversial. We therefore sought to determine whether Lp(a) has a clinically significant venous or arterial prothrombotic effect, carrying out a Mendelian randomization study.
Methods. An Lp(a) genetic score was calculated for each UK Biobank participant by summing the number of increasing alleles inherited at rs3798220 and rs10455872 variants, which are the main polymorphisms in the LPA gene influencing Lp(a) plasma values. To verify the procoagulant and prothrombotic effect of Lp(a) we performed the following analyses. First, we conducted a Mendelian randomization analysis to evaluate the causal effect of Lp(a) on the risk of venous thromboembolism (VTE), as a composite of deep vein thrombosis (DVT) and pulmonary embolism (PE), among participants in the UK Biobank. Then, we evaluated the causal effect of Lp(a) on the risk of myocardial infarction (MI) in the entire study sample. Finally, we repeated the latter analysis stratifying by two genetic scores that mimic the effect of antiplatelet and antithrombin therapies. The effect of increased Lp(a) on VTE and MI was assessed using Cox proportional hazard models adjusted for age, sex, and the first 10 principal components of ancestry, with age as the time scale, and expressed as hazard ratios (HR) and 95% confidence intervals (95%CI). The risk was estimated using both Lp(a) genetic score and measured Lp(a) concentrations (each 100 nmol/L increase in measured levels).
Results. Among 445,719 participants (mean age 57.3 years, 54% female), a total of 17,432 subjects had an incident VTE event, and 21,868 had a first MI. The median [IQR] level of Lp(a) [nmol/L] increased with increasing value of the genetic score (13.6 [6.2-35.0], 146.3 [104.8-200.2], 261.8 [190.2-336.0]), as expected. Lp(a) was not associated with the risk of VTE (HR: 1.00, 95%CI: 0.97-1.03,), neither with the risk of DVT (HR: 1.00, 95%CI: 0.96-1.05) or PE (HR: 1.00, 95%CI: 0.96-1.04) evaluated separately. By contrast, Lp(a) was strongly associated with the risk of MI (HR: 1.32 per 100 nmol/L higher Lp(a), 95%CI: 1.26-1.38), with the effect of increased Lp(a) concentrations on the risk of atherosclerotic events that did not change with the type of atherosclerotic event considered. Furthermore, despite the antithrombin score was associated with a dose-dependent step-wise decrease in the risk of VTE and the antiplatelet score was associated with a dose-dependent step-wise decrease in the risk of MI (Figure E), as expected from antiplatelet and antithrombin therapies, the effect of Lp(a) on the risk of MI was not diminished by either genetically determined thrombin or platelet inhibition, suggesting that the effect of Lp(a) against atherosclerotic disease is not modulated by these treatments.
Conclusions. Lp(a) does not have a clinically significant venous or arterial prothrombotic effect. Indeed, genetically determined and measured Lp(a) concentrations were not associated with thrombotic events. Moreover, the effect of increased Lp(a) levels on MI was not attenuated by antithrombotic therapies. Therefore, the increased risk of MI caused by elevated Lp(a) is unlikely to be reduced by treatment with an antiplatelet or antithrombin therapy, emphasising that drugs specifically targeting Lp(a) are extremely needed.

THE SODIUM-GLUCOSE COTRANSPORTER-2 (SGLT2) INHIBITORS REDUCE PLATELET ACTIVATION AND THROMBUS FORMATION BY LOWERING NOX2-RELATED OXIDATIVE STRESS: A PILOT STUDY

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Introduction. Sodium-glucose cotransporter-2 inhibitors or Gliflozins, the newest anti-hyperglycemic class, induce cardioprotective benefits in patients with type 2 diabetes (T2D). As platelet activation and oxidative stress play a key role in atherothrombotic-related complications, we hypothesized that gliflozins might modulate oxidative stress, platelet activation, and thrombus formation.

Methods. We performed an interventional open-label single-arm before-after study in 32 T2D patients on top of ongoing metformin therapy. The population was divided into two groups: treatment with GLP-1 receptor agonists (GLP-1RA, Group A) and gliflozins (Group B). Oxidative stress, platelet activation, and thrombus growth were assessed before and after 15 days of treatment.

Results. Compared to baseline, gliflozins treatment significantly decreased sNOX2-dp (-45,2%,p<0.001), H2O2 production (-53.4%,p<0.001), TxB2 (-33.1%,p<0.001), sP-selectin (-49.3%,p<0.001) and sCD40L levels (-62.3%,p<0.001) as well as thrombus formation (-32%,p<0.001), whereas potentiated antioxidant power(H-BA,+30.8%,p<0.001). Moreover, a significant difference in oxidative stress, platelet activation, and thrombus formation across groups A and B was found. In addition, in vitro study on stimulated platelets treated with gliflozins (10–30μM) showed a reduction in oxidative stress, platelet activation, and thrombus growth.

Conclusion. Our results showed that gliflozins have an antiplatelet and antithrombic activity related to NOX2 downregulation suggesting a new mechanism responsible for cardiovascular protection.

TREATMENT WITH PCSK9 INHIBITORS IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA LOWERS PLASMA LEVELS OF PLATELET-ACTIVATING FACTOR AND ITS PRECURSORS: A COMBINED METABOLOMIC AND LIPIDOMIC APPROACH

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Background. Familial hypercholesterolemia (FH) is characterized by extremely high levels of circulating low-density lipoprotein cholesterol (LDL-C) and is caused by mutations of genes involved in LDL-C metabolism, including LDL receptor (LDLR), apolipoprotein B (APOB), or proprotein convertase subtilisin/Kexin type 9 (PCSK9). Accordingly, PCSK9 inhibitors (PCSK9i) are effective in LDL-C reduction. However, no data are available on the pleiotropic effect of PCSK9i. To this end, we performed an untargeted metabolomics approach to gather a global view on changes in metabolic pathways in patients receiving treatment with PCSK9i.

Methods. FH patients starting treatment with PCSK-9i were evaluated by an untargeted metabolomics approach at baseline (before PCSK9i treatment) and after 12 weeks of treatment.

Results. 25 FH subjects were enrolled on maximal tolerated lipid-lowering therapy prior to study entry. After a 12 week treatment with PCSK9i, we observed an expected significant reduction in LDL-cholesterol levels (from 201.0±69.5 mg/dL to 103.0±58.0 mg/dL, p<0.001). The LDL-C target was achieved in 36% of patients. After peak validation and correction, after 12 weeks of PCSK9i treatment as compared to baseline, we observed increments in creatine (p-value =0.041), indole (p-value =0.045), and indoleacrylic acid (p-value= 0.045) concentrations. Conversely, significant decreases in choline (p-value =0.045) and phosphatidylcholine (p-value < 0.01) together with a reduction in platelet activating factor (p-value =0.041) were observed.

Conclusions. Taking advantage of untargeted metabolomics, we first provided evidence of concomitant reductions in inflammation and platelet activation metabolites in FH patients receiving a 12 week treatment with PCSK9i.

CHANGE OVER TIME OF LIPID PROFILE RELATES TO STEROID TREATMENT BUT NOT TO AN INFLAMMATORY STATE IN GRANULOMATOSIS WITH POLYANGIITIS (GPA)

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Introduction. Granulomatosis with polyangiitis (GPA) is a small vessel vasculitis. Anti-Neutrophils cytoplasm Antibodies (ANCA) characterize the disease in a large number. Inflammation of the vessel wall may induce multiple vascular damages, mainly in the lung and kidneys. Lipid and metabolic profile and cardiovascular risk are far to be determined in these patients. However, atherosclerosis is accelerated during vasa inflammation. Thus, Cardiovascular atherosclerotic disease (ASCVD) may represent a risk for patients' outcomes.The purpose of the study is to evaluate ASCVD risk and lipid profile in GPA over time during disease follow-up.
Methods. We retrospectively evaluated 37 patients (22 Females and 15 Males, aged 51.45 ± 17.15) who received a diagnosis of GPA (T0) according to the recent international guidelines. Patients were evaluated at 1 (T1) and 2 (T2) year follow-up. All patients were treated with high steroid dose followed by a one year tapering, associated to another immunosuppressant. Lipid profile included total cholesterol, HDL, LDL and Triacylglycerols. To evaluate inflammatory activity, we evaluate erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and neutrophil to lymphocyte ratio (NLR) at the same time points. ANOVA for repeated values was used to evaluate the trend over time and Tukey's multiple comparisons test was a second step evaluation.
Results. At one year follow-up there was an increase in total cholesterol compared to baseline (T0 181.60 ± 38.33 vs T1 259.50 ± 65.76 mg/dl, $p<0.05$) and T2 (T1 259.50 ± 65.76 mg/dl vs T1 186.30 ± 51.45 mg/dl, $p<0.05$). Similarly, LDL (T1 259.50 ± 65.76 mg/dl vs T0 117.70 ± 47.65 mg/dl, $p<0.05$; vs T2 95.00 ± 9.89 mg/dl) presents the same trend, while Triacylglycerols increased in T1 compared to baseline (T1 253.50 ± 216.20 mg/dl vs T0 112.20 ± 27.43 mg/dl, $p<0.05$), but no difference there was in T2 compared to T1 or T0. Moreover, no difference was found in HDL between the different time points. CRP was no different in the different time points, despite a reduction being noticed. On the contrary, we found a reduction at T2 but not in T1 in ESR (T1 259.50 ± 65.76 mg/dl vs T0 117.70 ± 47.65 mg/dl, $p<0.05$) and NLR (T1 259.50 ± 65.76 mg/dl vs T0 117.70 ± 47.65 mg/dl, $p<0.05$).
Conclusion. Our data suggest that a change in lipid profile may not relate to better control of inflammation. On the contrary, the increase in the first year of follow-up should be a consequence of steroid treatment needed to spread disease control. These data may be helpful in the evaluation of both cardiovascular disease and lipid metabolism due to the connection between the two parameters with vessel inflammation. Further studies are needed to better evaluate the cardiovascular effect of vasculitis and consequent treatment.

MACROPHAGE POLARIZATION MARKERS IN SUBCUTANEOUS, PERICARDIAL AND EPICARDIAL ADIPOSE TISSUE ARE ALTERED IN PATIENTS WITH CORONARY HEART DISEASE

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Background. Epicardial and pericardial adipose tissue (EAT, PAT) surround the heart, with EAT sharing the microcirculation with the myocardium, possibly presenting a distinct macrophage phenotype that might affect the inflammatory environment in coronary heart disease (CHD). This study aims to investigate the expression of genes in different AT compartments driving the polarization of AT macrophages towards an anti- (L-Galectin 9, CD206) or pro-inflammatory (NOS2) phenotype.
Methods. EAT, PAT and subcutaneous (SAT) biopsies were collected from 52 CHD patients undergoing coronary artery bypass grafting and 22 CTRLs undergoing aortic valve replacement. L-Galectin 9, CD206 and NOS2 AT gene expression and circulating levels were analysed through RT-PCR and ELISA, respectively.
Results. The measured markers were similarly expressed in all AT compartments in CHD and CTRLs, as also L-Gal9 and CD206 circulating levels, while NOS2 serum concentration was higher in CHD ($p=0.012$ vs CTRLs). In CTRLs, NOS2 expression was lower in EAT compared to SAT ($p=0.007$), while in CHD patients CD206 expression was lower in SAT and EAT compared to PAT ($p=0.003$, $p=0.006$, respectively). In CHD patients NOS2 expression in SAT correlated to that in PAT and EAT ($r=0.556$, $p=0.007$, both), suggesting an overall inflammatory milieu driven by CHD. CD206 expression correlated positively to L-Gal9 ($r=0.561$, $p<0.0001$) only in EAT. These associations weren't observed in CTRLs. Among CHDs, subjects with LDL-C>1.8mmol/l showed higher EAT and PAT NOS2 expression vs subjects with LDL-C<1.8mmol/l ($p<0.05$, both), possibly increasing the cardiac AT pro-inflammatory activation. BMI correlated with CD206 expression in SAT and PAT in CHD and CTRLs ($p<0.05$, all), and with L-Gal9 in EAT in CHD ($r=0.503$, $p=0.02$). Altogether, the lower EAT CD206 expression in CHD patients suggests a macrophage reprogramming towards a pro-inflammatory phenotype, also supported by the lower EAT NOS2 expression in CTRLs. Hence, EAT macrophage polarization might represent a promising field of investigation to target the altered inflammation in CHD.

EFFICACY AND SAFETY OF VASCULAR DOSE OF RIVAROXABAN IN PATIENTS WITH CARDIOVASCULAR DISEASE: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Background. Low dose rivaroxaban 2,5 mg twice daily (LDR) has been approved as secondary prevention in patients with coronary artery disease (CAD) and peripheral artery disease (PAD).
Aims. To assess the efficacy and safety of LDR in patients with CAD and/or PAD in RCTs.
Methods. Systematic review and meta-analysis of randomized controlled trials (RCTs) including CAD and/ or PAD patients treated with LDR. Efficacy endpoints were cardiovascular events (CVEs), myocardial infarction, stroke, all-cause and cardiovascular death. Any, major and fatal bleeding, and intracranial haemorrhage (ICH) were safety endpoints. Number needed to treat (NNT) and number needed to harm (NNH) were calculated for LDR+ASA vs ASA treatment.
Results. 9 RCTs were included with 45,836 patients: 34,276 with CAD and 11,560 with PAD. Overall, 4,247 CVEs and 3,082 bleedings were registered. LDR in association with either any antiplatelet drug or ASA alone reduced the risk of CVEs (Hazard Ratio [HR] 0.86, 95% Confidence Interval [95%CI] 0.78-0.94) and ischemic stroke (HR 0.68, 95%CI 0.55-0.84). LDR + ASA increased the risk of major bleeding (HR 1.71, 95%CI 1.38-2.11) but no excess of fatal bleeding or ICH was found. The NNT to prevent one CVE for LDR was 63 (43-103) and the NNH to cause a major bleeding was 107 (77-193).
Conclusion. LDR reduces CVEs and ischemic stroke in patients with CAD/PAD. There was an increased risk of major bleeding but no excess of fatal or ICH was found. LDR seems to have a favourable net clinical benefit compared to ASA treatment alone.

MORTALITY RISK OF ATRIAL FIBRILLATION PATIENTS WITH AN INDICATION TO STATIN TREATMENT BUT LEFT UNTREATED: INSIGHTS FROM THE START NATIONWIDE REGISTRY

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Background. Statins are mainstream drugs for cardiovascular prevention. Use of different statins in atrial fibrillation (AF) patients is barely known. We also investigated the association between underuse of statins and mortality risk in a large AF cohort.
Methods. 5,477 patients from the ongoing nationwide START registry were included. The prevalence of different statins was reported and the association with all-cause and cardiovascular mortality investigated. We also studied mortality risk of patients with an indication to but not taking statins.
Results. Mean age was 80.2 years, and 2,539 patients (46.4%) were women. Overall, 1,578 (28.8%) of patients were on statins. The most prescribed statins were atorvastatin (45.3%), simvastatin (35.4%) and rosuvastatin (15.3%). In a mean follow-up of 22.5±17.1 months, 491 all-cause and 106 cardiovascular deaths occurred. At multivariable Cox regression analysis, statin use was inversely associated with all-cause and cardiovascular mortality (HR 0.755, 95%CI 0.598-0.953, p=0.018 and HR 0.398, 95%CI 0.241-0.660, p<0.001). In our cohort, 24.1% of high cardiovascular risk patients were not taking statins despite an indication to treatment. Of these, 4.5% had more than one indication to statin. Under prescription of statins was associated with higher risk of all-cause mortality (multivariable HR 1.283, 95%CI 1.032-1.595, p=0.025) and cardiovascular death (HR 1.986, 95%CI 1.339-2.945, p=0.001).
Conclusions. AF patients with an indication to statins but left untreated disclose a high risk of all-cause mortality. Statin prescription should be implemented in the AF population to reduce the residual cardiovascular risk of these patients.

CARDIOVASCULAR RISK ASSESSMENT IN A COHORT OF CHILDREN WITH SEVERE PRIMARY HYPERCHOLESTEROLEMIA

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Introduction. Familial hypercholesterolemia (FH) is one of the most common genetic dyslipidemias characterized by marked hypercholesterolemia that exposes affected patients to a high risk of cardiovascular disease (CVD). The identification of cardiovascular risk factors starting from pediatric age allows the identification of those FH patients for whom earlier and more aggressive treatment should be reserved, as they are exposed to a higher risk.
Patients and Methods. We conducted an observational analysis of a cohort of 126 children and adolescents (9,8±3,5 years) with FH (median LDL-C value: 191.5 mg/dl). The aim is to define the main risk factors that can be identified in pediatric age, and we then attempted to stratify the analyzed population according to the identified risk factors. The first stratification was performed considering only LDL-C values and the score given according to the modified DLCN SCORE for Italian pediatric population. We subsequently added the most significant risk factors identified: presence of CVD or premature CVD (pCVD, before 55 and 60 years in male and female, respectively) in first- or second-degree relatives, Lp(a)>30 mg/dl, overweight or obesity, MTHFR TT genotype.
Results. Considering only LDL-C values, patients were stratified as follows: 25 at moderate risk, 16 at medium risk, 21 at high risk, and 64 at very high risk. Adding up the additional risk factors, patients were reclassified as: 19 moderate, 15 medium, 15 high risk, and 77 patients at very high risk. Overall, by applying the proposed score 24 patients (19% of the cohort) were reclassified to a higher risk class than using LDL-C value only.
Conclusions. We can speculate that a careful identification of risk factors could allow identifying patients who required more aggressive treatment starting from pediatric age.

MAFLD AND COMORBIDITIES: AN OBSERVATIONAL, RETROSPECTIVE STUDY OF PHENOTYPES AND DISEASE PREDICTORS

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Metabolic Associated Fatty Liver Disease (MAFLD) is characterized by the association of fatty liver with metabolic alteration. Aim of this study was to identify MAFLD's different phenotypes and the predictive power of clinical and biochemical variables towards the potential evolution to cirrhosis. We analysed 418 patients with newly diagnosed MAFLD gathering anthropometric and bio-humoral data, calculating steatosis (FLI, HSI) and fibrosis (FIB-4, APRI, NFS) indices, and measuring hepatic stiffness and fatty involvement (Controlled Attenuation Parameter, CAP) through FibroScan. In the MAFLD cohort, compared to non-diabetic overweight/obese and lean subjects, subjects with type 2 diabetes (T2D, n=68, 16.3%) were older (50.0±0.7, 48.2±1.5 and 57.7±1.5 years, p<0.0001), with higher waist circumference (103.4±10.5, 88.9±8.3 and 180.7±16.2 cm, p<0.0001) and slightly lower eGFR (98±1, 100±2 and 91±2 ml/min/1.73 m2, p=0.001). T2D showed higher BMI and triglycerides only with respect to lean subjects. No difference emerged among groups in term of sex, hepatic function (albumin, prothrombin time, platelets, bilirubin) and cytolisis (AST, ALT, GGT) indices, HDL and LDL-cholesterol, ferritin. Steatosis (FLI e HSI) and fibrosis (NFS) scores, hepatic stiffness and CAP were higher in obese and in T2D than lean; FIB-4 was higher in T2D; APRI did not differ among groups. In the whole cohort, the negative effect of BMI on hepatic stiffness was more marked in T2D than in non-T2D (p for interaction 0.001). Biochemistry performed 59 [33-74] months before MAFLD diagnosis was retrieved in 144 participants. In such subset, higher ALT (r=0.28, p=0.0007) and AST (r=0.33, p<0.0001), reduced platelets count (r=-0.20, p=0.02), older age (r=0.18, p=0.03) and higher FIB-4 (r=0.30, p=0.0008) were associated with increased liver stiffness at diagnosis. These data confirm that T2D and obese patients are characterized by a more advanced form of MAFLD, supporting the indication of a systematic use of FIB-4 for a deep screening of these high-risk individuals.

PLASMA HDL PATTERN, CHOLESTEROL EFFLUX AND CHOLESTEROL LOADING CAPACITY OF SERUM IN CARRIERS OF A NOVEL MISSENSE VARIANT (Gly176Trp) OF ENDOTHELIAL LIPASE

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Background. Loss of function variants of LIPG gene encoding endothelial lipase (EL) are associated with primary hyperalphalipoproteinemia (HALP), a lipid disorder characterized by elevated plasma levels of high density lipoprotein cholesterol (HDL-C). Objective. Aim of the study was the phenotypic and genotypic characterization of a family with primary HALP.
Methods. HDL subclasses distribution was determined by polyacrylamide gradient gel electrophoresis. Serum content of preβ-HDL was assessed by (2D)-electrophoresis. Cholesterol efflux capacity (CEC) of serum mediated by ABCA1, ABCG1 or SR-BI was assessed using cells expressing these proteins. Cholesterol loading capacity (CLC) of serum was assayed using cultured human macrophages. Next generation sequencing was used for DNA analysis. Plasma EL mass was determined by ELISA.
Results. Three family members had elevated plasma HDL-C, apoA-I and total phospholipids, as well as a reduced content of preβ-HDL. These subjects were heterozygous carriers of a novel variant of LIPG gene [c.526 G>T, p.(Gly176Trp)] found to be deleterious in silico. Plasma EL mass in carriers was lower than in controls. CEC of sera mediated by ABCA1 and ABCG1 transporters was substantially reduced in the carriers. This effect was maintained after correction for serum HDL concentration. The sera of carriers were found to have a higher CLC in cultured human macrophages than control sera.
Conclusion. The novel p.(Gly176Trp) variant of endothelial lipase is associated with changes in HDL composition and subclass distribution as well as with functional changes affecting cholesterol efflux capacity of serum which suggest a defect in the early steps of reverse cholesterol transport.

VOLANESORSEN TO TREAT SEVERE HYPERTRIGLYCERIDEMIA: A POOLED ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Background. Patient with severe hypertriglyceridemia (sHTG) are often refractory to lipid lowering therapy. Apolipoprotein (Apo) CIII inhibition could be promising to treat subjects with sHTG. The antisense oligonucleotide against APOC3 mRNA volanesorsen was recently introduced to treat sHTG. We performed a systematic review and meta-analysis of RCTs on efficacy and safety of volanesorsen as compared to placebo treatment in patients with severe HTG.
Methods. Studies were systematically searched in the PubMed, Web of Science, Scopus databases according to PRISMA guidelines. Last search performed on 07thFeb2022.
Results. Four studies showed significant reduction in TG after 3 months of treatment with volanesorsen as compared with placebo (MD: -73.9%; 95%CI: -93.5%, -54.2; P<0.001 I2=89.05%; P<0.001); VLDL-C level (MD: -71.0%; 95%CI: -76.6%, -65.4%; P<0.001 I2= 94.1 %; P<0.001); Apo-B48 level (MD: -69.03%; 95%CI: -98.59.4%, -39.47%; P<0.001, I2=93,51%; P<0.001); Apo-CIII level (MD: -80.0%; 95%CI: -97.5 %, -62.5; P<0.001 I2=94.1 %; P<0.001) with an increase in HDL-C level (MD: +45.92.5%, 95%CI: +37.24%, +54.60%; P<0.001 I2= 94.34%; P<0.001) and in LDL-C level (MD: +68.6%, 95%CI: +7.0%, +130.1%; P<0.001 I2=96.18%; P<0.001) without a significant elevation of Apo-B100 level (MD: +4.58%, 95%CI: -5.64%, +14.79%; P=0.380 I2= 95.09%; P<0.001) in 139 volanesorsen patients as compared to 100 placebo-treated controls. Most of adverse events were mild and related to local injection site reactions.
Conclusions. In patients with severe HTG, volanesorsen is associated with a significant reduction in TG, VLDL-C, Apo-B48, non-HDL-C, and increment of HDL-C as compared to placebo. Documented efficacy is accompanied by an acceptable safety profile.

PREVALENCE OF OBESITY AND LIPID PHENOTYPE IN A COHORT OF DYSLIPIDEMIC PATIENTS: A RETROSPECTIVE STUDY

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Background. Obesity (Body Mass Index or BMI> 30 kg/m^2), classified into 3 stages with increasing severity, is a chronic disease and is associated with multiple comorbidities including the metabolic syndrome characterized by atherogenic dyslipidemia, hypertension and fasting hyperglycemia. Dyslipidemias (hypercholesterolemias, hypertriglyceridemias and mixed dyslipidemias) are classified into primary and secondary and most of them determine an increase in cardiovascular risk.
Objectives. The study aim is to evaluate the prevalence of obesity in a cohort of dyslipidemic patients. Secondary objective is to evaluate the relationship between BMI and lipid phenotype.
Patients and Methods. A sample of 479 patients referred for the first time to the Dyslipidemias Outpatient Clinic of the San Martino Hospital (Genoa) from 2020 to 2022, selecting those who are overweight and obese and classifying them according to their BMI. Of these patients were recorded age, sex, blood pressure, blood tests (glycemia, total cholesterol, HDL, LDL, triglyceridemia).
Results. Of the dyslipidemic patients evaluated in the clinic 44.69% are normal weight, 33.4% overweight, so the prevalence of obesity is 21.91% (in general population 14% ISS data): 16.7% I degree, 3.54% II degree, 1.67% III degree. There is no significant correlation between the increase in BMI and the severity of dyslipidemia, on the contrary, higher lipid levels are found in overweight subjects in comparison to obese patients.
Conclusions. Prevalence of obesity in dyslipidemic patients is higher than in general italian population. Despite excess weight, the patient must always be framed from a lipidological point of view in order to correctly diagnose primary dyslipidemias (FH, polygenic hypercholesterolemia, FCHL), in particular those with high cardiovascular risk.

CARDIOVASCULAR RISK IN SARCOIDOSIS: A PROGNOSTIC STRATIFICATION MODEL BASED ON SUBCLINICAL ATHEROSCLEROSIS EVALUATION

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Sarcoidosis is a chronic granulomatous disease that can affect any organ and that can lead to increased risk of atherosclerosis and cardiovascular (CV) disease. The aim of our observational study was to define a prognostic stratification model of sarcoidosis patients based on CV risk assessment. A cohort of 53 sarcoidosis patients and a cohort of 48 healthy volunteers were enrolled. Sarcoidosis patients were divided in four subgroups based on different organ involvement; CV risk evaluation was performed through the analysis of hemodynamic and morphological parameters of common carotid ultrasound and using CV risk scores. Results showed CV risk scores significantly higher in sarcoidosis cohort than in the control group (Framingham score, American Heart Association/American College of Cardiology score and Heart score: p=0,008, p=0,000 and p=0,034, respectively). The assessment of common carotid doppler parameters showed the presence of subclinical atherosclerosis significantly more pronounced in sarcoidosis group: peak systolic velocity (PSV) and end diastolic velocity (EDV) were significantly lower in sarcoidosis cohort (p=0,045 and p= 0,017, respectively), whereas intima media thickness (IMT) showed higher values in sarcoidosis group than in controls (p=0,016). The analysis of sarcoidosis phenotypes showed no significative differences of CV risk among them when CV risk scores were considered, while partial differences emerged by evaluating surrogates of subclinical atherosclerosis. A relationship between CV risk score and carotid doppler ultrasound parameters was detected: EDV showed an inverse correlation with Framingham score (R=-0,275, p=0,004), whereas IMT showed a direct one (R=0,429; p=0,001); furthermore, an inverse correlation between PSV and EDV and illness duration (R=-0,298, p=0,030 and R=-0,406, p=0,002, respectively) was found, suggesting a higher CV risk in patients with a longer story of disease. Our study suggests a useful role of subclinical atherosclerosis evaluation in clinical and prognostic phenotyping of sarcoidosis patients.

PRO-INFLAMMATORY EFFECT OF ANGIOPOIETIN-LIKE 3 ON THP-1 MACROPHAGES

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Background and Aim. ANGPTL3 is an hepatokine acting as negative regulator of lipoprotein lipase (LPL) with its N-terminal domain. Besides this activity, the C-terminal domain of ANGPTL3 interacts with integrin αVβ3. Since integrins are involved in inflammation and in the initiation of the atherosclerotic plaque, the aim of our study was to evaluate the potential direct pro-inflammatory action of ANGPTL3 through the interaction of fibrinogen-like domain (FLD) and integrin αVβ3.
Methods. We utilized cultured THP-1 derived macrophages and evaluated their pro-inflammatory phenotype in response to treatment with human recombinant ANGPTL3 (hANGPTL3). By western blot, RT-qPCR, biochemical analysis and ELISA assays, we determined the expression of genes and proteins involved in lipid metabolism and inflammatory response as well as intracellular cholesterol and triglycerides levels.
Results. Incubation of THP-1 derived macrophages with 100ng/mL of hANGPTL3 increased the mRNA expression of proinflammatory cytokines IL-1β, IL-6 and TNFα (respectively 1.87±0.08, 1.35±0.11, 1.57±0.49 fold vs control), thus demonstrating the positive involvement of ANGPTL3 on inflammation in THP1 derived macrophages. The secretion of TNFα, determined by ELISA assay, was also induced by hANGPTL3 (1.98±0.4 fold vs control). The pro-inflammatory effect of hANGPTL3 was counteracted by the co-treatment with RGD peptide, inhibitor of integrin αVβ3, reducing the mRNA levels of IL-1β. Moreover, intracellular triglyceride and cholesterol concentration after incubation with hANGPTL3 increased respectively by 30% and 18% compared to control.
Conclusions. ANGPTL3 is an important liver-derived regulator of plasma lipoprotein metabolism, and overall, our results allow us to define an additional important role of ANGPLT3 in promoting the inflammatory response that leads to plaque formation through the integrin αVβ3. The originality of this work is the identification of a possible new pathophysiological action of ANGPTL3 in the context of atherosclerosis. Its localization within the atherosclerotic plaque could be linked to the disease even independently of the action on lipid metabolism. This new evidence could have significant implications in evaluating the efficacy of new anti-AGPTL3 therapies such as evinacumab and this needs to be further investigated.

SENESCENCE AND ATHEROSCLEROSIS: CHARACTERIZATION OF A REPLICATIVE SENESCENCE MODEL IN VASCULAR SMOOTH MUSCLE CELLS

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Cellular senescence is characterized by growth arrest, senescence-associated secretory phenotype (SASP), and oxidative stress. Accumulation of senescent vascular smooth muscle cells (SMCs) contributes to aging and cardiovascular disease. Senescent SMCs are present in atherosclerotic plaques and contribute to their instability. We aimed at establishing the molecular signatures of replicative senescence (RS) in SMCs.
Human aortic SMCs were serially passaged to represent different stages of RS and used from 5th to 7th passages (young cells) and from 15th to 17th passages (old cells). We measured SA-β-gal activity (a marker of senescence), genes, proteins, and long non-coding RNA (lncRNAs) expression by qPCR and western blot analysis, morphological and nuclear changes by immunofluorescence, and cell proliferation by cell counting.
More than 40% of old cells stained positive for SA-β-gal compared to 10% of young cells and showed an increased β-galactosidase-1 expression. Old cells have a diminished proliferation rate (doubling time of 42 hours compared to 29 hours in young cells), a migratory activity reduced by 50%, a downregulation of contractile markers (α-actin, calponin), but increased cell cycle inhibitors (p21/p16) expression. Senescent/old cells showed a flattened appearance and enlarged and irregular nuclei. The expression levels of LMNB1 and HMGB1 were downregulated in old cells, indicating an altered nuclear membrane. Old cells showed an increased expression of SASP molecules (e.g.NF-kB, IL1β, MMP-1,-2,-3), and of "Related glycolysis inhibitor and calcium channel regulator" (RRAD), recently associated in cellular senescence as a negative regulator. Also, a set of lncRNAs (PURPL, SENEBLOC, NEAT1, MIR31HG and ANRIL) was modulated in old cells. The modification of markers specific for the contractile state and migratory activity, together with low-level proliferation in old SMCs, could contribute significantly to inefficient plaque repair and instability. The detection of novel genes/lncRNAs deregulated in senescent SMCs could be helpful for future studies on potential anti-aging factors.

COMBINATION OF LIPOPROTEIN APHERESIS AND ALIROCUMAB THERAPY IN A PATIENT WITH ELEVATED LIPOPROTEIN(A) LEVELS AND RADIATION-INDUCED CARDIOVASCULAR DISEASE: A CASE REPORT

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Introduction. Lipoprotein apheresis (LA) is effective in acutely lowering concentration of low-density lipoprotein cholesterol (LDL-C) and lipoprotein(a). PCSK9 inhibitors have potential to improve performance of LA, dampening the lipid rebound effect and possibly reducing its frequency. However, no data are available on the effectiveness and safety of combination therapy in patients with radiation-induced atherosclerotic cardiovascular disease (ASCVD) and concurrent Lp(a)hyperlipoproteinemia.
Case presentation. We present the case of a 62 years-old male patient with non-familial hypercholesterolemia and Lp(a)hyperlipoproteinemia, complicated by symptomatic and recurrent ASCVD. Medical history included Hodgkin lymphoma treated with radiotherapy at age 28, middle cerebral artery stroke at age 46, unstable angina for critical stenosis of left anterior descending artery treated with percutaneous transluminal angioplasty (PTA) at age 49, unstable angina for intrastent restenosis at age 54, right carotid artery PTA for 80% stenosis at age 56. At baseline LDL-C was 150 mg/dl and Lp(a) 104 mg/dl. No xanthomas or corneal arcus were detected. Because of statin/ezetimibe intolerance, he was initially treated with LA, obtaining mean inter-apheresis LDL-C level of 136.2±14.9 mg/dl (-9.3%) and Lp(a) of 73.1±8.2 mg/dl mg/dl (-29.7%). Then alirocumab 150 mg/2 weeks was added, obtaining mean inter-apheresis LDL-C level of 59.1±9.9 mg/dl (-60.6%), proximal to the recommended target, and Lp(a) of 62.9±11.4 mg/dl (-39.5%). After 5 years of follow-up, he is still on biweekly LA and alirocumab, reporting no cardiovascular events or side effects, demonstrating a good efficacy and safety profile of combination therapy.
Conclusion. This report suggests that combination therapy with LA and PCSK9 inhibitors may have synergic effects on lipid levels, reducing concentration of LDL-C by approximately 60% and Lp(a) by almost 40% in our patient. Its relevance as a highly effective and safe treatment in patients with documented ASCVD, Lp(a)hyperlipoproteinemia and a history of thoracic radiation therapy warrants further investigation in larger studies.

REAL-WORLD USE OF PCSK9 INHIBITORS: ANALYSIS OF DATA FROM 180 PATIENTS AND 5 YEARS OF EXPERIENCE IN A SINGLE LIPID CENTRE

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Background. Elevated levels of low-density lipoprotein cholesterol (LDL-C) and lipoprotein(a) are well established cardiovascular risk factors. Clinical trials have shown that proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) are able to reduce LDL-C by 60% and Lp(a) by 25-30%. However, data regarding the use of PCSK9i in real-world practice are limited.
Methods. Monocentric observational cohort study aiming at evaluating the efficacy of PCSK9i therapy in reducing LDL-C and Lp(a) levels in a real-world setting.
Results. We recruited 180 patients (112 males, 68 females, mean±DS age 63.3±10.9 years): 35.6% had familial hypercholesterolemia (FH), 63.3% coronary heart disease, 7.2% stroke, 48.9% peripheral vascular disease, 67.2% hypertension, 40% impaired fasting glucose, 18.9% diabetes mellitus (DM), 23.9% multiple cardiovascular events, 57.8% statin intolerance. Baseline mean±DS LDL-C levels were 149.8±52.5 mg/dl. 35% of patients had hyperLp(a), median baseline levels 89 mg/dl [IQR 62-127]. FH patients had higher mean LDL-C levels (179.8 vs 133.2 mg/dl). Lower mean LDL-C levels were observed in patients with DM (134.5 vs 153.4 mg/dl) and hyperLp(a) (143.5 vs 160.5 mg/dl). LDL-C levels were reduced between -46.8% and -60.8%, while Lp(a) levels in hyperLp(a) patients between -2.8% and -12.3%. LDL-C target in very high-risk patients (<55 mg/dl) was reached in 60.4% at 3 months, 55.2% at 6 months, 58.2% at 12 months, 52.6% at 24 months, 52.3% at 36 months, 55.4% at 48 months, 56% at 60 months, 46.2% at 72 months. During follow-up, 14 patients suspended PCSK9i, 5 patients interrupted apheresis. Side effects were reported in 13 subjects, mostly injection-site reactions or flu-like symptoms.
Conclusion. We observed a significant reduction in LDL-C and a high percentage of patients achieving recommended targets, with results comparable to those reported in clinical trials. Lp(a) reduction was noticed, but in a less pronounced way. Even in real-world practice, PCSK9i represent an effective and safe therapy.

CHANGES IN HDL SUBFRACTIONS IN RELATION TO THE PRESENCE OF OVERWEIGHT, HYPERTENSION, AND METABOLIC SYNDROME IN A POPULATION OF WOMEN

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Background. Obesity, hypertension, and metabolic syndrome are comorbidities associated with increased cardiovascular disease (CVD) risk. Although there is an inverse correlation between HDL cholesterol (HDL-C) levels and CVD risk, this parameter is not sufficiently predictive. In contrast, HDL functionality, which in turn is closely related to the size of these particles, is emerging as a key factor in dictating their cardio-protective properties. Therefore, assessment of the concentration of HDL-C subfractions could provide more insights into the hypothesized protective activity of HDL-C.

Scope. To assess changes in HDL lipoprotein subfractions in relation to overweight, hypertension, and metabolic syndrome in a population of women.MethodsSeventy-two women aged 35-65 years (52±6), with 25,1±3,9 kg/m^2 and Caucasian race, were enrolled in this study. Fasting blood samples were collected to assess biochemical parameters. Serum HDL-C subfractions were quantified using the Lipoprint HDL System, which allows identification of 10 subclasses.

Results. The large HDL-C 1, 2, 3, 4 and 5 subfractions were found to be significantly higher in normal-weight women than in overweight women. Furthermore, nonhypertensive women showed significantly higher levels of HDL-C subfractions 1, 2 and 3 (large) and, in parallel, a decrease in HDL-C 10 (small) levels compared to women affected by hypertension. In parallel, HDL-C 1, 2, 3 and 4, were significantly more abundant among women without metabolic syndrome versus patients diagnosed with the metabolic syndrome, as opposed to HDL-C 10, which was higher in women with metabolic syndrome.

Conclusions. Conditions linked with impaired cardio-metabolic health are associated with a decrease of large HDL-C subfractions, suggesting that these subfractions correlate with better metabolic profiles and potentially lower cardiovascular risk. In contrast, an increase of the small HDL-C subfraction 10, which occurs in the presence of hypertension and metabolic syndrome, is associated with unfavourable cardio-metabolic profiles.

POSITIVITY OF STATIN-ASSOCIATED MUSCLE SYMPTOMS – CLINICAL INDEX IN A HYPERTENSIVE POPULATION CANDIDATED TO LIPID-LOWERING THERAPY BUT NOT TAKING STATINS

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Background. Statin use has been claimed to be associated with muscle-related symptoms, called SAMS (Statin-Associated Muscle Symptoms). The SAMS-Clinical Index (SAMS-CI) is an approved questionnaire to assess the probability that muscle symptoms are related to statin. Aim: evaluate the difference in prevalence and characteristics of muscle symptoms between hypertensive patients taking statins and hypertensive patients candidates for statins.

Methods. Observational study on 390 outpatients referred to our Hypertension Centre: 250 patients were already on statin therapy and 140 who took at least one other drug different from statins. Patients underwent a modified version of SAMS-CI for patients not taking statins in which the items referred to any other drug or nutraceutical taken by the patients.

Results. Mean age: 60.5±13.6 years; male prevalence: 53.8%; mean SAMS-CI score: 3.33±2.17 points. In the statin group, the main statins taken were rosuvastatin (52.0%) and atorvastatin (37.6%). Patient-reported episodes of muscle symptoms was reported by 50.8% of patients in the group taking statins and by 44.3% in the group not taking them (p=0.217). Within patients with reported episodes of muscle symptoms, a slightly higher score at SAMS-CI emerged in the statin group (3.6±2.4 vs 2.8±1.6 points, p=0.004). Regarding SAMS-CI items, no significant difference emerged in the localization of muscle pain (p=0.170) and timing of symptoms onset in relation to drug (p=0.067). A slightly higher score in the item "resolution timing of muscle symptoms after drug/statin withdrawal" was showed in the statin group (p=0.002).

Conclusion. In our study no significative differences emerged in the prevalence of patient-reported episodes of muscle symptoms between hypertensive patients taking statins and hypertensive patients not taking them. This finding is in line with the growing evidence that most subjective muscle-related adverse effects are misattributed to statins and occurring because of the nocebo/drucebo effect or due to other common conditions.

COMORBIDITY IN LIPOPROTEIN APHERESIS: THEIR ROLE IN THE ERA OF NEW LIPID-LOWERING THERAPIES

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Introduction. Lipoprotein apheresis (LA), decreasing the plasma and tissue pools of lipoproteins, plays a leading role in the management of severe hypercholesterolemia and in atherosclerosis prevention. Recent advances in lipid-lowering therapy can reduce the use of LA which, however, it remains an important therapeutic option such as the impact of comorbidities in effectiveness of therapeutic plan/outcomes.
Methods. Aim of this study was to retrospective evaluate Charlson Comorbidity Index (CCI), presence of major comorbidity and/or concomitant polypharmacy (definite as 5+ drugs daily assumptions) in patients with inherited dyslipidemias on chronic LA.
Results. Since 1994 a total of 83 patients (mean age 55±12 years, male 75%) was treated. In these subjects we recorded a progressive increase in the time-course of CCI / number-of-patient-ratio (from 4.00 to 5.00), consistent with a progressive increase in the care burden. In subjects with more than 5 years of LA treatment (38 patients, mean age 52±12 years, male 66%), we evaluated comorbidity and concomitant polypharmacy: at the end of observation time, they had higher CCI (3.5±1.6 vs 6.0±2.4; p<0.001), polypharmacy (18 vs 53; <0.001), anemia (0 vs 11; p<0.05), heart failure (0 vs 7; p< 0.05), peptic ulcer disease (6 vs 16; p<0.05) and benign prostatic hyperplasia (1 vs 10; p<0.05).
Conclusions. Even in the era of new lipid-lowering therapies, the LA treatment, considered for decades like a therapeutic "Cinderella", established itself as a safe and lifesaving intervention. Patients on chronic LA require a multidisciplinary approach to face their comorbidities and the apheresis unit's medical staff (doctors and nurses) play a pivotal role in creating a bridge with general practitioner and other specialists to overcome the clinical issues management.

PARADOXICAL MACES INCREASE IN LONG-TERM PCSK9-INHIBITORS THERAPY: A TUSCANY COST EFFECTIVENESS STUDY

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Aim. Proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) represent a breakthrough in the treatment of hypercholesterolemia as efficiently and safely reduce LDL-C and cardiovascular events. Aim of this study was to perform a multicentre prospective analysis on the effects of PCSK9i, introduced in Italy on 2017.
Methods. During the study period (July 2017 - February 2022) 246 patients (mean age 61±11 years, male 73%) were enrolled in the CERTI (Costo Efficacia Regione Toscana terapia Inibitori PCSK9) study (evolocumab 142/246; alirocumab 104/246). Lipid value before and after PCSK9i therapy, major cardiovascular events (MACE), intima-media thickness evaluation and adverse events (AEs) recorded during the follow-up were analysed.
Results. PCSK9i therapy allowed a significant improvement in patient's lipid profile (total cholesterol -35%, p<0.001; triglycerides -9%, p<0.05; LDL cholesterol -51%, p<0.001; Lp(a) levels -4%, p<0.05) maintained in the follow-up. No significant variation in intima-media thickness were observed.In the subgroup of patients with more than one year of PCSK9i therapy (165/246 patients) we observed:
1) a 66% reduction in MACEs compared to the year before recruitment,
2) a progressive increase in MACEs during the follow-up (MACEs event/rate at first year 0.08 vs MACEs event/rate at year 5: 0.47),
3) patients with late MACEs are older, with higher prevalence of hypertension, smoking habit and peripheral vascular disease. Moreover, during the follow-up, we recorded AEs in 31% of patients which led to reduction/discontinuation in back-bone lipid-lowering therapy.

Conclusions. Our data agree with the large evidences on effectiveness and tolerability of PCSK9i therapy, however, despite PCSK9i represent a good dyslipidaemias therapeutic option, our study show a progressive increase in MACEs during the follow-up.

DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA IN A LARGE COHORT OF ITALIAN GENOTYPED HYPERCHOLESTEROLEMIC PATIENTS

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Introduction. Familial Hypercholesterolemia (FH) is the most relevant genetic cause of early cardiovascular disease (CVD). FH is suspected when LDL-C levels exceed the 95th percentile of the population distribution, but family and clinical history support the diagnosis. Different scoring systems have been developed, as the Dutch Lipid Clinic Network (DLCN) score, used worldwide to diagnose FH. The aim of the study is to describe the characteristics of FH patients of a large cohort of more than eight hundred genotyped subjects enrolled in an Italian Lipid Clinic and evaluated the DLCN score performance applied retrospectively to the case study.
Materials and Methods. 836 hypercholesterolemic patients with LDL-C > 4.88 mmol/L were genotyped for FH causative mutations in the LDLR, PCSK and APOB genes. Relatives of mutated patients were also analyzed by cascade screening.
Results and Conclusions. Mutation carriers were younger, presented higher LDL-C and DLCN score and lower HDL-C levels in comparison with hypercholesterolemic (HC) noncarriers and presented a five-fold higher prevalence of previous CV events. Carotid US data available in 490 subjects (FH n=195, HC n=295), showed that mutation carriers had an odds ratio of 3.66 (1.43-10.24) for atherosclerotic plaques in comparison with noncarriers. Scoring system were evaluated by ROC analysis in 203 subjects without missing DLCN items and with available pre-therapy LDL-C levels, and LDL-C levels (A.U.C.=0.737) resulted more performing than the DLCN score (A.U.C.=0.662), even including carotid US data (A.U.C.=0.641) in a modified DLCN score version. The DLCN scoring systems failed to demonstrate a clear superiority in predicting FH mutations in comparison with the measure of LDL-C levels in a retrospective case study. The results enforce the need for more performant tools to detect FH.

NOVEL MISSENSE VARIANTS IN THE LMF1 GENE: IDENTIFICATION BY NEXT GENERATION SEQUENCING AND FUNCTIONAL CHARACTERIZATION

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Introduction. Hypertriglyceridemia (HTG) is a common form of dyslipidemia associated with an increased risk of cardiovascular disease and pancreatitis. The severe forms are characterized by very high plasma levels of triglycerides (TG) (>1000 mg/dL -11.2 mmol/l). Monogenic autosomal recessive forms are characterized by homozygous or compound heterozygous loss-of-function mutations of genes involved in the intravascular lipolysis of the triglyceride-rich lipoproteins, namely lipoprotein lipase (LPL), apolipoprotein C2 (APOC2), apolipoprotein A5 (APOA5), glycophosphatidylinositol (GPI)-anchored high-density lipoprotein-binding protein 1 (GPIHBP1), lipase maturation factor 1 (LMF1), and glycerol-3-phosphate dehydrogenase 1 (GPD1). LMF1 has been shown to be essential for the maturation of both LPL and hepatic lipase (HL) to their fully functional forms.
Materials and Methods. We performed Next Generation Sequencing (NGS) analysis on Ion GeneStudio S5 Plus to study the coding exons and intron/exon boundaries of genes affecting the main pathways of triglyceride synthesis and metabolism.
Results and Conclusion. In the majority of subjects no functionally relevant mutations in the LPL, APOC2, APOA5, GPIHBP1 genes were detected. Four patients were found to be carriers of unknown missense variants in LMF1 gene: a) one compound heterozygous carrier for c.787C>T (p.His263Tyr) and c.1381C>T (p.Arg461Cys); b) one homozygous carrier for c.874 G>A (p.Gly292Arg). The other two were heterozygous carriers for c.1351 C/T (p.Arg451Trp) and c.428 C/T (p.Thr143Met) respectively. A functional analysis was carried out to assay LMF1 activity, protein expression and specific activity. The results showed that the Arg461Cys and Gly292Arg dramatically impair LMF1 function, the Arg451Trp does not have an impact, whereas His263Tyr and Thr143Met exhibit moderate effects.

BIGLYCAN INVOLVEMENT IN HEART FIBROSIS: MODULATION OF ADENOSINE 2A RECEPTOR IMPROVES DAMAGE IN CARDIAC FIBROBLASTS

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Cardiac fibrosis is a common pathological feature of different cardiovascular diseases characterized by an aberrant deposition of extracellular matrix (ECM) proteins in the cardiac interstitium and myofibroblasts differentiation stimulated by Transforming Growth Factor (TGF)β. Biglycan (BGN), a small leucine-rich proteoglycan, plays a key role in matrix assembly and phenotypic control of cardiac fibroblasts. Moreover, BGN is involved in the pathological cardiac remodelling through TGF-ß binding. BGN is also acknowledged as a biomarker of atherogenic process, and its role in atherogenesis is currently investigated. Adenosine receptors (ARs), and in particular A2AR, may play a key role in stimulating fibrotic damage through collagen production/deposition. For this reason, A2AR modulation could be though useful tool to manage cardiac fibrosis and reduce fibrotic scar deposition in heart tissue. Therefore, the aim of the present study was to investigate the possible crosstalk between A2AR modulation and BGN in an in vitro model of TGF-β-induced fibrosis. Immortalized Human Cardiac Fibroblasts (IM-HCF) were stimulated with TGF-β (10 ng/ml) for 24 hours to induce a fibrotic phenotype. After TGF-β stimulus, cells were treated with two different A2AR antagonists, Istradefylline (10 µM) and ZM241385 (1 µM) for additional 24 hours. Both A2AR antagonists were able to regulate the oxidative stress induced by TGF-β through intracellular reactive oxygen species (ROS) reduction. Moreover, Collagen1a1, MMPs 3/9, BGN, caspase-1 and IL-1β gene expression appeared to be markedly decreased following A2AR antagonists treatment. The results obtained for Collagen1a1 and BGN were also confirmed when protein expression was evaluated; phospho-Akt protein levels were also reduced following Istradefylline and ZM241385 use, thus suggesting that collagen production involves AKT recruited by A2AR.These results suggest that A2AR modulation might be an effective therapeutic option to reduce the fibrotic processes in the heart. The potential role of this pathway in atherogenesis and atherosclerotic vascular damage should be further investigated.

DIETARY FATTY ACID QUALITY IS AT THE NEXUS BETWEEN IL-18 CIRCULATING LEVELS AND INSULIN RESISTANCE

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Metabolic inflammation not only represents a key feature of obesity, but has also been implicated in the pathogenesis of its comorbidities, including type 2 diabetes. In this regard, the pro-inflammatory cytokine IL-18 has been consistently linked with obesity and insulin resistance. As such, it represents a biomarkers of the metabolic aberrations underpinned by obesity and possibly one of the effectors of metabolic inflammation on insulin resistance. Despite dietary lipids representing a major player in shaping inflammatory responses, their impact on IL-18 circulating levels remains to be fully elucidated. Thus, the aim of this study was to investigate how saturated, unsaturated fatty acids and their ratios affected IL-18 circulating levels. Using a cross-sectional study design a total of 403 individuals aged 66±5 years and with a BMI of 26.5±3.7kg/m2 were characterised with regard to their metabolic health status and their dietary intakes. Particularly, insulin resistance was assessed using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and the metabolic syndrome defined according to the National Cholesterol Education Program-Adult Treatment Panel III criteria. Dietary intakes were investigated using 24h recalls. Additionally, circulating IL-18 levels were measured using an enzyme-linked immunosorbent assay (ELISA).First, it was confirmed the relationship between IL-8 and impaired metabolic health in our cohort. Indeed, IL-18 correlated positively with insulin resistance ($p<0.001$) and individuals with a HOMA-IR >2.5 displayed higher circulating IL-18 levels compared to subjects with a lower HOMA-IR ($p<0.001$). The same held true for the metabolic syndrome, with higher IL-18 plasma levels occurring in individuals affected by the metabolic syndrome ($p<0.001$). In terms of the effect of dietary lipids, the intake of saturated fatty acids tended to positively correlate with IL-18 ($p=0.068$). On the contrary, the monounsaturated/saturated fatty acid ($p<0.001$), omega-3/saturated fatty acid ratio ($p<0.001$) and the intake of eicosapentaenoic ($p=0.0445$) as well as docosahexaenoic ($p=0.006$) acids correlated negatively with IL-18. However, this cytokine did not correlate with total energy, carbohydrate, total lipid or fibre intake ($p>0.05$). Not surprisingly, considering the Mediterranean diet being low in saturated and high in monounsaturated and omega-3 fatty acids, adherence to this dietary pattern also negatively correlated with IL-18 levels ($p=0.044$). Finally, individuals with a HOMA-IR >.5, apart from having higher IL-18 levels, consumed a lower ratio of monounsaturated/saturated fatty acid and omega-3/saturated fatty acids.Thus, the downregulation of IL-18 may be key in underpinning, at least in part, the beneficial metabolic effects of substituting monounsaturated or omega-3 for saturated fatty acids with this cytokine potentially representing a biomarker linking dietary lipids and metabolic outcomes.

STRENUOUS PHYSICAL EXERCISE INDUCES AN INCREASE IN HIGH DENSITY LIPOPROTEIN CHOLESTEROL IN A POPULATION OF OBESE MEN

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Background. Regular exercise increases HDL-cholesterol (HDL-C), but changes induced by a single bout of strenuous physical exercise such as half-marathon or marathon race remain to be fully elucidated. Indeed, the studies conducted so far only focused on athletes or normal weight subjects.
Aim. To evaluate changes in HDL-C induced by strenuous exercise in a population of trained obese men.
Methods. Seventeen obese men aged 40±6 years and with BMI 31.3±2.8 kg/m^2, were enrolled in this study. Participants were trained for 6 months, in preparation to a race of 21.1, 30 or 42.2 km depending of their physical conditions. Fasting blood samples were collected before (pre-race), immediately after (post-race) and three days after (3- post-race) the race to measure biochemical parameters. Heart rate, average speed, maximal aerobic speed (MAS) and VO2 max were measured or estimated during the race.
Results. No changes were observed in the serum levels of total cholesterol, LDL-C, triglycerides, glucose and insulin. Instead, the concentration of HDL-C and cortisol were significantly increased after the race ($p<0.001$ and $p=0.001$, respectively). Cortisol levels returned to baseline three days after the race, HDL-C remained elevated even at 3-post-race relative to pre-race levels ($p=0.041$). Correlation analysis showed a positive association between Δ HDL-C and the MAS (Coef. Pearson= 0.511, $p=0.036$) and VO2max (Coef. Pearson= 0.549, $p=0.022$). Non association was found between Δ HDL-C and race distance or pre-race BMI. Stepwise regression analysis indicated that only MAS was an independent predictor of Δ HDL-C ($\beta=1.475$, $R2=0.261$, $p=0.036$).
Conclusions. Strenuous exercise induced an increase of HDL-C in trained obese men that could be predicted by MAS.

PLASMA LEVELS OF PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 ARE INVERSELY ASSOCIATED WITH N-TERMINAL PRO B-TYPE NATRIURETIC PEPTIDE IN OLDER POPULATION

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Background and Aims. Cardiac natriuretic peptides (NPs) exert several effects on lipid metabolism. Higher NPs levels are likely to be associated with a favorable lipid profile. In in vitro studies, NPs have been found to modulate low-density lipoprotein receptor (LDLR) trafficking by preventing proprotein convertase subtilisin/kexin type 9 (PCSK9) overexpression. The aim of our study is to investigate a possible association between plasma levels of PCSK9 and N-terminal pro B-type natriuretic peptide (NT-proBNP) in vivo.
Methods. We performed a cross-sectional study on 160 consecutive older male and female patients hospitalized for medical conditions. Patients taking lipid-lowering drugs and patients with an admission diagnosis of acute heart failure were excluded. Fasting blood samples were collected after clinical stabilization of the acute illness, the day before discharge.
Results. The mean age was 87.8±6.4 years with a female prevalence (62.5%). The median NT-proBNP was 2340 (814-5397) pg/mL. The mean plasma PCSK9 was 275.2±113.2 ng/mL. We found an inverse correlation between plasma PCSK9 and NT-proBNP ($r=-0.280$; $p=0.001$). This association was confirmed after taking into account NT-proBNP tertiles (plasma PCSK9 levels: 317.4±123.6 ng/mL in the first tertile, 283.3±101.8 ng/mL in the second tertile, 231.3±99.0 ng/mL in the third tertile, $p=0.001$) and even after an adjustment for confounding factors (beta=−0.361, $p=0.001$ for ln(NT-proBNP); beta=−0.330, $p=0.001$ for NT-proBNP tertiles). The strength of the correlation between plasma PCSK9 and NT-proBNP was likely greater in patients affected by type 2 diabetes mellitus ($r=-0.483$; $p=0.006$) and in male patients ($r=-0.431$, $p=0.001$).
Conclusion. The inverse association found between PCSK9 and NT-proBNP plasma levels in our real-life clinical study supports the hypothesis that NPs may play a role in cholesterol metabolism, possibly through an inhibitory action on circulating PCSK9 concentrations, thus increasing the availability of LDLR.

GENETIC PREDISPOSITION PROFILE UNDERLYING FAMILIAL HYPERCHOLESTEROLEMIA

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Background. As many familial hypercholesterolemia (FH) subjects (about 60%) did not demonstrate functional mutations in major candidate genes (LDLR, APOB, PCSK9, LDLRAP1), we assessed FH patients genetic profile by high-throughput sequencing (HTS).
Methods. We analysed 90 FH patients[adults with possible/probable/definite FH according to Dutch Lipid Clinic Network Score (DLCN)]. Targeted HTS (57 genes including those involved in lipid metabolism, supposed to be involved in dyslipidaemia, pharmacogenetics of statins, related to FH polygenic forms, HDL and triglycerides related diseases) was assessed by Illumina technology.
Results. Among 90 patients, 41 carried a rare variant in LDLR gene, whereas 49 patients were LDLR-negative. Talmud score evaluation (Talmud 2013) showed a higher mean value in patients without LDLR mutations, with respect to LDLR-positive (0.972±0.207 vs 0.941±0.175). HTS analysis revealed that 14 LDLR mutation-positive patients also carried likely pathogenetic/uncertain significance mutations in APOB or LDLRAP1 genes. In patients without LDLR mutations, at least 2 rare variants were identified in 24 patients (49%), and at least 3 rare variants were identified in 18 patients (37%). In these patients, a total of 117 rare variants with uncertain significance/conflicting interpretation of pathogenicity have been identified in 44 different genes (APOB, PCSK9, LDLRAP1, ABCB1, ABCG2, ABCG5, ABCG8, ANGPTL3, APOA4, CELSR2, CETP, CREB3L3, DAB2, GCKR, GHR, HFE, ITIH4, LCAT, LIPC, LIPI, LMF1, LPA, LPL, LRP1, MTTP, NPC1, NYNRIN, PON1, PP1R17, SCARB1, SLCO1B1, SLC12A4, SREBF1, SREBF2, SLC22A1, EPHX2, GPD1, OSBPL5, STAP1, ABCA1, DGAT1, INSIG2, NPC1L1, APOA5). Among FH patients, 29 were younger than 18 yrs. Among adults, LDL-cholesterol levels were comparable between LDLR-positive and LDLR-negative group, whereas in younger subjects significantly higher LDL-cholesterol levels were observed among LDLR-positive. As concerns DLCN score, performed in adult population, significantly higher values in subjects carrying LDLR mutation were found.
Conclusions. Present data support the involvement of multiple loci beyond LDLR gene in the modulation of lipid profile, as well as cardiovascular risk. Expansion of genetic analysis might allow a better comprehension of the role of further major/modifier genes, as well as of accumulation of common small-effect LDL-C raising alleles in determining LDL-C levels and cardiovascular events.

IMPACT OF ASIALOGLYCOPROTEIN RECEPTOR AND MANNOSE RECEPTOR DEFICIENCIES ON MURINE PLASMA N-GLYCOME PROFILES

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Background. Recent studies have found that glycan moieties can modulate the progression of atherosclerosis due to the functional impact on cytokines and other inflammatory mediators implicated in the disease. Glycan-binding receptors carry out the clearance of circulating glycoproteins based on the selective affinity for the glycan moiety. Two well described receptors for their selective recognition and clearance of circulating glycoproteins, are the asialoglycoprotein receptor (ASGPR) which recognizes galactose and N-acetylgalactosamine and the mannose receptor C-type 1 (MRC1) which recognizes mannose, fucose and N-acetylglucosamine. In mice models, lack of ASGR1 and MRC1 promotes an improved cardiometabolic phenotype. Yet, the role of those receptors on modulating the glycome and the potential role of glycans in CVD has not been well exported.
Methods. ASGR1-/-, MRC1-/- mice and their WT littermates, were fed in a high-fat diet (45% Kcal fat) for 20 weeks. Plasma was collected and processed for N-glycan release and for linkage-specific sialic acid derivatization. Ultra-high-resolution matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry (MALDI-FTICR-MS) was used for glycan assessment and MassyTool software was used for spectra analysis.
Results. In both plasma from ASGR1-/- and MRC1-/- mice, 78 glycan compositions were assigned. The most prevalent structures were complex glycans, represented for more than 80% by di- and triantennary glycans, with almost complete galactosylation. No change is observed on the galactose in ASGR1-/- mice neither in mannose in MRC1-/-. ASGR1-/- presented a 32% increase in O-acetylation compared to the WT mice (WT 8.2%±0.2%, ASGR1-/- 10.8%±0.4%, P-value<0.001). MRC1 deficient mice did present a 24% reduction in core fucosylation (WT 34.3%±2.2%, MRC1-/- 26.2%±1.1%, P-value=0.002). Also, a trend towards reduction in antennary fucosylation was observed for MRC1-/- mice (WT 12.8%±1.1%, MRC1-/- 10.4%±0.4%, not significant).
Conclusions. This study suggests that a tight control of the glycome is so important for an organism that significant redundance exists in terms of plasma glycoprotein clearance receptors with glycan-epitope specificity. Whether those glycan changes can predict the cardiometabolic state of the disease is under investigation.

LONG-TERM ADVERSE EFFECT OF LIVER STIFFNESS ON GLYCAEMIC CONTROL IN TYPE 2 DIABETIC PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE: A PILOT STUDY

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Background. Currently, there is limited data regarding the long-term effect of liver stiffness on glycaemic control in patients with type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD).
Methods. We prospectively followed an outpatient sample of 61 consecutive post-menopausal women with T2DM and NAFLD, who had baseline data on liver ultrasonography and Fibroscan®-measured liver stiffness (LSM) in 2017 and who underwent follow-up in 2022. Hemoglobin A1c (HbA1c) was measured both at baseline and follow-up.
Results. At baseline, 52 patients had NAFLD (hepatic steatosis) alone and 9 had NAFLD with coexisting clinically significant fibrosis (defined as LSM ≥7 kPa on Fibroscan®). At follow-up, 16 patients had a worsening of glycaemic control (arbitrarily defined as a HbA1c increase ≥0.5% from 2017 to 2022). The prevalence of NAFLD and coexisting significant fibrosis at baseline was at least three times greater among patients who developed worse glycaemic control at follow-up, compared with those who did not (31.3% vs. 8.9%; p=0.030). In logistic regression analysis, the presence of NAFLD and significant fibrosis was significantly associated with an approximately 4.5-fold increased likelihood of developing worse glycaemic control at follow-up (odds ratio 4.66, 95% confidence interval 1.07-20.3), even after adjustment for age, body mass index and baseline use of some glucose-lowering agents that may positively affect NAFLD and liver fibrosis.
Conclusions. Our results suggest that the presence of Fibroscan®-assessed significant fibrosis was associated with a higher risk of developing worse glycaemic control in post-menopausal women with T2DM and NAFLD.

THE AGING OF NEUTROPHILS IS A CRITICAL DETERMINANT OF HIGH FAT DIETS-INDUCED METABOLIC ALTERATIONS

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Aim. High-Fat diet (HFD) promotes metabolic alterations and hyper-activation of myeloid precursors. Neutrophils, the main short-living innate immune responders, physiologically age in circulation, thanks to the signaling of CXCR2 after being released as "fresh" from the bone marrow (BM) by the signaling of CXCR4. We here studied whether the aging is accelerated by HFD and whether it contributes to the development of metabolic alterations.
Methods. We immunophenotyped and metabolically profiled mice with constitutively aged neutrophils (conditional CXCR4 deletion by Cre recombinase on MRP8; CXCR4fl/flCre+), mice with constitutively fresh neutrophils (CXCR2fl/flCre+) and wild-type mice (WT) fed 20 weeks a HFD versus a standard diet (Standard Fat Diet "SFD").
Results. Metabolic alterations developed after feeding a HFD WT resulted in a striking infiltration of neutrophils in liver and visceral adipose tissue (VAT) as compared to SFD.CXCR4fl/flCre+ mice were affected by comparable metabolic alterations to those observed in WT while, CXCR2fl/flCre+ mice were protected from obesity, hepatic steatosis and displayed insulin sensitivity. This phenotype observed of CXCR2fl/flCre+ mice associated with more hepatic utilization of fatty acids and was explained by reduced infiltration of CXCR2fl/flCre+ in the liver, eliciting less inflammatory pathways. Besides, CXCR4fl/flCre+ neutrophils also infiltrated significantly more in VAT, resulting into local inflammation and impaired proresolving skewing of inflammatory macrophages (M1). Notably, these effects were abrogated in presence of CXCR2fl/flCre+ neutrophils, which infiltrated less in VAT and reduced the accumulation of M1 macrophages. In humans Cxcl1 (CXCR2 ligand) and Cxcl12 (CXCR4 ligand) were related to circulating neutrophils counts, accumulation of visceral adiposity in abdominal area and hepatic steatosis indices.
Conclusion. The aging of neutrophils appears as a critical determinant for the development of HFD-induced metabolic alterations and could represent a future target against metabolic diseases.

PCSK9 PLASMA LEVELS ARE ASSOCIATED WITH MECHANICAL VASCULAR IMPAIRMENT IN FAMILIAL HYPERCHOLESTEROLEMIA SUBJECTS WITHOUT A HISTORY OF ATHEROSCLEROTIC CARDIOVASCULAR DISEASE: RESULTS OF SIX-MONTH ADD-ON PCSK9 INHIBITOR THERAPY

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Proprotein convertase subtilisin/kexin type-9 (PCSK9) is a key regulator of low-density lipoprotein (LDL) metabolism and of low-density lipoprotein receptor (LDLR) degradation. Changes in PCSK9 plasma levels have been recorded in subjects who were prescribed lipid lowering therapy (LLT). Few data exist regarding the role of PCSK9 in vascular damage. The aim of the study was to evaluate the impact of PCSK9 plasma levels on pulse wave velocity (PWV), as well as the effect of PCSK9 inhibitors (PCSK9-i) on circulating PCSK9 concentration and PWV in a cohort of heterozygous familial hypercholesterolemia (HeFH) subjects. Biochemical analyses and PWV assessment were performed at baseline (T0), after 6 months of high-efficacy statin plus ezetimibe (T1) and after 6 months add-on therapy with PCSK9- i (T2). PCSK9 levels were evaluated in 26 selected HeFH subjects at T0, T1 and T2. 26 subjects were enrolled as healthy controls for the reference value for PCSK9 plasma levels. PWV values decreased at each time point in HeFH subjects after starting LLT (8.61 ± 2.4 m/s, –8.7%; $p<0.001$ vs. baseline at T1, and 7.9 ± 2.1 m/s, –9.3; $p < 0.001$ vs. both T1 and baseline). This was correlated to PCSK9 levels ($r = 0.411$, $p = 0.03$). PCSK9 levels increased on statin/EZE therapy (+42.8% at T1) while it decreased after PCSK9-i was started (–34.4% at T2). We noted a significant relationship between PCSK9 levels and PWV changes at T1 and T2. In conclusion, PCSK9 levels were associated with baseline PWV values in HeFH subjects; moreover, we found that PCSK9 level variations seemed to be correlated with PWV changes on LLT. A longer observation time and wider sample size are needed to assess the potential role of PCSK9 plasma levels on the vascular function and remodeling, and to clarify the effects of PCSK9-i in these pathways.

KIDNEY DISEASE, A NEW COMPONENT OF FCS PHENOTYPE: PRELIMINARY EVIDENCE FROM A COHORT STUDY

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Background. Familial Chylomicronemia Syndrome (FCS) is a very rare monogenic autosomal recessive disorder of lipid metabolism determining severe hypertriglyceridemia (HTG). The hallmarks of FCS are fasting chylomicronaemia (TGs levels greater than 885 mg/dl), poor response to conventional lipid lowering medication and high risk of acute pancreatitis. It has been reported also kidney complication in FCS, but the data are sparse.
Aim and methods. The study population comprises 20 patients defined as FCS by NGS sequencing that have been included in the LIPIGEN study. Among this, 11 were females and 9 males with a median age of 51 years (IQR 38.2-63.7). Clinical and biochemical information were collected retrospectively. In most cases (85%) patients were carrying homozygous mutations in LPL. The majority (85%) of them had experienced at least one episode of acute pancreatitis (AP) that was recurrent in 12 patients (75%). Glomerular filtration rate (eGFR) was estimated with CKD-EPI and diagnosis of CKD was performed based on the more recent Kidney Disease Improving Global Outcomes (KDIGO) guideline. Hyperfiltration was defined as an increase in eGFR greater than 75° percentile. Proteinuria was defined as protein in the urine spot ≥30 mg/dl or ≥ 150 mg/day in the 24-hour urine sample.
Results. The median GFR values was 99.5 (IQR 93.8-113.7) ml/min. Four (20.0%) patients had hyperfiltration whereas 3 (15.0%) were exhibiting an eGFR below 90 ml/min. Overall, 5 (25%) have had proteinuria in at least one occasion. Among hyperfiltrating, two had also proteinuria in at least one occasion during life. One patient with eGFR below 90 ml/min and proteinuria had a biopsy-proven diagnosis of glomerulonephritis. In two patients, kidney data were missing. The impairment in kidney function was independent from age, diabetes, hypertension, median TGs, AP, sex.
Conclusions. In our cohort, 9 out of 20 patients (45%) had evidence of renal impairment. Further studies are needed to better clarify if kidney disease might be a hallmark of FCS in broader population and understand the patho-physiological mechanism, if any.

THROMBOCYTOPENIA AS A POSSIBLE HALLMARK OF FCS PHENOTYPE: PRELIMINARY EVIDENCE FROM A COHORT STUDY

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Background. Familial Chylomicronemia Syndrome (FCS) is a rare monogenic autosomal recessive disorder of lipid metabolism determining severe hypertriglyceridemia (HTG). A recent study reported the occurrence of spontaneous, transient thrombocytopenia in 55.2% of FCS patients. As the use of volanesorsen, a novel FCS treating drug, has been associated with thrombocytopenia, the relationship between FCS and low platelets counts should be firmly estabilshed. To this aim, we have retrospectively evaluated the spontaneous variation of platelet counts in a cohort of patients with FCS.
Methods. The population comprises 20 FCS patients (16 index cases) equally distributed among sex (F/M 11/9). Most were Caucasian (95) and carrying homozygous mutation in LPL (85%). The median age at enrolment was 51 years (IQR 38.2-63.7) and 17 patients (85%) had experienced cumulatively 65 episodes of acute pancreatitis (AP). None had history of atherosclerotic cardiovascular disease, but one patient has had Tako-Tsubo. Two patients reported history of skin and pancreatic cancer, respectively. The occurrence of thrombocytopenia was defined as mild, moderate, or severe if platelet count (PLTs) were below 140000, 100000 or 50000, respectively.
Results. Across the study population, the median PLT count was 180,225 platelet/mcL (IQR 158,404-213,624). During follow-up, 8 (44.4%) patients experienced at least one episode of mild and 1 (5%) of moderate thrombocytopenia. None had severe thrombocytopenia. The median on treatment TG levels in the whole cohort was 1309 (IQR 820-1701) mg/dl Changes in platelets counts did not correlate with variation of TG nor was associated with history of AP.
Conclusions. The present analysis confirmed that thrombocytopenia might be a clinical characteristics of FCS phenotype. No association with changes of TG levels was detected suggesting the other mechanisms not involved in TG-rich lipoprotein metabolism might be involved.

PARAOXONASE-1 AND MYELOPEROXIDASE ACTIVITIES ARE IMPAIRED IN MENOPAUSE WOMEN AFFECTED BY DIABETES: A PILOT STUDY

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Background. Type 2 diabetes (T2D) is a metabolic disease characterized by an increased risk for cardiovascular diseases (CVDs). Women in menopause and affected by T2D have a higher risk of cardiovascular events, that may be related to an impaired atheroprotective function of High Density Lipoprotein-cholesterol (HDL) particles. These pleiotropic activities are mostly related to HDL accessory proteins, like Paraoxonase-1 (PON-1, antioxidant) and myeloperoxidase (MPO, pro-oxidant).
Aims: To determine whether menopause in the presence of diabetes impairs the atheroprotective function of HDL as assessed by PON-1 and MPO activities.
Methods. A total of 148 women (n=63 pre-menopause/no diabetes, n=62 post-menopause/no diabetes, n=23 post-menopause/yes diabetes) were included in this pilot study. The activity of PON-1 and MPO were evaluated by spectrophotometric or spectrofluorimetric assays in serum samples from the subjects.
Results. Pre- and post-menopause women did not differ in PON-1 and MPO activities. Post-menopause women with diabetes showed decreased PON-1 and increased MPO activities compared to pre-menopause/no diabetes ($P<0.05$). MPO was positively correlated with the duration of menopause only in women with diabetes ($r=0.395$, $P<0.05$), whereas PON-1 was negatively correlated with the duration of the disease ($r=-0.470$, $P<0.05$). By separating post-menopause diabetic women in two groups depending on the duration of menopause (<10 years or >10 years), we found that a longer duration of menopause was associated with lower levels of PON-1 and higher activity of MPO ($P<0.05$).
Conclusion. Menopause seems able to negatively affect HDL atheroprotective functions in conjunction with diabetes.

EFFECTS ON LIPIDS AND PHARMACOKINETICS PARAMETERS OF PCSK9I MONOCLONAL-ANTIBODIES IN A REAL LIFE SETTING HIGH CARDIOVASCULAR RISK PATIENTS

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Aim. To investigate PCSK9-inhibitor mAbs kinetics and possible correlations between blood levels of total, free and monoclonal antibodies (mAbs)-bound forms of PCSK9 in a group of high CV risk patients.
Methods. Blood samples were obtained from 56 patients (32 men, 24 women) 7 days after administration of PCSK9-i mAbs (Evolocumab or Alirocumab), and again after a wash-out period of 3-4 weeks or before starting therapy. Full lipid profile and total/free-PCSK9 plasma levels were measured by two ELISA assays: a standard ELISA assay for total PCSK9, and in house developed ELISA assay for free-PCSK9. The treatment effects were evaluated as Δ and Δ% of the means. Data were analyzed by paired t-test and the Wilcoxon test.
Results. PCSK9 mAbs decreased TC by 38%; LDL-C by 53%; TG by 17%; non-HDL-C by 50%, and Lp(a) by 15% ($p<0.05$ for all variables); HDL-C increased by 5%. On treatment circulating total PCSK9 values increased by 72% ($p<0.05$), and free-PCSK9 decreased by 20% ($p<0.05$) with some differences between the two drugs. Four patients were "hypo-responders" with an LDL-C reduction <15%.
Conclusions. In a real-life setting, mAbs effects on LDL-C, non-HDL-C and Lp(a) were comparable to those observed in large clinical trials (FOURIER, ODYSSEY OUTCOMES). Interestingly, PCSK9 plasma values measured 3-4 weeks after last injection show a significant residual effect of PCSK9 mAbs. It is plausible to hypothesize the development of an algorithm using total/free/bound PCSK9 assays to define both adherence to therapy and hypo-responders patients.

GENETIC PROFILE OF FAMILIAL HYPERCHOLESTEROLEMIA AND RESPONSE TO LIPID LOWERING THERAPY

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Background. Familial hypercholesterolemia (FH) is the most frequent Mendelian disorder among genetic diseases and is characterized by the plasma accumulation of cholesterol in the form of LDL.The early genetic diagnosis of FH is essential in the fight against atherosclerosis also thanks to biotechnological drugs with monoclonal antibodies.
Aim. The aim of the study was to evaluate the efficacy of therapy with PCSK9i drugs, both in terms of overall reduction of the lipid profile and in terms of reaching the LDLc target, in a population of patients with a clinical phenotype suggestive of familial hypercholesterolemia (FH), confirmed by genetic testing. In particular, we stratified patients on the base of genotype to study the correlation with the great phenotypic variability (both in terms of the severity of plasma LDLc levels and the prevalence of major cardiovascular events related to atherosclerosis) and with the response to therapy.
Methods. The subjects involved in the study (195 subjects, divided into three groups: Group I = patients with confirmed FH, Group II = patients negative to the genetic test and Group III = patients with high cardiovascular risk) were evaluated with clinical and laboratory parameters and the lipid profile was analyzed at baseline and 1 month after the introduction of lipid-lowering therapy (with Statins, Ezetimibe and PCSK9i alone or in various combinations). Furthermore, within the population affected by genetically confirmed FH (90 patients), the molecular profile of the various "major candidate genes" implicated in the disease (LDLR, APOB, PCSK9, LDLRAP1, LIPA) was examined and the correlation was analyzed between the different mutations found and the changes in the lipid profile before and after treatment.
Results. In the analyzed population (patients with FH + patients with high CV risk), the PCSK9i biotechnological drugs show good efficacy. Patients with confirmed FH appear to be more responsive to Alirocumab than to Evolocumab (54% vs 44% reduction in LDLc), while those at high risk appear to be more responsive to Evolocumab compared to Alirocumab (67% vs 52% reduction in LDLc).The most responsive patients to PCSK9i drug therapy are those carrying mutations on the PCSK9 gene (overall reduction of LDLc, after treatment, by 64%), as expected based on the intrinsic mechanism of the molecule. Patients less responsive to PCSK9i drugs are those carrying mutations on the LDLRAP1 gene (overall LDLc reduction, after treatment, by 30%); in only one case this mutation was present as a single one, whereas in most cases it was associated with other genes' mutations (double heterozygosity in 12 out of 13 patients with mutation of LDLR).Patients with mutations on the other genes (LDLR, APOB, LIPA), on the whole, are on average responsive to treatment with PCSK9i (LDL reduction of about 40-50%).
Conclusions. The efficacy of PCSK9i drugs could be conditioned by the each individual's "genetic pedigree"; therefore, in daily clinical practice, therapeutic choices should also converge towards "tailored therapy" interventions, related to the mean expected response to the different treatments we could choose.

PLASMA EXCHANGE REDUCES LIPOPROTEIN X IN FAMILIAL LCAT DEFICIENCY

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Familial LCAT deficiency (FLD) is a rare recessive disease of lipid metabolism with no cure. The major cause of morbility and mortality in FLD is renal failure due to the nephrotoxicity of an abnormal lipoprotein, called lipoprotein X (LpX). Therapeutic plasma exchange (TPE) was tested in a FLD patient for the first time to remove LpX and to prevent kidney failure. The plasma exchange was performed once per week for 8 weeks using 5% human albumin solution. Plasma samples were collected at baseline and after 1 and 2 months of TPE, immediately before plasma exchange, and 4 months after TPE interruption. A complete lipid-lipoprotein profile was determined using a Roche Integra c311 analyzer. Cholesterol esterification rate (CER) and LCAT activity were tested by in-house assays. The 1.020-1.063 g/mL lipoprotein fraction was separated at each timepoint by ultracentrifugation and analyzed by fast performance liquid chromatography (FPLC).The patient was genotyped as a compound heterozygote of the Leu187Pro and Thr270Ile mutations in LCAT gene; CER and LCAT activity were null, confirming the FLD phenotype. After 1 month of treatment, all plasma lipids decreased and the unesterified/total cholesterol ratio (UC/TC) decreased from 0.84 to 0.74, suggesting a partial removal of LpX. TPE produced a remodelling of the lipoprotein profile, reducing circulating LpX as confirmed by FPLC. Unfortunately, after 8 weeks kidney function worsened and TPE was stopped; the lipoprotein profile 4 months after TPE interruption showed a subsequent reoccurrence of LpX.The present results demonstrate that TPE induces lipoprotein remodelling in FLD by reducing nephrotoxic LpX. When the therapy was stopped, due to worsening of the kidney function, lipoprotein abnormalities reoccurred. In conclusion, plasma exchange could represent an option to normalize lipoprotein profile in LCAT deficiency. More investigations are needed to clarify the effects on renal outcomes.

PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 AND PLASMA/ CEREBROSPINAL FLUID LIPIDS IN PATIENTS WITH COGNITIVE DECLINE

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Cerebral cholesterol homeostasis impairment seems related to the pathogenesis of Alzheimer's disease (AD). We previously found higher levels of Proprotein convertase Subtilisin/Kexin type 9 (PCSK9) in the cerebrospinal fluid (CSF) of AD patients compared to control subjects. This study aims to evaluate the levels of PCSK9, as well as several markers of cholesterol metabolism, in both CSF and plasma of subjects with different degrees of cognitive decline. The 83 subjects were divided into three groups: patients with AD (AD; n=27), patients with mild cognitive impairment that furtherly developed AD at follow-up (MCI-AD; n=28), patients with mild cognitive impairment stable at follow-up (MCI; n=28). PCSK9 levels were determined with ELISA assay. CSF and plasma cholesterol concentration was determined with a fluorometric assay; oxysterols (24-, 25-, 27-hydroxycholesterol, OHC) were quantified through liquid chromatography associated with mass spectrometry.PCSK9 levels in CSF were similar in all three groups, but higher compared to a control group analyzed in our previous work. Among AD, PCSK9 levels were increased in carriers of the ε4 isoform of apolipoprotein E (ApoE) compared to non-carriers (+38%, $p<0,05$). In AD we found a positive correlation between CSF and plasma levels of PCSK9 ($r=0.521$; $p=0.004$), particularly robust in ε4 carriers ($r=0.755$; $p=0.001$). The concentrations of cholesterol and oxysterols were comparable among the groups, both in plasma and in CSF. Only in ε4 carriers, a negative correlation between 24-OHC and PCSK9 levels in CSF was observed ($r=-0.34$; $p=0.03$).In conclusion, our data suggest that PCSK9 levels may be already increased since the early phases of the disease, particularly in the presence of the ε4 isoform of ApoE. The plasma/CSF positive correlation of PCSK9 levels suggests a major permeability of blood brain barrier occurring specifically in AD. Moreover, the relationship between 24-OHC and PCSK9 supports an involvement of this protein in sterol homeostasis that is worth to be further investigated.

RNA-SEQ ANALYSIS OF HUMAN ATHEROSCLEROTIC PLAQUES AND THEIR ADJACENT REGIONS: A FOCUS ON LNCRNAS

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Aim. Atherosclerosis is a chronic inflammatory disease of the arterial wall. Pro-inflammatory factors and lipoprotein accumulation trigger the development of atherosclerotic plaques. In the last decade the role of non-coding RNAs in the physiopathology of this disease has been emerging, but long non-coding RNAs (lncRNAs) and their association with atherosclerosis is still poorly investigated. In this contest, this work has been developed with the aim to evaluate the role of lncRNAs in plaque development and find novel therapeutic and/or diagnostic biomarkers.
Materials and Methods. Human carotid atherosclerotic plaques and their respective adjacent regions (with a lower lesion grade) from 15 patients undergoing carotid endarterectomy were collected (30 samples). All samples were homogenized with liquid nitrogen and immediately processed for RNA extraction, by a combined protocol with Trizol and MirVana kit. After quality check, NGS libraries were prepared using the Illumina Stranded Total RNA Prep kit. The sequencing reactions were performed on the Illumina NextSeq550Dx instrument.
Results. After a quality check, Dragen RNA bioinformatic tool by Illumina was used for the alignment, the RNA quantification and the quality controls. The iDEP.96 web-based bioinformatic tool was used for the differential expression analysis. Clustering analysis showed a clear separation between the two sample groups, highlighting 214 downregulated and 756 upregulated genes in the plaques respect to the adjacent regions. Moreover, we found 108 upregulated and 42 downregulated lncRNAs in the plaques respect to their adjacent regions; among these, SAMMSON and LINC00670 have been already reported as downregulated, whereas HAGLR, LINC01480, LINC00528, IFNG-AS1, and HAGLROS as upregulated in cardiovascular diseases.
Conclusions. Even if these preliminary data need to be further evaluated by a functional point of view, also by increasing the sample number, they suggest a different expression pattern of lncRNAs in plaques samples that may be considered as potential biomarkers/therapeutic targets.

ASSOCIATION BETWEEN CIRCULATING LEVELS OF LIPOPROTEIN(A) AND RISK OF CORONARY ARTERY DISEASE, CEREBROVASCULAR DISEASE AND PERIPHERAL ARTERY DISEASE IN A DYSLIPIDEMIC POPULATION

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Background. Hyperlipoproteinemia (a) represents a widespread public health problem: levels of Lp(a) greater than 30 mg/dL have been found in 10-30% of the world population. High Lp(a) levels are a strong, causal and independent risk factor for atherosclerotic cardiovascular disease (ASCVD) through different pathogenetic mechanisms.
Objective. Investigation of associations between circulating Lp(a) levels, risk of coronary (CAD), cerebrovascular (CVD) and peripheral (PAD) artery diseases. Methods: in this retrospective case-control study 519 dyslipidemic outpatients were included between January 2019 and June 2022. We collected lipid profile, Lp(a) concentration and Dutch Lipid score.
Results. 519 dyslipidemic patients in total, 282 female (54%) and 237 male (46%), aged 53–70 years. 268 patients (52%) were cases with Lp(a)>30 mg/dL, and 251 were controls (48%) with Lp(a)<30 mg/dL. Cases showed higher levels of Lp(a) (8 mg/dL vs 88.1 mg/dL), while controls have higher concentration of total cholesterol, LDLc and lower levels of HDL. Subjects developing ASCVD were 101 (19%), with a higher prevalence among cases (21% vs 18%). Instead, controls have a higher prevalence of CVD (48% vs 35%), while cases of CAD and PAD were 45% vs 37% and 20% vs 3%, respectively. Moreover, individual vascular events were analyzed by gender, and we observed that high Lp(a) levels are significantly associated with incidence of PAD in women (p-value 0.024), but it is no longer significant in men (p-value 0.635).
Conclusions. Our study confirms a strong association between Lp(a) levels and PAD. Our analysis highlights the role of gender difference, in particular women with hyperlipoproteinemia (a) exhibit a greater risk of developing PAD than men. In this context we may propose an ecographical screening to identify presence of PAD in women with higher levels of Lp(a).

A LATE DIAGNOSIS OF FAMILIAL CHYLOMICRONEMIA SYNDROME

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Background. Familial Chylomicronemia Syndrome (FCS) is a rare genetic disease characterized by severe hypertriglyceridemia (sHTG) non-responding to standard lipid-lowering therapy (LLT). The worst clinical manifestation of FCS is HTG-induced acute pancreatitis because of its severity and potentially life-threatening consequences.
Case Report. A 70 years-old man was referred to our lipid-clinic with sHTG refractory to diet and LLT, diabetes mellitus type 2 with micro and macrovascular complications and severe chronic iperCPKemia. He reported chronic abdominal pain, steatorrhea. At physical examination the patient was asthenic, with hypotrophic muscular masses, legs pitting edema, mild ascites, mild pleural effusion. Patient was hospitalized and blood tests confirmed the sHTG and malnutrition compatible alteration. The abdominal CT-scan showed a complete substitution of pancreatic tissue by adipose tissue and laboratory examination demonstrated a severe reduction of exocrine pancreatic function. Genetic test showed a pathogenetic homozygous mutation in lipoprotein-lipase gene (LPL c.844G>T p.(Glu282)). An appropriate therapy was settled, including Volanesorsen, an antisense oligonucleotide inhibitor of apolipoprotein CIII.
Conclusions. Delayed diagnosis of FCS led to exocrine pancreatic impairment that resulted in a severe protein energy malnutrition complicated by severe sarcopenia and anasarcatic state. The presented case shows that rare genetic disorder, despite the typical presentation is reported in childhood, may also be diagnosed in adult age. A delayed diagnosis could determine both the development of irreversible complications and retard an adequate treatment.

META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS COMPARING THE IMPACT OF LIPID-LOWERING THERAPIES ON C-REACTIVE PROTEIN LEVELS

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Lipid-lowering therapies (LLTs) may have an effect also on inflammatory mediators, with potentially better clinical outcomes. However, evidence from clinical studies is conflicting. Our study aimed to assess the effect of LLTs on C-reactive protein (CRP), in addition to lipid reduction. We conducted a meta-analysis according to the PRISMA guidelines, and databases were searched from inception to June 2022. Inclusion criteria were:
1) randomized controlled trials (RCTs) in humans, phase II, III, or IV;
2) English language;
3) reporting the effects on CRP levels;
4) with intervention duration of more than 3 weeks;
5) and sample size over than 100 subjects.

Meta-regression analysis was conducted to assess the correlation between mean absolute change in low-density lipoprotein cholesterol (LDL-C) or triglyceride (TG) and CRP. A total of 173,939 subjects from 47 RCTs were included in our meta-analysis. An additional -0.65 mg/L (95%CI -0.87 to -0.43) absolute reduction of CRP concentration was observed for statins. CRP was also decreased by -0.24 mg/L (95%CI -0.37 to -0.11) and -0.26 mg/L (95%CI -0.52 to -0.01) with ezetimibe and omega-3 fatty acids (omega3FAs), respectively. In addition, a -0.40 mg/L (95%CI -1.17 to 0.38) decrease in CRP level was observed in patients treated with fibrates, although not statistically significant. A slight increase of CRP concentration was found for PCSK9 inhibitors (0.06 mg/L [95%CI -0.04 to 0.15]) and CETP inhibitors (0.05 mg/L [95%CI 0.00 to 0.10]), but none of these differences were statistically significant. Meta-regression did not show a significant correlation between changes in CRP and LDL-C or TG across LLTs even after adjustment by age, sex, and intervention duration (LDL-C, slope: 0.0048, P=0.1536; TG, slope: 0.0015, P=0.7420).In conclusion, among LLTs, statins, ezetimibe, and omega3FAs seemed to reduce serum CRP concentration. The impact of this anti-inflammatory effect in terms of cardiovascular prevention needs further investigation.

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