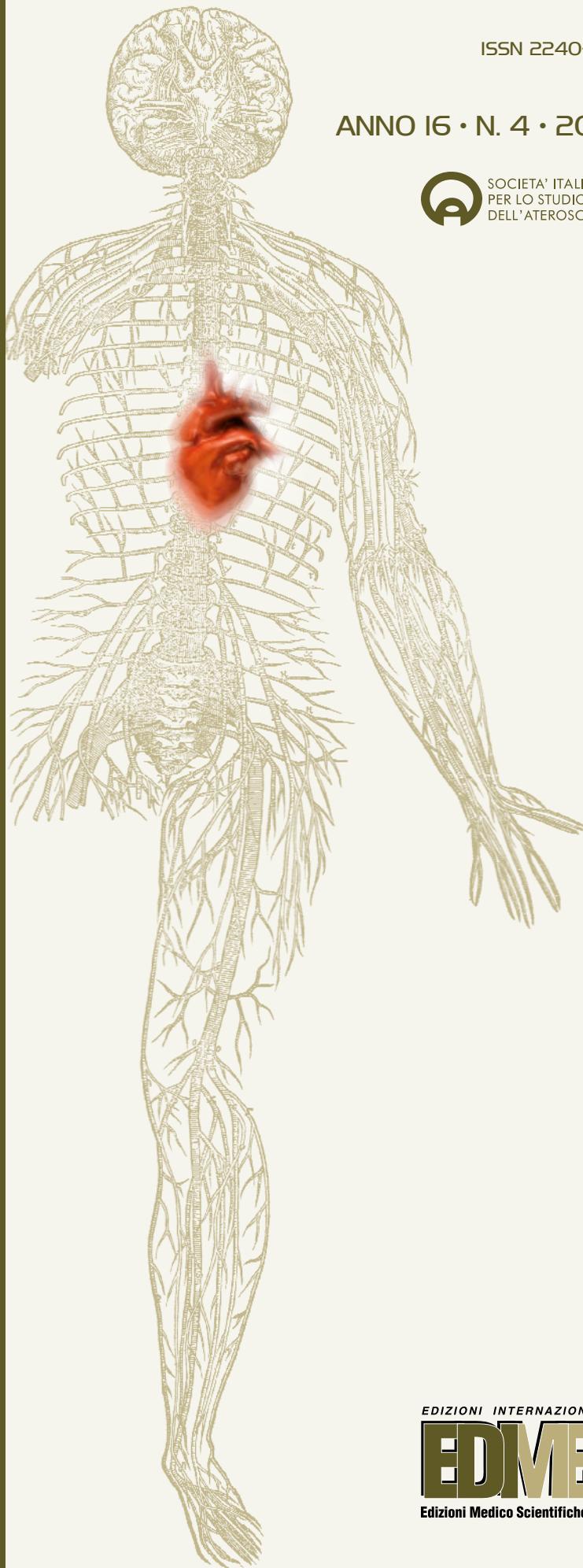
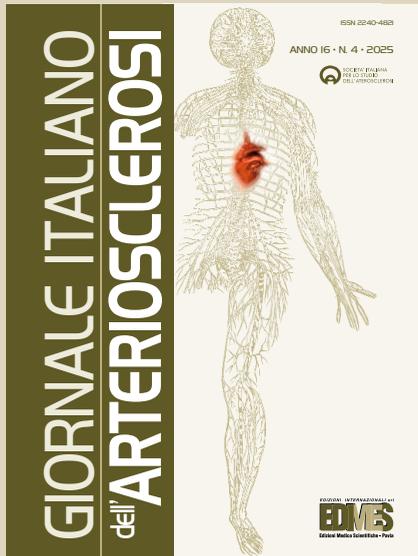




# GIORNALE ITALIANO dell'ARTERIOSCLEROSI





Rivista ufficiale della Società Italiana  
per lo Studio dell'Aterosclerosi (SISA)

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di Andreas Vesalius (Basilea, 1543)

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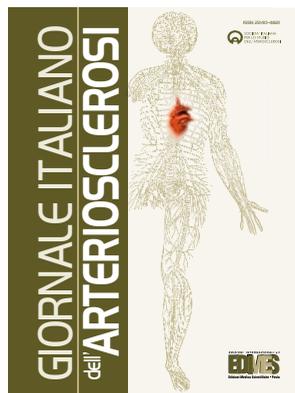
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 PER LO STUDIO  
 DELL'ATEROSCLEROSI

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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:

- revisioni critiche di argomenti di grande rilevanza nel campo dell'arteriosclerosi sia per quanto riguarda gli aspetti di base che gli aspetti clinico-applicativi;
- quesiti relativi agli argomenti trattati per una verifica di auto apprendimento;
- opinioni di esperti qualificati sui nuovi sviluppi delle conoscenze sull'arteriosclerosi;
- lavori originali relativi ad aspetti di ricerca sanitaria nell'ambito dell'arteriosclerosi e delle malattie ad essa correlate.

### TIPOLOGIA E STRUTTURA DEGLI ARTICOLI

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

Le tabelle dovranno pervenire in formato editabile (word, excel, txt, ecc...).

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### Lavori originali

I lavori originali saranno sottoposti a processo di "peer review". La lunghezza del testo non deve superare

le 4.000 parole (esclusa la bibliografia) ma incluso l'abstract, con un massimo di 4 figure o tabelle.

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Citazione di articoli su riviste: Es. 1: Austin MA, Hutter CM, Zimmern RL, Humphries SE. Familial hypercholesterolemia and coronary heart disease. Am J Epidemiol 160: 421-429, 2004. Es. 2: Humphries SE, Whittall RA, Hubbart CS et al. Genetic causes of familial hypercholesterolemia in patients in the UK: a relation to plasma lipid levels and coronary heart disease risk. J Med Genet 43: 943-949, 2006  
 Citazioni di capitoli di libri Assmann G, von Eckardstein A, Brewer H. Familial anaphalipoproteinemia: 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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Le rassegne devono includere in appendice un questionario di autoapprendimento relativo all'argomento affrontato nella rassegna.

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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

## ■ SISA LIPID ACADEMY

### **Report SISA LIPID ACADEMY – Modena 2025**

L'articolo riassume le attività svolte nell'ambito della nona Edizione del corso avanzato di Lipidologia Clinica della SISA (SISA Lipid Academy) rivolto alla formazione di nuovi specialisti nel campo della lipidologia clinica e della prevenzione cardiovascolare. La nona edizione della Lipid Academy ha adottato un nuovo formato didattico basato sulla partecipazione ad attività on line ed in presenza. A maggio ed aprile, otto corsi propedeutici interattivi a distanza hanno introdotto le principali tematiche che sono state successivamente approfondite nell'ambito di un corso in presenza tenutosi a Modena il 10-11 luglio. Il corso, che ha avuto 40 partecipanti e docenti leader nel campo della lipidologia clinica e delle malattie cardiovascolari, ha offerto un aggiornamento di alto livello nel campo delle lipoproteine e dell'aterosclerosi. I 4 corsi propedeutici e le relazioni frontali tenute a Modena sono disponibili sul sito web della SISA Academy.

### **Report SISA LIPID ACADEMY – Modena 2025**

*This article summarizes the activities carried out as part of the ninth edition of the SISA Advanced Course in Clinical Lipidology (SISA Lipid Academy), aimed at training new specialists in the field of clinical lipidology and cardiovascular prevention. The ninth edition of the Lipid Academy adopted a new teaching format based on participation in online and residential activities. In May and April, eight interactive online introductory courses introduced the main topics, which were then explored in depth in a residential course held in Modena on July 10-11. The course, which attracted 40 participants and featured leading instructors in the field of clinical lipidology and cardiovascular disease, offered a high-level update on the field of lipoproteins and atherosclerosis. The four introductory courses and the lectures given in Modena are available on the SISA Academy website.*

## Presentazione del numero

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### ■ CONGRESSI REGIONALI

#### **Congresso Regionale SISA Lazio**

In questo numero del Giornale ha il suo esordio una nuova rubrica dedicata alla descrizione dei principali argomenti trattati nell'ambito dei Congressi regionali della SISA. Il format delle presentazioni prevede un breve riassunto delle relazioni tenute al congresso e una figura riassuntiva delle principali tematiche trattate da ciascuna di esse. Il principale obiettivo della rubrica è una maggiore diffusione delle attività delle sezioni regionali che realizzano congressi annuali di contenuto scientifico molto rilevante ma, tuttavia, poco diffuso a livello nazionale. In particolare, un obiettivo è quello di offrire ai più giovani l'opportunità di ottenere una maggiore visibilità a livello della comunità scientifica nazionale.

La nuova rubrica ha inizio con la pubblicazione di un documento che riassume le principali tematiche trattate nel congresso regionale della SISA Lazio che si è tenuto a Roma il 26 settembre 2025.

#### **SISA Lazio Regional Conference**

*This issue of the Journal debuts a new column dedicated to describing the main topics covered at SISA's regional conferences. The presentation format includes a short summary of the papers presented at the conference and a figure summarizing the main topics covered by each. The primary objective of the column is to better publicize the activities of the regional sections that organize annual conferences with highly relevant scientific content, yet which are not widely held at the national level. One goal is to offer younger participants the opportunity to gain greater visibility within the national scientific community.*

*The new column begins with the publication of a document summarizing the main topics covered at the SISA Lazio regional conference held in Rome on September 26, 2025.*

SISA LIPID ACADEMY

# REPORT SISA LIPID ACADEMY MODENA 2025

## Report SISA LIPID ACADEMY Modena 2025

A cura di **GIOVANNI PENNISI, FRANCESCO DI GIACOMO BARBAGALLO, ROBERTO SCICALI**

*Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Catania*

La SISA Lipid Academy rappresenta un'iniziativa di eccellenza promossa dalla Società Italiana per lo Studio dell'Arteriosclerosi, pensata per formare e supportare le nuove generazioni di medici e specialisti. Attraverso un percorso dinamico che integra attività online e in presenza, l'Academy offre l'opportunità di approfondire i temi più attuali della lipidologia e della prevenzione cardiovascolare. I corsi, caratterizzati da un approccio interattivo e multidisciplinare, sono guidati da figure di riferimento nazionali, garantendo ai partecipanti un aggiornamento di alto livello e un confronto diretto con esperti riconosciuti nel panorama medico-scientifico.

Il 10 aprile 2025 si è tenuto il primo webinar della 9<sup>a</sup> Edizione del corso avanzato di Lipidologia Clinica, aperto dalla relazione del Professor A.B. Cefalù che ha affrontato e introdotto le malattie del trasporto lipidico e fisiopatologia delle LDL. L'analisi è stata avviata prendendo in esame l'epidemiologia delle patologie cardiovascolari, con particolare attenzione all'osservazione e alla possibilità di individuare tali mani-



*Indirizzo per la corrispondenza*

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festazioni cliniche anche in età pediatrica: tale approccio consente di comprendere meglio non solo l'incidenza e la distribuzione di queste condizioni, ma anche i potenziali fattori di rischio precoci. I fattori genetici rivestono un ruolo centrale nell'approccio clinico e nello sviluppo di terapie innovative. Mutazioni in geni chiave come LDLR, ApoB, PCSK9, CAV1 e PE-CAM1 influenzano significativamente il profilo lipidico e la funzione endoteliale. Tra le principali condizioni di interesse vi è l'ipercolesterolemia familiare (FH), disordine ereditario caratterizzato da elevati livelli di LDL fin dall'infanzia e correlato a mutazioni in LDLR, PCSK9, ApoB o LDLRAP1. La diagnosi è eseguita mediante l'ausilio del Dutch Lipid Clinic Network Score (DLCN) che tramite parametri clinico-laboratoristici permette un inquadramento chiaro del paziente. È stato inoltre sottolineato come la durata dell'esposizione a livelli elevati di colesterolo incida in modo determinante sulla progressione della malattia cardiovascolare: un carico lipidico prolungato anticipa di decenni l'insorgenza di eventi acuti aterosclerotici. Infine, si è trattato il processo di transitosi delle LDL nello spazio sub-endoteliale come il *primum movens* della patologia aterosclerotica, processo fisiopatologico che inizia con un accu-

mulo lipidico e cellulare nello spessore vascolare in un contesto infiammatorio asettico e si evolve in una placca aterosclerotica, esitando in patologia cardiaca ischemica acuta.

La presentazione successiva, tenuta dal Professor Davide Noto, ha esplorato la fisiopatologia delle ipertrigliceridemie primitive, esaminando l'incidenza e le caratteristiche cliniche. È stata posta l'attenzione anche sulla differenza qualitativa dei chilomicroni, poiché il loro potere aterogeno dipende dalle dimensioni e dal contenuto di colesterolo. Uno degli aspetti centrali trattati nella presentazione ha riguardato la distinzione tra le principali cause di ipertrigliceridemia. Da un lato, si riscontra un incremento della produzione, legato a un'eccessiva sintesi epatica di VLDL, generalmente conseguente a un surplus di substrati. Dall'altro lato, si osserva una ridotta clearance, dovuta a un deficit quantitativo o funzionale della lipoprotein lipasi (LPL), enzima deputato all'idrolisi dei trigliceridi e al rimodellamento delle lipoproteine. Tale condizione è frequentemente correlata a mutazioni di LPL e, più raramente, a carenze di cofattori essenziali quali ApoC-II, ApoC-III e ApoA-V. È stata inoltre evidenziata l'importanza di ANGPTL3 come bersaglio terapeutico di un farmaco di recente introduzione, l'Evinacumab.

Il secondo evento online della Sisa Academy si è tenuto giorno 17/04/2025, aprendosi con la relazione della Professoressa Laura Calabresi che ha trattato la fisiopatologia delle HDL. È stata descritta la struttura molecolare delle particelle HDL e il loro metabolismo, annoverando poi il trial AIM-HIGH e gli studi di randomizzazione mendeliana che hanno nel tempo confermato e consolidato il ruolo non solo non impattante dell'HDL-c ma anche la sua funzione cardio-protettiva. Sono stati menzionati anche gli studi di Copenhagen che approfondiscono la funzione protettiva delle HDL nei confronti della patologia ischemica cardiaca con decremento dell'incidenza degli eventi solo per valori iniziali di HDL molto bassi. È stato poi approfondito il ruolo biologico delle HDL, partendo



## Report SISA LIPID ACADEMY – Modena 2025

dal trasporto inverso di colesterolo e terminando con la sua funzione antiossidante, passando per la protezione endoteliale mediato dall'NO e la modulazione dell'immunità cellulare. L'ultima parte della relazione è stata dedicata a una panoramica sui difetti genetici a carico di HDL, condizioni in cui si annoverano ipo-alfalipoproteinemie, caratterizzate da bassi livelli di HDL e generalmente clinicamente evidenti, e iper-alfalipoproteinemie, con alti livelli di HDL e manifestazioni cliniche meno evidenti. È stata, soprattutto, posta attenzione sulle prime menzionate, trattandone gli aspetti genotipici, fenotipici e clinici. La relazione, infine, si è chiusa con un approfondimento sull'enzima LCAT, che sulla base del substrato genotipico mutato può causare la Fish-Eye Syndrome o deficit familiare totale di LCAT, condizioni accomunate da un accumulo di colesterolo non esterificato che si deposita a livello corneale e, nelle forme di deficit totale, si somma ad anemia e insufficienza renale ma senza un effettivo aumento del rischio cardiovascolare. Ad oggi non c'è ancora terapia per quadri di ipo-alfalipoproteinemie ma si sta studiando l'ipotesi della somministrazione di HDL sintetiche quali CSL-112 e CER-001.

La fisiopatologia della Lp(a) è stata affrontata invece dalla Professoressa Giulia Chiesa, che ha descritto la struttura molecolare, facendo cenno dell'ancora non del tutto chiaro processo di sintesi e catabolismo e delle ipotesi attualmente in fase di studio. È stata sottolineata anche l'omologia di alcune componenti proteiche tra plasminogeno e Lp(a), informazione che ha permesso di ipotizzare una correlazione filogenetica tra l'uno e l'altra e ha messo in evidenza che il gene in questione si è generato in esseri viventi in stadi evolutivi molto avanzati come primati e uomo. È stata riportata una correlazione tra il numero di domini Kringle-IV e quantità circolante di Lp(a) confermata da studi di popolazione, il che pone l'attenzione sul fatto che, essendo geneticamente determinato, il dosaggio plasmatico non necessita di controlli seriati nel tempo. La funzione di Lp(a) nella



fisiopatologia dell'aterosclerosi è determinata dal fatto che, essendo a tutti gli effetti una LDL altresì capace di trasportare fosfolipidi ossidati, ha un ruolo pro-infiammatorio favorente il passaggio di lipidi nel sub-endotelio e, data l'analogia strutturale col plasminogeno, probabilmente anche protrombotico. È stata menzionata una review del gruppo di studio del Professor Kronenberg che ha evidenziato come i livelli di Lp(a) correlino in modo lineare con il rischio relativo cardiovascolare anche per bassi valori della lipoproteina indipendentemente dai fattori di rischio di base. Infine, la relazione si è chiusa con l'analisi di un limite laboratoristico dell'Apo(a) Isoform-Sensitive Assay, metodica che tende a sovra o sottostimare i valori di Lp(a) in base alla grandezza dell'isoforma. Pertanto, è consigliato eseguire il dosaggio di Lp(a) mediante Apo(a) Isoform-Intensive Assay che si è rivelata una metodica più precisa.

L'incontro online dell'08/05/2025 si è aperto con la relazione del Professor Matteo Pirro, che ha analizzato i fattori di rischio dell'aterosclerosi, illustrandone la distribuzione globale e sottolineando come le patologie cardiovascolari costituiscano la principale causa di morte a livello mondiale, con un impatto significativo della stessa aterosclerosi sulla mortalità cardiaca. È stata posta l'attenzione sulla differenza di

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prevalenza e mortalità negli scorsi 30 anni e la previsione dei futuri 25: si prevede che il trend mostrerà un aumento della prevalenza ma una riduzione della mortalità, proprio grazie all'ausilio della diagnosi sempre più precoce e trattamenti farmacologici sempre più efficienti. Iperensione, diabete e ipercolesterolemia sono condizioni che determinano per la maggior parte il rischio cardiovascolare di pazienti in tutte le fasce d'età. Il focus della relazione è stato posto infine sui criteri utilizzati per definire tale un fattore di rischio e per promuoverlo a fattore causale.

Definito il profilo di rischio, l'intervento successivo è stato tenuto dalla Professoressa Maria Del Ben che, ricollegandosi ai fattori di rischio ha sottolineato quanto il primo passo di vera prevenzione sia adottare uno stile di vita sano ed equilibrato, adottando una dieta corretta, interrompendo l'abitudine tabagica e facendo attività fisica. La relatrice ha fatto riferimento anche a una nuova condizione patologica che è stata recentemente definita: Disordine Metabolico Sistemico, un cluster di alterazioni metaboliche ad alta mortalità con eziologia eterogenea, tra cui si annoverano ipertensione arteriosa, MASLD, prediabete, insulino resistenza, dislipidemia aterogena, infiammazione sistemica, malattia renale cronica e insufficienza cardiaca. È stata poi analizzata la dieta come forma primo passo nella prevenzione, analizzando contenuto e vantaggi di diversi regimi dietetici: dieta mediterranea, DASH diet e dieta vegetariana, tutte accomunate dallo stesso principio di riduzione di cibi processati e sale e un aumento del consumo di cibi vegetali. In particolare, è stato citato il Seven Country Study, una pietra miliare della ricerca dietologica che ha analizzato le abitudini alimentari di diversi paesi, tra cui l'Italia, concludendo che i paesi in cui le abitudini alimentari includevano alimenti che oggi sono parte fondamentale della dieta mediterranea, nata e definita proprio alla luce dei risultati di questo studio e il cui ruolo ha assunto sempre più importanza grazie a studi

successivi. È stato infine trattato il ruolo dell'attività fisica nella riduzione del rischio cardiovascolare e incidenza di tumori e citato lo studio ARIC inerente all'effetto patologico a lungo termine sul bilancio globale del rischio, chiudendo così i lavori della giornata.

L'ultimo webinar della Sisa Academy si è svolto in data 27/05/2025 ed è stato aperto dalla Professoressa Chiara Pavonello che ha approfondito la fisiopatologia della placca aterosclerotica. La relazione è iniziata con una panoramica sull'epidemiologia e la storia naturale della placca aterosclerotica con l'esame dell'anatomia della lesione aterosclerotica. È stata poi suddivisa la fisiopatologia in 3 punti principali: inizio, progressione e complicanze. L'inizio vede come assoluti protagonisti LDL, endotelio e infiammazione, poiché dalla loro interazione inizia l'accumulo delle LDL stesse che sviluppano eterogenee forme di alterazione della struttura molecolare. È stata poi discussa la progressione della placca aterosclerotica, processo a cui contribuiscono diversi processi, tra cui l'attivazione dell'inflammasoma da parte dei cristalli di colesterolo, lo switch fenotipico delle cellule muscolari lisce in cellule macrofago-simili e la regolazione pro-infiammatoria linfociti-T mediata. Infine, la relazione si è chiusa con una panoramica sulle complicanze della patologia aterosclerotica: rottura ed erosione.

La seconda relazione della giornata è stata presentata dal Professor A.L. Catapano che ha discusso delle terapie ipolipemizzanti in uso per la riduzione del rischio CV. Il primo gruppo di molecole trattate è quello delle statine, molecole che hanno un bilancio rischio-beneficio decisamente a favore del beneficio sia per quanto riguarda la gestione del rischio cardiovascolare nonostante il profilo diabetologico tenda a peggiorare. È stato citato lo studio SAMSON che mette in luce quanto la mialgia, evento avverso tanto discusso, in realtà si manifesti molto meno frequentemente di quanto riferito dai pazienti. È stato poi trattata la farmacodinamica dell'eze-timibe, farmaco che è stato rivoluzionario nella

## Report SISA LIPID ACADEMY – Modena 2025

prevenzione cardiovascolare come dimostrato dallo studio IMPROVE-IT, e i vantaggi della terapia di combinazione rispetto all'utilizzo di statina ad alta intensità in monoterapia. Un maggior focus è stato fatto sul PCSK9 come target per la riduzione dell'LDL-c, nominando farmaci come Alirocumab ed Evolocumab (mABs), supportati dagli studi ODISSEY e FOURIER, e Inclisiran (siRNA), supportato invece dagli studi ORION; di queste molecole sono stati valutati vantaggi e svantaggi. L'ultimo farmaco analizzato è stato l'acido bempedoico.

Gli otto webinar precedentemente menzionati hanno costituito un'introduzione al 9° Corso Avanzato SISA di Lipidologia Clinica, svoltosi a Modena il 10 e 11 luglio 2025.

I lavori congressuali si sono aperti giorno 10 Luglio 2025 con la relazione del Professor Maurizio Averna sulle ipocolesterolemie, in cui si sono analizzate prevalenza e caratteristiche cliniche per rispondere all'interrogativo: "Sono condizioni rare?". In prima istanza è stata definita la condizione patologica, analizzandone forme primarie, la cui causa è genetica, e secondarie, eterogeneo gruppo di condizioni dal patologico al para-fisiologico tra cui rientrano patologia epatica, cachessia paraneoplastica, dieta e alcolismo. È stata poi fatta una panoramica sull'epidemiologia, facendo notare quanto nello spettro delle ipocolesterolemie ci siano patologie rare come l'abetalipoproteinemia e ipobetalipoproteinemia familiare omozigote, ma anche decisamente più frequenti come il deficit di PCSK9 e ANGPTL3. Si è posto l'accento anche sulla classificazione delle ipobetalipoproteinemie sulla base del meccanismo patogenetico: difetti di secrezione e/o assemblaggio dovuti a carenza o difetti strutturali di apoB, aumentato catabolismo causati da mutazioni a carico di

PCSK9, ANGPTL3 o LDL-R. Di importanza rilevante è il fenotipo clinico della patologia soprattutto quando severo o mild, dato che l'espressione patologica spazia da una totale asintomaticità a un quadro clinico con interessamento multiorgano. È stata fatta notare una costante del quadro patologico: la riduzione del rischio cardiovascolare associato a steatosi



epatica. Infine, si è trattato il tema della comorbidità con steatosi epatica, diabete, cancro e patologia cardiovascolare.

I lavori della I Sessione sono proseguiti con una controversia: "È meglio avere il colesterolo molto basso?" a cui i discenti sono stati invitati a votare.

Le ragioni del SI sono state discusse dal Professor Claudio Bilato che ha evidenziato come, date le forti evidenze provenienti da pietre miliari della ricerca lipidologica come lo studio FOURIER o l'ODISSEY, valori molto bassi di LDL-c riducono drasticamente la mortalità cardiovascolare, evidenza supportata anche da evidenze portate da studi di morfologia di placca in pazienti in trattamento con inibitori del PCSK9. È stato citato anche lo studio FOURIER-OLE, che dimostra un profilo di sicurezza cardiovascolare maggiore in pazienti che

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sono arrivati a bassi target di LDL-c. In chiusura è stato menzionato un dato del ODISSEY OUTCOMES trial: considerando che la mortalità dei pazienti che assumono Alirocumab per un anno e poi interrompono è ancora inferiore rispetto a quella dei pazienti in trattamento cronico con lo stesso farmaco, si potrebbe valutare un cambiamento nell'approccio terapeutico dalla strategia STRIKE EARLY, STRIKE STRONG alla strategia PULSE-CHASE.

Le ragioni del NO, invece, sono state discusse dalla Professoressa Francesca Carubbi che



ha posto l'attenzione su un aspetto secondario sulla questione, vale a dire qual è la causa scatenante del basso valore di colesterolo. Ha inoltre valutato quale componente di colesterolo sia conveniente avere basso, poiché un valore di HDL-c molto basso ha evidenza di essere un fattore prognostico negativo, al contrario di LDL-c. Ha sottolineato quanto sia di fondamentale importanza identificare la causa dell'ipocolerolemia, poiché livelli ridotti di colesterolo possono essere secondari non solo a terapie ipolipemizzanti, ma anche a patologie epatiche, difetti genetici infezioni, infiammazioni o CKD. Infine, è stato valutato come numerose metanalisi abbiano confermato l'effetto neuroprotetti-

vo delle statine nelle patologie neurodegenerative come Alzheimer e demenza, citando anche studi in corso come lo STAREE e il PREVENTABLE.

Al termine della controversia, i discenti sono stati invitati a esprimere nuovamente il proprio voto in risposta all'interrogativo posto all'inizio della sessione.

I lavori sono quindi proseguiti con l'attività interattiva intitolata "Quando il segno guida non guida (Peep Hole)", durante la quale i membri della Faculty hanno affiancato i partecipanti nella risoluzione di un caso clinico presentato dalla Commissione.

La I Sessione si è conclusa con un momento dedicato a Domande e Risposte, volto ad approfondire e chiarire i contenuti delle relazioni precedentemente esposte.

Successivamente, i lavori congressuali sono ripresi con la II Sessione, centrata sull'attività interattiva "Diagnosi per Pattern Recognition", che ha stimolato il confronto e l'applicazione pratica delle conoscenze acquisite.

I lavori congressuali della prima giornata sono continuati con la III Sessione, "Esperienze dei Partecipanti - Parte 1", durante la quale alcuni discenti hanno presentato casi clinici di particolare interesse, successivamente discussi e commentati in un clima di partecipazione e approfondimento condiviso e si sono infine conclusi con la IV Sessione, in cui si sono tenute le prime rotazioni della Whispering session che ha visto la partecipazione di cinque relatori, i quali hanno trattato tematiche eterogenee. La Professoressa Angelina Passaro ha trattato le dislipidemie secondarie, evidenziandone le principali cause e modalità di gestione; la Professoressa Cristina A. Pederiva ha illustrato l'approccio diagnostico all'ipercolesterolemia familiare in età pediatrica, sottolineando l'importanza dello screening precoce; la Professoressa Laura D'Erasmus ha discusso l'intolleranza alle statine, presentando strategie terapeutiche alternative oggi disponibili; il Professor Alberto Zambon ha analizzato il ruolo della riduzione

dei trigliceridi nella prevenzione cardiovascolare; infine, il Professor Francesco Angelico ha approfondito le nuove definizioni di MASLD e MASH, con riferimento ai criteri diagnostici e alle implicazioni metaboliche.

Il giorno successivo i lavori congressuali sono cominciati con la V Sessione – La pratica clinica apertasi con le “Esperienze dei Partecipanti – Parte 2”.

Ad avviare la VI Sessione è stato il Professor Alberto Corsini che ha trattato il futuro delle strategie ipolipemizzanti, iniziando con una panoramica delle ormai superate strategie di trattamento ipolipemizzante e soffermandosi sulle evidenze che ad oggi suggeriscono in prima istanza la terapia di associazione Statina + Ezetimibe. Sono stati poi passati in rassegna i farmaci dell’armamentario attualmente in uso con tutti i rispettivi vantaggi sulla prevenzione cardiovascolare e sono stati poi aggiunti dei farmaci tutt’ora in via di sviluppo che agiscono sulla pathway di PCSK9: Lerodalcibep (proteina di fusione) somministrato sottocute, Enclitide (peptide macrociclico) ed AZD0780, entrambi somministrabili per os. Un problema persistente è quello del rischio residuo che alcuni pazienti, nonostante i trattamenti polifarmacologici, mantengono. Tra le molecole in fase di studio è stato menzionato anche l’Icosapentaetile (EPA), considerabile a tutti gli effetti un farmaco in virtù degli studi che sono stati condotti, tra cui è stato citato il REDUCE-IT, e l’Obicetrapib, molecola interessante con duplice meccanismo di secrezione intestinale di colesterolo e regolatoria sulla Cholesteryl Ester Transfer Protein (CETP) che verrà introdotto in commercio con associazione fissa con Ezetimibe dati gli effetti di additività dimostrati dallo studio ROSE-II. Uno spiccato interesse hanno suscitato gli ASO (come Pelacarsen) e i siRNA (come Olpasiran) capaci di ridurre i valori di Lp(a) di oltre l’80% o le OSM (come Muvalaplin, farmaco per os), capace di ridurre fino al 50%. Infine, la relazione si è chiusa con una panoramica di farmaci con target ANGPTL3 (Evi-

nacumab, Zodasiran, Solbisiran) e apoC-III (Volanesorsen, Olezarsen e Plozasiran).

La relazione del Professor Corsini ha aperto le porte a una controversia: “C’è davvero bisogno di nuovi farmaci?”.

Le ragioni del SI sono state affrontate dal prof Giuseppe D. Norata. Per espressa volontà dell’autore i contenuti non sono divulgabili.

Le ragioni del NO, invece, sono state approfondite dal Professor Aldo P. Maggioni che ha analizzato lo studio BRING-UP Prevenzione, studio italiano multicentrico che ha incluso quasi 5000 pazienti. È emerso che ottimizzando le terapie in modo paziente-centrico la percentuale di pazienti a target di LDL è passato dal 33% al 60% in un arco di tempo piuttosto ristretto.

Al termine della controversia i discenti, analogamente alla precedente, sono stati invitati a votare nuovamente.

Successivamente è stata proposta un’attività interattiva dal titolo “Quando è necessario ridurre il rischio CV associato a LDL-C e Lp(a). L’inibizione di PCSK9: una sola strategia per due obiettivi sfida all’AI” in cui i discenti, sotto guida della Faculty, hanno sfidato l’IA nella gestione e risoluzione di un caso clinico.

In seguito, si è aperto lo spazio di domande e risposta con i relatori della VI Sessione.

La VII Sessione è stata aperta dal Prof Aldo P. Maggioni che ha tenuto una relazione sulla metodologia di lettura critica dei trial clinici. Il primo studio analizzato è il FOURIER, in cui è stato fatto notare non sia stata menzionata la riduzione del rischio assoluto (ARR) né del Number Needed to Treat (NNT). Si è posta l’attenzione su quali sono i metodi per riportare l’efficacia di un trattamento: Relative Risk Reduction (RRR), ARR, NNT, Average Duration of Life Gained (ADLG). Un altro aspetto importante è la cauta analisi dei sottogruppi, per stratificare l’efficacia del trattamento. È stato inoltre portato l’esempio dello studio TOPCAT per sottolineare quanto il livello di rischio di base influenzi i risultati. Sono stati presentati anche i due studi PLATO (Ticagrelor) e CLEAR SI-

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NERGY (Colchicina), di cui sono stati evidenziati dei dubbi attualmente in fase di studio per provare la veridicità e la verificabilità dei dati ricavati nel tempo sui farmaci in questione. È stato lanciato un messaggio finale, in chiusura, sulla lettura e interpretazione dei trial: c'è sempre da imparare anche da quelli con risultati neutri o negativi.

I lavori sono proseguiti con l'VIII Sessione in cui si è tenuta la terza parte di esperienze dei partecipanti.

Seguiva poi la IX Sessione con le ultime rotazioni di Whispering Session.

I lavori congressuali sono ripresi con la lettura del Professor Alberico L. Catapano sulla stratificazione del rischio cardiovascolare che ha aperto la X Sessione. È stato evidenziato come il fattore temporale influisca sull'esposizione a determinati livelli di colesterolo, come dimostrato da studi basati su dati reali. Un ruolo emergente sembra essere svolto dalla proteomica nella comprensione del singolo individuo, al fine di colmare il divario tra lo studio teorico e la pratica clinica. In conclusione, è stato sottolineato il ruolo causale delle LDL, la capacità predittiva dell'esposizione cumulativa, che rappresenta un eccellente metodo per personalizzare lo studio del paziente, e il ruolo delle -omiche, in particolare della proteomica, per stratificare i pazienti.

Avviandosi alla chiusura del 9° Corso Avanzato SISA di Lipidologia Clinica, viene posto un ultimo interrogativo: LDL-c è il Boss?

Le ragioni del SI sono state portate dal Professor Marcello Arca, che ha iniziato la relazione con un'analisi del contenuto della placca aterosclerotica, il cui principale componente è l'LDL-c, che viene intrappolato all'interno della parete arteriosa diventando, previa ossidazione, materiale captabile dai macrofagi. È stato fatto notare che esiste una correlazione statistico-epidemiologica, come precedentemente sottolineato dal Professor Catapano, tra colesterolemia LDL e tempo di esposizione. Viene da sé

che in presenza di mutazioni che aumentano l'esposizione a elevati livelli di LDL-c il rischio cardiovascolare aumenta, cosa che non accade nei casi di mutazioni che la riducono. Infine, è stato menzionato lo studio PACMAN-AMI per supportare l'argomento, poiché tale studio dimostra che una riduzione del colesterolo LDL migliora il profilo di rischio nei pazienti esaminati.

Le ragioni del NO sono state affrontate dal Professor Alberto Zambon che ha presentato una relazione in cui analizza i fattori che insieme all'LDL-c impattano sul rischio cardiovascolare. A supporto della tesi, sono stati presentati gli studi FOURIER (Evolocumab) e ODYSSEY-OUTCOMES (Alirocumab), studi che hanno messo in luce il ruolo dei trigliceridi nella patologia cardiovascolare. È stata poi posta l'attenzione sulla Lp(a), definita come un fattore di rischio indipendente per la patologia cardiovascolare. Lp(a) ha potere aterogeno, a parità di numero di molecole, superiore alla particella LDL. Altro fattore impattante citato è l'infiammazione, che ha evidenze, nonostante i bassi livelli di LDL, di aumentare il rischio. Infine, è stata evidenziata la caratteristica comune a LDL, trigliceridi e Lp(a): la presenza di ApoB, che è stato riconosciuto come il tratto predominante che spiega la relazione eziologica tra lipoproteine e rischio cardiovascolare; pertanto, alla luce di quanto detto, può essere considerato "il boss".

Le sessioni congressuali sono proseguite con un'attività interattiva in cui i partecipanti hanno avuto il compito di affrontare un quesito clinico: "In quale contesto è prioritario intervenire sul peso corporeo rispetto alla gestione dei lipidi?". Tale esercitazione ha richiesto di individuare i principali snodi decisionali, favorendo un approccio critico e basato sull'evidenza.

Infine, si è tenuta l'ultima sessione di domande e risposte, seguita dalla XI Sessione, dedicata alle "Esperienze dei partecipanti", con la quale si sono ufficialmente conclusi i lavori congressuali.

*Giornale Italiano dell'Arteriosclerosi 2025; 16 (4): 15-36*

## CONGRESSI REGIONALI

# CONGRESSO REGIONALE SEZIONE LAZIO ROMA - 26 SETTEMBRE 2025

A cura di **DANIELE PASTORI**

*Dipartimento di Scienze Mediche e Cardiovascolari, Sapienza Università di Roma*

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8 CREDITI  
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*Indirizzo per la corrispondenza*

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Congresso Regionale SISA Lazio

## TARGET TERAPEUTICI. LI STIAMO RAGGIUNGENDO?

**ARTURO CESARO***Università della Campania «Luigi Vanvitelli»; AORN Sant'Anna e San Sebastiano, Caserta*

Le evidenze disponibili dimostrano in modo concorde che solo una minoranza dei pazienti trattati con terapia ipolipemizzante raggiunge i livelli di LDL-C raccomandati dalle linee guida europee. Dati provenienti da ampi registri, tra cui lo studio SANTORINI, confermano che la maggior parte dei soggetti ad alto o altissimo rischio cardiovascolare permane al di sopra dei valori soglia, anche a causa di una prescrizione ancora insufficiente di terapie di combinazione. Questa condizione espone i pazienti a un rischio persistente e significativamente aumentato di eventi cardiovascolari maggiori.

È pertanto necessario un approccio terapeutico personalizzato, guidato da principi chiari:

- 1) la stratificazione accurata del rischio;
- 2) la definizione del target terapeutico;
- 3) la valutazione della distanza dal target;
- 4) la scelta del farmaco o della combinazione più efficace, secondo il principio “trattare meglio e più precocemente”.

L'analisi di un caso clinico ha permesso di evi-

denziare come la sola monoterapia con statina a basso dosaggio risulti spesso inadeguata per il raggiungimento degli obiettivi terapeutici, rendendo necessario il ricorso precoce a terapie di combinazione. Le recenti opzioni farmacologiche – ezetimibe, inibitori di PCSK9, inclisiran e acido bempedoico – consentono una riduzione incrementale dei livelli di LDL-C e un impatto clinico misurabile sugli outcome cardiovascolari.

Infine, pur osservandosi un aumento quasi triplo nella prescrizione delle combinazioni terapeutiche tra il 2018 e il 2022, la gestione dei pazienti dopo sindrome coronarica acuta rimane subottimale, con meno del 40% dei pazienti a target alla terza visita. In conclusione, raggiungere e mantenere i target di LDL-C rappresenta oggi una priorità clinica assoluta. Per farlo, è indispensabile adottare un approccio proattivo, intensivo e personalizzato, capace di ridurre in modo concreto il rischio cardiovascolare residuo nella popolazione ad alto rischio.

### LDL-C Treatment Gap



- Most high-risk patients remain persistently above guideline-recommended LDL-C thresholds

- Patients with LDL-C below thresholds remained there for only brief time periods

- Insufficient prescribing of combination LLTs by clinicians elevates the risk for ASCVD events

- High-risk patients whose LDL-C remains persistently above recommended thresholds have a significantly increased risk of major CV events

Family Heart Database™.  
LLT, lipid lowering therapy; ASCVD, atherosclerotic cardiovascular disease

## TERAPIA DI ASSOCIAZIONE, ACIDO BEMPEDOICO, INIBITORI DEL PCSK9: QUANDO, COME E A CHI

MATTEO PIRRO

Università degli Studi di Perugia, Sezione di Medicina Interna,  
Angiologia e Malattie da Arteriosclerosi

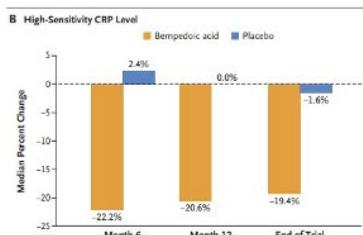
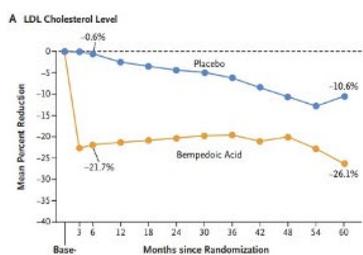
La crescente prevalenza delle malattie cardiovascolari e la loro proiezione verso il 2050 rendono imprescindibile un approccio di prevenzione basato su una gestione integrata e personalizzata dei fattori di rischio, tra cui l'ipercolesterolemia rappresenta uno dei principali determinanti. Le recenti proiezioni epidemiologiche mostrano come, nonostante i progressi terapeutici, il carico globale di mortalità cardiovascolare rimarrà elevato, richiedendo strategie farmacologiche più efficaci e meglio tollerate.

In quest'ottica, il piano di prevenzione cardiovascolare deve fondarsi su tre pilastri fondamentali: identificazione precoce dei fattori di rischio, stratificazione accurata del rischio glo-

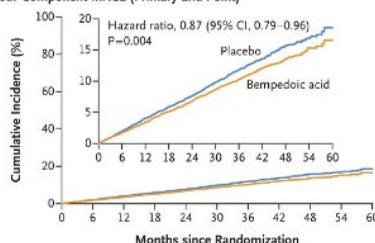
bale e raggiungimento intensivo dei target terapeutici di LDL-C. Le linee guida ESC 2025 ribadiscono la necessità di adottare approcci sinergici che combinino più classi farmacologiche, al fine di massimizzare la riduzione del rischio residuo nei pazienti ad alto e altissimo rischio cardiovascolare.

L'associazione tra statine, ezetimibe, acido bempedoico e inibitori di PCSK9 rappresenta oggi la strategia più efficace per ottenere riduzioni additive del colesterolo LDL, fino e oltre il 70%. In particolare, l'acido bempedoico, grazie al suo meccanismo d'azione epato-selettivo, offre un profilo di tollerabilità favorevole, risultando particolarmente indicato nei pazienti con intolleranza alle statine o con necessità di ulte-

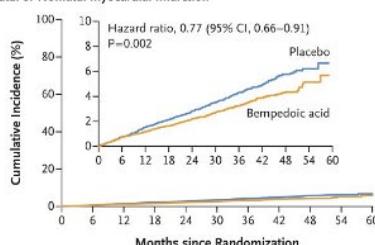
### Bempedoic Acid and Cardiovascular Outcomes in Statin-Intolerant Patients



Four-Component MACE (Primary End Point)



Fatal or Nonfatal Myocardial Infarction



Nissen SE, et al. N Engl J Med. 2023 Apr 13;388(15):1353-1364

rioni riduzioni lipidiche. I dati provenienti dagli studi CLEAR Outcomes e dalle meta-analisi più recenti hanno dimostrato una significativa riduzione degli eventi cardiovascolari maggiori (MACE) nei soggetti trattati, senza incremento degli eventi avversi gravi.

Parallelamente, gli inibitori di PCSK9 – inclusi alirocumab, evolocumab e più recentemente inclisiran – si confermano sicuri ed efficaci nel lungo periodo, con effetti consistenti sulla riduzione di mortalità e incidenza di MACE, sia nei pazienti in prevenzione secondaria sia in quelli con sindrome coronarica acuta. L'introduzione precoce di questi farmaci, in associazione alla terapia di base, ha mostrato benefici sia sul profilo lipidico sia sull'infiammazione residua, contribuendo a una migliore prognosi cardiovascolare.

Un aspetto cruciale rimane la sostenibilità economica e la scelta appropriata del paziente: le terapie devono essere non solo efficaci, ma anche accessibili, combinabili e mirate ai soggetti più complessi, come quelli con diabete, insufficienza renale cronica o ipercolesterolemia familiare.

In conclusione, la disponibilità di un arsenale terapeutico ampio e sicuro rappresenta un privilegio che impone un uso razionale e precoce delle combinazioni ipolipemizzanti. Solo attraverso un approccio integrato, basato su personalizzazione, sinergia farmacologica e aderenza alle linee guida, sarà possibile raggiungere e mantenere i target di LDL-C, riducendo in modo significativo il rischio cardiovascolare residuo e migliorando la prognosi dei pazienti più fragili.

## IPERTRIGLICERIDEMIA: NUOVE OPZIONI TERAPEUTICHE

LAURA D'ERASMO

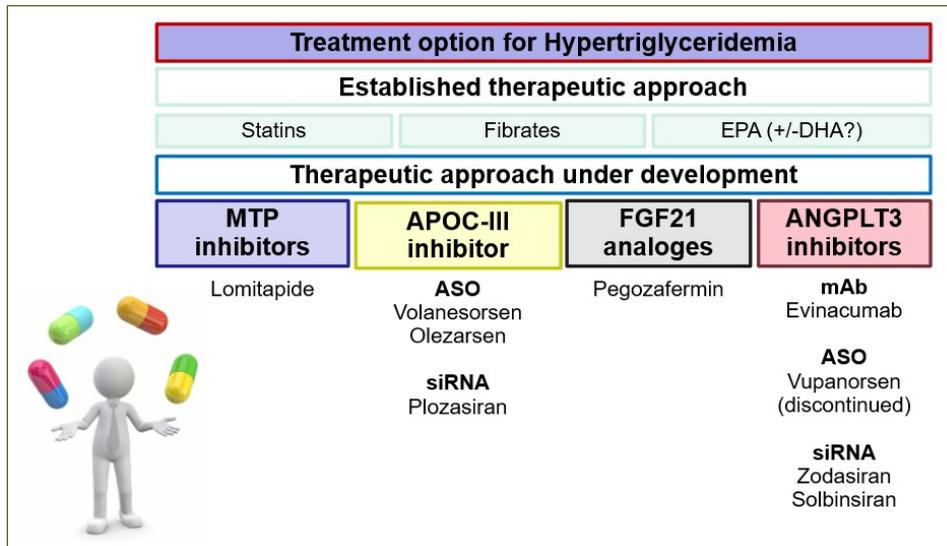
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Negli ultimi anni, l'ipertrigliceridemia è passata dall'essere considerata un semplice marker di rischio cardiovascolare a un vero e proprio fattore causale di aterosclerosi. Le evidenze genetiche e cliniche hanno infatti dimostrato che le lipoproteine ricche in trigliceridi, in particolare i loro remnant contenenti apolipoproteina B, contribuiscono alla formazione e alla progressione della placca ateromasica attraverso meccanismi di ritenzione lipidica, infiammazione e disfunzione endoteliale. Questo ha portato a riconsiderare i trigliceridi non solo come parametro metabolico da monitorare, ma come target terapeutico fondamentale nella prevenzione cardiovascolare.

La terapia tradizionale dell'ipertrigliceridemia si basa su statine, fibrati e acidi grassi omega-3. Le statine rappresentano la prima linea, poiché riducono sia il colesterolo LDL sia i trigliceridi,

con una diminuzione media compresa tra il 15 e il 30%. I fibrati, agonisti dei recettori PPAR- $\alpha$ , abbassano in modo efficace i trigliceridi e aumentano l'HDL-colesterolo, ma i loro benefici in termini di riduzione degli eventi cardiovascolari maggiori risultano limitati. Anche gli omega-3, in particolare le combinazioni di EPA e DHA, hanno mostrato una riduzione dei trigliceridi a dosi elevate, ma i risultati dei principali studi clinici sono stati eterogenei ed hanno fornito risultati contrastanti.

Un'importante novità è rappresentata dall'introduzione dell'EPA purificato nella forma di icosapent etile (IPE), una molecola stabile e ad alta biodisponibilità che ha rivoluzionato l'approccio terapeutico all'ipertrigliceridemia. Lo studio REDUCE-IT ha dimostrato che, nei pazienti in trattamento con statine e con trigliceridi compresi tra 135 e 499 mg/dL, l'aggiunta di



IPE 4 g al giorno riduce del 25% il rischio di eventi cardiovascolari maggiori, indipendentemente dai livelli basali di trigliceridi o di colesterolo LDL. Lo studio EVAPORATE ha poi confermato un effetto favorevole sulla placca coronarica, con riduzione della componente lipidica e aumento della stabilità. Il meccanismo d'azione dell'IPE sembra multifattoriale: oltre alla riduzione dei trigliceridi, l'aumento del rapporto EPA/AA (acido arachidonico) modula i processi infiammatori, riduce lo stress ossidativo e stabilizza le membrane cellulari.

Secondo la determinazione AIFA del 5 dicembre 2024, l'icosapent etile è prescrivibile e rimborsabile per pazienti adulti con ipertrigliceridemia lieve-moderata (135-499 mg/dL) in terapia stabile con statine, con malattia cardiovascolare accertata o con diabete mellito associato ad almeno un ulteriore fattore di rischio, e con valori di LDL-C sotto controllo. La posologia approvata è di 4 grammi al giorno, suddivisi in due somministrazioni ai pasti.

Il profilo di sicurezza dell'IPE è complessivamente buono. È stato osservato un aumento del rischio di fibrillazione atriale, soprattutto nei soggetti con anamnesi positiva per aritmie o fattori predisponenti, come età avanzata, ipertensione o cardiopatia strutturale. Tale effetto

appare dose-dipendente e richiede un monitoraggio clinico attento nei pazienti più a rischio. È stato inoltre segnalato un incremento marginale del rischio di sanguinamento ma senza un aumento significativo degli eventi emorragici maggiori.

Parallelamente, la ricerca sta esplorando nuovi orizzonti terapeutici con farmaci innovativi che agiscono su specifici target metabolici dei trigliceridi, come gli inibitori di APOC-III (volanesorsen, olezarsen, plozasiran), gli analoghi del FGF21, gli inibitori di ANGPTL3 e la lomitapide. Queste molecole, alcune già in uso sperimentale nelle forme genetiche severe come la sindrome da chilomicronemia familiare, promettono un approccio sempre più personalizzato nella gestione delle dislipidemie complesse.

In conclusione, l'ipertrigliceridemia rappresenta oggi un obiettivo terapeutico centrale per la riduzione del rischio cardiovascolare residuo. L'icosapent etile, grazie alla solidità delle evidenze cliniche e alla recente approvazione regolatoria, costituisce una nuova e importante opzione nella pratica clinica, mentre le terapie emergenti lasciano intravedere ulteriori sviluppi verso una medicina sempre più mirata e meccanicisticamente fondata.

## STRATEGIE DI TRATTAMENTO NEL PAZIENTE AD ALTO RISCHIO CARDIOVASCOLARE

ILARIA BARCHETTA

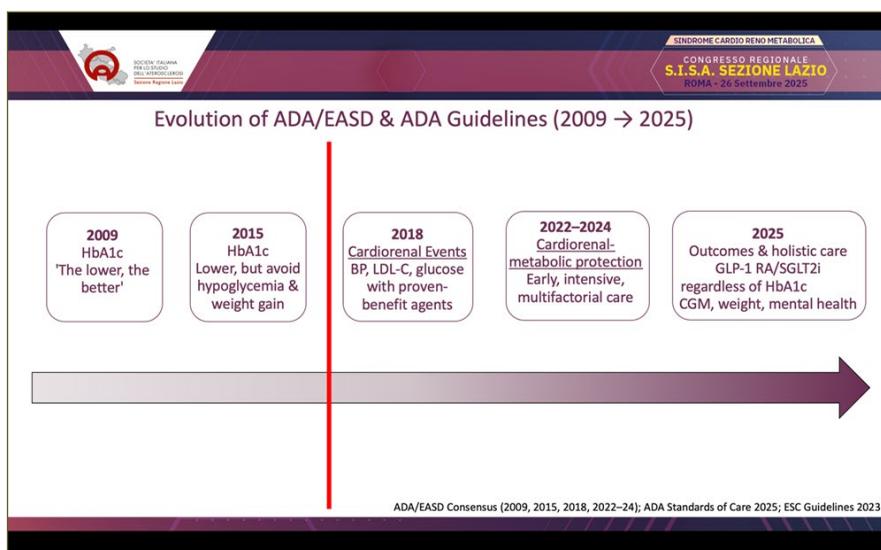
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Il diabete mellito di tipo 2 (T2D) è una patologia cronica a elevato rischio cardiovascolare e renale, caratterizzata da una complessa interazione tra disfunzione metabolica, infiammazione cronica e danno d'organo progressivo. La maggior parte dei pazienti con T2D presenta almeno un fattore di rischio cardiovascolare maggiore, e un approccio terapeutico focalizzato esclusivamente sul controllo glicemico si è dimostrato insufficiente nel ridurre la morbilità e la mortalità correlate alla malattia. Le evidenze emerse dai principali trial di outcome cardiovascolare (CVOTs) hanno ridefinito i paradigmi terapeutici, dimostrando che gli agonisti del recettore del GLP-1 (GLP-1 RA) e gli inibitori del cotrasportatore sodio-glucosio di tipo 2 (SGLT2i) conferiscono benefici cardiovascolari e renali significativi, indipendenti dalla riduzione dell'HbA1c. Tali risultati hanno spostato l'obiettivo del trattamento dal semplice controllo metabolico alla protezione d'organo, con particolare attenzione al cuore e al rene come principali bersagli della malattia

diabetica. Le linee guida internazionali più recenti (ADA/EASD 2025; ESC/EASD 2023) sottolineano la necessità di un approccio precoce e multifattoriale, in cui il paziente sia al centro di una strategia terapeutica integrata. Tale approccio include la correzione simultanea dei fattori di rischio cardiovascolare – ipertensione, dislipidemia, obesità e disfunzione renale – unitamente alla promozione di comportamenti salutari e alla personalizzazione del trattamento farmacologico. Il concetto emergente di “cardiorenal-metabolic protection” rappresenta quindi il nuovo paradigma della cura del diabete: una medicina di precisione orientata alla prevenzione delle complicanze, alla salvaguardia funzionale degli organi bersaglio e al miglioramento della qualità e dell'aspettativa di vita del paziente.

### Key Points

- Il diabete tipo 2 è una condizione cardio-reno-metabolica che richiede un approccio terapeutico olistico e multifattoriale.



- GLP-1 RA e SGLT2i offrono protezione cardiovascolare e renale indipendente dal controllo glicemico.
- L'obiettivo terapeutico si evolve dalla riduzione dell'HbA1c alla prevenzione del danno d'organo e alla protezione integrata del paziente.

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## TRATTAMENTO DELL'OBESITÀ NEL PAZIENTE CON E SENZA DIABETE

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La relazione analizza in dettaglio il trattamento dell'obesità, sia nei pazienti con diabete che in quelli senza, evidenziando l'impatto significativo di questa condizione sulla salute cardiovascolare. L'obesità è definita come un eccesso di grasso corporeo che comporta rischi per la salute, con il body mass index - BMI come parametro diagnostico, sebbene con importanti limitazioni: non distingue tra massa grassa e magra, non considera la distribuzione del grasso e non fornisce informazioni sullo stato metabolico.

L'obesità è strettamente correlata a un aumento del rischio di malattie cardiovascolari, come dimostrato da numerosi studi preclinici e clinici. Anche in assenza di diabete, ipertensione o dislipidemia, il sovrappeso e l'obesità aumentano il rischio di eventi cardiovascolari. Inoltre, è stato introdotto il concetto di sindrome cardiorenale-metabolica (CKM, Cardiovascular-Kidney-Metabolic), che evidenzia il ruolo dell'obesità come prima alterazione patologica determinante per l'insorgenza e la progressione della malattia renale cronica e delle malattie cardiovascolari.

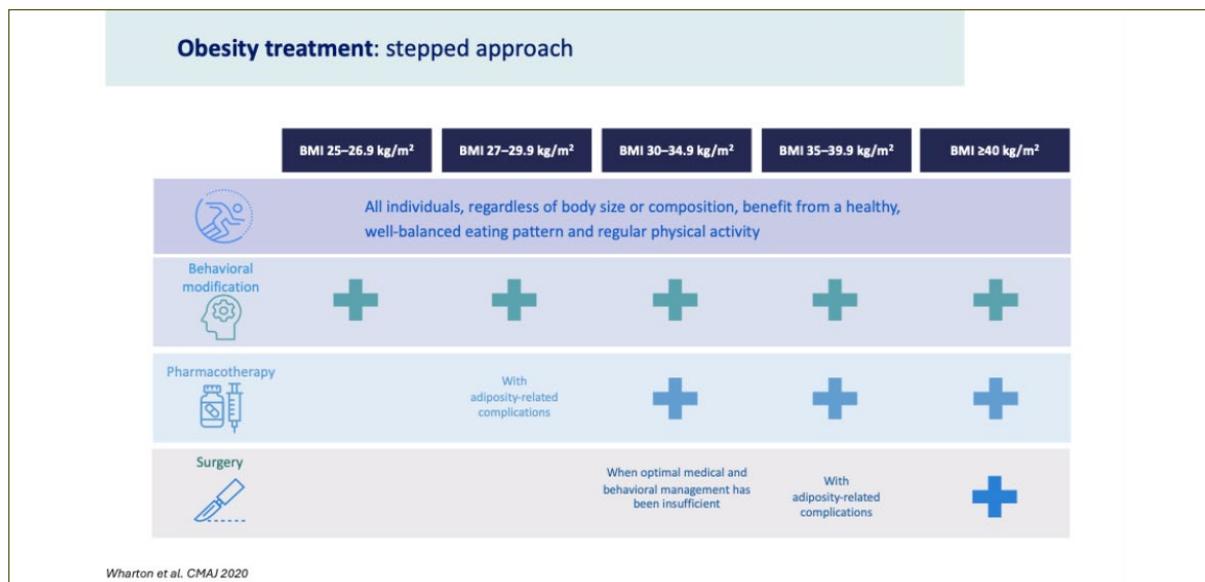
Il trattamento dell'obesità segue un approccio graduale: modifiche dello stile di vita, farmacoterapia e chirurgia bariatrica. La dieta equilibra-

ta e l'attività fisica regolare sono fondamentali, ma spesso insufficienti nel lungo termine a causa di adattamenti metabolici che favoriscono il recupero del peso. L'obiettivo iniziale è una riduzione del 10% del peso corporeo in 4-6 mesi.

Tra i farmaci disponibili, semaglutide 2,4 mg (agonista del recettore per il GLP-1) ha mostrato nei trial STEP una riduzione del peso corporeo fino al 15% in 68 settimane, con benefici aggiuntivi nella prevenzione cardiovascolare, come dimostrato dallo studio SELECT. Tirzepatide, doppio agonista dei recettori per il GIP e per il GLP-1, ha mostrato, negli studi SURMOUNT, di indurre una perdita di peso fino al 22.5% circa in 72 settimane. Nel confronto diretto dello studio SURMOUNT-5, tirzepatide (10 mg o 15 mg a settimana) ha superato semaglutide (1.7 mg o 2.4 mg a settimana) in efficacia, con una maggiore riduzione del peso corporeo.

La chirurgia bariatrica rimane un'opzione efficace nei casi di obesità grave, con procedure come il bypass gastrico e la sleeve gastrectomy. Infine, la ricerca sta esplorando nuove molecole come CagriSema (combinazione di cagrilintide e semaglutide), MariTide (Maridebart cagrilintide) e Bimagrumab (anticorpo contro il

## Congresso Regionale SISA Lazio



recettore dell'activina), che promettono ulteriori progressi nella lotta contro l'obesità. In sintesi, l'obesità è una pandemia cardiometabolica e la disponibilità di terapie farmacologi-

che sempre più efficaci rappresenta una svolta nella sua gestione. Il percorso terapeutico deve essere personalizzato e continuo, evitando interruzioni che favoriscono il recupero ponderale.

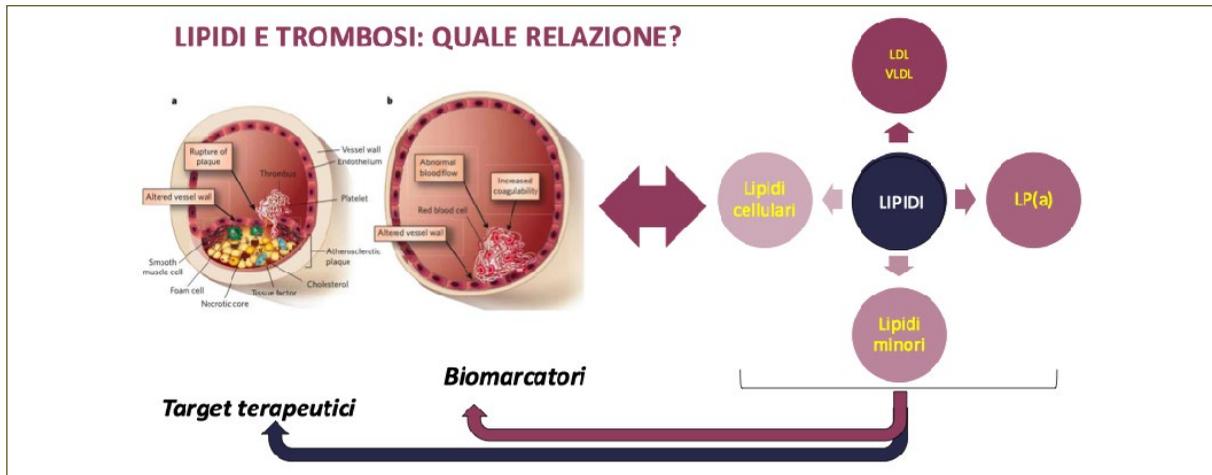
## LIPIDI E TROMBOSI: QUALE RELAZIONE?

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I lipidi costituiscono una classe eterogenea di composti con molteplici funzioni biologiche: sono componenti fondamentali delle membrane cellulari, riserve energetiche e molecole coinvolte nei meccanismi di segnalazione intracellulare. Lipidi e lipoproteine giocano un ruolo diretto nei processi trombotici, poiché influenzano l'attivazione piastrinica e modulano sia le vie procoagulanti che anticoagulanti. In particolare, il ruolo delle lipoproteine nella regolazione della trombosi è ben documentato. Le LDL e le VLDL favoriscono la generazione di trombina e inibiscono i meccanismi di fibrinolisi. Le LDL, inoltre, promuovono l'attivazione e l'aggregazione piastrinica, stimolando la secrezione dei granuli. Alcune sottopopolazioni

di LDL, come le LDL desialilate, sono state associate indipendentemente a un aumentato rischio sia di ictus trombotico che emorragico. Un possibile meccanismo coinvolge la loro maggiore suscettibilità all'ossidazione, che si traduce in una significativa potenza procoagulante e in un'elevata produzione di trombina, superiore a quella indotta dalle LDL native. Particolare attenzione merita la Lipoproteina(a) [Lp(a)], il cui ruolo protrombotico è supportato da numerose evidenze. Lp(a) possiede proprietà antifibrinolitiche e può aumentare la sintesi dell'inibitore dell'attivatore del plasminogeno (PAI-1), favorire la formazione di reti di fibrina più dense e resistenti alla lisi, attivare le piastrine, stimolare l'ossidazione dei fosfolipidi,



incrementare l'espressione del fattore tissutale e inibire l'attività dell'inibitore della via del fattore tissutale (TFPI). Tuttavia, nonostante questi effetti, il ruolo trombogenico di Lp(a) è ancora oggetto di dibattito.

Anche alcuni lipidi minori presenti nel plasma in concentrazioni ridotte influenzano in modo significativo la coagulazione. Le acilcarnitine a catena lunga, ad esempio, circolano a livelli compresi tra 1 e 4  $\mu\text{mol/L}$  e aumentano in particolari condizioni metaboliche. Studi in vitro hanno evidenziato che molte di queste molecole esercitano un'attività anticoagulante, e i loro livelli risultano significativamente ridotti nei soggetti con trombosi venosa profonda.

Un altro lipide coinvolto nei meccanismi trombotici è l'acido lisofosfatidico (LPA), prodotto dall'idrolisi della lisofosfatidilcolina (LPC). LPA,

rilasciato dalle piastrine attivate, stimola l'aggregazione piastrinica e promuove il rilascio di trappole extracellulari neutrofiliche (NETs), contribuendo alla stabilizzazione del trombo.

In conclusione, le alterazioni qualitative e quantitative del profilo lipidico plasmatico contribuiscono in maniera significativa all'aumento del rischio trombotico. Pertanto, il monitoraggio e la modulazione dei lipidi circolanti rappresentano un aspetto cruciale nella prevenzione delle malattie cardiovascolari.

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## COMORBIDITÀ CARDIO-METABOLICHE NEL PAZIENTE CON FIBRILLAZIONE ATRIALE

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I pazienti affetti da fibrillazione atriale (FA) presentano un'elevata prevalenza di comorbidità come l'ipertensione arteriosa sistemica, il

diabete mellito, l'abitudine tabagica o la broncopneumopatia cronico-ostruttiva (BPCO), e la presenza di progressi eventi cardio- e cere-

brovascolari (1). Tali comorbidità sono strettamente associate alla FA con un rapporto bidirezionale: da un lato, i suddetti fattori clinici rappresentano un fattore di rischio per la nuova insorgenza di FA, con un rischio progressivo in base al numero delle comorbidità presenti e non trattate. D'altro canto, la presenza di comorbidità aumenta il rischio di eventi cardiovascolari di natura ischemica, oltre ai ben noti rischi tromboembolici. In particolare, circa il 44% delle ospedalizzazioni nei pazienti affetti da FA è associato a cause cardiovascolari (2), di cui una delle cause è l'infarto del miocardio (3). Tale associazione è dovuta essenzialmente alle caratteristiche dei pazienti con FA come il rischio di embolizzazione sistemica che può coinvolgere anche le arterie coronarie e il rischio di episodi ad elevata risposta ventricolare che aumentano il fabbisogno di ossigeno e il rischio di sviluppare infarto del miocardio di tipo 2 e da numerosi fattori di rischio e comorbidità cardiovascolari (3). Alla luce della complessità terapeutica necessaria ad un'ottimale gestione clinica di questi pazienti, sono stati sviluppati diversi strumenti clinici (ABC pathway, AF-CARE e SOS) (4) che permettono di attuare una presa in carico olistica di tali pazienti, con un approccio disciplinare e integrato. Tali strumenti (4) sono stati sviluppati per ridurre il rischio di ictus tromboembolico attraverso un ottimale controllo della terapia anticoagulante orale, il rischio di riacutezza dei sintomi associati alla FA attraverso una strategia di controllo del ritmo o della frequenza cardiaca, e infine il rischio cardiovascolare residuo e gli eventi di natura cardiovascolare per mezzo di un attento controllo delle comorbidità cardiovascolari. Sebbene gli studi clinici abbiano dimostrato l'efficacia di questi strumenti nella gestione dei pazienti con FA, la loro applicazione nella pratica clinica quotidiana risulta scarsa con un'adesione a un ottimale regime terapeutico in solo il 20% dei pazienti trattati. In conclusione, la FA presenta numerose comorbidità car-

diovascolari che aumentano il rischio di eventi cardiovascolari e mortalità, tuttavia una gestione integrata e multidisciplinare per la gestione di questi pazienti volta a ridurre mortalità e morbilità è scarsamente applicata nella comune pratica clinica.

### Key Points

- La fibrillazione atriale (FA) è gravata da multiple comorbidità cardiovascolari che espongono non solo ad un aumentato rischio tromboembolico, ma anche ad un aumentato rischio di eventi ischemici come l'infarto del miocardio ed a frequenti ospedalizzazioni.
- Numerose strategie pluridisciplinari (ABC pathway, AF-CARE, SOS) sono state sviluppate per un'ottimizzazione della gestione clinica, delle comorbidità e della terapia.
- Nonostante l'approccio multidisciplinare raccomandato, i pazienti con FA risultano attualmente sotto-trattati con circa il 20% dei pazienti con ottimale gestione della patologia stessa, della sintomatologia e delle comorbidità.

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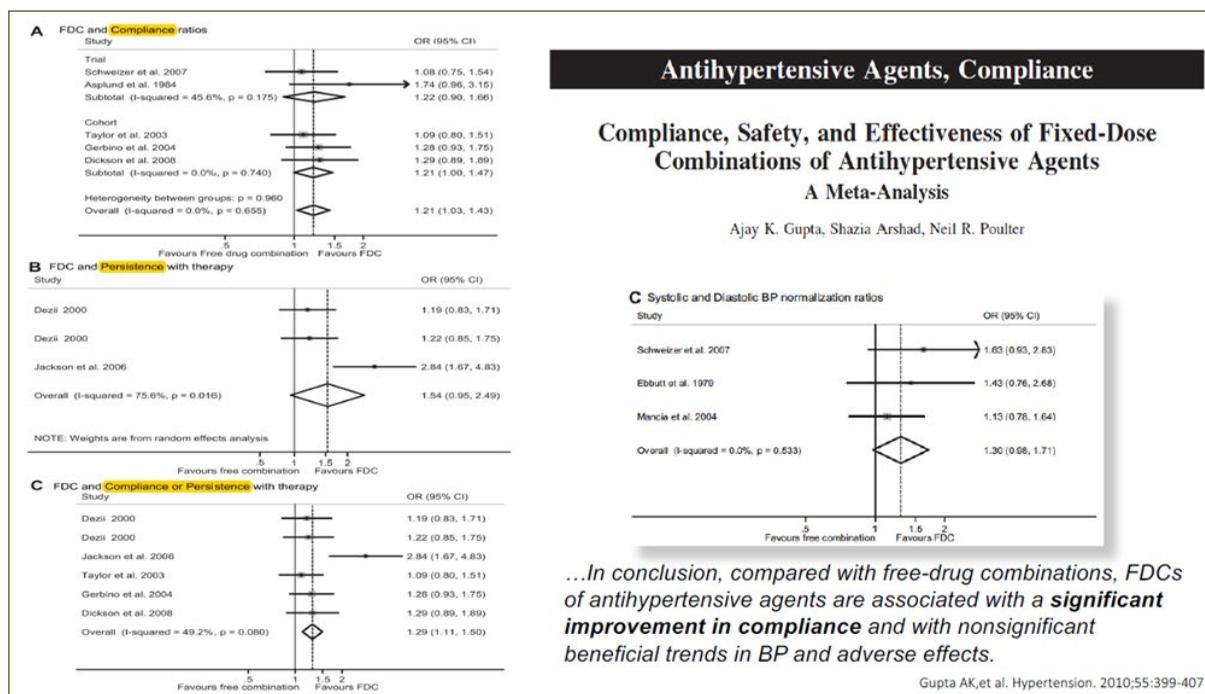
# TERAPIA DI ASSOCIAZIONE PER L'IPERTENSIONE ARTERIOSA: SI PUÒ MIGLIORARE L'ADERENZA?

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L'aderenza alla terapia rappresenta un elemento cardine nella gestione dell'ipertensione arteriosa e nella prevenzione cardiovascolare. Tuttavia, in Italia, analogamente a quanto osservato in ambito lipidologico, solo circa il 50% dei pazienti affetti da ipertensione e scompenso cardiaco aderisce correttamente al trattamento. In un contesto scientifico sempre più orientato verso lo sviluppo di nuove strategie di prevenzione, l'inerzia terapeutica e la scarsa aderenza emergono come fattori determinanti nell'aumentare il rischio di eventi cardiovascolari maggiori. La complessità terapeutica, spesso inevitabile nei pazienti con polipatologie, costituisce una delle principali cause di ridotta aderenza: la necessità di assumere numerosi farmaci in momenti diversi della giornata favorisce la discontinuità terapeutica e può simula-

re forme di apparente ipertensione resistente. Le linee guida internazionali, pur con alcune differenze tra le diverse società scientifiche, raccomandano per la maggior parte dei pazienti l'impiego di terapie di combinazione in associazione preconstituita, al fine di semplificare lo schema terapeutico e migliorare l'aderenza. Tale approccio consente di agire contemporaneamente su diversi meccanismi fisiopatologici dell'ipertensione arteriosa, riducendo i fenomeni di compensazione che spesso limitano l'efficacia delle monoterapie. Come ricordava Everett Koop, "nessuna terapia è efficace nei pazienti che non la assumono": ridurre la complessità, migliorare la tollerabilità e rendere più comoda la somministrazione rappresentano strategie essenziali per aumentare la persistenza nel tempo. Le combinazioni a dose fissa



(Fixed-Dose Combination, FDC) hanno dimostrato di migliorare significativamente l'aderenza, la persistenza e il controllo pressorio. Ulteriore evoluzione di questo concetto è la polipillola, che consente di integrare in un'unica formulazione più principi attivi rivolti a diversi aspetti della prevenzione cardiovascolare. Lo studio SECURE ha dimostrato la superiorità della polipillola rispetto alla sommini-

strazione separata dei singoli farmaci, in termini di aderenza terapeutica e outcome cardiovascolari. In conclusione, le combinazioni a dose fissa e la polipillola rappresentano strumenti fondamentali per migliorare l'aderenza, semplificando il trattamento, aumentando la tollerabilità e contribuendo a una strategia di prevenzione cardiovascolare più completa ed efficace.

## 2025 FOCUSED UPDATE OF THE 2019 ESC/EAS GUIDELINES FOR THE MANAGEMENT OF DYSLIPIDAEMIAS

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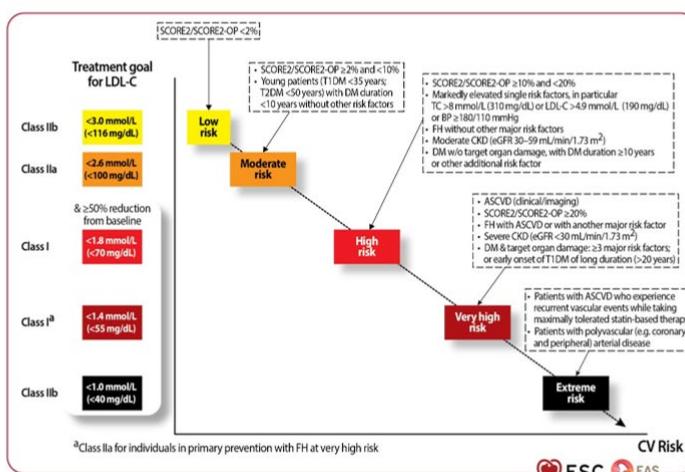
L'aggiornamento 2025 delle Linee Guida ESC/EAS per la gestione delle dislipidemie conferma, sulla base di solide evidenze genetiche, epidemiologiche e cliniche, il ruolo causale del colesterolo LDL (LDL-C) nell'aterogenesi. Il beneficio cardiovascolare è direttamente proporzionale alla riduzione assoluta e alla durata dell'esposizione a livelli più bassi di LDL-C, se-

condo il principio "the lower, the better". Tutti i farmaci ipolipemizzanti – statine, ezetimibe e inibitori di PCSK9 – mostrano un'efficacia comparabile in rapporto all'entità della riduzione del LDL-C, con un profilo di sicurezza favorevole anche a valori inferiori a 1.0 mmol/L (40 mg/dL).

La valutazione del rischio cardiovascolare glo-

**Figure 1**

Treatment goals for low-density lipoprotein cholesterol across categories of total cardiovascular risk.



2025 Focused Update of the 2019 ESC/EAS Guidelines for the management of dyslipidaemias (European Heart Journal; doi: 10.1093/eurheartj/ehaf190)

bale si basa sui sistemi SCORE2 (per soggetti di età <70 anni) e SCORE2-OP (≥70 anni), che stimano il rischio di eventi cardiovascolari fatali e non fatali a 10 anni. In base al rischio stimato, i pazienti vengono classificati in:

- *Rischio moderato*: rischio <5%; target LDL-C <2.6 mmol/L (<100 mg/dL);
- *Rischio alto*: rischio 5-10%; target <1,8 mmol/L (<70 mg/dL);
- *Rischio molto alto*: rischio >10% o presenza di malattia cardiovascolare documentata, diabete con danno d'organo, o insufficienza renale cronica grave; target <1,4 mmol/L (<55 mg/dL);
- *Rischio estremo*: pazienti con evento cardiovascolare recidivante (non più necessariamente entro due anni come indicato nelle linee guida 2019) nonostante trattamento otti-

male; target <1.0 mmol/L (<40 mg/dL).

Le nuove raccomandazioni incoraggiano un approccio terapeutico intensivo e precoce, orientato al rischio complessivo piuttosto che all'eziologia, con l'obiettivo di ottenere la massima riduzione del LDL-C precoce e sostenuta nel tempo. Sono inoltre introdotti fattori modificatori del rischio, tra cui i livelli di lipoproteina(a) e la durata dell'esposizione cumulativa a LDL-C elevato, da considerare in fase di stratificazione e personalizzazione del trattamento.

Infine, le Linee Guida 2025 promuovono l'utilizzo di strumenti digitali, come l'app ESC Pocket Guidelines, che integra algoritmi, calcolatori e schemi terapeutici aggiornati, a supporto di una gestione clinica basata sull'evidenza e finalizzata alla riduzione dell'incidenza di eventi cardiovascolari nella popolazione europea.

## DISLIPIDEMIA NEL PAZIENTE ONCOLOGICO: LA CERCHIAMO?

MASSIMILIANO CAMILLI

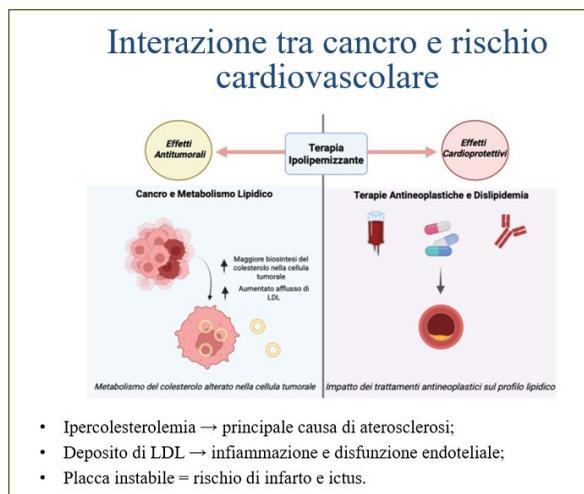
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Il tema affrontato riguarda il ruolo del colesterolo nel paziente oncologico, con particolare attenzione alla sua duplice valenza: da un lato fattore di rischio cardiovascolare, dall'altro possibile elemento coinvolto nei processi di tumorigenesi e progressione neoplastica.

Il colesterolo rappresenta una molecola essenziale per la fisiologia cellulare, in quanto componente strutturale delle membrane, precursore di ormoni steroidei, vitamina D e acidi biliari. Tuttavia, l'alterazione del metabolismo del colesterolo — in particolare la sovraregolazione della via del mevalonato e del recettore per le LDL — è stata associata a un incremento della proliferazione cellulare, all'attivazione di vie di segnalazione pro-oncogene (PI3K/AKT e Wnt/ $\beta$ -catenina) e a una maggiore aggressività e resistenza terapeutica dei tumori.

Parallelamente, è noto come la dislipidemia rappresenti un determinante primario di aterosclerosi e disfunzione endoteliale, configurandosi dunque come fattore di rischio condiviso fra patologie cardiovascolari e neoplastiche. Inoltre, numerosi farmaci antineoplastici inducono alterazioni del profilo lipidico e danno vascolare, accentuando la necessità di una valutazione integrata del rischio cardiovascolare nei pazienti oncologici.

Le statine assumono in questo contesto un ruolo di particolare rilievo: oltre alla riduzione dei livelli di LDL-C, esse esercitano effetti pleiotropici di tipo anti-infiammatorio, anti-angiogenetico e stabilizzante di placca. I risultati del trial STOP-CA e di successive meta-analisi hanno evidenziato un potenziale beneficio nella prevenzione della cardiotoxicità da antracicline.



Un ulteriore ambito di interesse riguarda gli inibitori di PCSK9, che mostrano promettenti effetti antitumorali e di potenziamento dell'im-

munoterapia, grazie alla modulazione dell'espressione di MHC-I e alla riduzione dell'immuno-evasione tumorale. Le prospettive future includono inoltre l'acido bempedoico, che presenta un profilo farmacologico favorevole e un possibile ruolo antinfiammatorio, pur richiedendo conferme cliniche dedicate nella popolazione oncologica.

In conclusione, il colesterolo si conferma come un nodo biologico e clinico centrale nell'intersezione tra cancro e malattie cardiovascolari. La dislipidemia rimane tuttavia sottodiagnosticata e sottotrattata nei pazienti oncologici. È quindi fondamentale promuovere un approccio multidisciplinare alla cardio-oncologia, integrando strategie di prevenzione e trattamento mirate a ottimizzare contemporaneamente la prognosi cardiovascolare e quella oncologica.

## TRATTAMENTO DELLA TROMBOSI NEL PAZIENTE ONCOLOGICO: QUALI OPZIONI?

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Il tromboembolismo venoso (TEV) associato a cancro (Cancer-Associated Thrombosis, CAT) è una complicanza frequente e clinicamente rilevante nei pazienti con patologia oncologica: è associata a morbilità, ritardi o modifiche della terapia oncologica e a un aumento della mortalità. La sua incidenza è aumentata in questi anni, per effetto della maggiore sopravvivenza dei pazienti oncologici, dell'uso crescente di nuovi trattamenti antineoplastici, della diagnosi più frequente di TEV incidentale e della diffusione degli accessi venosi centrali. Tutto ciò rende cruciale un approccio terapeutico basato su evidenze e personalizzato per il singolo paziente. La patogenesi della CAT è multifattoriale: produzione tumorale di fattori protrombotici, infiammazione sistemica, danno endoteliale, immobilità, interventi chirurgici, cateteri venosi

centrali ed effetto procoagulante di alcuni trattamenti antitumorali. Inoltre, la contemporanea presenza di trombocitopenia o alterazioni della coagulazione – indotte dalle terapie e dalla neoplasia stessa – rende il bilancio rischio/beneficio dell'anticoagulazione più complesso. Prima dell'avvento degli Anticoagulanti Orali Diretti (AOD), le eparine a basso peso molecolare (EBPM) sono divenute lo standard per la terapia del TEV in pazienti oncologici, grazie a trial clinici randomizzati che mostrarono una riduzione statisticamente significativa delle recidive tromboemboliche e di sanguinamento maggiore. I trial che portarono a questa modifica di paradigma terapeutico furono principalmente il trial CLOT1 (dalteparina vs warfarin) del 2003 ed il trial CATCH2 (tinzaparina vs warfarin) del 2015 (1-3).

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Trombocitopenia	Trattamento del tromboembolismo venoso (TEV)
Grado 1-2 Piastrine < 100 to 50 x10 <sup>9</sup> /L	<ul style="list-style-type: none"> <li>• Continuare la anticoagulazione a dose terapeutica</li> <li>• Se la conta piastrinica è instabile, preferire l'utilizzo di Eparina a Basso Peso Molecolare (EBPM)</li> </ul>
Grado 3 Piastrine < 50 to 25 x10 <sup>9</sup> /L	<ul style="list-style-type: none"> <li>• Non utilizzare gli Anticoagulanti Orali Diretti (AOD)</li> <li>• In caso di alto rischio tromboembolico e piastrinopenia stabile, considerare EBPM a dose ridotta del 50% e stretto monitoraggio delle piastrine</li> <li>• In caso di TEV acuto (&lt;1 mese), considerare la trasfusione di <i>pool</i> piastrinico a utilizzo di EBPM a dose terapeutica una volta raggiunta una conta piastrinica &gt;40-50x10<sup>9</sup>/L.</li> </ul>
Grade 4 Piastrine < 25 x10 <sup>9</sup> /L	<p>Interrompere la terapia anticoagulante</p> <ul style="list-style-type: none"> <li>• In caso di TEV acuto (&lt;1 mese), considerare la trasfusione di <i>pool</i> piastrinico a utilizzo di EBPM a dose terapeutica una volta raggiunta una conta piastrinica &gt;40-50x10<sup>9</sup>/L; se il <i>target</i> di conta piastrinica non è raggiunto, ma la conta piastrinica è 25-40 x10<sup>9</sup>/L, una dose ridotta di EBPM potrebbe essere considerata.</li> <li>• In caso di trombosi venosa profonda catetere-relata acuta (&lt;1 mese), il catetere dovrebbe essere rimosso (valutando la fattibilità di posizionare un catetere venoso centrale a livello di un'altra sede, se essenziale)</li> <li>• Se l'anticoagulazione non risulta possibile, in caso di trombosi venosa profonda prossimale degli arti inferiori acuta (&lt;1 mese), considerare il posizionamento di un filtro cavale.</li> </ul>

boembolismo venoso o sanguinamento maggiore. In maniera analoga a rivaroxaban, edoxaban mostrò un tasso inferiore di recidiva tromboembolica, ma in presenza di un aumento del rischio di sanguinamento maggiore, in particolar modo nei pazienti con neoplasia gastrointestinale secondo una successiva sotto-analisi (5).

Infine, il Trial Caravaggio (6) (apixaban vs dalteparina) pubblicato nel 2020 ha documentato la non-inferiorità in termini di ricorrenza trombotica di apixaban rispetto a dalteparina, in assenza di un significativo aumento di

Nell'ultimo decennio le strategie di trattamento del TEV si sono ampliate, con il progressivo studio dell'utilizzo di AOD (rivaroxaban, edoxaban, apixaban), anche nella popolazione oncologica. Il Trial Clinico Randomizzato SELECT-D3 (rivaroxaban vs dalteparina) pubblicato nel 2018 ha infatti mostrato una riduzione delle recidive tromboemboliche nel braccio di trattamento con rivaroxaban, sebbene accompagnato con un aumento dei sanguinamenti clinicamente rilevanti. Da questo punto di vista, è interessante notare come venne notato un eccesso di sanguinamenti maggiori nei pazienti con neoplasie esofago-gastriche, tale da indurre negli sperimentatori la necessità di sospendere l'arruolamento di questo sottogruppo a rischio (4).

Successivamente, il Trial Hokusai VTE Cancer4 (edoxaban vs dalteparina) pubblicato nel 2018 ha mostrato una non-inferiorità di edoxaban rispetto a dalteparina per quanto riguardava l'endpoint composito di ricorrenza di trom-

sanguinamenti maggiore. Questo venne confermato anche nella sottopopolazione con tumori gastrointestinali, fornendo una evidenza favorevole per apixaban come opzione terapeutica in questo contesto.

Nel trattamento del tromboembolismo venoso associato a cancro è mandatoria la valutazione del bilancio tra rischio tromboembolico e rischio emorragico, individualizzando la scelta nel singolo paziente. Gli AOD presentano una buona efficacia e sicurezza in questa popolazione, sebbene debba essere prestata una particolare cautela nei pazienti affetti da neoplasia gastrointestinale o genito-urinaria non resecata. In questi sottogruppi di pazienti, le EBPM rappresentano una opzione terapeutica più sicura a parità di efficacia.

Altri aspetti rilevanti e di comune riscontro nella pratica clinica sono i temi delle interazioni farmacologiche e della gestione della terapia anticoagulante durante piastrinopenia.

Gli AOD sono soggetti al metabolismo epatico

tramite il citocromo CYP3A4 e sono substrati della glicoproteina P (P-gp). Numerosi trattamenti antineoplastici sono inibitori/induttori di CYP3A4 o modulanti della P-gp e possono quindi alterare i livelli plasmatici degli anticoagulanti orali diretti, aumentando in maniera variabile il rischio trombotico o emorragico.

Nella scelta della terapia anticoagulante, è opportuno tenere conto delle possibili interazioni farmacologiche presenti tra terapia oncologica e terapia antitrombotica. Per la gestione di questa problematica sono disponibili numerosi strumenti online, che consentono una rapida verifica della presenza di interazioni farmacocinetiche o farmacodinamiche. Un'ulteriore difficoltà può essere incontrata nel paziente che sviluppa nel corso della patologia oncologica un quadro di piastrinopenia. Una proposta operativa di gestione della terapia antitrombotica nel paziente oncologico piastrinopenico è riportata in tabella, in accordo con le Linee Guida della European Hematology Association (7). Infine, nel paziente oncologico, il bilancio dei rischi tromboembolico ed emorragico deve essere necessariamente rivalutato periodicamente, con una cadenza di 3-6 mesi, al fine di determinare la persistenza di malattia oncologica attiva, la tolleranza alla terapia anticoagulante e/o la possibilità di utilizzare una terapia anticoagulante orale diretta a dose ridotta in prevenzione secondaria del tromboembolismo venoso.

#### Key Points

- Il trattamento del tromboembolismo venoso associato a cancro richiede una valutazione individualizzata del rischio trombotico ed emorragico.
- Gli Anticoagulanti Orali Diretti sono una strategia terapeutica sicura ed efficace in questa popolazione, con necessità di cautela nei pazienti con neoplasia gastrointestinale o genito-urinaria non resecata.
- La trombocitopenia e le interazioni farmacologiche con la terapia oncologica richiedono una costante attenzione al fine di minimizzare il rischio trombotico ed emorragico nel paziente che presenta queste condizioni.
- Il bilancio rischi-benefici della terapia anticoagulante deve essere rivalutato periodicamente, con una cadenza di 3-6 mesi.

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## LE EPARINE NEL TRATTAMENTO DELLA TROMBOSI ASSOCIATA A CANCRO: QUALI EVIDENZE

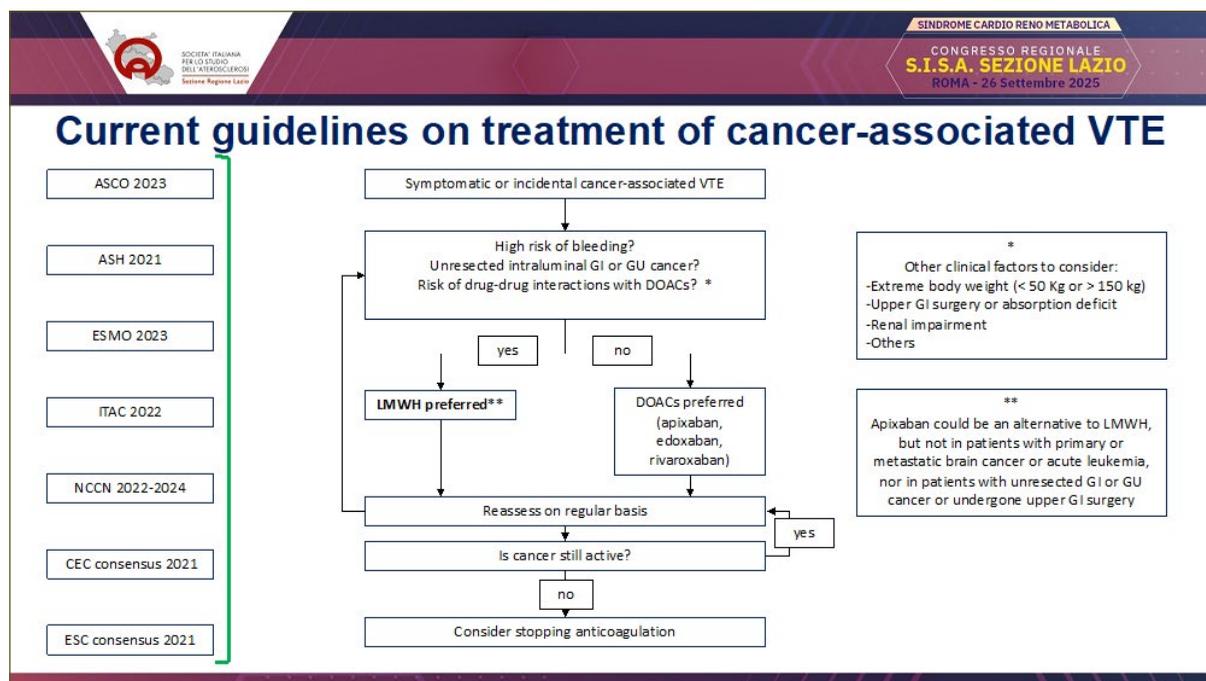
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La trombosi associata a cancro (CAT) è un insieme di entità cliniche tra loro anche molto distinte accomunate dal punto di vista fisiopatologico da una disregolazione dei pathways emostatici in corso di malattia neoplastica. Non si tratta soltanto di tromboembolismo venoso, l'entità clinica più comune e per la quale esiste il maggior numero di evidenze scientifiche, ma anche di condizioni meno comuni per quanto potenzialmente catastrofiche come il tromboembolismo arterioso e alcune sindromi sistemiche complesse come la coagulazione intravascolare disseminata, la trombosi microangiopatica, la malattia venocclusiva, l'endocardite trombotica non batterica, e la sindrome da anticorpi antifosfolipidi. Le eparine costituiscono da oltre un secolo un cardine indiscusso della terapia anticoagulante. Nella sua accezione più

ampia il termine eparine, indica distinte classi di polisaccaridi a diverso peso molecolare e conseguentemente diversa farmacocinetica, accomunati dalla capacità di inibire la cascata coagulativa principalmente a livello del fattore X e del fattore II. La classe più utilizzata è indiscussamente quella delle eparine a basso peso molecolare, commercializzate per lo più in preparazioni farmaceutiche ottenute dalla depolimerizzazione di forme non frazionate di estrazione animale.

Le eparine trovano di diritto un proprio spazio nella gestione della CAT per una serie di ragioni. In primis, perchè le evidenze scientifiche attuali (trials clinici randomizzati controllati, metanalisi di trials clinici randomizzati controllati e studi real world) dimostrano che le eparine sono superiori ai dicumarolici e almeno tan-



to efficaci quanto i DOACs nel trattamento del tromboembolismo venoso associato a cancro, ma al tempo stesso più sicure rispetto ai DOACs in termini di rischio di sanguinamenti minori. In secondo luogo, perché esistono diverse situazioni cliniche nelle quali l'impiego delle eparine è consolidato come standard of care sebbene non sempre sulla base dei risultati di grandi trials clinici randomizzati controllati (es. il trattamento del tromboembolismo venoso associato a dispositivi intravascolari, il trattamento di casi selezionati di trombosi venosa splancica, il trattamento e la profilassi del tromboembolismo venoso associato a cancro nel paziente pediatrico, alcuni casi di profilassi del tromboembolismo arterioso nella fibrillazione

atriale associata a cancro, la profilassi e il trattamento di alcune sindromi sistemiche, come la coagulazione intravascolare disseminata in casi selezionati, l'endocardite trombotica non batterica e la sindrome da anticorpi antifosfolipidi in casi selezionati). In terzo luogo, perché le eparine offrono, nel trattamento della CAT, potenziali opportunità terapeutiche aggiuntive legate ai loro possibili effetti antineoplastici, riconducibili principalmente a meccanismi antiproliferativi, immunomodulatori, e anti-angiogenetici.

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## DALLA SINDROME METABOLICA ALLA SINDROME CRM: COSA CAMBIA

DANIELE PASTORI

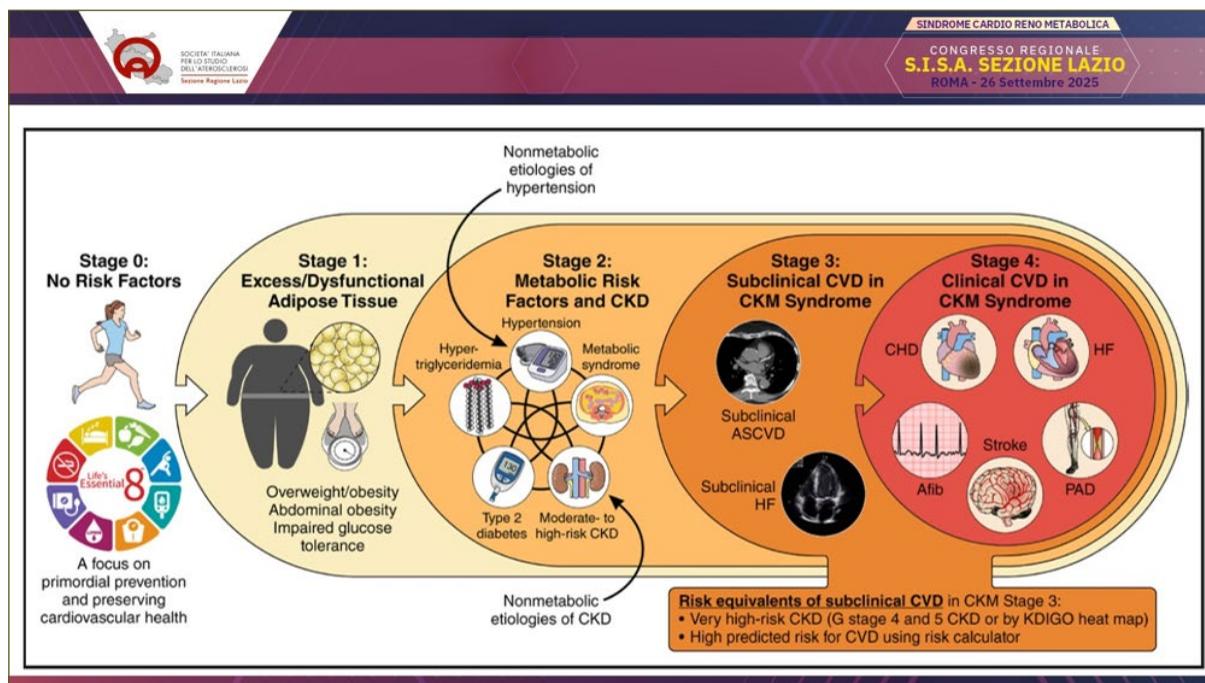
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La relazione affronta in modo approfondito il concetto di sindrome cardio-renale-metabolica (CRM), una condizione sistemica che rappresenta l'evoluzione concettuale della precedente sindrome cardio-renale. Essa descrive un continuum fisiopatologico nel quale le alterazioni metaboliche, la malattia renale cronica (MRC) e le disfunzioni cardiovascolari interagiscono in maniera bidirezionale, amplificando reciprocamente il rischio di eventi avversi e la progressione del danno d'organo. La CRM include sia i soggetti con fattori di rischio metabolici o renali che predispongono a malattie cardiovascolari, sia coloro che presentano già una patologia cardiovascolare la cui evoluzione è complicata da disturbi metabolici o renali.

Vengono inoltre evidenziati numerosi fattori di rischio aggiuntivi che contribuiscono all'insorgenza o all'aggravamento della sindrome CRM, tra cui le condizioni infiammatorie croniche

(psoriasi, artrite reumatoide, lupus, HIV), le disuguaglianze socioeconomiche, i disturbi del sonno e della salute mentale, nonché fattori specifici legati al sesso, come la menopausa precoce o la sindrome dell'ovaio policistico. Questi elementi sottolineano la complessità del quadro clinico e la necessità di un approccio multidimensionale alla diagnosi e alla gestione. La presentazione introduce inoltre una classificazione in stadi (dallo stadio 1, caratterizzato da disordini metabolici sistemici, fino agli stadi più avanzati), utile per definire la gravità del rischio e impostare strategie di prevenzione e trattamento personalizzate. I dati provenienti da ampie coorti, come la UK Biobank, evidenziano una significativa prevalenza delle fasi iniziali della CRM nella popolazione europea e un chiaro incremento della mortalità complessiva associato alla progressione della sindrome. In conclusione, la diagnosi di CRM richiede

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una valutazione globale e integrata del paziente, che includa parametri metabolici, renali, ecocardiografici e infiammatori, secondo i criteri proposti dall'EAS. Tale approccio consente di individuare precocemente le fasi subcliniche della malattia e di intervenire in maniera mira-

ta. Nei casi di mancata risposta terapeutica o progressione non spiegata, è raccomandato il referral a specialisti di area (nefrologo, epatologo, cardiologo), in un'ottica di collaborazione interdisciplinare per la gestione ottimale del rischio cardio-metabolico e renale.

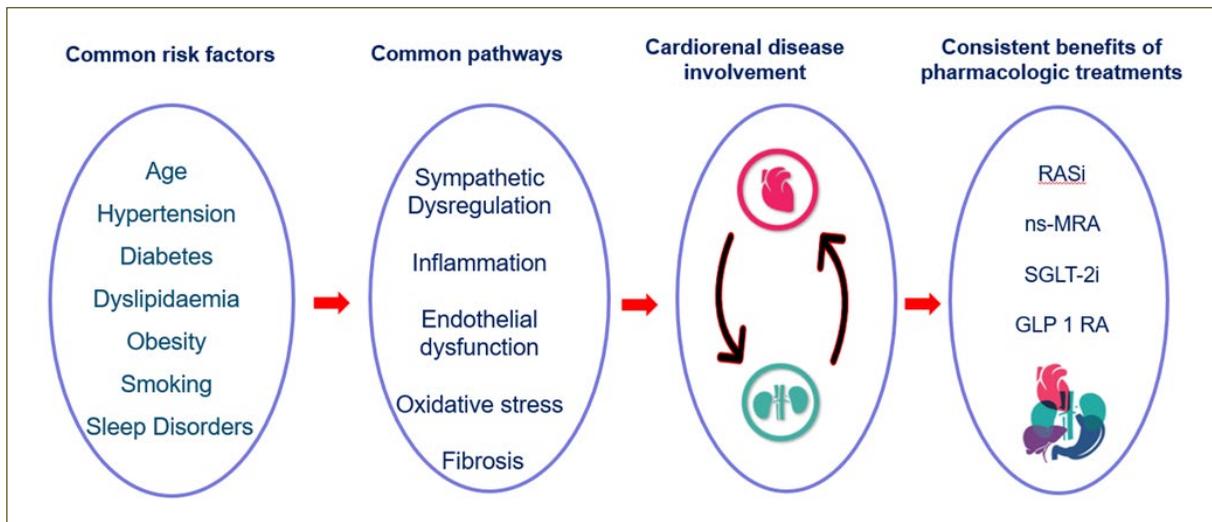
## OPZIONI DI TRATTAMENTO PER LA SINDROME CARDIO-RENALE-METABOLICA

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La sindrome cardio-renale-metabolica (CRM) è una malattia sistemica caratterizzata da interazioni fisiopatologiche tra fattori di rischio metabolici (obesità, diabete mellito di tipo 2, dislipidemie), malattia renale cronica (CKD) e sistema cardiovascolare, con un severo impatto in termini di mortalità e morbilità. Un ruolo cruciale è svolto dal tessuto adiposo in eccesso e/o disfunzionale responsabile dello stato pro-infiammato-

rio, dello stress ossidativo, dell'insulino-resistenza e della disfunzione vascolare. La classificazione in cinque stadi facilita l'identificazione precoce della malattia al fine di mettere in atto strategie terapeutiche per ridurre il rischio cardiovascolare globale. Gli aspetti principali su cui bisogna intervenire sono condivisi e raccomandati dalle principali linee guida delle comunità scientifiche (AHA, ACC, ESC) e suddivisi in:



**Figura 1** - Fattori di rischio, meccanismi patogenetici e terapia nella sindrome CKM.

RASi: *renin-angiotensin system inhibitors*; ns-MRA: *non-steroidal selective mineralocorticoid receptor antagonist*; SGLT-2i: *Sodium-glucose co-transporter 2 inhibitors*; GLP1 RA: *Glucagon-like peptide-1 receptor agonists*.

- *Stile di vita:* attività fisica (circa 150 minuti a settimana) e riduzione dell'apporto calorico al fine di ridurre BMI e circonferenza addominale, fondamentale negli stadi precoci della sindrome CRM per rallentare la progressione di malattia e ridurre gli outcomes cardiovascolari.
- *Terapia farmacologica:* indispensabile negli stadi più avanzati (secondo e terzo) per la gestione di iperlipidemia, ipertensione arteriosa, malattia renale cronica e diabete prediligendo strategie farmacologiche cardio e nefro protettive capaci di rallentare la progressione di CKD e ridurre la mortalità per eventi cardiovascolari fatali e non fatali. Nello stadio ancora più avanzato fortemente raccomandato il controllo delle comorbidità tipiche dei pazienti con sindrome CRM (diabete mellito tipo II, fibrillazione atriale, cardiopatia ischemica, scompenso cardiaco cronico, arteriopatia obliterante degli arti inferiori, anemia multifattoriale). Oltre ai benefici indiscussi di farmaci noti e ampiamente utilizzati come gli Inibitori dell'Enzima di Conversione dell'Angiotensina (ACEi) e i Bloccanti del Recettore dell'Angiotensina II (ARB) e

gli antagonisti del cotrasportatore sodio-glucosio 2 (SGLT2i), un ruolo importante è stato affidato anche agli agonisti recettoriali del glucagone-like peptide-1 (GLP-1) e al finerenone (Figura 1). Il finerenone è un antagonista selettivo non steroideo dei recettori dei mineralocorticoidi, indicato per il trattamento dei pazienti adulti con CKD associata a diabete di tipo 2. Grazie ai suoi effetti pleotropici, anti-fibrotici e antinfiammatori, è in grado di ostacolare la progressione della CKD e delle malattie cardiovascolari.

- *Determinanti socio-sanitari:* rappresentati dalle condizioni sociali, economiche, ambientali e culturali) promuovono uno stile di vita sano con particolare attenzione e sensibilizzazione al tema dell'obesità, informazione ed educazione all'identificazione della sindrome CRM, gestione interdisciplinare e accesso alle terapie.

In conclusione, possiamo affermare che l'obiettivo primario nei pazienti affetti da sindrome CRM è quello di abbattere il rischio cardiovascolare globale agendo sulle varie comorbidità mediante strategie terapeutiche combinate, volte a contrastare le disfunzioni multiorgano.

### Key Points

- La Sindrome CRM è un disturbo sistemico interconnesso che coinvolge Metabolismo (obesità, diabete), Malattia Renale Cronica e Sistema Cardiovascolare.
- Il management si basa sul cambiamento dello stile di vita, terapia farmacologica avanzata (con farmaci cardio/nefro protettivi come SGLT2 inibitori, antagonista non steroidei dei recettori dei mineralocorticoidi, GLP1 RA) e determinanti socio-sanitari.
- L'obiettivo primario è ridurre drasticamente il rischio cardiovascolare globale attraverso

strategie terapeutiche combinate e gestione delle comorbidità.

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## SINDROME CHILOMICRONEMICA FAMILIARE: LUCI ED OMBRE DEI NUOVI FARMACI

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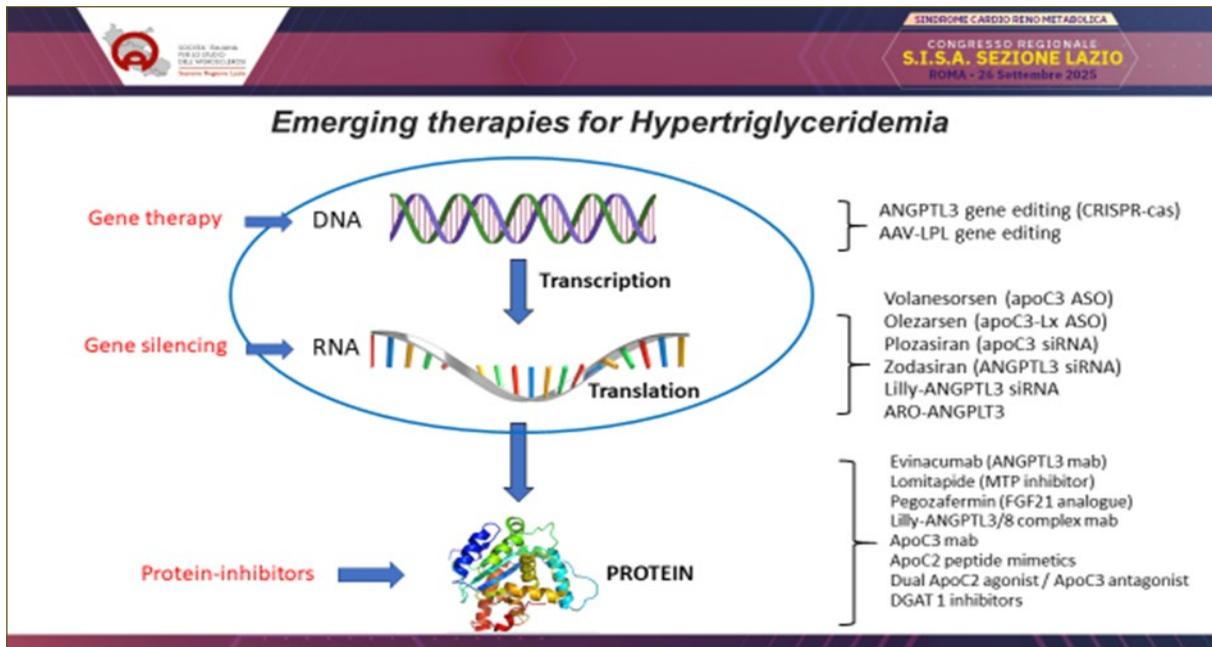
La Sindrome Chilomicronemica Familiare (FCS) è una rara malattia monogenica a trasmissione autosomica recessiva, causata da mutazioni bialleliche nei geni coinvolti nella lipolisi intravascolare (LPL, APOC2, APOA5, LMF1, GPIHBP1). Il deficit del sistema lipolitico determina una marcata riduzione della clearance dei chilomicroni, con livelli plasmatici di trigliceridi spesso superiori a 1000-2000 mg/dL e un elevato rischio di pancreatite acuta ricorrente, la complicanza più temibile e potenzialmente letale (1).

Le terapie convenzionali (fibrati, omega-3, niacina, statine) si dimostrano inefficaci, poiché incapaci di ridurre significativamente la trigliceridemia o di prevenire le complicanze. Per anni, la sola misura di controllo è stata una dieta rigidamente ipolipidica (<15-20 g/die di grassi), spesso insufficiente a mantenere un adeguato controllo della trigliceridemia.

Negli ultimi anni, l'introduzione di farmaci innovativi ha radicalmente modificato lo scenario terapeutico. Volanesorsen (Waylivra®), un oli-

gonucleotide antisense diretto contro l'mRNA epatico di APOC3, nello studio di fase 3 APPROACH2, ha determinato una riduzione media dei trigliceridi del 70-80% e una marcata diminuzione dell'incidenza di pancreatite. Tuttavia, nello studio è stata documentata una riduzione della conta piastrinica in una quota significativa di pazienti in terapia con Volanesorsen, talora fino a <100.000/ $\mu$ L e, raramente, <25.000/ $\mu$ L, evento risultato reversibile con sospensione o differimento delle somministrazioni. Pur in assenza di eventi emorragici, tale evenienza ha reso necessario un attento e regolare monitoraggio ematologico, con protocolli di controllo ravvicinati e aggiustamento posologico individualizzato.

Lomitapide, inibitore della microsomal triglyceride transfer protein (MTP), rappresenta un ulteriore approccio terapeutico per il trattamento della FCS. Riducendo la secrezione epatica di VLDL e la formazione intestinale dei chilomicroni, il farmaco consente una marcata riduzione dei trigliceridi plasmatici. Nello studio



LOCHNES3, ha determinato una riduzione mediana dei trigliceridi di circa il 70% e un significativo miglioramento del profilo lipidico, con assenza di nuovi episodi di pancreatite durante il periodo di trattamento. Tuttavia, l'inibizione cronica della MTP si associa a un rischio di accumulo di grasso intraepatico e incremento delle transaminasi, eventi generalmente reversibili ma potenzialmente evolutivi verso fibrosi in caso di esposizioni prolungate. Sebbene tali effetti riflettano il meccanismo d'azione del farmaco, la loro comparsa impone un rigoroso monitoraggio epatico, con controlli biochimici e strumentali periodici. Nonostante queste limitazioni, lomitapide si conferma una terapia efficace nel controllo dell'ipertrigliceridemia severa, rappresentando un'opzione per pazienti con FCS refrattaria o intollerante ad altre strategie farmacologiche.

Le prospettive future si orientano verso nuove terapie mirate ai principali regolatori del metabolismo dei trigliceridi, come gli inibitori di ApoC3 e ANGPTL3 o gli analoghi di FGF21, con l'obiettivo di garantire una più efficace e sicura gestione della FCS e di ri-

durare in modo duraturo il rischio di pancreatite ricorrente.

#### Key points

- La FCS è una forma grave e refrattaria di ipertrigliceridemia che necessita di approcci terapeutici innovativi.
- Le terapie innovative Volanesorsen e Lomitapide si confermano altamente efficaci nel controllo della severa ipertrigliceridemia nella FCS, pur richiedendo un rigoroso monitoraggio del profilo di sicurezza.

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# HOW TO: L'INTERPRETAZIONE DEL REFERTO GENETICO NELLA IPERCOLESTEROLEMIA FAMILIARE ETEROZIGOTE

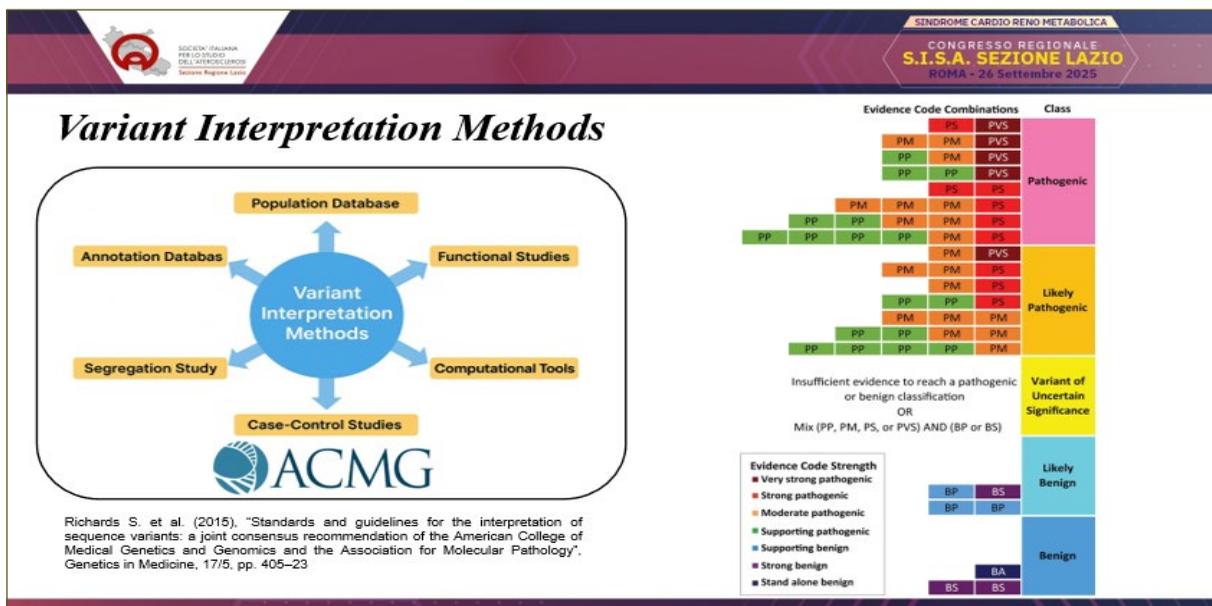
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L'ipercolesterolemia familiare eterozigote (HeFH) è il più comune disordine genetico del metabolismo lipidico, caratterizzato da elevate concentrazioni plasmatiche di colesterolo LDL e da un aumentato rischio di malattia cardiovascolare aterosclerotica precoce (ASCVD) [1,2]. Oltre il 90% dei casi di FH è causato da varianti patogenetiche nel gene del recettore delle lipoproteine a bassa densità (LDLR), mentre le varianti nei geni dell'apolipoproteina B (APOB) e della proproteina convertasi subtilisina/kexina di tipo 9 (PCSK9) rappresentano rispettivamente il 5–10% e meno dell'1% dei casi [3,4]. Il test genetico è oggi considerato il pilastro della diagnosi di FH. Sebbene esistano quadri di classificazione standardizzati, come le linee guida ACMG/AMP e gli adattamenti del ClinGen FH Expert Panel [5,6], che forniscono criteri strutturati per valutare la patogenicità delle

varianti, l'interpretazione clinica rimane complessa. Tra i fattori che contribuiscono a tale difficoltà vi sono lo screening familiare a cascata incompleto e la limitata disponibilità di dati funzionali, che aumentano il numero di varianti di significato incerto (VUS).

Questa presentazione mira a fornire indicazioni sull'interpretazione dei referti genetici per la FH. Inoltre, attraverso esempi pratici, verranno discusse strategie per l'interpretazione dei risultati molecolari nella FH, in particolare quando viene identificata una VUS. Lo screening familiare a cascata dovrebbe sempre essere eseguito, poiché fornisce prove fondamentali di segregazione che possono guidare la riclassificazione delle varianti. Infine, sono in corso sforzi per integrare i dati funzionali nella pratica clinica, con l'obiettivo di migliorare ulteriormente l'accuratezza della diagnosi molecolare della FH.



Congresso Regionale SISA Lazio

## PALLIATIVE CARDIOVASCULAR CARE: LA GESTIONE DEL FINE VITA NEL PAZIENTE CARDIOVASCOLARE

**NICHOLAS COCOMELLO***Dipartimento di Scienze Mediche e Cardiovascolari - Sapienza Università di Roma*

Le cure palliative hanno come obiettivo principale il miglioramento della qualità di vita del paziente e dei suoi caregiver, intervenendo non solo sugli aspetti fisici della malattia, ma anche su quelli emotivi, psicologici e spirituali. Si tratta di un approccio globale che mira a ridurre l'impatto complessivo della malattia cronica, offrendo sostegno e accompagnamento lungo tutto il percorso assistenziale.

Questa visione integrata trova oggi un rinnovato fondamento scientifico. Nell'agosto 2025 la European Society of Cardiology (ESC) ha pubblicato nuove linee guida che mettono in luce il legame tra benessere mentale e salute cardiovascolare. Secondo il documento, la presenza di stressori psicosociali, come solitudine, difficoltà economiche o stress lavorativo, non solo aumenta il rischio di sviluppare patologie cardiovascolari, ma accelera anche la progressione delle malattie già presenti, fino agli stadi più avanzati.

Le patologie cardiache che possono trarre beneficio dalle cure palliative sono numerose: tra le più comuni troviamo lo scompenso cardiaco, la cardiopatia ischemica, la fibrillazione atriale e le valvulopatie. In questi contesti, l'intervento palliativo si articola attorno a quattro pilastri fondamentali: gestione dei sintomi, miglioramento della qualità di vita, supporto psicologico e spirituale, e assistenza nel percorso di lutto.

Fornire un'assistenza di questo tipo richiede al personale sanitario competenze specifiche: abilità comunicative, capacità decisionali condivise e una solida propensione al lavoro interdisciplinare. Infatti, l'approccio palliativo ottimale prevede la collaborazione di diverse figure professionali: clinici, specialisti palliativisti, farmacisti clinici, infermieri, psicologi e assistenti sociali, ciascuno con un ruolo ben definito nel prendersi cura del paziente nel suo insieme.

Tradizionalmente, le cure palliative venivano attivate in fase avanzata di malattia, quando il paziente presentava sintomi refrattari, ricoveri ricorrenti o difficoltà nella gestione domiciliare. Tuttavia, le nuove raccomandazioni dell'American Heart Association (AHA) propongono un cambiamento di paradigma: l'attivazione precoce delle cure palliative, già al momento della diagnosi di una patologia cardiovascolare avanzata, anche in fase di stabilità clinica.

Questo approccio proattivo consente di educare il paziente riguardo alla propria condizione, alle possibili evoluzioni e alle decisioni cliniche che potrebbe trovarsi ad affrontare nel tempo. In tal modo, le cure palliative diventano non solo uno strumento di accompagnamento nella fragilità, ma una parte integrante della medicina moderna, orientata al benessere complessivo della persona.

# 39° CONGRESSO NAZIONALE S.I.S.A.

ROMA, 23/25 NOVEMBRE 2025



SOCIETÀ ITALIANA  
PER LO STUDIO  
DELL'ATEROSCLEROSI



## RIASSUNTO DELLE COMUNICAZIONI PRESENTATE AL 39° CONGRESSO NAZIONALE S.I.S.A.

### COMUNICAZIONI ORALI

#### THE MITOCHONDRIAL ENZYME CARBAMOYL PHOSPHATE SYNTHETASE 1 MODULATES LDLR EXPRESSION: INSIGHTS FROM *IN VIVO* AND *IN VITRO* STUDIES

Maria Giovanna Lupo<sup>1</sup>, Giorgia Marodin<sup>2</sup>, Ilaria Rossi<sup>3</sup>,  
Lorenzo Da Dalt<sup>4</sup>, Martina La Spina<sup>5</sup>, Cinzia Franchin<sup>5</sup>,  
Andrea Rasola<sup>6</sup>, Giorgio Arrigoni<sup>5</sup>, Gerald S. Lipshutz<sup>6</sup>,  
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**Background and Aim:** LDL receptor (LDLR) is the main regulator of LDL-cholesterol plasma levels. Our aim was to identify proteins associated with differently expressed levels of LDLR.

**Methods:** HuH7 cells were incubated with fluo-LDLs and sorted by FACS in 2 subpopulations expressing high and low levels of LDLR. The two identified populations were challenged with 10% or 0.4%FBS and proteomic analysis was performed. CPS1, a mitochondrial protein catalyzing the first and step of the urea cycle, has been found differentially expressed in the two FBS culturing conditions and has been knocked-down by siRNA in HuH7 cells. HuH7 cells were challenged with increasing concentrations of simvastatin and CPS1 mRNA levels were evaluated. In the same cellular system, LDLR was silenced as well. RT-qPCR and WB assays were performed to check for CPS1 (and other urea cycle enzymes) expression upon LDLR silencing, and for LDLR (and LDLR-turnover related proteins) expression upon CPS1 silencing. Livers mRNAs samples from adult Ldlr KO and Pcsk9 KO mice were checked for Cps1 expression levels vs WT, while livers mRNA samples from newborn Cps1 KO mice were checked for Ldlr, Pcsk9, ApoB, Vldlr, and Fasn expression levels vs matched WT.

**Results:** In HuH7 High cells vs Low cells proteomic analysis, Gene-Ontology search of DEPs revealed an enrichment in mitochondrial proteins and mitochondrial (or mitochondria-related) pathways among the upregulated proteins, with an increase at 0.4%FBS. CPS1 was the most upregulated mitochondrial protein among the upregulated proteins both in standard culturing conditions (10%FBS) and in nutrients-deficient conditions (0.4%FBS), in the latter being more stressed the upregulation compared to the former, suggesting a SREBP-dependent modulation as for cholesterol-related proteins. The positive modulation of simvastatin on CPS1 expression confirmed this hypothesis. By silencing LDLR mRNA in HuH7 we didn't observe any significant changes in CPS1 mRNA and protein levels, data confirmed in Ldlr KO and Pcsk9 KO mice livers. In addition, the other enzymes involved in

the urea cycle were not affected by LDLR silencing in HuH7 cells. Conversely, by knocking-down CPS1 in HuH7 cells we observed a significant decrease in LDLR mRNA (-42%, p<0.01) and protein (-57%, p<0.01) levels, as well as a significant decrease in PCSK9 mRNA levels (-40%, p<0.05) and a non-significant decrease in PCSK9 protein levels (-20%), and a significant decrease in HMG-CoA reductase (-65%, p<0.05) and SREBP-2 (-60%, p<0.05) mRNAs levels. These changes in LDLR and LDLR-related modulators were confirmed *in vivo* on mRNA livers samples from newborn Cps1 KO mice, where Ldlr transcript levels significantly dropped down about 99% compared to WT (p<0.05), and Pcsk9, Fasn, ApoB, and Vldlr were significantly decreased of 99% (p<0.05), 98% (p<0.001), 97% (p<0.05), and 90% (p<0.001), respectively.

**Discussion and Conclusions:** Our data show a strong modulation of LDLR by the mitochondrial matrix protein CPS1, paving the way to new possible therapeutic scenarios in cholesterol dysfunctional diseases and for atherosclerosis, especially considering that GWAS identified CPS1 associated to serum lipid levels (PMID:31938413) and coronary artery disease (PMID:26822151).

#### GLP-1 RECEPTOR AGONISTS IN PERIPHERAL ARTERY DISEASE: SYSTEMATIC REVIEW, META-ANALYSIS, AND FUNCTIONAL EVIDENCE

Andrea Boccatonda<sup>1</sup>, Damiano D'Ardes<sup>2</sup>, Alice Brighenti<sup>1</sup>,  
Paola Simeone<sup>2</sup>, Maria Teresa Guagnano<sup>2</sup>, Francesca Santilli<sup>2</sup>,  
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**Background:** People with peripheral artery disease (PAD) face high risks of major adverse limb events (MALE) and cardiovascular events (MACE). We synthesized evidence on glucagon-like peptide-1 receptor agonists (GLP-1RA) in PAD.

**Methods:** We conducted a protocol-driven systematic review and meta-analysis of comparative studies (observational cohorts and randomized trials). We extracted adjusted hazard ratios (HRs) and pooled them using inverse-variance methods on the log-HR scale. Risk of bias was assessed with ROBINS-I for observational studies and RoB 2 for RCTs; certainty was graded with GRADE.

**Results:** Across three comparative cohorts, pooled HR for MALE was 0.56 (95% CI 0.50–0.62), indicating a 44% lower risk with incretin-based therapy versus non-use. Two cohorts contributed to MACE, yielding a pooled HR of 0.87 (95% CI 0.85–0.89), consistent with a modest cardiovascular benefit. Heterogeneity was moderate for MALE (Q=4.96; I<sup>2</sup>≈60%) and higher for MACE (Q=6.58; I<sup>2</sup>≈85%). Findings were robust in sensitivity analyses (random-effects vs fixed-effect; leave-one-out), and directionally consistent after excluding a surgical post-bypass cohort.

**Conclusions:** In adults with PAD, GLP-1RA therapy is associated with fewer limb events and a modest reduction in cardiovascular risk in observational cohorts, alongside clinically meaningful improvements in walking capacity, perfusion, and quality of life in an RCT. These findings support the therapeutic potential of GLP-1RA as part of comprehensive PAD care.

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 ABSTRACT
 

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**IMPACT OF INNER MITOCHONDRIAL PROTEIN ON HEPATIC LIPID METABOLISM AND ATHEROSCLEROSIS PROGRESSION**

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**Background/Aim:** Optic atrophy 1 (OPA1) is an inner mitochondrial membrane protein that regulates mitochondrial fusion, cristae structure, and organelle tethering. Through these functions, OPA1 influences energy homeostasis, sterol and bile acid biosynthesis, and lipid handling in the liver and extrahepatic tissues. Here, we investigated the impact of OPA1 modulation on hepatic metabolism, dietary lipid utilization, and atherosclerosis progression.

**Methods:** Mouse models with OPA1 overexpression (Opa1 Tg) or hepatocyte-specific OPA1 knockout (Opa1LKO) on LDLR KO background were fed with Western-type diet (WTD). Metabolic phenotyping included indirect calorimetry, insulin and glucose tolerance tests (ITT, GTT), lipid tolerance tests (LTT), histology, and multi-omics analyses (proteomics). The metabolic impact of OPA1 was also investigated on a non-LDLR KO background of mice on chow and High Fat Diet (HFD).

**Results:** OPA1 overexpression on LDLR KO background significantly increases hepatic steatosis, enhances cholesterol and lipoprotein production, resulting in higher circulating cholesterol levels. Conversely, Opa1LKO mice exhibited impaired bile acid conjugation, reduced dietary lipid absorption, and decreased circulating cholesterol, leading to protection from liver steatosis and improved glucose homeostasis. Importantly, Opa1 deficiency limited atherosclerotic plaque development, highlighting bile acid-mediated modulation of lipid metabolism as a key mechanism. In contrast, OPA1 overexpression promoted plaque formation, though plaques appeared more stable, likely due to OPA1-dependent effects on vascular cells, which displayed a more contractile rather than synthetic phenotype.

**Conclusion:** Both OPA1 overexpression and OPA1 hepatocyte deficiency models demonstrate that OPA1 is a central regulator of hepatic and systemic lipid metabolism. By controlling bile acid production, lipoprotein handling, and mitochondrial dynamics, OPA1 modulates not only liver steatosis but also the progression of atherosclerosis. These findings identify OPA1 as a potential therapeutic target for metabolic and cardiovascular diseases.

**Funding:** Supported by the European Atherosclerosis Society (EAS) with a competitive grant to Lorenzo Da Dalt (2023).

**SEX DIFFERENCES IN INFLAMMATORY BIOMARKERS DURING LONG-TERM EVOLOCUMAB THERAPY**

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**Introduction and Objectives:** Atherosclerotic cardiovascular disease remains a predominant cause of morbidity and mortality worldwide, driven by complex interactions between lipid metabolism and chronic inflammation. While Evolocumab, a proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor, is established in lowering low-density lipoprotein cholesterol (LDL-C) and cardiovascular risk, its long-term effects on inflammatory biomarkers – and potential sex-specific responses – are not fully understood. This study aimed to elucidate the impact of prolonged Evolocumab therapy on inflammation markers in a real-world cohort, focusing on sex-related differences.

**Methods:** We analyzed data from 202 hypercholesterolemic patients (111 men, 91 women) treated with Evolocumab for at least 36 months. Key inflammatory indices, including the monocyte-to-high-density lipoprotein cholesterol ratio (MHR) and platelet-to-monocyte ratio (PMR), were assessed longitudinally alongside traditional lipid parameters.

**Results:** Significant sex-related differences emerged in inflammatory profiles: men exhibited consistently higher MHR levels at baseline ( $p=0.010$ ) and throughout follow-up ( $p<0.001$ ), whereas women showed persistently elevated PMR values ( $p<0.001$ ). Intriguingly, a strong inverse correlation was observed between lymphocyte count and lipoprotein (a) levels in women ( $r_s=-0.885$ ,  $p<0.001$ ), a pattern absent in men, suggesting distinct immunometabolic mechanisms.

**Conclusions:** Our findings reveal pronounced biological sex differences in inflammatory responses to long-term Evolocumab therapy, highlighting the need to incorporate sex-specific considerations in cardiovascular risk management and treatment monitoring. These novel insights pave the way for personalized therapeutic strategies and call for further investigation into the clinical significance of inflammation in lipid-lowering treatment outcomes.

## ABSTRACT

## SENESCENT VASCULAR SMOOTH MUSCLE CELLS PROMOTE INTRAPLAQUE ANGIOGENESIS VIA SASP: UNCOVERING THE VEGFA-VEGFR2 AXIS AS A THERAPEUTIC TARGET

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**Introduction:** Atherosclerosis, the leading cause of cardiovascular disease, progresses from fatty streaks to unstable plaques that may rupture, triggering thrombotic events. A hallmark of plaque instability is intraplaque angiogenesis, the formation of fragile, leaky neovessels that promote, inflammation, plaque growth and hemorrhage. While hypoxia is a known trigger, additional pro-angiogenic mechanisms remain unclear. As an age-related disease, atherosclerosis is characterized by the accumulation of senescent vascular smooth muscle cells (VSMCs), which, despite being non-proliferative, are metabolically active and adopt a senescence-associated secretory phenotype (SASP) that promotes chronic inflammation and tissue remodeling. Although the detrimental roles of senescent VSMCs in atherosclerosis are increasingly recognized, it remains unexplored whether SASP secreted by senescent VSMCs could stimulate intraplaque angiogenesis and subsequent haemorrhage.

**Aim:** We aim to investigate the interplay between VSMC senescence, SASP and intraplaque angiogenesis. Moreover, we want to evaluate the effect of simvastatin on this process, and test whether targeting specific SASP-related pathways may represent more effective therapeutic strategies.

**Results:** Re-analysis of publicly available bulk RNASeq datasets of human carotid atherosclerosis revealed that senescence markers such as CDKN1A, CDKN2A and GIL1 were significantly upregulated in unstable versus stable plaques. Interestingly, by using immunohistochemistry, we found that senescent VSMCs were significantly more abundant in human unstable carotid atherosclerotic plaques than in stable ones. To investigate further, we developed two in vitro models of senescent VSMCs: terminal replicative (or old cells) and doxorubicin-induced (or stress-induced). Surprisingly, we found that both in vitro senescent models produced significantly more pro-angiogenic molecules (like VEGFa) compared young control cells. Next, we exposed HUVECs endothelial cells to senescent VSMC-conditioned media. This led to increased endothelial proliferation, migration, and tube formation compared to conditioned media from young VSMCs. Since the majority of patients with atherosclerosis are on statin therapy, we next investigated whether statins influence the composition of the SASP secreted by senescent VSMCs. Notably, simvastatin-treated senescent VSMCs secreted significantly less pro-angiogenic factors, especially VEGFa, when compared to control. Treatment

of HUVECs with conditioned medium from simvastatin-treated senescent VSMCs resulted in decreased angiogenic response. While statins can attenuate the pro-angiogenic effects of senescent VSMCs in vitro, intraplaque angiogenesis persists in patients undergoing carotid endarterectomy despite chronic statin therapy, underscoring the need for novel therapeutic strategies. Therefore, since our results showed an increase in VEGFA production by senescent VSMCs, we blocked VEGFR2 in HUVECs, to test whether VEGFA was a key mediator of the observed pro-angiogenic effect. Blocking VEGFR2 suppressed the senescent VSMCs SASP pro-angiogenic effects (migration, proliferation and tube formation), confirming VEGFA/VEGFR2 as key pro-angiogenic trigger.

**Conclusions:** Overall, our findings show that VSMC senescence actively contributes to plaque progression and instability, mainly through a pro-angiogenic secretory phenotype. While simvastatin, reduces pro-angiogenic activity in vitro, it does not fully suppress it in vivo, as intraplaque angiogenesis persists in statin-treated patients. By identifying VEGF/VEGFR2 as a central mediator of this process, our study underscores the need for therapeutic strategies beyond lipid-lowering agents to effectively target vascular senescence and its pathological consequences.

## LP(a) AS AN INDEPENDENT PREDICTOR OF PREMATURE CARDIOVASCULAR RISK IN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLAEMIA

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**Background:** Lipoprotein(a) [Lp(a)] is an independent, genetically determined cardiovascular risk factor. Its role in paediatric risk stratification, especially in familial hypercholesterolaemia (FH), remains incompletely defined.

**Methods:** We retrospectively analysed 451 children and adolescents (aged 2–19 years; 49.1% males) with clinical or genetic diagnosis of FH, followed at the Paediatric Dyslipidaemia Centre, San Paolo Hospital, Milan. Clinical, anthropometric, biochemical, and genetic data were collected. Plasma Lp(a) was measured immunometrically. Family history of premature cardiovascular disease (pCVD) was defined as myocardial infarction or ischemic stroke in first- or second-degree relatives <55 years (men) or <60 years (women). Associations between Lp(a) and pCVD were assessed by univariate and multivariate logistic regression analyses.

**Results:** Lp(a) distribution was highly skewed, with the 80th percentile at 52 mg/dl. A positive family history for pCVD was reported in 43% of children. Mean Lp(a) levels were significantly higher in those with positive vs negative family history (46.2±39.6 vs 17.6±22.6 mg/dl, p<0.05). Very high Lp(a) (≥80 mg/dl) was more frequent in patients with positive family history (8.2% vs 1.6%). Multivariate regression confirmed elevated Lp(a) (>30 mg/dl) as an independent predictor of pCVD (OR 8.7; 95%CI 5.5–13.6; p<0.01), regardless of sex, age, anthropometric or lipid profile variables. No significant correlations were found between Lp(a) and LDL-C or other lipid parameters.

**Conclusions:** In paediatric FH patients, elevated Lp(a) levels are strongly associated with a family history of premature cardiovascular disease. Lp(a) measurement in childhood may enhance cardiovascular risk stratification and support early preventive strategies, including reverse cascade screening in relatives.

## ABSTRACT

## LIPID PROFILE AND MANAGEMENT OF DYSLIPIDEMIA IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE: EXPERIENCE FROM THE CENTER OF LECCE

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Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are associated with an increased cardiovascular risk. Epidemiological studies have shown that patients with IBD have a higher prevalence of atherosclerotic disease and an increased risk of arterial ischemic events, particularly myocardial infarction (1-3).

The pathophysiological mechanisms underlying this increased risk are multifactorial. Chronic systemic inflammation contributes to endothelial dysfunction and accelerates atherosclerosis. Moreover, infection and inflammation can alter lipoprotein metabolism, leading to changes in plasma lipid concentrations. Corticosteroids, commonly used in IBD, are associated with dyslipidemia (5), while tofacitinib has been shown to increase lipid levels. In contrast, anti-TNF $\alpha$  agents may have a more favorable metabolic profile (4). Despite these associations, evidence on lipid profiles in IBD remains scarce.

We present our clinical experience with a small cohort of IBD patients, aiming to highlight the need for structured cardiovascular risk management in this population. This is a descriptive, single-center case series of seven consecutive IBD patients referred to the Lipid Clinic of Lecce.

The cohort included 7 patients, mean age 54 years. 3 had CD (2 men, 1 woman), and 4 had UC (3 men, 1 woman). At referral, 6 patients were receiving biologic therapy: 2 adalimumab, 1 ustekinumab, 1 tofacitinib, and 2 upadacitinib. One patient discontinued tofacitinib due to intolerance. All patients were on mesalazine.

At baseline, none of the patients were at LDL-C targets recommended by ESC/EAS guidelines. The mean LDL-C was 138 mg/dL and the mean triglyceride was 207 mg/dL. Four patients were started on statin therapy, two received bempedoic acid due to statin intolerance and one patient with isolated hypertriglyceridemia was successfully treated with omega-3 fatty acids. Lipid levels improved in all treated patients. No patient required therapy with PCSK9 inhibitors or inclisiran, having achieved the target.

Our little experience confirms that dyslipidemia is frequent and insufficiently controlled in IBD patients. The observed elevation in LDL-C and triglycerides reflects both the inflammatory burden of IBD and the potential metabolic impact of therapies. Dedicated screening programs and tailored lipid-lowering strategies should be integrated into long-term management of IBD.

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## A NOVEL CASE OF FAMILIAL COMBINED HYPOLIPIDEMIA DUE TO A MUTATION OF ANGPTL3 GENE

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**Background:** Loss-of-function (LOF) variants of the ANGPTL3 gene lead to reduced plasma lipids in mice through increased activity of lipoprotein lipase and endothelial lipase. In humans, ANGPTL3 deficiency caused by homozygous LOF variants results in profound hypolipidemia involving all major lipoproteins, a condition termed familial combined hypolipidemia (FHL2), which is not typically associated with overt clinical manifestations.

**Aim:** To report a new case of FHL2 due to ANGPTL3 deficiency.

**Methods:** The proband is a 27-year-old male (BMI 22.4 kg/m<sup>2</sup>), with unremarkable clinical history except for tonsillectomy. Lipid profile: TC 65 mg/dl, HDL-C 29 mg/dl, TG 27 mg/dl, LDL-C 31 mg/dl, ApoA-I 47 mg/dl, ApoB 36 mg/dl. Liver ultrasound showed no evidence of steatosis. Relatives had normal lipid profiles. Next-generation sequencing (NGS) was performed using the Esoma Esteso WES\_v2 kit (Sophia Genetics) on the Nova-seq (Illumina) platform, targeting ABCA1, ANGPTL3, APOA1, APOB, MTTP, and PCSK9.

**Results:** No mutations were identified in candidate genes for hypobeta- or hypoalpha-lipoproteinemia. The proband was homozygous for ANGPTL3 LOF mutations (p.I19LfsX22/p.N147X), previously reported by Pisciotta L. et al. (*Circ Cardiovasc Genet* 2012; 5:42-50). This variant is listed in dbSNP (rs398122987, frequency 0.015%) and in ClinVar (VCV000091865.3) and Franklin as pathogenic.

**Conclusions:** This case confirms that complete ANGPTL3 deficiency is associated with recessive hypolipidemia characterized by reduced ApoB and ApoA-I levels. The absence of hepatic steatosis or clinical symptoms supports previous observations that FHL2 is a benign biochemical phenotype with potential relevance for cardiovascular protection.

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**ABSTRACT**


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**GLIFLOZINES AS ADD-ON TO ARNI IN ECHOCARDIOGRAPHIC, SARCOPENIC AND OXIDATIVE STRESS PARAMETERS IN ELDERLY PATIENTS WITH CHRONIC HEART FAILURE**

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Sarcopenia, defined as a progressive and generalized skeletal muscle disorder associated with increased risk of adverse outcomes including falls, fractures, and mortality, is commonly observed in patients with heart failure (HF). Sarcopenia significantly influences HF progression and has been linked to increased risk of all-cause mortality and major adverse cardiovascular events. Sodium-glucose co-transporter 2 inhibitors (SGLT2i), initially developed for type 2 diabetes mellitus treatment, demonstrated protective effects against HF-related events and cardiovascular mortality. This study aimed to evaluate the effects of add-on SGLT2i therapy for 12 months in elderly patients with HF<sub>rEF</sub> and sarcopenia already treated with sacubitril/valsartan (ARNI) on clinical, echocardiographic, laboratory parameters, functional abilities, muscle performance, and quality of life. We enrolled 147 outpatients (131 males, 16 females; mean age 72.5±6.9 years). All patients had diagnoses of sarcopenia and HF<sub>rEF</sub> according to ESC guidelines and were treated with sacubitril/valsartan for at least 12 months at baseline. Patients received dapagliflozin or empagliflozin 10 mg/day according to guideline recommendations. Comprehensive evaluations were performed at baseline and after 12 months, including clinical assessment, laboratory tests, electrocardiography, and color Doppler echocardiography. Sarcopenia was assessed using SARC-F questionnaire, handgrip strength test, and appendicular skeletal muscle mass evaluation. Comprehensive geriatric assessment included Mini-Mental State Examination (MMSE), Activities of Daily Living (ADL), Instrumental Activities of Daily Living (IADL), Geriatric Depression Scale (GDS), and Short Physical Performance Battery (SPPB). Quality of life was evaluated using Minnesota Living with Heart Failure Questionnaire and Kansas City Cardiomyopathy Questionnaire. Echocardiographic parameters were assessed using advanced techniques including speckle tracking for global longitudinal strain (GLS) evaluation. After 12 months of SGLT2i treatment, significant improvements were observed across multiple parameters. Clinical improvements included enhanced inflammatory profile with decreased high-sensitivity C-reactive protein (p<0.0001) and uric acid levels (p<0.0001). Oxidative stress parameters significantly decreased: NOX-2 (p<0.0001) and 8-isoprostane (p<0.0001). Platelet activation biomarkers also improved: sP-selectin (p<0.0001) and glycoprotein-VI (p<0.0001). Echocardiographic parameters demonstrated significant improvements including increased left ventricular ejection fraction (LVEF) (p<0.0001), cardiac index (p<0.0001), and global longitudinal strain p<0.0001). Functional capacity significantly improved as demonstrated by enhanced MMSE scores (p<0.0001), SPPB (p<0.0001), and quality of life measures. Linear regression analysis revealed that Δ8-isoprostane was the major predictor of ΔCI, accounting for 13.3% of its variation, while Δ8-isoprostane was the main predictor of ΔSPPB, justifying 54.6% of its variation.

This study demonstrates that adding SGLT2i therapy to sacubitril/valsartan treatment in elderly patients with HF<sub>rEF</sub> and sarcopenia results in comprehensive improvements across clinical, hemodynamic, and functional parameters. The significant reductions in oxidative stress and platelet activation biomarkers, coupled with improvements in cardiac function, sarcopenia-related parameters, and quality of life measures, highlight the synergistic therapeutic benefits of this combination therapy. The strong predictive relationship between oxidative stress reduction and improvements in both cardiac index and physical performance suggests that SGLT2i may exert beneficial effects through antioxidant mechanisms. These findings support the clinical utility of SGLT2i as add-on therapy in this vulnerable patient population, potentially addressing both cardiac dysfunction and sarcopenia simultaneously.

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**MAGNESIUM DEPLETION SCORE IS ASSOCIATED WITH ARTERIAL STIFFNESS: DATA FROM THE BRISIGHELLA HEART STUDY**

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**Background and Aims:** The Magnesium Depletion Score (MDS) estimates magnesium deficiency risk by integrating dietary intake and physiological losses. This study evaluated the association between MDS and arterial stiffness in a rural Mediterranean population.

**Methods:** We analyzed data from 2,048 participants (49.2% men, 50.8% women) in the Brisighella Heart Study. MDS and arterial stiffness parameters – Augmentation Index (AIx) and carotid-femoral Pulse Wave Velocity (cfPWV) – were assessed using validated methods. Multiple regression models adjusted for age and mean arterial pressure included sex, smoking, physical activity, BMI, heart rate, fasting glucose, LDL-C, triglycerides, serum uric acid, eGFR, and MDS.

**Results:** An MDS ≥2 was observed in 51.6% of participants, more often in men (p<0.001). Higher MDS was significantly associated with increased AIx and cfPWV in both sexes (p < 0.001). MDS remained an independent predictor of AIx (β=0.087, p=0.011) and cfPWV (β=0.131, p=0.013) after adjustment.

**Conclusion:** Higher MDS values correlate with greater arterial stiffness, suggesting that magnesium imbalance may negatively affect vascular health.

**ABSTRACT**
**THE GENETIC SPECTRUM OF FAMILIAL HYPERCHOLESTEROLEMIA: INSIGHTS FROM A 57-GENE PANEL MUTATIONAL ANALYSIS IN AN ITALIAN REFERRAL CENTER**

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**Background:** Familial Hypercholesterolemia (FH) is an autosomal disorder predominantly linked to pathogenic variants in four key genes: LDLR, APOB, PCSK9, and LDLRAP1. Loss-of-function mutations in LDLR account for 60% to 80% of cases, while alterations in APOB and PCSK9 are responsible for about 5%-10% and 1% of cases, respectively. Many definite FH (20-40%) or possible/probable/definite FH subjects (about 60%) did not demonstrate functional mutations in major candidate genes.

A polygenic origin has been proposed, as studies have shown that multiple genetic variants beside major FH genes, when present together, could modulate severity and presentation of FH phenotype. To better characterize the genetic basis of FH in such patients, we performed targeted high-throughput sequencing (HTS).

**Methods:** We analyzed 177 subjects diagnosed with possible, probable, or definite FH according to the widely used Dutch Lipid Clinic Network Score (DLCN) diagnostic algorithm. Targeted HTS was performed using a 57-gene panel via Illumina MiSeq technology. This panel included genes involved in lipid metabolism, dyslipidaemias, lipid-lowering drug pharmacogenetics, polygenic forms of FH. Variants identified were classified according to American College of Medical Genetics (ACMG) criteria (2015).

**Results:** We identified 72 rare variants in the LDLR gene across 67 individuals. Sixty of them had shown uncertain significance (VUS)/likely pathogenic (LP)/pathogenic (P) variants and we considered them as LDLR-positive. The remaining 117 patients were LDLR-negative, including 110 with no LDLR variants and 7 carrying benign (B) or likely benign (LB) variants. When comparing the clinical characteristics of the two groups, individuals with LDLR-positive mutations showed significantly higher levels of total cholesterol and LDL [Total: 306.00 (276.50-354.50) vs 287.00 (246.00-322.00); p=0.012; LDL-C: 229.00 (197.75-263.75) vs 205.00 (180.25-243.00); p=0.008], as well as a higher average Dutch score [6.00 (4.00-7.00) vs 4.00 (4.00-5.00); p=0.00001] compared to LDLR-negative subjects.

Among LDLR-positive patients, only 6 had variants exclusively in LDLR while 54 patients harbored at least one rare variant in other genes within the panel, of whom 15 had B/LB/VUS variants in the other major FH genes (APOB, PCSK9, LDLRAP1). Among LDLR-negative patients, a high proportion carried multiple rare variants: 71.8% had at least two and 52.1% had three or more rare variants across the 56-gene panel. Specifically, 36 rare variants were found in APOB, 4 in PCSK9 and 4 in LDLRAP1 alongside 270 in 46 genes of remaining 53. Patients with variants in both LDLR and other genes exhibited a trend of higher LDL-cholesterol levels compared to those with variants solely in LDLR [230.5 (200.8-265.3) vs 195.5 (169.0-229.5); p=0.087]. Focusing on genes mainly associated to monogenic and polygenic FH (APOB, PCSK9, LDLRAP1, APOE, ABCG5, ABCG8, CELSR2,

HFE, MYLIP, SLC22A1, NYNRIN e ST3GAL4) out of 57 gene panel, patients with variants in both LDLR and at least two other genes exhibited a higher DLCN score compared to other LDLR-positive patients [8 (6-9) vs 6 (4-7), p=0.036].

**Conclusions:** These findings highlight the complexity of FH genetics, suggesting the possible contribution of rare genetic variants burden in modulation of clinical phenotype also in LDLR+ monogenic FH.

**A FRAMESHIFT APOA5 VARIANT IN A PATIENT WITH SEVERE HYPERTRIGLYCERIDEMIA: CLINICAL AND MOLECULAR CHARACTERIZATION**

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**Introduction:** Hypertriglyceridemia (HTG) is a prevalent form of dyslipidemia that is strongly associated with an increased risk of cardiovascular disease and pancreatitis. Current guidelines define normal fasting plasma TG levels as <1.7 mmol/L (150 mg/dL), while levels ranging from 2 to 10 mmol/L (175-885 mg/dL) and >10 mmol/L (885 mg/dL) classify mild-to-moderate and severe hypertriglyceridemia, respectively. Very severe HTG, defined as TG levels >20 mmol/L (>1770 mg/dL), is extremely rare, with a prevalence of only 0.014%. Autosomal recessive monogenic forms typically involve loss-of-function mutations in key genes that regulate triglyceride metabolism, such as LPL, APOC2, APOA5, GPIIIBP1, and LMF1.

**Materials and Methods:** We report a case of a 44-year-old man with a family history of dyslipidemia, Crohn's disease, and atrial fibrillation. Laboratory tests revealed fluctuating triglyceride levels ranging from 400 to 1000 mg/dL (peak values ~789-1000 mg/dL), total cholesterol ~184 mg/dL, HDL-C 33 mg/dL, and no documented episodes of pancreatitis. Targeted next-generation sequencing (NGS) was employed to analyze the coding regions and intron-exon junctions of genes implicated in the synthesis and metabolic pathways of triglycerides. Molecular and genetic analyses identified APOA5: c.427delC (p.Arg143AlafsTer57) in heterozygosis, a loss-of-function frameshift variant (Pathogenic/Likely Pathogenic) and ANGPTL8: c.13G>A (p.Ala5Thr) in heterozygosis, a missense variant with MAF ~0.0048, classified as likely benign/modifier.

**Results and conclusion:** The APOA5 frameshift variant likely accounts for the observed severe lipid phenotype. Although ANGPTL8 p.Ala5Thr is not considered pathogenic, it may act as a modifier of triglyceride metabolism in the presence of APOA5 deficiency, comorbidities, and lifestyle factors. Clinical implications include the need for close monitoring, prevention of pancreatitis, and optimization of pharmacological and dietary management, with potential consideration of emerging therapies. This case highlights the importance of genetic testing in patients with severe HTG, allowing the identification of pathogenic variants (APOA5) and possible modifying alleles (ANGPTL8). A molecular diagnosis can guide risk stratification and support a more personalized therapeutic approach.

## ABSTRACT

## LIPIDOMIC ANALYSIS OF FH PATIENTS WITH AND WITHOUT PATHOGENIC VARIANTS: SPHINGOMYELINS AS POTENTIAL CARDIOVASCULAR RISK FACTORS

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**Introduction:** Familial Hypercholesterolemia (FH) is a disease mainly caused by pathogenic variants in three genes (LDLR, APOB and PCSK9). It is characterized by high levels of LDL-cholesterol (LDL-c) and increased cardiovascular risk. Despite pathogenic variants in these genes are a well-established cause, not all patients with clinical suspicion of FH carry either pathogenic or uncertain significance variants (USV) in the main causative genes (FH/V-/USV-). We aim to evaluate the lipidomic and metabolomic differences between heterozygous FH patients (HeFH) compared to FH/V-/USV- ones and to healthy controls. **Methods:** Genetic characterization of 39 FH patients was performed by NGS to identify FH-causative variants (Devyser's FH kit). Untargeted high-resolution mass spectrometry (UHPLC-Q-Exactive-MS)-based lipidomics and nuclear magnetic resonance-based metabolomics were performed on plasma samples of FH patients (20 HeFH and 19 FH/V-/USV-) and controls (n=22).

**Results:** Multivariate analysis (PLS-DA) revealed group-level separation, suggesting differences in the circulating lipidome between HeFH, FH/V-/USV- and controls. Most of the identified lipid classes were higher in both FH groups compared to controls. Several lipids showed differences between HeFH and FH/V-/USV- patients, particularly sphingomyelins (SM). Among the SM significantly different between HeFH and FH/V-/USV- patients, several showed correlations with biochemical data in both FH groups. In particular, SM(d44:4) and SM(d32:0) were strongly and positively correlated with LDL-c (respectively,  $r=0.79$  and  $0.81$ ;  $p<0.001$ ) in FH/V-/USV- patients. In HeFH patients, a similar correlation coefficient was observed only for SM(d44:4) ( $r=0.84$ ;  $p<0.001$ ), whereas a lower correlation was observed for SM(d32:0) ( $r=0.52$ ;  $p<0.001$ ).

Multivariable regression analysis, adjusted for age, sex and lipid-lowering therapy, confirmed the association of several SM with the presence of a pathogenic variant. ROC curve analysis demonstrated that all SM species had AUC values greater than 0.7, confirming the discriminatory power of these lipid species. Only trends of difference in metabolite levels were observed between the two FH groups.

**Conclusions:** SM are increased in both FH groups compared to controls. Furthermore, their levels are higher in HeFH patients than in FH/V-/USV- ones, showing the importance of SM metabolism in presence of pathogenic variants in FH-causative genes. Due to the recent evidence of SM atherogenic role (1), our results can provide an explanation of the increased cardio-

vascular risk of FH patients with pathogenic variants, independently from LDL-c levels (2). The identified signature could improve risk stratification and personalized therapeutic strategies.

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## ANGPTL3 AND PCSK9 LEVELS REGULATE THE CELLULAR SWITCH BETWEEN LIPOLYSIS AND LIPOGENESIS THROUGH STAT3

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**Background and Aim:** ANGPTL3 and PCSK9 are known to form a complex in response to nutrients. Our group has previously proved that the genetic overexpression of ANGPTL3 and PCSK9 modulates the uptake and secretion of lipoproteins and impacts cellular lipid accumulation. In this project, we searched for intracellular pathways that determine the modulation in lipid accumulation in response to the modulation of ANGPTL3 or PCSK9 levels.

**Methods:** We used a liver cellular model (HepG2). HepG2 cells were transfected with ANGPTL3 or PCSK9 overexpressing plasmid and ANGPTL3 or PCSK9 siRNA. Two different proteome profilers (intracellular kinase and tyrosine kinase receptors) were used to identify the intracellular activated pathways in the different conditions of ANGPTL3 and PCSK9 expression modulation. Chromatin immunoprecipitation (ChIP) for the identified activated STAT1 and STAT3 were performed for key genes in lipogenesis (ACACA; ACLY; ELOVL5) and lipolysis (CPT1a; CPS1; GPD2).

**Results:** At the proteome profiler, ANGPTL3 OE cells show an activation of the IGF-1 receptor and PLC-gamma and STAT3. PCSK9 OE cells show the activation of different TRK receptors and the intracellular activation of STAT1. STAT1 and STAT3 were selected as targets for ChIP enrichment analysis for selected genes of lipolysis and lipogenesis. ANGPTL3 siRNA determines an 80-fold enrichment for CPS1 and GPD2 for both STAT1 and STAT3; ANGPTL3 OE activates STAT3 but is not enriched in the investigated genes. PCSK9 siRNA determines a 50-fold enrichment for ACACA for only STAT3; PCSK9 OE activates STAT1 predominantly and enriches the ELOVL5 gene consistently.

**Conclusion:** The balance between ANGPTL3 and PCSK9 levels is crucial for the switch between lipolysis and lipogenesis through STAT3. The downregulation of ANGPTL3 activates STAT3 and determines the expression of lipolytic genes, whereas, the downregulation of PCSK9 activates lipogenesis.

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**ABSTRACT**


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**COMPARISON OF POSTPRANDIAL LIPOPROTEIN METABOLISM IN PATIENTS WITH METABOLICALLY AND GENETICALLY-DRIVEN NAFLD**

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**Introduction:** Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent condition characterized by hepatic fat accumulation and an increased risk of cardiovascular disease. This risk may vary by disease etiology, with metabolic and genetic forms showing distinct pathophysiological features. The I148M PNP-*LA3* variant is the strongest genetic determinant of NAFLD, acting independently of metabolic risk factors. Postprandial lipid levels are key determinants of both NAFLD and cardiovascular risk. During the postprandial period, changes in intestinally and hepatically derived TG-rich lipoproteins (TRLs) can be measured in the circulation, providing a dynamic reflection of atherogenic lipoprotein fluxes. However, postprandial TRL measurements have never been compared between metabolic and genetic NAFLD.

**Project Aims:** To assess differences in TRL dynamics after an high-fat meal during the 8-hour postprandial phase in adults with metabolic versus genetic NAFLD.

**Materials and Methods:** This study included three groups: 4 subjects with PNP-*LA3* wild-type genotype, NAFLD and metabolic syndrome (group M), 7 blood donors carrying M148M PNP-*LA3* genotype with NAFLD (group G) and 6 blood donors, PNP-*LA3* wild-types and without NAFLD (controls). Diabetes and lipid-lowering therapy were exclusion criteria. All participants underwent clinical evaluation, anthropometry, and NAFLD assessment by ultrasound (US). Liver stiffness was measured by Acoustic Radiation Force Impulse (ARFI) elastography. Laboratory tests were performed at baseline and after the fat load; postprandial changes were quantified by 8h area under the curve (AUC) analysis.

**Results:** The three groups were matched for age. Compared with group G, group M showed higher adiposity (BMI 32.1±4.1 vs 24.9±2.0 kg/m<sup>2</sup>, P=0.02 and WC 113.5±5.0 vs 93.0±11.0 cm, P=0.01), fasting glucose (102.0±4.7 vs 89.8±10.2 mg/dL, P=0.05), remnant cholesterol (35.0±15.4 vs 19.0±4.8 mg/dL, P=0.04), and lower HDL-C (52.0±8.6 vs 65.5±10.2 mg/dL, P=0.058), consistent with a metabolic profile. Transaminases and steatosis severity were comparable between the metabolic and genetic groups, while ARFI values tended to be higher in group M (4.6±2.7 vs 2.8±2.2 Kpa, P=0.07). Controls resembled group G in most parameters. At baseline, total TG levels were highest in group M (160±41.4 mg/dL), intermediate in group G (103.0±17.4 mg/dL), and lowest in controls (70.1±12.6 mg/dL, P<0.01). After the fat load, group M showed a marked postprandial response in total TG, peaking at 319±85.6 mg/dL at 4h and remaining high at 8h (208.5±56.4 mg/dL). In contrast, Group G increased modestly, peaking at 206.3±92.6 mg/dL at 6h and returning to 128.4±40.7 mg/dL at 8h. Control subjects, showed the lowest curve, with TG levels remaining close to baseline (70-111 mg/dL) through-

out the 8h observation. Similar patterns were observed in TRL-TG concentrations across all groups and at all time points. These differences in magnitude and timing translated into a significantly reduced postprandial lipemic response in group G compared with group M, as reflected by lower AUCs for total TG (-39%, P=0.011), TRL-TG (-41%, P=0.01), and apoB100 (-21.3%, P=0.04).

**Conclusions:** Metabolic NAFLD was associated with an amplified postprandial lipemia, likely reflecting increased TRL production from insulin resistance and visceral adiposity. In contrast, genetic NAFLD showed an attenuated response, consistent with impaired VLDL secretion in PNP-*LA3*-driven steatosis. These preliminary results underscore the differential impact of the aetiology of NAFLD on postprandial lipid metabolism.

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**ETHNIC DIFFERENCES IN HDL QUANTITY, QUALITY, AND POTENTIAL ASSOCIATIONS WITH CORONARY HEART DISEASE RISK**

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**Background and Aims:** Ethnic differences in HDL cholesterol (HDL-c) may contribute to disparities in coronary heart disease (CHD) risk, but the clinical interpretation of HDL-c and its metabolomic subfractions across populations remains unclear.

**Methods:** We examined HDL-c quantity and quality in relation to ethnicity and CHD risk in UK Biobank. HDL-c and nine metabolomic HDL measures (reflecting particle size and lipid composition, considered proxies of HDL functionality) were analyzed in relation to CHD using Cox models stratified by ethnicity. Linear regression assessed differences in HDL-c by ethnicity. Models were sequentially adjusted for sociodemographic, clinical, adiposity, and physical activity variables.

**Results:** After adjustment, HDL-c concentrations were modestly lower in South Asian (-0.091 mmol/L) and Black (-0.025 mmol/L) participants compared to White participants. These differences were substantially attenuated after further adjustment for adiposity and physical activity. HDL-c was inversely associated with CHD across all ethnic groups, with the strongest association in South Asian (HR per SD: 0.40 [0.29–0.54]) compared with White participants (HR: 0.64 [0.61–0.67]). Several HDL subfractions – particularly large HDL particles and average HDL diameter – showed stronger inverse associations with CHD in South Asian participants compared to other ethnic groups.

**Conclusions:** Lower HDL-c concentrations in South Asian and Black vs White participants appear largely driven by differential activity and/or adiposity measures. In South Asian individuals, HDL quantity and quality measures may be more strongly predictive of risk, warranting further investigation into their clinical and biological utility in diverse populations.

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 ABSTRACT
 

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**ROLE OF DYSLIPIDEMIA IN ALAGILLE SYNDROME ON CARDIOVASCULAR AND RENAL OUTCOMES**

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Alagille syndrome (ALGS) is a rare, autosomal dominant disease characterized by abnormal development of intrahepatic bile ducts, heart, arteries and kidneys due to disrupted Notch signaling. Although hypercholesterolemia is a hallmark feature of ALGS, lipid and lipoprotein profiles have been studied only in small patient cohorts. The aim of the study is to characterize the lipid and lipoprotein profiles in ALGS and explore their relationship to disease phenotype with a particular focus on liver involvement, and to assess the impact of dyslipidemia on renal and cardiovascular outcomes. Twenty-five subjects affected by ALGS were enrolled with a median age of 13 years (IQR: 4.0–16.75), 20 patients with native livers and 5 liver transplant recipients. ALGS patients with native liver exhibited a distinctive lipid profile, characterized by high total cholesterol (TC, 42%), elevated free cholesterol (FC, 47.6%) and phospholipids (PL, 52%). Most patients also had elevated HDL-cholesterol (81%, median 68 mg/dl [54–115]) and LDL-cholesterol (LDL-C, 33%). LpX was identified in 62% of the cohort and was absent in patients without cholestasis ( $p < 0.001$ ). LpX-positive patients exhibited higher FC/TC ratios (0.29 vs. 0.24,  $p = 0.008$ ), and LpX presence was associated with markers of cholestatic liver disease. ALGS patients had IMT and PWV values exceeding the 95th percentile for age in 60% and 38% of cases, respectively. IMT and PWV values were not associated with dyslipidemia or LpX. The presence of LpX was not linked to proteinuria or chronic kidney disease. *In vitro*, plasma from LpX-positive patients induced significant podocyte necrosis ( $p = 0.03$ ). Plasma containing both LDL and LpX, further caused podocyte necrosis ( $p = 0.030$ ) and apoptosis ( $p = 0.050$ ), compared to LDL from LpX-negative patients. Additionally, LpX reduced the expression of NPHS2, encoding podocin ( $p = 0.005$ ). In conclusion the obtained results showed that ALGS patients present with a distinctive LpX-driven dyslipidemia, characterized by elevated LDL-C, FC, HDL-C and PL. This dyslipidemia reflects altered cholesterol homeostasis, likely due to increased FC release from the cholestatic liver and reduced LpX catabolism due to low LCAT activity. ALGS-associated dyslipidemia does not appear atherogenic. However, *in vitro* findings indicate a nephrotoxic role for LpX, highlighting its potential contribution to renal complications in ALGS.

**ANGPTL3 DEFICIENCY ALTERS HEPATIC METABOLISM AND FUNCTION UNDER FASTING SIGNALING**

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**Background and Aim:** ANGPTL3 deficiency causes hypolipidemia and enhances triglycerides fluxes from the circulation to the tissues during fasting and the postprandial phase due to constitutive lipases activation. This project aims to unravel the connection between hypolipidemia-derived metabolic alterations and possible hepatic or systemic responses according to substrate availability in ANGPTL3 deficiency.

**Methods:** Angptl3-deficient (KO) mice and WT littermates were fed chow diet for 16 weeks. Metabolic rates were assessed *in vivo* by indirect calorimetry and lipid, glucose, insulin and pyruvate/lactate tolerance tests and lipoprotein production assay were performed. Metabolomics, bulk RNA sequencing and spatial transcriptomics were performed *ex vivo*.

**Results:** Angptl3KO mice were hypolipidemic postprandially and after an oral fat load. Surprisingly, Angptl3KO mice showed lower rates of hepatic lipoprotein production 4 hours after Poloxamer injection, and this was not mirrored by an increase of hepatic or ectopic fat deposit. Glucose and insulin tolerance were not different, although Angptl3KO mice showed increased gluconeogenesis after injection of pyruvate/lactate. Data from indirect calorimetry in KO mice show a reduction in the Respiratory Exchange Ratio to a more aerobic oxidative metabolism, with a shift in substrate preference at the end of the postprandial phase. Metabolomics analysis on liver samples confirmed an hypercatabolic phenotype. This hepatic reprogramming was unravelled with a whole liver RNA sequencing, where Angptl3KO mice faced an upregulation of both anabolic and catabolic pathways related to fatty acids, cholesterol and glucose. This led to the hypothesis that Angptl3KO mice could exploit nutrients differently compared to controls. An altered substrate use was coupled to altered hepatocytes zonation in the liver lobule, as observed with spatial transcriptomics analysis.

**Conclusion:** Lack of ANGPTL3 exerts deep hepatic metabolic and functional reprogramming especially during fasting, that may, in turn, alter other organs and tissues metabolic functions.

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**ABSTRACT**


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**LIPOPROTEIN-BOUND PCSK9: STRUCTURAL AND FUNCTIONAL INSIGHTS**

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**Introduction:** Circulating PCSK9 interacts with the LDLR (LDL-Receptor) thus promoting its degradation and blunting the uptake of LDL from the circulation. In this context, anti-PCSK9 monoclonal antibodies (mAbs) and small interfering RNAs (siRNAs) are drugs approved for the treatment of hypercholesterolemia. We and others have demonstrated that a fraction of total circulating PCSK9 associates with LDL. The purpose of our research is to understand the biological basis of the PCSK9-lipoprotein interaction.

**Methods:** A three-layered iodixanol gradient was used to isolate lipoproteins fractions from patients' plasma before and after drug treatments. The lipoprotein composition was studied by spectrophotometric, lipidomic and proteomic approaches. The lipoprotein dimension was analyzed by TEM (Transmission Electron Microscope). PCSK9 subspecies and secretion mechanism were studied using Western Blot and cell culture techniques.

**Results:** Plasma LDL-C (LDL-Cholesterol) levels decreased from 103±50 to 42±17 mg/dL after siRNA treatment (n=20, p<0.05) and from 126±50 to 57±31 mg/dL after mAbs therapy (n=30, p<0.05). Circulating total PCSK9 decreased from 616±152 to 271±124 ng/mL after siRNA (n=30, p<0.05), while plasma PCSK9 levels increased from 503±180 to 4337±1245 ng/mL (n=20, p<0.05) after mAbs. Independent of the therapy, PCSK9-bound to LDL was on average 15% (n=50) and the same result was observed in control subjects. Immunoblot analysis demonstrated that PCSK9 binds to LDL with its active form. Lipidomic and LC-MS analyses showed that PCSK9 associates with a subfraction of LDL that has a lower density and contains higher amounts of ApoE, ApoCs and triglycerides than "classical" LDL (IDL-like). The TEM analysis of the dimension of the lipoprotein fractions bound with PCSK9 confirmed this observation.

**Conclusions:** Our study identified a LDL subfraction more buoyant than classical LDL (IDL-like lipoprotein) that is involved in binding circulating PCSK9. Our observations led us to hypothesize that the active form of PCSK9 may enter the bloodstream in association with VLDL, which are metabolized to IDL. Although the therapies significantly modify the total circulating levels of LDL and PCSK9, the percentage of PCSK9-bound LDL remains rather constant. This finding suggests that the PCSK9-Lipoprotein interaction is non-saturable. Ongoing experiments will determine whether the lipoprotein-bound form of PCSK9 modulates LDL receptor activity.

**INVESTIGATING PCSK9 AS A THERAPEUTIC TARGET IN ALZHEIMER'S DISEASE: EVIDENCE FROM PATIENT-DERIVED ASTROCYTES**

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a master regulator of lipid metabolism in the brain and has been implicated in Alzheimer's disease (AD). In this regard, increased PCSK9 levels have been found in cerebrospinal fluid (CSF) of AD patients. Beyond cholesterol homeostasis, it has also been demonstrated that PCSK9 contributes to apoptosis, neuroinflammation, and mitochondrial functions, all processes that drive AD pathology. This study aims to characterise PCSK9 expression in astrocytes derived from AD patients and to assess the impact of its pharmacological inhibition. Human astrocytes were differentiated from induced neural progenitor cells (iNPCs) reprogrammed from fibroblasts obtained from skin biopsies of AD patients and matched controls (CTRL). PCSK9 and its precursor pro-PCSK9 were evaluated by western blot (WB) analysis under basal and hypoxic conditions, the latter mimicking disease-related stress. Cells were also exposed to a newly synthesised PCSK9 inhibitor (MR-3), previously shown to inhibit PCSK9 in neuroblastoma cell lines. Effects on PCSK9 expression were evaluated by WB analysis, ATP production through colorimetric assay, and mitochondrial parameters including mitophagy, membrane potential, and the number of functional mitochondria, evaluated through images techniques. PCSK9 and pro-PCSK9 expression was significantly higher in AD-derived astrocytes compared to CTRL-derived astrocytes (+47%, p<0.05; +41%, p>0.05, respectively). Under hypoxia condition, a further, though not statistically significant, increase was detected only in the AD group. Treatment with MR-3 compound markedly reduced PCSK9 expression in AD lines (-85% vs basal condition, p<0.0001), but not in the CTRL lines (p>0.05). Moreover, incubation with PCSK9 inhibitor did not affect ATP levels, as index of the absence of compound toxicity, both in AD and CTRL astrocytes compared to the basal condition (p>0.05). Similarly, mitochondrial functionality assessment showed no significant differences in terms of mitophagy, membrane potential, or in the number of functional mitochondria across treatment groups (p>0.05). These preliminary findings indicate that PCSK9 is upregulated in AD astrocytes, corroborating its involvement in the disease pathogenesis. PCSK9 inhibitor MR-3, that resulted non-toxic at the tested concentration led to an effective and significant inhibition of PCSK9 expression, which, interestingly, was detected in AD-derived astrocytes. Further studies, elucidating the underpinning mechanisms, will be required to better define the role of PCSK9 as a therapeutic target in neurodegeneration.

## ABSTRACT

## NEW SMALL MOLECULES INHIBITORS OF PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) AS POTENTIAL PHARMACOLOGICAL TOOL IN ALZHEIMER'S DISEASE: *IN VITRO* STUDIES

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**Background and Aim:** The proprotein convertase subtilisin/kexin 9 (PCSK9), beyond regulating plasma cholesterol through the degradation of the hepatic LDL receptor (LDLR), is expressed in the brain, where it may play a role in the pathogenesis of Alzheimer's disease (AD). We previously demonstrated that PCSK9 negatively affects astrocyte-to-neuron cholesterol transport, enhances  $\beta$ -amyloid ( $A\beta$ )-induced neurotoxicity and the neuroinflammatory response in cerebral cell models. Consistently, PCSK9 ablation in AD mice improves cognitive function and reduces  $A\beta$  deposition and neuroinflammation. This study aims to investigate the potential protective effects of small, lipophilic PCSK9 inhibitors molecules on AD-related parameters in an in vitro model of cultured neurons.

**Methods:** Four compounds - MR3 and MR546 (chemotype 1) and MR533 and MR644 (chemotype 2), which were previously selected based on their ability to inhibit PCSK9 in HepG2 cells, were tested in human neuroblastoma IMR-32 cells and compared to a known PCSK9 inhibitor, 7030B-C5. PCSK9 expression/secretion (WB, ELISA), the expression of PCSK9 target receptors LDLR and LRP1 (WB), and  $A\beta$ -induced neurotoxicity (MTT assay) were evaluated. The anti-inflammatory effect was assessed by evaluating RelA/NF- $\kappa$ B p65 expression (WB).

**Results:** At the concentration of 10  $\mu$ M, MR3, MR546, and 7030B-C5 significantly reduced PCSK9 expression (MR3: -44%, MR546: -19%, 7030B-C5: -34%,  $p < 0.01$  vs basal) and secretion (MR3: -49%, MR546: -33%, 7030B-C5: -49%,  $p < 0.05$  vs basal). MR533 and MR644 only reduced PCSK9 secretion (MR533: -37%, MR644: -46%,  $p < 0.05$  vs basal) without affecting protein expression. All compounds increased LDLR expression (MR3: +20%, MR546: +9%, MR533: +26%, MR644: +19%, 7030B-C5: +26%  $p < 0.05$  vs basal), responsible for neuronal cholesterol uptake, while only MR3 and the reference compound significantly induced LRP1 expression (MR3: +7%, 7030B-C5: +15%,  $p < 0.01$  vs basal), responsible for neuronal  $A\beta$  clearance. All compounds significantly ameliorated  $A\beta$ -induced neurotoxicity (MR3: +10%, MR546: +7%, MR533: +17%, MR644: +16% and 7030B-C5: +22% vs  $A\beta$ -treated neurons,  $p < 0.05$ ) and reduced p65 activation (MR3: -27%, MR546: -16%, MR644: -12% and 7030B-C5: -40%,  $p < 0.0001$  and  $p < 0.01$  vs basal).

**Conclusion:** The pharmacological inhibition of PCSK9 using novel small, lipophilic molecules exerts a favourable impact on  $A\beta$ -induced neurotoxicity and neuroinflammation in vitro. This effect appears to be related to a reduction in PCSK9 secretion rather than the inhibition of its intracellular expression. Activity on the PCSK9 target receptors LDLR and LRP1 may positively impact neuronal cholesterol metabolism and  $A\beta$  clearance, respectively. These data could pave the way for further research into the pharmacological inhibition of PCSK9 as a novel, brain-directed strategy for counteracting AD.

## FUNCTIONAL CHARACTERIZATION OF VARIANTS OF UNCERTAIN SIGNIFICANCE (VUS) IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA (FH) USING A FLOW CYTOMETRY ASSAY

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**Background:** The definitive diagnosis of familial hypercholesterolemia (FH) is based upon the identification of variants in FH-related genes, mainly in the low-density lipoprotein receptor (LDLR) gene. However, many variants are of uncertain significance (VUS), whose functional evaluation is only based in silico prediction.

**Aim:** To assess the functional effect of LDLR VUS found in FH patients using a flow-cytometry based assay in autologous activated CD4<sup>+</sup> T-lymphocytes.

**Methods:** Twenty unique LDLR VUS identified in 36 FH patients were tested. In addition, six LDLR variants whose deleterious effect was well defined were included as positive controls. Patients' PBMCs were isolated and stimulated with  $\alpha$ CD3/ $\alpha$ CD28 conjugated microbeads in LPDS for 60 hours to achieve maximum up-regulation of LDLR. Quantification of cell surface LDLR expression, LDL binding and uptake was assessed by flow-cytometry using Bodipy-labeled LDL for the binding and uptake and using anti-LDLR mAb for the expression. Results were expressed as the ratio of the mean fluorescence intensity (gMFI) of patients' activated CD4<sup>+</sup> T-lymphocytes to gMFI of negative control cells. LDLR activity was also evaluated by in vitro validation by overexpressing LDLR with either the wild-type sequence or one of the five mutated versions in HEK293T cell lines. VUS were classified defective if they showed <85% of control activity in at least one of functional parameters, according to the ClinGen FH Expert Panel Specifications.

**Results:** Fifteen (75%) LDLR VUS showed deleterious effects in at least one of LDLR functional parameters while 5 variants showed normal expression, binding and uptake. The c.940\_940+14del, c.1007A>G, c.1530\_1532del and c.\*34C>T variants (n=4; 21.5%) disrupted the entire LDLR cycle, while others caused defects in LDLR expression (n=5; 35.7%) or binding/expression or binding/uptake (n=6; 42.8%). In vitro functional validation in HEK293T cells of c.367T>C, c.1007A>G, c.1530\_1532del, c.2282C>T and c.2479G>A variants demonstrated that all of them impaired receptor function, confirming flow cytometry results.

**Conclusions:** These results indicate that functional evaluation of LDLR VUS by using a flow cytometric assay could be very helpful to perform a conclusive diagnosis in a large percentage of FH patients carrying LDLR VUS. Therefore, this method could become part of the diagnostic workup of these patients.

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 ABSTRACT
 

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## PCSK9 KO MICE UNDER STANDARD OR UREMIC DIET REGIMENS SHOWED AN ACCUMULATION OF TRIGLYCERIDES IN KIDNEYS

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**Background and Aim:** Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) is a key regulator of cholesterolemia, primarily due to its ability to bind and send to irreversible degradation hepatic LDL receptors. It is also known that PCSK9 is expressed, albeit at different levels, in extrahepatic tissues, kidney included. Given these premises, the aim of this study is to investigate the potential local effects of PCSK9 under physiological and kidney damage conditions taking advantage of a Pcsk9 full knock-out (KO) mouse model.

**Methods:** Thirty-one wild-type (WT) and thirty-three KO mice were randomized to receive either a control diet (no phosphate, no adenine, 19% protein) or uremic diet (2% phosphate, 0.5% adenine, 9.5% proteins) for the first 5 weeks, upon which the mice on uremic diet were switched to high-phosphate diet (2% phosphate, no adenine, 9.5% proteins) for the last 5 weeks of treatment. At the time of sacrifice, blood, liver, and kidney samples were collected. Serum biochemical assays were performed to measure cholesterol and triglycerides. The tissues were used for histological analysis to assess kidney damage and lipid accumulation and for qualitative/quantitative lipidomics (analysis of fatty acid from total lipid extract, mass and composition of free fatty acid, triglycerides, cholesterol, cholesteryl esters) by using gas chromatography (n=4 each tissue). All lipidomics data were normalized both to tissue wet weight and to total protein content (BCA assay).

**Results:** The sera lipid profile revealed a significant reduction in cholesterol (CTRL diet (mg/dl): WT 79,44±15,85, KO 58,14±33,15. Uremic diet (mg/dl): WT 97,72±28,49 KO 66,8±20,09) and a significant increase in circulating triglycerides (CTRL diet (mg/dl): WT 60,57±33,31, KO 134,1±68,13. Uremic diet (mg/dl): WT 42,79±19,38 KO 88,33±39,77) in Pcsk9 KO mice, regardless of diet. Histological analysis on cortex and medulla renal specimens showed the presence of renal damage in mice fed with uremic diet, exacerbated in KO mice vs WT. Lipidomic analysis of the kidneys confirmed a significant accumulation of triglycerides in Pcsk9 KO mice, independent of dietary conditions. In contrast, hepatic triglyceride levels remained largely unchanged. We also analyzed total fatty acid, free fatty acid, free cholesterol, cholesterol ester in renal and liver tissue and found that they have remained largely unchanged between mice WT and KO in both diets.

**Discussion:** These findings suggest that PCSK9 may play a pivotal role in regulating renal lipid metabolism. Specifically, data indicate that the observed increase in circulating and, most intriguingly, renal triglycerides occur under both dietary conditions (standard and uremic), implying that this accumulation is associated with the absence of PCSK9, suggesting a potential

mechanistic link between PCSK9 deficiency and altered lipid handling in the kidney. To further investigate the mechanisms underlying biological processes responsible for this phenomenon, future experiments will focus on in vitro studies employing both human hepatic and human renal cell models to assess whether there will be differences between mice and human and how this could impact clinical patients.

## PALMITOLEIC ACID ATTENUATES PALMITATE-INDUCED LIPOTOXICITY AND INSULIN RESISTANCE IN HUMAN MYOTUBES

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**Background:** Palmitic acid (PA), a dietary saturated fatty acid largely consumed in Western diet, is known to induce lipotoxicity, oxidative stress, and metabolic dysfunction in the skeletal muscle. In contrast, palmitoleic acid (PO), a monounsaturated fatty acid with lipokine properties, has been linked to improved carbohydrate and lipid metabolism and anti-inflammatory effects.

**Aim:** This study evaluated whether PO could counteract PA-induced metabolic dysfunction in human myotubes, focusing on insulin signalling.

**Methods:** Immortalized human myotubes were treated for 24 hours with 250 μM PA, 250 μM PO, and an equimolar combination of both (250 μM+250 μM). Insulin-induced phosphorylation of protein kinase B (Akt) and stearoyl-CoA-desaturase (SCD), an enzyme involved in the synthesis of unsaturated fatty acids, was assessed by Western blot. Neutral lipid accumulation was measured by Oil Red O staining. Gene expression of IL-6, PPAR<sub>γ</sub>, and PPAR<sub>α</sub> was analysed by qPCR. Differences between treatments were assessed using one-way ANOVA followed by Tukey's post hoc test. Statistical analyses were performed with Graph-Pad Prism for Windows.

**Results:** PA significantly impaired insulin signalling by reducing AKT phosphorylation; this effect was partially reverted by PO. Furthermore, PA upregulated SCD protein levels, an effect partially normalized by PO co-treatment. Oil Red O staining revealed that both PA and PO increased intramyocellular neutral lipid accumulation, but PO promoted more compact droplets, suggesting a more efficient storage pattern compared with the diffuse lipid accumulation throughout the cytoplasm induced by PA. At the transcriptional level, PA markedly increased the expression of IL-6, indicating a pro-inflammatory effect with this effect being, albeit partially, inhibited by PO. Additionally, while PA increased PPAR<sub>γ</sub> expression, PO had no effect on PPAR<sub>γ</sub> expression, either alone or in combination with PA. By contrast, PPAR<sub>α</sub> expression was unchanged across treatments.

**Conclusions:** PO mitigates the detrimental metabolic effects of PA in human myotubes, improving insulin sensitivity and promoting safer lipid storage within compact droplets. These effects suggest that PO may buffer lipotoxic stress by redirecting PA towards neutral lipid storage and away from harmful species such as ceramides.

ABSTRACT

**EFFICACY OF EVINACUMAB BY GENOTYPE AND LOW-DENSITY LIPOPROTEIN RECEPTOR FUNCTION IN PATIENTS WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLAEMIA: A SUBANALYSIS FROM THE ELIPSE OPEN-LABEL EXTENSION STUDY**

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**Background:** Homozygous familial hypercholesterolaemia (HoFH) is a rare genetic disorder primarily caused by variants in both alleles of the gene encoding the low-density lipoprotein receptor (LDLR). Patients with severe loss of function LDLR variants have the highest plasma LDL-cholesterol (LDL-C) levels and are at the greatest risk for experiencing an early major cardiovascular event. This subanalysis of the ELIPSE open-label extension (OLE) study assessed the efficacy of evinacumab, an angiopoietin-like 3 inhibitor, by genotype and LDLR function in patients with HoFH.

**Methods:** Open-label, single-arm, phase 3 trial (NCT03409744) in patients aged  $\geq 12$  years with HoFH on stable lipid-lowering therapies receiving evinacumab 15 mg/kg intravenously every 4 weeks. Homozygosity was defined as bi-allelic semi-dominant hypercholesterolaemia – monogenic (same variant) or digenic (different variants), in the LDLR alleles. Null/null variants in LDLR were defined as variants resulting in  $< 15\%$  LDLR activity.

**Results:** A total of 116 patients enrolled in the OLE. At baseline, 53 (45.7%) patients had monogenic variants and 41 (35.3%) patients had digenic variants in LDLR. Mean (SD) LDL-C levels were reduced by -51.3% (23.3) and -54.1% (16.9) by Week 8 of evinacumab treatment in the monogenic and digenic groups, respectively, and remained low through Week 104 (-39.8% [41.0] and -45.5% [24.5], respectively). At baseline, 36 (31.0%) patients had null/null variants. Evinacumab treatment also resulted in rapid and sustained decreases in mean LDL-C levels in these pa-

tients (Week 104 null/null: -45.1% [24.6]). A similar response to evinacumab was observed in their non-null/null comparators (n=78) over 104 weeks.

**Conclusions:** In this large cohort of patients with HoFH, evinacumab treatment resulted in sustained LDL-C reduction in patients with HoFH irrespective of genotype or LDLR function.

**LOMITAPIDE-INDUCED FATTY LIVER IS A REVERSIBLE CONDITION: EVIDENCE FROM FAMILIAL CHYLOMICRONEMIA SYNDROME**

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Familial chylomicronemia syndrome (FCS) is a rare autosomal recessive disorder characterized by severe hypertriglyceridemia. It is caused by loss-of-function variants in the genes encoding the lipoprotein lipase (LPL) enzyme and its cofactors, which severely impair the hydrolysis of triglycerides (TG). Its main complication is represented by acute pancreatitis (AP), a potentially life-threatening condition.

Conventional TG-lowering therapies are poorly effective in FCS, thus requiring the search of novel treatments. Lomitapide, an inhibitor of microsomal triglyceride transfer protein (MTP), has demonstrated efficacy in reducing TG levels in FCS. However, it is associated with hepatic side effects, namely liver fat accumulation. Here we present a case study of a 71-year-old female patient with genetically confirmed FCS, baseline TG level of 2300 mg/dL (25.97 mmol/L) and a history of AP, who was treated with lomitapide for almost 5 years.

The treatment allowed a marked reduction of TG (about 90%) and no recurrence of AP. Remarkably, the lowest TG value recorded during follow-up was 24 mg/dL, further underscoring the profound lipid-lowering efficacy of lomitapide. However, hepatic monitoring during treatment revealed a progressive worsening of liver fat accumulation as detected by magnetic resonance imaging (MRI), which was associated with pronounced increases in liver transaminases and liver stiffness (up to 15 kPa). Due to these hepatic adverse events, it was decided to discontinue therapy with lomitapide. An MRI scan repeated after 70 days of drug withdrawal revealed complete resolution of fatty liver disease associated with normalization of liver stiffness (4.1 kPa) and liver transaminases. Subsequently, lomitapide therapy was reintroduced at a carefully monitored dose, and the patient is currently under follow-up without new hepatic or clinical complications. This case provides on the longest real-world experiences with lomitapide in FCS, illustrating its extraordinary potential in reducing triglycerides and its potential ability to prevent pancreatitis, while also highlighting the critical need for vigilant hepatic monitoring. Beyond the individual case, it adds novel evidence to the current debate on the place of lomitapide in FCS management, where efficacy must be carefully balanced against long-term liver safety.

## ABSTRACT

## EXPANDED CLINICAL EXPERIENCE OF CHILDREN WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLAEMIA (HOFH) UNDER 5 YEARS OF AGE TREATED WITH EVINACUMAB THROUGH COMPASSIONATE USE PROGRAMS

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**Background:** Globally, there are limited pharmacologic options available to treat children <5 years old living with HoFH, a rare genetic disorder characterized by extremely elevated LDL-C levels from birth. We previously reported that initiation of evinacumab, an angiopoietin-like 3 inhibitor, before 5 years old resulted in large reductions in LDL-C levels in 3 compassionate use (CU) patients with HoFH. Here, we present longer-term results from these patients, and include data from 3 additional CU patients, to provide further clinical insight into a patient population with a high unmet need.

**Methods:** This case series includes 6 children <5 years old treated with evinacumab 15 mg/kg IV Q4W through 2 CU programs; two children transitioned to commercially available drug during the treatment period. Caregivers provided informed consent prior to treatment initiation. LDL-C levels were compared before and during treatment and serious adverse events (SAEs) were monitored.

**Results:** Data from 2 males and 4 females are included. Most (5/6, 83%) were receiving multiple lipid-lowering therapies at baseline. All initiated evinacumab before 5 years old (range, 1.1-4.4 years). LDL-C levels were substantially reduced in all patients after initiating evinacumab treatment (range, 32.9-77.3%), with reductions sustained up to the last reported dose (range, 16-130 weeks). Plasmapheresis was reduced from biweekly in one child receiving it at baseline to monthly after 34 weeks of treatment. Xanthomas regressed over time in the 5 patients with baseline presentation. No SAEs were reported. Findings were consistent with approved indications for patients ≥5 years old with HoFH.

**Conclusions:** Longer-term and additional data collected for up to 130 weeks in a real-world setting adds to a growing body of evidence which suggests that evinacumab administration is safe and results in clinically meaningful reductions in LDL-C levels in very young patients for whom other therapies are not approved, and for whom apheresis poses several challenges.

## REAL-WORLD EXPERIENCE OF EFFICACY AND SAFETY OF EVINACUMAB COMBINED WITH LOMITAPIDE IN PEDIATRIC PATIENTS WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: A 24-WEEK OBSERVATIONAL STUDY HIGHLIGHTING LDL-C REDUCTION AND APHERESIS WITHDRAWAL

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**Background:** Homozygous familial hypercholesterolemia (HoFH) is a rare genetic disorder characterized by extremely elevated plasma low-density lipoprotein cholesterol (LDL-C) levels and a markedly increased risk of premature atherosclerotic cardiovascular disease. Conventional lipid-lowering therapies, including statins, ezetimibe, and lipoprotein apheresis, often fail to achieve adequate LDL-C reductions in these patients. Evinacumab, a fully human monoclonal antibody that inhibits angiopoietin-like 3 (ANGPTL3), has emerged as a novel therapeutic option capable of reducing LDL-C levels and it is approved for the treatment of patients with HoFH. Objective The aim of this study was to evaluate the percentage reduction in LDL-C levels achieved with evinacumab combined with lomitapide therapy in paediatric patients with severe hypercholesterolemia who were refractory to standard-of-care lipid-lowering therapy (LLT), including lipoprotein apheresis (LA), in real-world clinical practice.

**Methods:** Four pediatric patients with genetically confirmed HoFH (mean age 10.75 years; 2 males, 2 females) were enrolled in this single-center, observational study. Evinacumab was administered intravenously at a dose of 15 mg/kg every four weeks for a median duration of 24 weeks. At each visit, clinical assessments and blood sampling were performed to monitor lipid profiles and to evaluate liver and kidney function for safety monitoring.

**Results:** Four HoFH pediatric patients were treated with evinacumab for up to 24 weeks. At diagnosis, the mean pre-treatment LDL-C concentration was 804 ± 60 mg/dL (mean ± standard deviation, SD). At the beginning of evinacumab treatment, all patients had discontinued LA and were receiving LLT, including lomitapide, which had been started at least one year previously. Evinacumab decreased LDL-C from 314±91 mg/dL at baseline to 137±30 mg/dL at week 24, with a mean LDL-C percentage reduction of 53±20%. This represents an additional 43% reduction compared to Lomitapide. Reductions were evident as early as week 4 and were maintained throughout the 24-week treatment period. Evinacumab was generally well tolerated with no serious treatment-related adverse events or treatment discontinuations reported in any patient. No cardiovascular events were recorded during the study period.

**Conclusions:** Evinacumab, used in combination with lomitapide, achieved a substantial and clinically relevant reduction in LDL-C levels and showed promise as an effective LDL receptor-independent option for pediatric patients with HoFH. This therapeutic approach allowed the permanent withdrawal of lipoprotein apheresis, underscoring evinacumab's potential to transform the clinical management of HoFH.

## ABSTRACT

## EFFECTS OF ANGPTL3 INHIBITION ON LIPIDS, LIPOPROTEINS, APOLIPOPROTEINS, AND BIOMARKERS: A META-ANALYSIS OF CONTROLLED RANDOMIZED TRIALS

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**Background and Aims:** Angiopoietin-like protein 3 (ANGPTL3) is a key regulator of lipid metabolism, influencing triglyceride-rich lipoproteins, low-density lipoprotein cholesterol (LDL-C), and apolipoprotein homeostasis. Inhibition of ANGPTL3 has been proposed as a promising approach to reduce residual cardiovascular risk, particularly in patients with familial hypercholesterolemia (FH) or persistent dyslipidaemia despite optimal treatment. Pharmacological strategies to inhibit ANGPTL3 include monoclonal antibodies (mAbs, evinacumab), antisense oligonucleotides (ASOs, vupanorsen), and small interfering RNAs (siRNA, zolasiran and solbinsiran). We conducted a meta-analysis of randomized controlled trials (RCTs) to provide a comprehensive evaluation of the metabolic effects of ANGPTL3 inhibitors.

**Methods:** A systematic search of PubMed, EMBASE, Web of Science, CENTRAL, and ClinicalTrials.gov was performed through July 2025. Eligible studies were phase II or III RCTs comparing ANGPTL3 inhibitors against placebo. Outcomes included total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), non-HDL cholesterol (non-HDL-C), very-low-density lipoprotein cholesterol (VLDL-C), remnant cholesterol (RC), triglycerides (TG), apolipoprotein B (ApoB), apolipoprotein A1 (ApoA1), apolipoprotein C3 (ApoC3), lipoprotein(a) [Lp(a)], as well as ANGPTL3 and C-reactive protein (CRP). Pooled estimates of percentage change from baseline were obtained using fixed- and random-effects models. When significant heterogeneity was discovered (as determined by Cochrane's Q test and the I<sup>2</sup> statistic), the results from the random-effects model were presented. Subgroup analysis was performed based on the mechanism of action (mAbs, ASOs, siRNA).

**Results:** Nine RCTs encompassing 1,254 participants were included. ANGPTL3 inhibition significantly reduced levels of TC (-32.8%), LDL-C (-21.6%), HDL-C (-20.1%), non-HDL-C (-31.5%), VLDL-C (-40.6%), RC (-72.7%), TG (-47.1%), ApoB (-19.9%), ApoA1 (-18.3%), ApoC3 (-53.5%), and Lp(a) (-11.5%). ANGPTL3 inhibitors also markedly reduced circulating ANGPTL3 protein (-70.7%), while no significant effect was observed on high-sensitivity CRP. Subgroup analyses demonstrated greater reductions in LDL-C, ApoB, non-HDL-C, and TC with evinacumab compared to the other groups, whereas small interfering RNAs produced more pronounced VLDL-C lowering than vupanorsen.

**Conclusions:** This meta-analysis highlights that ANGPTL3 inhibition provides broad lipid-lowering effects, with particularly pronounced reductions in triglyceride-rich lipoproteins. Because this mechanism is independent of LDL receptor (LDLR) function, ANGPTL3 inhibition represents a promising therapeutic option for patients with dyslipidaemia, especially those with residual cardiovascular risk despite standard treatments or with genetic disorders impairing LDLR activity, such as familial hyper-

percholesterolemia. The potential benefit of the marked reduction in RC, compared with ApoB lowering, remains to be fully evaluated. Large, long-term clinical trials are warranted to confirm both the cardiovascular benefits and the safety profile of this innovative approach.

## WEIGHT EXCESS IN A COHORT OF PEDIATRIC SUBJECTS WITH FAMILIAL HYPERCHOLESTEROLEMIA

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**Background:** Overweight and obesity are modifiable risk factors for atherosclerotic cardiovascular disease. Weight excess incidence is epidemically increasing worldwide in paediatric subjects. Heterozygous Familial Hypercholesterolemia (HeFH) is present in 1 out of 250 subjects in the general population, and it is already present, detectable and treatable in children and adolescents. The evaluation of weight excess prevalence in pediatric individuals with HeFH is an issue of utmost importance in terms of risk stratification and coronary heart disease (CHD) prevention. The aim of this observational, retrospective, monocentric study is to evaluate the prevalence of weight excess in a cohort of pediatric patients with hypercholesterolemia, referred to the Centre for Pediatric Dyslipidemias, Pediatrics and Neonatology Unit, Guglielmo da Saliceto Hospital, Piacenza.

**Methods:** 75 pediatric subjects with HeFH, not on lipid lowering treatment, evaluated as first access at the Pediatric Lipid Clinic at Piacenza City Hospital from January 2015 to January 2025, were included in the study. At baseline, anthropometric parameters (weight, height, body mass index BMI) and complete lipid profile were evaluated.

**Results:** Mean age (mean±sd) was 10.4±4.2 years, range 2-17 years. 31/75 participants (41.3%) were male. 9/75 participants (12%) and 7/75 (9.3%) were overweight or obese, respectively. At baseline, lipid profile of the study participants, expressed as median (25th-75th percentile) was as follow: total Cholesterolemia 245 mg/dL (224-280), LDL-C 167 mg/dL (149-193), HDL-C 55 mg/dL (48-66), triglycerides 89 mg/dL (70-114), non-HDL-C 188 mg/dL (168-219). No statistically significant difference were found in lipid profile according to BMI classes.

**Conclusions:** The results of our study confirm that weight excess is common in patients with HeFH. Our cohort displays lower overweight percentage, but higher obesity percentage than European pediatric patients with FH (4% obese and 15% overweight, EAS data for Western Europe). BMI distribution is similar to those of Okkio alla Salute survey participants, i.e. Italian school children aged 8-10 years. The result of our study are unexpected, as children with HeFH belong to families with at least one member with HeFH, thus they should be already aware of healthy heart lifestyle. Weight management and prevention of weight excess is a call to action for all pediatric subjects, especially if they are at increased CHD risk due to the presence of HeFH.

ABSTRACT

**REAL-WORLD MANAGEMENT OF SEVERE HYPERTRIGLYCERIDEMIA: INSIGHTS FROM THE PADUA LIPID CLINIC – HOW CAN WE DO BETTER?**

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**Background and Aim:** The Study Hyper triglyceridemia is characterized by elevated triglyceride levels in the blood and an increased risk of acute pancreatitis and atherosclerotic cardiovascular disease (ASCVD). Lifestyle modifications and available triglyceride-lowering therapies, particularly volanesorsen, have significantly changed the therapeutic approach, leading to a reduction in the incidence of acute pancreatitis as well as cardiovascular risk, with improvements in clinical outcomes. This study analyzes the evolution of therapeutic management in patients with severe hyper triglyceridemia followed at the Lipid Clinic of the University of Padua and the impact of triglyceride-lowering therapies and triglyceride control on the incidence of acute pancreatitis and new cardiovascular events.

**Materials and Methods:** This study included 12 subjects with severe hyper triglyceridemia (M=8, F=4; age 48.5±13.2 years, triglycerides >1000 mg/dL), of whom 7 had genetically diagnosed familial chylomicronemia syndrome, enrolled in the LIPIGEN-SHTG registry. The average follow-up was 5.5±8.6 years, during which 75% of the original cohort was followed.

**Results.** In this population of patients with severe hyper triglyceridemia, the mean triglyceride levels at baseline were 1577±1628 mg/dL. Of these, 66% were on a dietary therapy with less than 20 g of fat/day, 50% were on a combined therapy with fenofibrate 145 mg and omega-3 fatty acids, and 25% were not receiving any therapy. During the follow-up, there was a progressive intensification of triglyceride-lowering therapy: the percentage of patients on treatment increased to 92%, with 66% on combined therapy with fenofibrate 145 mg and omega-3 fatty acids and 25% on volanesorsen 285 mg therapy. The mean triglyceride concentration at the last follow-up visit were reduced to 537±339 mg/dL, corresponding to a mean reduction of 55±31% compared to pre-treatment levels (p<0.05). The prevalence of ASCVD in the studied cohort was 25% (n=3), with an incidence of new ASCVD events during follow-up of 8.3%. Furthermore, the prevalence of acute pancreatitis at baseline was 75%, whereas the incidence of new pancreatitis cases at the last follow-up visit was 25%. At the last follow-up visit, patients on combination therapy with fenofibrate 145 mg, omega-3 fatty acids, and volanesorsen 285 mg showed a lower average reduction in triglyceride levels compared to patients not on volanesorsen therapy (208 vs. 351 mg/dL), although this difference did not reach statistical significance (p=0.67).

**Conclusions:** In patients with hyper triglyceridemia followed at our Lipid Clinic Center, intensification of triglyceride-lowering therapy, particularly with the use of volanesorsen, led to a reduction in triglyceride levels. The low incidence of new cardiovascular events and acute pancreatitis during follow-up supports the effectiveness of combination triglyceride-lowering therapy in reducing negative outcomes in this high-risk population.

**EXOME ANALYSIS OF PATIENTS WITH A CLINICAL SUSPECT OF FAMILIAL HYPERCHOLESTEROLEMIA: IDENTIFICATION OF RARE VARIANTS IN ABCG5 AND ABCG8**

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**Aim:** Familial hypercholesterolemia (FH) is a disease mainly caused by pathogenic variants in LDLR, APOB and PCSK9 genes. Pathogenic variants are not present in all patients with a clinical suspect of FH. Rare variants in genes causing different dyslipidemias characterized by increased LDL-cholesterol levels (FH-phenocopies) could be an alternative genetic cause. Sitosterolemia is an FH-phenocopy in which the molecular defect is the decreased excretion of phytosterols, including sitosterol, due to the presence of biallelic pathogenic variants in the causative genes, resulting in increased plasma levels of cholesterol, xanthomas, carotid plaque, and cerebrovascular events. We aim to report rare variants in sitosterolemia causing genes (ABCG5 and ABCG8), identified by exome sequencing in FH patients without FH-causative variants.

**Patients and Methods:** Twenty patients with clinical suspicion of FH without pathogenic variants and even without uncertain significance variants in FH-causative genes (LDLR, APOB, PCSK9, APOE, LDLRAP1) were analyzed by exome sequencing (WES – Agilent SureSelect Human All Exon V6). A preliminary analysis was conducted analyzing an in-silico panel of 39 genes associated with dyslipidemias. The pathogenicity classification of variants was carried out according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

**Results:** Patients carrying variants in ABCG5 and ABCG8 were 3/20 (15%). All variants were identified at heterozygous status. One patient carried two USVs one in each gene: ABCG5 c.293C>G - p.(Ala98Gly) and ABCG8 c.1370C>G - p.(Ala457Gly). Patient 2 had a null pathogenic variant in ABCG8 c.1083 G>A - p.(Trp361\*). Patient 3 had a USV in ABCG5 c.182G>A - p.(Arg61Gln). Most of ABCG5 and ABCG8 variants were classified as USVs given the poor availability of pathogenicity evidence, such as functional data and previous reports in affected patients (rare disease). The only pathogenic variant was identified in a patient with tendon xanthomas.

**Conclusion:** Due to the high prevalence of rare variants in sitosterolemia-causative genes among patients with a clinical suspect of FH, it is recommended to include ABCG5 and ABCG8 in the NGS panel for FH diagnosis. This deeper analysis allows better therapeutic management, identifying patients that should avoid the use of vegetal sterols. Furthermore, NGS is considered simpler and more accessible than the measurement of phytosterols, which is not routinely performed and is carried out by only a few laboratories in Italy and around the world.

## ABSTRACT

## SW872-DERIVED HUMAN ADIPOCYTE MODELS: CHARACTERIZATION AND APPLICATION TO STUDY CARDIOMETABOLIC PATHOPHYSIOLOGY

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Adipose tissue dysfunction is central to the pathophysiology of metabolic syndrome, type 2 diabetes mellitus, fatty liver disease, and cardiovascular complications. However, there is still no widely accessible human adipocyte model that reproduces human adipose dysfunctions, creating a major gap in cardiometabolic research. The human liposarcoma-derived SW872 cell line provides a valuable alternative to develop dysfunctional phenotypes under altered metabolic conditions. Establishing and characterizing such models is essential to create translational platforms for cardiometabolic research. In addition, once validated, these models can also be applied to investigate the effects of natural extracts with nutraceutical properties as well as of environmental pollutants. This project aims to characterize dysfunctional SW872-derived adipocyte models and assess their potential for exploring pathophysiological mechanisms of cardiometabolic diseases. Human SW872 adipocytes were used to establish two dysfunctional models by oleic acid treatment for 7 days (SW872-OA) and by 17-day spontaneous differentiation (SW872-AUTO). In these models, lipid accumulation, glucose uptake, oxidative stress, and metabolomic changes were assessed, together with alterations in gene expression and glucose transporter localization. In addition, bioenergetic profiles, including mitochondrial respiration and glycolytic activity, were determined. Both SW872-OA and SW872-AUTO cell models showed dysfunctional traits compared to control SW872 cells. More specifically, Oil Red O staining and quantification revealed increased lipid droplet accumulation (\*\* $p < 0.001$ ). Cytofluorimetric analysis has shown increased oxidative stress (\*\* $p < 0.01$ ) and impaired glucose (-2-NBDG) uptake (\*\* $p < 0.001$ ). Consistent with reduced glucose uptake, immunostaining of main glucose transporters, expressed by human adipocytes (e.g., GLUT4 and GLUT1), revealed lack of translocation to the plasma membrane. GLUT4 was detected in the vesicular compartment around nuclei, whereas GLUT1 expression was dramatically lost in SW872-OA and SW872-AUTO compared to SW872 cells. 1H-NMR-driven metabolomic analysis highlighted a distinct profile in SW872-OA compared to SW872 cells, according to cellular lipid and low molecular weight metabolite (LMWM) content, in addition to LMWM pattern of the cell culture media. Furthermore, Seahorse analyses showed reduced oxygen consumption rate cells by -13.6% and 21.5%, respectively in SW872-OA and SW872-AUTO cells. However, ATP production was not affected in both models. Consistent with these data, spare respiratory capacity (\* $p < 0.05$ ) and coupling efficiency (\*\* $p < 0.01$ ) increased in SW872-AUTO cells. Gene expression analysis (qRT-PCR) further showed changes in both models consistent with a shift toward adipogenic differentiation. These findings highlight SW872-derived adipocyte models as promising human cell-based systems to study dysfunctional adipocytes in the context of cardiometabolic disease. Overall, these models represent a valuable translational platform to advance research on disease mechanisms and preventive strategies.

## ANGPTL3 DOWNREGULATION ENHANCES COAGULATION FACTOR V SECRETION BY HUH7 CELL LINE

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**Background and Aim:** Angiopoietin-like protein 3 (ANGPTL3) is a hepatokine that regulates plasma lipid metabolism through inhibition of lipoprotein lipase (LPL) and endothelial lipase (EL), and it is targeted by the evinacumab, a monoclonal antibody approved for the treatment of homozygous familial hypercholesterolemia. Previous unpublished RNA-seq studies on ANGPTL3 silenced human hepatocarcinoma cell line (HuH7) from our lab identified LMAN1 (lectin, mannose-binding 1) transcript as strongly downregulated in silenced cells compared to control cells, data confirmed via RT-qPCR and Western Blot. LMAN1 is a shuttle protein that complexes with MCFD2 (multiple coagulation factor deficiency protein 2) to transport coagulation factors V (FV) and VIII (FVIII) from the endoplasmic reticulum (ER) to the Golgi, and loss-of-function mutations on LMAN1 gene causes the combined deficiency of FV and FVIII (F5F8D). Given this premises, the aim of this study is to investigate a potential crosstalk between ANGPTL3 and coagulation FV regulation, a mechanism that remains poorly understood, and thus if genetic or pharmacological inhibition of ANGPTL3 may affect the production of coagulation factor V in HuH7 cells.

**Methods:** The human hepatocarcinoma cell line HuH7 was transfected with validated ANGPTL3-targeting siRNA or a scrambled siRNA control using Lipofectamine 3000 for 48h, upon which Western Blotting, ELISA assay and RT-qPCR were performed to check for protein and transcript expression levels of ANGPTL3, as well as FV secretion rates into the cell culture medium. In a separate set of experiments, cells were treated with five increasing doses of evinacumab (0.625µg/ml > 10µg/ml) for 48h, and the aforementioned assays were performed downstream.

**Results:** Following 48h of transfection with ANGPTL3-targeting siRNA, FV secretion showed a modest, but statistically significant increase compared to scramble-transfected cells (+14%,  $p < 0.05$ ). By inhibiting the secreted ANGPTL3 through evinacumab, a consistent and observable upward trend in factor V secretion was detected (+25% at 5µg/ml and +20% at 10µg/ml vs untreated cells), although this increase did not reach statistical significance.

**Conclusions:** Taken together, these preliminary results suggest a possible modulation between ANGPTL3 and FV that warrant further investigations, including disentangling the biological network that orchestrates the observed effects and a possible effect of ANGPTL3 pharmacological interventions on coagulation cascade. A possible involvement of LMAN1 remains an open question since our result contrast with what observed in Lman1-deficient mice and in patients carrying LMAN1 loss-of-function mutations.

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 ABSTRACT
 

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## LIPODYSTROPHY AS A RARE AND NEGLECTED CAUSE OF SEVERE HYPERTRIGLYCERIDEMIA: A CASE REPORT

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**Aim:** Familial chylomicronemia syndrome (FCS) is characterized by severe hypertriglyceridemia (usually triglycerides >1000 mg/dL in multiple measurements) causing eruptive xanthomas and recurrent acute pancreatitis. Lipodystrophy syndromes are rare disorders characterized by abnormal or selective loss of adipose tissue, together with severe metabolic complications including insulin resistance, diabetes mellitus, and severe hypertriglyceridemia. These conditions are often underdiagnosed due to their heterogeneous clinical presentation and rarity. We aim to report a case highlighting the importance of considering lipodystrophy in the differential diagnosis of patients presenting with severe hypertriglyceridemia.

**Patients and Methods:** The patient is a 28-year-old woman with severe hypertriglyceridemia in different measurements (>3000 mg/dL), cutaneous xanthomas, recurrent pancreatitis and diabetes. First analysis was performed by amplification and traditional sequencing of exons and intronic flanking regions of the main genes; also, multiplex ligation-dependent probe amplification (MLPA) was performed. Next generation sequencing (NGS) was performed by capture of target regions (exons and intronic flanking regions) by SureSelectXT-HS2 Target Enrichment System (Agilent Technologies), followed by illumina MiSeq platform (2x150bp) (illumina Inc., San Diego, CA, USA). Data Analysis was conducted by Seqr (Broad Institute). Rare variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al. 2015). The Population database (GnomAD v4.1.0) was used to establish the minor allele frequency of the identified variants. The variants were also checked in databases of variant-disease association (HGMD and ClinVar) to verify if previous reports. In silico tools were used to predict the potential functional impact of the identified variants.

**Result:** The patient was suspected of FCS and was initially screened for variants in the most frequent causative genes (LPL, APOC2 and APOA5). No rare variants (including copy number variants in LPL) were identified in these genes. The patient was then analysed by NGS for variants in 16 lipid-related genes, including all the FCS-causative genes, resulting again resulted negative for rare variants. After the implementation of a panel for lipodystrophy diagnosis, the patient was further analysed and the missense variant c.1069C>A - p.(Pro357Thr), in exon 7 of PPARG gene was identified. This gene is causative of familial Partial Lipodystrophy, type 3, a disease with an autosomal dominant inheritance. Variant was confirmed by Sanger sequencing. The variant was extremely rare and never reported in population and variant-disease databases. In silico prediction tools consistently classified the variant as damaging. Based on these data and according to the ACMG guidelines the variant was classified

as Likely pathogenic. The patient was diagnosed with lipodystrophy, allowing more appropriate therapeutic management.

**Conclusions:** The experience with this patient highlights the importance of considering lipodystrophies as additional cause of severe hypertriglyceridemia. Expanded gene panel, including lipodystrophy-causative genes, should be considered for analysis of patients with potential FCS in order to perform an accurate differential diagnosis.

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## DRUG-RELATED HEPATOTOXICITY RISK OF CARDIOVASCULAR DRUGS IN ELDERLY PATIENTS WITH ATRIAL FIBRILLATION: INSIGHTS FROM THE NATIONWIDE ITALIAN START REGISTRY

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**Aims:** Atrial fibrillation (AF) patients frequently require polypharmacy, increasing the risk of drug-drug interactions and liver toxicity. For this reason, aim of our study was to investigate the use of drugs potentially associated with liver injury in anticoagulated AF patients.

**Methods:** We applied the LiverTox classification to AF patients from the START registry, categorizing them based on the use of LiverTox A drugs (high risk of liver toxicity). Logistic regression was used to calculate the odds ratios (ORs) and 95% confidence intervals (95%CI) for factors associated with the use of these drugs and their impact on liver function tests (LFTs).

**Results:** Median age was 81 years with 46% of women. A total of 40,355 distinct drugs were prescribed to 8,215 patients, with a median of 5 drugs per patient (IQR 3–7). Overall, 3,416 (41.6%) received at least one LiverTox A drug. These patients were more often male, overweight, smokers, hypertensive, diabetic, with cardiovascular or cerebrovascular history, and on polypharmacy. The most commonly prescribed LiverTox A drugs were statins, amiodarone, and allopurinol, both in the overall population and within subgroups stratified by major cardiovascular conditions. In multivariable logistic regression, among LiverTox A drugs, amiodarone (OR 1.61, 95%CI 1.29–2.00) and methimazole (OR 1.83, 95%CI 1.04–3.03) were associated with elevated LFTs. Among frequently prescribed drugs, warfarin, furosemide, ramipril, lansoprazole, and canrenone were also associated with increased LFTs. Direct oral anticoagulants, compared to warfarin, showed a lower risk of increased LFTs after adjustment for confounders.

**Conclusion:** Many AF patients are treated with drugs at high risk of hepatotoxicity. A tailored therapy could improve clinical outcomes reducing the risk of liver toxicity and elevated LFTs.

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 ABSTRACT
 

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**METABOLIC RESILIENCE OF SIMPSON-GOLABI-BEHMEL SYNDROME (SGBS) ADIPOCYTES TO PALMITIC ACID OVERLOAD REFLECTS A SECRETOME THAT DOES NOT IMPAIR SKELETAL MUSCLE INSULIN SIGNALLING**

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**Background:** Excess intake of long-chain saturated fatty acids, particularly in the form of palmitic acid (PA), is considered a major contributor to metabolic impairments. Adipocytes are able to buffer excess energy by storing it in the form of triglycerides in intracellular lipid droplets. However, it is currently unknown whether merely increasing intracellular lipid storage in adipocyte is sufficient to hamper their endocrine function leading to the release of a pro-insulin resistance secretome.

**Aim:** The aim of this study was to evaluate whether nutrient overload in the form of PA affected adipocyte metabolic health and if this was coupled with the release of a secretome able to modulate insulin signalling in human skeletal muscle cells.

**Methods:** Simpson-Golabi-Behmel Syndrome (SGBS) adipocytes were treated with 1000  $\mu$ M PA for 96 hours. Thereafter, insulin signalling was assessed by evaluating the phosphorylation of protein kinase B (AKT) and AKT substrate 160 kDa by Western blot. This technique was also employed to assess the activation of the NF- $\kappa$ B signalling pathway. The expression of IL-6 and IL-1 $\beta$  was quantified by real-time qPCR, while secreted IL-6 and adiponectin were measured by ELISA. To assess adipocyte-myotube crosstalk, SGBS adipocytes were cultured and differentiated on transwell inserts with 0.3  $\mu$ m pore size membranes and exposed to PA as already described. After the treatment, adipocyte-containing inserts were placed over differentiated human myotubes, allowing indirect cell-to-cell communication for 6 hours. Finally, myotube insulin signalling at the AKT and AS160 level was evaluated as reported for adipocytes. Data are expressed as mean  $\pm$  standard error (SEM). Differences between groups was evaluated using Student's t-test, with  $p < 0.05$  considered as statistically significant.

**Results:** Following 96 hour treatment, PA was taken up by adipocyte and readily incorporated into lipid droplets as confirmed by their enlargement. Despite their hypertrophic expansion, adipocytes displayed a preserved insulin-mediated phosphorylation and activation of Akt and its substrate AS160. This was coupled with the absence of NF- $\kappa$ B proinflammatory signalling activation. Consistent with the absence of an inflammatory response, the 96-hour PA treatment failed to induce the expression of the pro-inflammatory cytokines IL-6, and IL-1 $\beta$  in SGBS adipocytes. In line with the preserved insulin signalling and the lack of pro-inflammatory responses, the secretome of PA-treated adipocytes did not impair insulin-stimulated Akt and AS160 phosphorylation in myotubes compared with controls.

**Conclusion:** SGBS adipocytes adapt to PA overload by safely storing excess fatty acids in lipid droplets without developing overt dysfunction. This metabolic resilience is mirrored in their secretome, which fails to disrupt skeletal muscle insulin signalling.

**PROSPECTIVE EVALUATION OF CARDIOVASCULAR OUTCOMES IN RELATION TO LIPOPROTEIN(a) CONCENTRATIONS IN A COHORT OF PATIENTS WITH AORTIC VALVE STENOSIS**

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**Background:** Calcific aortic stenosis is the most common degenerative valvular disease in western countries, marked by progressive fibro-calcific remodeling of the valve, high rates of cardiovascular events (CV), and increased mortality. Lipoprotein(a) [Lp(a)] is an emerging risk factor linked to elevated incidence of CV events. Its role in the progression of aortic valve disease and its impact on CV outcomes remains debated.

**Aim of the study:** To evaluate the impact of Lp(a) levels on cardiovascular outcomes in patients with moderate to severe aortic valve stenosis.

**Methods:** A prospective observational study was conducted on 148 patients with moderate to severe aortic valve stenosis from the Cardiology Departments of Ca' Fondello Hospital in Treviso and the High-Specialty Rehabilitation Hospital of Motta di Livenza (TV). Baseline data included medical history, anthropometric, pharmacological, biochemical, and echocardiographic parameters. Patients were divided into two groups based on Lp(a) levels using a 62 nmol/L (30 mg/dL) cut-off. Cardiovascular events (acute myocardial infarction, stroke/TIA) and deaths (MACE) were recorded during a mean follow-up of 6.25 $\pm$ 3.0 years.

**Results:** The cohort had a mean age of 82.4 $\pm$ 7.6 years, with 44.6% prevalence of female sex. Hypertension was present in 84.9% of subjects, dyslipidemia in 60.3%, and diabetes mellitus in 24.7%. Patients with Lp(a) >62 nmol/L (n=46, 32.9%) showed higher dyslipidemia prevalence (82.2% vs 50.0%,  $p < 0.001$ ) and more frequent statin use (77.8% vs 47.9%,  $p < 0.001$ ). No significant differences were found in other analyzed parameters. The overall MACE incidence was 52%. Cox multivariable regression showed no significant association between Lp(a) levels and outcomes. Reduced renal function (GFR < 60 ml/min/1.73 m<sup>2</sup>) was significantly associated with event risk (HR 2.55;  $p = 0.002$ ), while valve replacement reduced the risk of MACE (HR 0.46;  $p = 0.007$ ).

**Conclusions:** In aortic valve stenosis patients, Lp(a) >62 nmol/L correlated with higher dyslipidemia prevalence and statin use, suggesting a pronounced cardio-metabolic risk profile. However, Lp(a) was not significantly linked to increased MACE incidence during follow-up, suggesting a limited contribution of this lipoprotein to the determination of cardiovascular outcomes in patients with advanced-stage valvular disease. Aortic valve replacement effectively reduced MACE risk, while reduced renal function was associated with adverse outcomes.

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**ABSTRACT**


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**LP(a) AND PREVALENCE OF AORTIC VALVE CALCIFICATION IN A FRENCH COHORT OF PATIENTS WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA**

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**Background:** Lipoprotein(a) [Lp(a)] has recently emerged both as a marker and a potential causal factor in the pathogenesis of aortic valve calcification (AoVC). Consequently, concern has been raised about the role of Lp(a) in high cardiovascular risk populations such as patients with heterozygous familial hypercholesterolemia (HeFH). However, the independent contribution of Lp(a) burden to AoVC in HeFH patients, separate from other established cardiovascular risk factors, remains insufficiently explored. This study aims to evaluate the prevalence of AoVC in patients with HeFH, specifically investigating whether Lp(a) levels or cumulative Lp(a) burden may serve as independent determinants of the presence and severity of AoVC in a cohort of HeFH patients without previous cardiovascular events. Secondary objectives include the identification of additional factors associated with the development and severity of AoVC in this population.

**Material and Methods:** A total of 246 were selected for the present study. All enrolled patients had a genetically confirmed diagnosis of HeFH. AoVC was investigated by computer tomography for the quantification of aortic valve calcium score according to the Agatston method. Lp(a) determination was carried out by immunoturbidimetric assays. Lp(a) burden was calculated by adding up the first ever measured Lp(a) multiplied by the age of the patient at first measure and the Lp(a) measured during the study visits during follow-up. Statistical analysis was performed using SPSS v. 29.0.1.0.

**Results:** The population consists of mainly female subjects (64.5%), with a median age of 47.1 years old. The majority of patients had a pathogenic variant of the LDLR gene (90.7%). A positive aortic valve calcium score was detected for 41.9% of patients, with a median score of 75. This cut-off was used to differentiate two subgroups to stratify calcification severity. No significant difference in Lp(a) values and Lp(a) burden were detected among patients with or without AoVC [19 mg/dL (8-156), 24 mg/dL (8-196), p-value=0.207 for Lp(a) values, 932 mg/dL (331-8208), 1074 mg/dL (293-8345), p-value=0.542 for Lp(a) burden]. Among patients with AoVC, there was no difference between those having a calcium score ≤75 or >75 units in either Lp(a) values [20 mg/dL (8-125), 17 mg/dL (8-156), p-value=0.977] or Lp(a) burden [957 (343-6540), 845 (331-8208), p-value=0.377]. Two separate

linear regression models showed that LDL-C burden and age were identified as determinants of the presence AoVC presence (AoVC score >0) and severity (AoVC score >75 units).

**Conclusions:** In genetically confirmed HeFH patients in primary cardiovascular prevention, neither Lp(a) or Lp(a) burden were associated with AoVC presence and severity. Age and cumulative LDL-C burden remain the key determinants of both the presence and progression of AoVC in this patient cohort. Incorporating valvular imaging into the assessment of these patients may offer new opportunities for risk stratification and therapeutic monitoring, considering the aortic valve as an early target of lipid-mediated damage, particularly in individuals with high cumulative LDL-C burden, for whom LDL-C control remains the priority.

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**IDENTIFICATION OF NOVEL miRNAs INVOLVED IN LIPID METABOLISM THROUGH INTEGRATED MIRNOMIC AND LIPIDOMIC ANALYSIS IN MICE**

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**Background and Aims:** The miRNomic and lipidomic profiles of mice with specific genotypes/phenotypes were integrated with a novel approach, with the aim of increasing our knowledge on the mutual interplay between miRNAs and lipids, and thus to discover previously uncharacterized miRNAs, potentially playing a role in lipid metabolism.

**Methods:** miRNomic and lipidomic analyses were performed in wild-type, Pcsk9 and Ldlr knockout mice fed normal laboratory diet or Western diet. Small RNA was extracted from liver, brain, duodenum, jejunum, ileum and abdominal white adipose tissue and quantified by RNAseq. Lipid species were quantified by high-throughput mass-spectrometry in liver, aorta and plasma. miRNA expression levels were tested for correlations with each lipid measurement in different samples. Highly correlated, uncharacterized miRNAs were subjected to testing in vitro in murine hepatoma Hepa1-6 cells. For each miRNA to be tested, cells were transfected with the miRNA mimic, the miRNA inhibitor and a non-target control. After 24-hours incubation, the cellular content of cholesterol and triglycerides was measured. For each miRNA, at least three independent experiments were carried out.

**Results:** Correlation analyses between miRNA expression levels and lipid concentrations in the different experimental conditions led to the selection of miRNAs potentially playing a major role in the regulation of lipid levels. Correlations mainly clustered in liver. Among selected miRNAs, some were already known to be related to lipid metabolism (miR-33, miR-210 and miR-21a) whereas others, including miR-431-5p, miR-434-3p, miR-434-5p and miR-677-5p had never been associated to lipid changes before. In vitro experiments allowed to highlight a potential role of miR-431-5p and miR-677-5p in the modulation of total cholesterol and triglyceride concentrations.

**Conclusions:** This study, bridging miRNomic and lipidomic data in well characterized mouse models, allowed to identify novel miRNAs potentially playing a role in the modulation of lipid levels.

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**ABSTRACT**


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**LIPOPROTEIN APHERESIS  
AND LIPOPROTEIN(a): REAL-WORLD  
DATA FROM PADUA**

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**Background:** Elevated plasma concentrations of lipoprotein (a) [Lp(a)] are a causal, independent, and underrecognized risk factor for atherosclerotic cardiovascular disease. High Lp(a) levels accelerate atherogenesis, promote plaque instability, and increase atherothrombotic potential, thereby predisposing to recurrent cardiovascular (CV) events. Current pharmacological options to lower Lp(a) are limited, and lipoprotein apheresis (LA) remains the most effective therapeutic approach in patients with very high Lp(a) concentrations and progressive ASCVD despite optimal medical therapy. Aim. The study aimed to assess the efficacy of LA in lowering plasma Lp(a) levels and preventing recurrent CV events in a cohort of very high-risk patients. Secondary endpoints included changes in other lipid profile parameters and overall treatment tolerability.

**Methods:** This retrospective, single-center, open-label study included 8 patients (7 men, 1 woman; mean age 57.1±7.7 years) with markedly elevated Lp(a) and recurrent CV events despite maximal lipid-lowering therapy and optimized lifestyle. For each patient, demographic data, ASCVD history, lipid profile, and ongoing treatments were collected. During follow-up pre- and post-LA session lipid values, CV events, and adverse reactions (AR) were recorded.

**Results:** All patients had a history of atherosclerotic coronary artery disease, with multivessel involvement documented in 7 cases (87.5%). Peripheral arterial disease was present in 3 patients (37.5%), with moderate-to-severe carotid artery stenosis in 2 cases and lower-limb obstructive arteriopathy in 1 case. In addition, one patient had experienced an ischemic stroke and another had aortic valve stenosis. The first CV event occurred at a relatively early age in all patients, with a mean onset of 52.8±10.6 years (range 34-66). The mean duration of LA treatment was 4.5 years (range 1-12, with a mean interval between sessions of 10.7±4.8 days (range 7-21)). 6 patients underwent DALI apheresis, while 2 were treated with HELP. Median Lp(a) levels decreased from 291 to 117 mg/dL pre- to post-LA, corresponding to a reduction of -58.5% (p<0.001). The time-averaged median Lp(a) concentration was 243 mg/dL, representing a -15.5% reduction compared with baseline (p<0.001). Mean LDL-c decreased from 45.6 to 17.8 mg/dL post-session (-59%, p<0.001), with a time-averaged mean concentration of 38.0 mg/dL (-15.9%, p<0.001). Prior to initiation of LA, 22 CV events had occurred over 34 observed patient-years, corresponding to an incidence of 0.69 events/patient-year. During LA (38 observed patient-years), only one acute myocardial infarction was recorded, resulting in a significant reduction in the annualized event rate to 0.03 events/patient-year (p<0.01). LA was generally well tolerated, with an AR rate of 1.1% per session and a mean incidence of 0.57 AR/patient-year.

**Conclusions:** LA proved effective in achieving a significant and sustained reduction in Lp(a) and LDL-c levels, confirming the overall efficacy of the procedure. LA treatment was associated

with a marked reduction in the incidence of CV events compared with the pre-treatment period. The procedure demonstrated a favourable tolerability profile, supporting its role as an established therapeutic option for patients at extremely high ASCVD risk. Nevertheless, LA remains an invasive, time-consuming and resource-intensive intervention, highlighting the need for novel pharmacological strategies currently under development.

**STUDY ON THE EFFICACY OF siRNA-BASED THERAPY IN THE MODULATION OF CERAMIDE SYNTHESIS**

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**Background and Aim:** Since increased plasma ceramide levels have recently emerged as a pro-atherogenic factor, selective modulation of hepatic ceramide biosynthesis might represent a novel therapeutic approach to reduce cardiovascular risk.

**Methods:** The efficacy of siRNA-based treatments (siPOOLS<sup>®</sup>) on ceramide level modulation was tested by targeting single enzymes or couples of enzymes playing key roles in ceramide metabolism. In vitro, the effects of siRNAs on target gene expression and lipidome changes were evaluated in Hepa1c1c7 murine hepatocytes (96 hours after treatment, 6 nM). For in vivo studies, siPOOLS<sup>®</sup> were encapsulated in lipid nanoparticles (LNPs) and first tested for hepatic tropism by confocal microscopy (single intraperitoneal injection of Cy5-labelled LNPs, 2 mg/kg, in C57Bl/6 male mice). In vivo gene silencing efficacy, effects on plasma and liver lipidome, and histological analyses were assessed after four intraperitoneal injections (2 mg/kg every 72 hours) in 8-week-old C57Bl/6 male mice.

**Results:** In vitro validation identified siRNAs effective in reducing target gene expression and lowering ceramide levels in hepatocytes. Biodistribution studies showed that siRNA-LNPs accumulated primarily in the liver and in the spleen. In vivo testing showed variable silencing efficacy of siRNA-LNPs, ranging from -17% (Sptlc1) to -80% (Sptssa), depending on the target and the combination/dose administered. Despite a promising silencing effect, lipidomic analyses of plasma and liver did not show effective modulation of ceramide levels. Treated mice displayed extramedullary hematopoiesis in the spleen and, in a few cases, hepatic necrosis.

**Conclusions:** The siRNA-LNP approach resulted in efficient silencing but did not translate into plasma ceramide modulation. A second strategy, consisting of N-acetyl galactosamine-conjugated siRNAs, will be tested in vitro and in vivo, with the aim of achieving higher efficacy and reduced toxicity.

## ABSTRACT

## ROLE OF LIPOPROTEIN APHERESIS TO SLOW DOWN THE AORTIC VALVE STENOSIS IN HIGH-LIPOPROTEIN(a)

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**Background:** In 1960s, in a pioneering way, plasmapheresis was used to treat children with homozygous familial hypercholesterolemia (HoFH). Over the years we have moved on to increasingly selective methods and nowadays, lipoprotein apheresis (LA) selectively removes atherogenic Apo-B lipoproteins from the blood: the main component of LDL-cholesterol, VLDL-cholesterol and Lipoprotein (a). Nowadays, despite the new lipid-lowering drugs, LA maintain its therapeutic role especially in HoFH, in severe hypercholesterolemia without achieved LDL-cholesterol target on maximally tolerated lipid-lowering therapies and in subjects with high levels of Lipoprotein (a) where LA is the proven therapeutic option. Moreover, hyper-Lp(a) in familial hypercholesterolemia increase the cardiovascular events and are associate with aortic valve stenosis. The aim of this study was to evaluate the progression of aortic valve disease in subjects of chronic LA treatment.

**Methods:** In our Site, Lipoapheresis Unit is allocated in a cardiopulmonary tertiary-level department. Since 1994, we performed more than 500 treatment/year and a total of 99 patients (43 current patient and 56 former patients). LA procedures were performed in agreement to guidelines and manufacturer's instructions with a median inter-apheresis interval of 14 [10-14] days, treating 4000ml plasma/session for subjects in HELP system or 1.5 patient plasma volume/session with the other systems and LA techniques in use are: dextran-sulphate absorption from plasma (Liposorber®-LA MA-03 systems; Kaneka, Osaka, Japan), heparin-induced LDL precipitation apheresis (HELP®, Plasmate Futura®; B. Braun, Melsungen, Germany), immunoadsorption (TheraSorb™ - LDL pro Adsorber, Miltenyi biotec, Bergisch Gladbach, Germany) in base on clinical characteristic of patients. To evaluate the therapeutic role of LA in aortic valve stenosis progression related to high-lipoprotein (a), patients with more than 3 years of chronic LA therapy where retrospective evaluated: have been identified 47 patients (mean age 52±12 years, male 72%) with suitable echocardiographic follow-up.

**Results:** Subjects (30/47) with hyper-Lp(a) had a more severe aortic valve disease at the beginning of LA treatment (mild/moderate aortic valve disease 7/30 vs. 0/17; p=0.029). During the follow-up (10 [5-14] years) a progressive increase in aortic peak velocity (baseline 1.45±0.42 m/sec vs. follow-up 1.78±0.92 m/sec - p <0.001) was recorded in overall subjects. Moreover, in subjects with hyper-Lp(a) the progression of aortic disease is similar to subjects with normal lipoprotein(a) values: Δ aortic peak velocity (m/sec) respectively 0.14 [0.30 - 0.55] vs. 0.20 [0.07 - 0.39]; p = 0.928. However, starting from more damaged valves, two cases with baseline moderate aortic valve stenosis were undergone to aortic valve replacement, even after 15 years from the start of LA therapy.

**Conclusion:** Even in the era of new lipid-lowering therapies, patients with hyper-Lp(a) may not be identified early and develop aortic valve disease. However, LA remains a safe and life-saving treatment also able to slow down the aortic valve stenosis progression.

## BICUSPID AORTIC VALVE: GENETIC CHARACTERIZATION OF THE KIV2 LPA POLYMORPHISM IN MODULATING CLINICAL COMPLICATIONS

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**Background:** Bicuspid aortic valve (BAV) is the most common congenital cardiopathy affecting 0.5 to 2.0% of the general population. BAV is associated with different complications such as valvular aortic stenosis, calcification, aortic insufficiency and thoracic aortic aneurysm. Beyond hemodynamic valvular impairment, dyslipidemia and elevated lipoprotein (a) [Lp(a)] levels also favour BAV disease progression and complications. Lp(a) levels are known to be under strict genetic control (trait heritability >90%) and are largely influenced by LPA Kringle IV type 2 (KIV2) size polymorphism, which inversely correlates with Lp(a) levels. In this study, we characterized LPA KIV2 repeats, using digital droplet PCR (ddPCR) approach in a cohort of Italian BAV patients.

**Methods:** One hundred nine subjects of Caucasian origin referred to the Regional Referral Center for Marfan syndrome and related disorders and to the Advanced Molecular Genetics Laboratory (Atherothrombotic Diseases Center, Careggi University Hospital, Florence) were enrolled. Patients were divided into two groups according to different clinical settings: 1) patients undergoing valve replacement surgery (VRS, n=68) and 2) patients not undergoing valve replacement surgery (NVRS, n=41).

**Results:** NVRS and VRS patients showed, respectively, significant differences in median age (IQR) [45 (28-52) vs 66 (57-75), p<0.001] and in classical cardiovascular risk factors such as dyslipidaemia [11 (16.2%) vs 19 (46.3%), p=0.0009], hypertension [20 (29.4%) vs 26 (63.4%), p=0.0007] and smoking habit [7 (10.3%) vs 23 (56.1%), p=0.0001], as well as a higher prevalence of BAV-associated complications (aortic stenosis, calcification and root/ascending aorta dilatation) in the VRS patient group, as expected. We also observed a significant difference in KIV2 repeats median number between the two groups [13 (8.1 - 22.6) in NVRS vs 33.6 (19.1 - 42.9) in VRS, p<0.001]. As concerns KIV2 repeat evaluation according to BAV clinical complications, NVRS patients with complications showed a trend towards reduction in KIV2 repeats number with respect to NVRS patients without complications [stenosis: 10.9 (7.1 - 19.9) vs 13.2 (9.2 - 24.4), p=0.341; calcification: 12.6 (7.2 - 20.3) vs 15 (9.88 - 24.5), p=0.319; root and/or ascending aorta dilatation: 11.9 (7.0 - 18.4) vs 19.4 (11.4 - 26.0), p=0.032]. In VRS patients, instead, no significant difference in the distribution of KIV2 repeats, according to the presence or absence of BAV clinical complications, was found.

**Conclusions:** Our analysis suggests that in NVRS patients, younger and with a lower prevalence of traditional cardiovascular risk factors than VRS ones, higher levels of genetically determined lipoprotein (a) might contribute to the development of BAV complications and in turn to a worse prognosis. Data observed in VRS patients might be likely due to the not negligible impact of cardiovascular risk factors burden in influencing the phenotype severity.

ABSTRACT

## PCSK9 LOSS-OF-FUNCTION VARIANTS LOWER LDL CHOLESTEROL WITHOUT INCREASING CHRONIC LIVER DISEASE RISK

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**Background:** Inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9) with anti-PCSK9 monoclonal antibodies (alirocumab and evolocumab) or with small-interfering RNAs (inclisiran) lowers plasma low-density lipoprotein cholesterol (LDL-C) levels, a major risk factor for the development of atherosclerotic cardiovascular disease (ASCVD). Pharmacologic inhibition of PCSK9 has shown a favorable hepatic safety profile so far, although long-term effects remain to be established. Preclinical studies and observational data suggested a potential link between the common missense p.Arg46Leu (R46L) variant in PCSK9 and susceptibility to chronic liver disease (CLD). Whether this effect extends to rare loss-of-function (LOF) variants in PCSK9 remains undetermined. We aimed to assess the association of lifelong genetic inhibition of PCSK9 with plasma liver enzymes and CLD by using LOF variants in PCSK9 as genetic instruments.

**Methods:** We studied the association of inactivating PCSK9 variants with lipid levels and liver outcomes in 280 carriers and 408,782 non-carriers from the UK Biobank. CLD was defined as a composite of alcoholic and non-alcoholic fatty liver disease, cirrhosis, and liver cancer. Kaplan–Meier curves and multivariable Cox proportional hazards regression models adjusted for covariates were used to estimate the lifetime and 15-year risk of incident CLD.

**Results:** Among 409,062 UK Biobank participants (median [25th–75th] age 56 [48–62] years; 177,425 [43%] male), we identified 280 carriers of inactivating variants in PCSK9 and 408,782 non-carriers. Compared with non-carriers, carriers had lower levels of total cholesterol (–12.6%,  $P=1.5 \times 10^{-30}$ ), LDL-C (–26%,  $P=9.5 \times 10^{-47}$ ), triglycerides (–10%,  $P=0.01$ ), and apoB (–15%,  $P=1.1 \times 10^{-30}$ ). At baseline, 6.4% of PCSK9 LOF variant carriers and 5.15% of non-carriers had a diagnosis of diabetes ( $P=0.33$ ). Variants in PCSK9 were not associated with higher circulating liver enzyme levels [alanine aminotransferase [ALT] 19.6 [15.0–26.9] U/L,  $P=0.91$ ; aspartate aminotransferase [AST] 24.5 [21.0–29.5] U/L,  $P=0.19$ ;  $\gamma$ -glutamyltransferase [GGT] 26.9 [18.2–37.5] U/L,  $P=0.73$ ]. For the composite outcome of CLD, diagnoses were reported in 5 of 280 (1.8%) PCSK9 carriers and in 5,946 of 404,322 (1.5%) non-carriers ( $P=0.63$ ). After adjustment for confounders, PCSK9 LOF variants were not associated with CLD risk (OR 1.03, 95% CI 0.37–2.82;  $P_{adj}=0.95$ ). These results were confirmed over 15 years of follow-up, during which PCSK9 carriers showed no significant association with the risk of CLD events (HR 1.73, 95% CI 0.43–3.20;  $P_{adj}=0.74$ ). Importantly, no deaths from CLD were recorded among carriers.

**Conclusions:** These results demonstrate that the genetic inhibition of PCSK9 due to LOF variants is not associated with an increased risk of CLD in the UK Biobank cohort. These findings are consistent with the safety data of randomized clinical trials with anti-PCSK9 monoclonal antibodies and inclisiran, which have found no significant changes in liver enzymes.

## MARKERS OF INFLAMMATION AND HYPOFIBRINOLYSIS ARE ASSOCIATED WITH COGNITIVE DYSFUNCTION AND MOTOR PERFORMANCES IN ATRIAL FIBRILLATION PATIENTS ON ORAL ANTICOAGULANT THERAPY: INSIGHTS FROM THE STRAT-AF STUDY

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**Background:** Atrial fibrillation (AF) is the most common supraventricular arrhythmia and one of the most commonly encountered heart conditions in clinical practice. Emerging evidence suggests a significant role of inflammation in the pathogenesis of AF. Population studies have also suggested an association between AF and cognitive impairment and dementia. The aim of this study is therefore to assess, in a population of AF patients on oral anticoagulant therapy, the association between circulating biomarkers involved in the pathogenesis of AF and the cognitive and motor performances of the enrolled patients.

**Methods:** The Strat-AF study is an observational, prospective, single-center, hospital-based study enrolling elderly patients with AF. Results refer to 180 subjects who underwent a complete clinical, biochemical, cognitive, and functional evaluation.

**Results:** At multivariate logistic regression, Clot Lysis Time (CLT) and circulating levels of von Willebrand Factor (vWF) remained significantly associated with pathological performances at the Stroop test (expressed as execution time) [OR 95% CI 1.54 (1.02-2.35),  $p=0.042$  and 1.75 (1.08-2.82),  $p=0.023$ , respectively]. With regard to the Short Physical Performance Battery (SPPB), the circulating levels of IL-8 remained significantly associated with the clinical endpoint [OR 95% CI 2.19 (1.13-4.25),  $p=0.020$ ].

**Conclusions:** Our results suggest a potential innovative tool able to identify AF patients at risk of worse prognosis in terms of cognitive and motor performances. The clinical relevance of these results is due to the fact that we have no efficient methods to predict a deterioration in the cognitive performance and, consequently, the possible onset of dementia in AF patients undergoing oral anticoagulant therapy.

## ABSTRACT

## OLINK PLASMA PROTEOMICS DISCRIMINATES PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA FROM UNAFFECTED INDIVIDUALS

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**Introduction:** Familial hypercholesterolemia (FH) is an inherited disease characterized by lifelong elevated low-density lipoprotein cholesterol (LDL-C) and increased risk of premature atherosclerotic cardiovascular disease. Despite available diagnostic tools, such as the Dutch Lipid Clinic Network (DLCN) score, many individuals remain undiagnosed or misclassified, particularly when family history is absent and genetic testing is unavailable. Proteomics has emerged as a promising strategy for enhanced cardiovascular phenotyping, capturing pathophysiological effects of LDL-C accumulation, including inflammation and vascular injury. We investigated whether Olink's proximity extension assay-based proteomics could reveal a plasma protein signature able to distinguish FH patients from unaffected individuals and assessed its performance against current diagnostic criteria. **Methods:** Plasma samples were obtained from 704 healthy participants in the PLIC cohort and 127 FH patients from the LIPIGEN registry. Data were generated using three Target 96 panels (Cardiovascular II, Cardiovascular III, Inflammation) and harmonized by bridging 16 overlapping samples. Assays with >30% of values below the limit or detection were excluded. Final dataset was randomly split into training (70%, n=582) and test (30%, n=249) sets with balanced covariates. Feature screening was performed using linear models adjusted for age, sex, LDL-C, and lipid-lowering therapy, with differentially expressed proteins identified with FDR-corrected p-values and retained for downstream modelling. Logistic regression with Least Absolute Shrinkage and Selection Operator (LASSO) penalization was implemented, with lambda hyperparameter tuned by 10-fold cross-validation using area under the curve (AUC) as primary metric. Class imbalance was addressed by applying inverse-frequency weighting during training. Preprocessing (median imputation and z-score scaling) was applied independently within each fold to avoid data leakage. Robustness of selection was evaluated through 1,000 bootstrap iterations, retaining proteins present in ≥80% of runs. These were refitted in an unpenalized logistic regression to obtain unbiased estimates. Model performance was evaluated on the test set and compared against DLCN and LDL-C as predictors of FH, with optimal classification threshold chosen using Youden's J statistic. **Results:** Feature screening identified 111 differentially expressed proteins out of 231. LASSO with bootstrapping yielded an initial 8-protein panel, which after refitting was reduced to a stable 6-protein signature (HSP27, tPA, TRAP, TIE2, APN, TNFRSF9). The 6-protein model showed superior fit (Akaike Information Criterion = 252.3 vs. 252.8 for the 8-protein model; likelihood ratio test p = 0.18) and was retained for validation. In the test set, it achieved an AUC of 0.972 (95% CI 0.953–0.992), accuracy of 0.908, sensitivity of 0.868, specificity of 0.915, precision of 0.647, F1-score of 0.742, and Matthews correlation coefficient of 0.698. Compared with benchmarks, the model was not statistically superior to DLCN score (AUC 0.962, p=0.71) or LDL-C (AUC 0.918, p=0.11). Likelihood ratio testing confirmed that add-

ing the proteomic model to DLCN improved prediction (AUC 0.996, p=0.01).

**Conclusions:** Plasma proteomics enabled the development of an interpretable model capable of discriminating FH patients from unaffected controls. These findings highlight the relevance of proteomic biomarkers as complementary tools for FH diagnosis. It remains to be determined whether our model can also discriminate between genetically confirmed and clinically diagnosed FH.

## ULTRASOUND EVALUATION OF CAROTID PERIVASCULAR ADIPOSE TISSUE THICKNESS AS A POTENTIAL MARKER OF ATHEROSCLEROSIS AND CARDIOVASCULAR RISK

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**Introduction:** Perivascular adipose tissue (PVAT) is widely recognized as a metabolically active organ that regulates arterial function through autocrine and paracrine pathways and possibly play a role in the development of atherosclerotic cardiovascular (CV) disease through dysfunctional signals. Accordingly, PVAT thickness at different arterial districts could serve as a marker of atherosclerosis and CV risk. The aims of our study were 1) to evaluate the feasibility a non-invasive, ultrasound-based approach for measuring carotid PVAT thickness (cPVATt), and 2) to evaluate the association between cPVATt, carotid atherosclerosis burden, and CV risk.

**Methods:** We conducted an observational, cross-sectional, pilot study including outpatients referred to our tertiary lipid clinic. Carotid PVAT was evaluated bilaterally by ultrasonography using a 10-MHz multifrequency linear probe positioned at the base of the neck in contact with clavicle, perpendicular to the skin and in a transverse orientation. The mean distance between the common carotid adventitia and the sternocleidomastoid muscle anteriorly and the longus colli muscle posteriorly, measured on both the right and the left sides, was used as a cumulative measure of cPVATt. Carotid atherosclerosis was evaluated by determining common carotid intima-media thickness (IMT) and by assessing the presence of atherosclerotic plaque (defined as IMT >1.5 mm at either common carotid or internal carotid) and the percentage of stenosis. Individual CV risk was estimated through SCORE2 and SCORE2-OP algorithms.

**Results:** A total of 465 patients (mean age 74±14 years, 51% male) were included in the study. The median value of cPVATt was 0.68 (0.58-0.85) cm. Significant direct correlations emerged between cPVATt and body mass index (r=0.170, p<0.001), waist circumference (r=0.224, p<0.001), neck circumference (r=0.269, p<0.001), uric acid (r=0.106, p=0.031), triglycerides (r=0.095, p=0.048), and hs-CRP (r=0.202, p=0.019). A significant inverse correlation was observed between cPVATt and HDL cholesterol (r=-0.152, p=0.001). No correlation was observed between cPVATt and any measure of carotid atherosclerotic burden. There was a significant increase in cPVATt across CV risk categories (p for trend=0.043).

**Conclusions:** The present study preliminarily demonstrates the feasibility of an ultrasound approach for assessing cPVAT, the

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reliability of cPVATt as a measure of adiposity and its potential value as a marker of increased CV risk. However, it does not show any significant correlation between cPVATt and carotid atherosclerotic burden. Future studies should 1) compare information obtained from cPVATt and fat attenuation index from computer tomography of the carotid district, 2) evaluate the relationship between cPVATt and carotid plaque composition, and 3) assess the prospective association between cPVATt and CV risk.

### SUBPHENOTYPING APPROACH IN INDIVIDUALS AT ELEVATED RISK OF DIABETES IDENTIFIES PERSONS WITH INCREASED ARTERIAL STIFFNESS, IRRESPECTIVE OF THE CLASSICAL CLASSIFICATION OF GLUCOSE TOLERANCE

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**Background and Aims:** Prediabetes is associated with increased cardiovascular (CV) risk and incidence of diabetes. However, this condition is quite heterogeneous and a new subphenotyping approach for persons at elevated risk of diabetes was proposed to improve the stratification of the risk of diabetes onset and its complications. This study aimed to evaluate arterial stiffness across these subtypes.

**Methods:** 377 persons without prior diagnosis of diabetes and CV events underwent physical examination and review of clinical history, smoking status, and medications;<sup>2</sup> classical glucose tolerance assessment through oral glucose tolerance test (OGTT), fasting glucose and HbA1c dosage;<sup>3</sup> CV risk assessment through tonometry measured arterial stiffness (i.e. pulse wave velocity – PWV –, augmentation index – AugI –, and subendocardial viability ratio – SEVR) and intima-media thickness (IMT) evaluation. Participants with incident diabetes (n=61) and with missing data for subphenotyping (n=57) were excluded. Thus, 259 participants were assigned to one of the following subtypes according to sex, body mass index, waist circumference (WC), fasting glucose, fasting insulin, glucose and insulin 2h-post-OGTT, triglycerides, and HDL cholesterol:<sup>1</sup> Low-risk;<sup>2</sup> Very-low-risk;<sup>3</sup> Beta-cell-failure;<sup>4</sup> Low-risk/obese;<sup>5</sup> High-risk/insulin-resistant/fatty-liver;<sup>6</sup> High-risk/visceral-fat/nephropathy. A multiple regression analysis adjusting for major confounders was performed to evaluate the association between subtypes and PWV.

**Results:** The overall study population showed a mean age of 47.88±11.03 years and 148 individuals (57.1%) were females. The 20% and 75.5% of individuals in subtypes 5 and 6, respectively, were normal glucose tolerant (NGT) when the classical definition was applied. Subtype 5 (8.88±1.83 m/s) showed higher PWV in comparison to subtypes 1 (7.29±1.69 m/s), 2 (7.14±2.49 m/s), and 4 (7.49±1.47 m/s) (all P<0.05). Subtype 6 (8.07±1.67 m/s)

showed nominally higher PWV in comparison to subtypes 1, 2, and 4 and a lower SEVR than subtype 3 (145.47±28.08 vs 165.62±35.50%, P=0.033). No differences were identified for AugI. After adjusting for age, sex, BMI, WC, antihypertensive therapy, systolic blood pressure, LDL cholesterol, active smoking, and HbA1c, subtype 5 (β=1.79 [0.70-2.88], P=0.001) and 6 (β=1.09 [0.02-2.16], P=0.047) were associated with higher PWV. **Conclusions:** Subtypes 5 (High-risk/insulin-resistant/fatty-liver) and 6 (High-risk/visceral-fat/nephropathy) of prediabetic metabolism exhibit subclinical vascular damage and higher CV risk. Early screening for CV disease, as well as personalized clinical interventions, in these subtypes, irrespective of classical glucose tolerance classification, could be important to mitigate adverse CV outcomes.

### IMPACT OF BEMPEDOIC ACID ON HEPATIC STEATOSIS, NON-ALCOHOLIC STEATOHEPATITIS, AND FIBROSIS: A CLINICAL EVALUATION USING ADVANCED ELASTOSONOGRAPHY TECHNIQUES

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**Introduction:** Bempedoic acid is an innovative oral drug that lowers LDL cholesterol levels and also provides benefits in glycometabolic control and systemic inflammation. While its impact on cardiovascular risk is well-documented, the effects of bempedoic acid on liver diseases, such as hepatic steatosis, non-alcoholic steatohepatitis (NASH), and hepatic fibrosis, remain under investigation. Recent studies, including those published in Cell in 2025, have begun exploring the potential of bempedoic acid in treating hepatic steatosis. These studies suggest that bempedoic acid may suppress diet-induced hepatic steatosis independently of ATP-citrate lyase (ACL), an enzyme crucial for fatty acid and cholesterol synthesis in the liver. This mechanism implies that bempedoic acid could reduce hepatic steatosis by targeting specific metabolic processes beyond its effect on LDL cholesterol levels. However, additional data are needed to confirm its efficacy and safety in this context. Our study aims to evaluate the effects of bempedoic acid on hepatic steatosis and fibrosis using advanced techniques like hepatic elastosonography, which will monitor liver stiffness and correlate elastosonographic parameters with traditional biomarkers such as GOT, GPT, GammaGT, and fibrosis indices FIB-4 and FLI.

**Study Objectives:** The primary objective of this study is to assess the impact of bempedoic acid on hepatic steatosis, steatohepatitis, and hepatic fibrosis. This will be achieved through abdominal ultrasonography and hepatic elastosonography performed "blind" by two separate radiologists. Liver stiffness will be measured using parameters like Shear Wave Elastography (SWE), Shear Wave Dispersion (SWD), and Attenuation Imaging (ATI), which will be correlated with traditional biomarkers such as GOT, GPT, GammaGT, and FIB-4 and FLI indices. Secondary objectives include evaluating changes in lipid levels (C-LDL, non-HDL, apoB), inflammatory markers (hsCRP), and glycometabolic function (HOMA Index).

**Methodology:** This study will involve 100 patients with dyslipidemia, metabolic syndrome, and familial hypercholesterolemia, treated with either bempedoic acid or statins. Patients will be enrolled at the Ambulatory Unit of Metabolic Disease Patho-

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physiology of the University Hospital of Siena. Each patient will undergo a comprehensive clinical evaluation, including monitoring lipid levels (C-LDL, non-HDL, apoB), hepatic enzymes (GOT, GPT, GammaGT), uric acid, creatinine, and glycometabolic parameters (HOMA Index). The instrumental part of the study will include abdominal ultrasonography and TSA color Doppler echography performed at baseline, three, six, and twelve months.

Regarding hepatic elastosonography, measurements will be conducted in a “blind” manner by two separate operators from the UOC Radiology of Siena. The operators will not be informed of whether the patient is receiving bempedoic acid or statins, minimizing potential biases associated with knowledge of the treatment. Each operator will independently take measurements of SWE, SWD, and ATI parameters. Subsequently, the quality and accuracy of the measurements will be assessed by comparing the operators’ readings to ensure the reliability of the results.

**Impact and Data Transferability:** Bempedoic acid could significantly impact the management of hepatic steatosis, steatohepatitis, and fibrosis by potentially reducing the progression to advanced liver disease. While studies on the effectiveness of bempedoic acid in hepatic steatosis are still in development, preliminary data, such as those recently published in *Cell*, suggest that the drug may positively influence lipid metabolism and inflammatory control, providing possible benefits for liver health. Hepatic elastosonography, using advanced parameters like SWE, SWD, and ATI, will allow precise monitoring of liver stiffness and correlate changes with traditional biomarkers. These data will help clarify the potential role of bempedoic acid in managing non-alcoholic liver diseases and preventing cardiovascular events, particularly in patients with metabolic syndrome and liver comorbidities. Moreover, bempedoic acid has a favorable economic profile compared to injectable therapies like PCSK9 inhibitors, making it a potentially cost-effective therapeutic option for primary prevention and management of dyslipidemia.

**Expected Results:** We expect that treatment with bempedoic acid will lead to a significant reduction in hepatic steatosis, steatohepatitis, and hepatic fibrosis, as measured by abdominal ultrasonography and hepatic biomarkers. Hepatic elastosonography should reveal a reduction in liver stiffness, measured by SWE, SWD, and ATI parameters, which will be correlated with fibrosis (FIB-4) and steatosis (FLI) scores. Additionally, we anticipate improvements in glycometabolic parameters such as fasting glycemia, Glycated Haemoglobin, HOMA Index and a reduction in cardiovascular risk, monitored through hsCRP. The results of this study could provide valuable evidence on the efficacy of bempedoic acid in treating non-alcoholic liver diseases and preventing cardiovascular complications.

**Conclusions:** This study will explore the efficacy of bempedoic acid in treating hepatic steatosis, steatohepatitis, and hepatic fibrosis using advanced methodologies such as hepatic elastosonography and FIB-4 and FLI indices. While studies on the drug in this context are still under development, preliminary data suggest potential benefits. The “blind” design with two separate operators will ensure the reliability of the results, minimizing potential biases from treatment knowledge. The data collected could support the adoption of bempedoic acid as a therapeutic option for managing liver and cardiovascular comorbidities, while also providing a valuable tool for the prevention and management of dyslipidemia in patients with metabolic syndrome.

## SURVIVORS FROM CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA SHOW AN INCREASED LONG-TERM CARDIOVASCULAR IMMUNO-METABOLIC RISK

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**Background and Aims:** Acute lymphoblastic leukaemia (ALL) is the most common haematological cancer in children, treated by chemotherapy as first line of treatment, followed by total body irradiation and hematopoietic stem cell transplantation (TBI-HSCT) for high-risk paediatric patients or in case of relapse. While these therapies have extended life expectancy reaching a survival rate above 90%, an increased cardiovascular and metabolic risk has been reported in ALL survivors. This project aims to explore the metabolic and inflammatory sequelae in ALL survivors after TBI-HSCT, specifically investigating how these effects might impact mature immune cells derived from transplanted stem cells.

**Methods:** 14 disease-free cALL survivors (11M/3F) who underwent TBI-HSCT (identified as recipients), and 14 matched sibling donors (3M/11F) were selected according to specific inclusion and exclusion criteria. Each recipient-donor couple were profiled for medical anamnesis and clinical assessment and were subjected to a blood collection, for immunophenotyping, transcriptomic and telomere length analysis. Furthermore, patients and donors underwent an ultrasound study of the supra-aortic trunks to determine the intima media thickness (IMT).

**Results:** No differences have been detected in anthropometric characteristics (cohort median age: 23.7; specifically 23.9 for the donors and 23.5 for the recipients group; median years since transplantation: 10.8), however dyslipidaemia, characterized by elevated levels of cholesterol (157.5 mg/dl, SE±9.194 vs 171.9 mg/dl, SE±10.43) and triglycerides (65.57 mg/dl, SE±5.216 vs 105.0 mg/dl, SE±11.31), increased insulin (8.457 mUI, SE±1.870 vs 15.23 mUI, SE±2.945) and HOMA index (1.587, SE±0.3653 vs 2.98, SE±0.6225) were reported in recipients compared to matched donors. Metabolic Syndrome was diagnosed in 14.3% of recipients and linear regression analysis of C-IMT showed an accelerated carotid thickening (0.0083 vs 0.0034) compared to donors. Vascular dysfunction was associated to an increased levels of pro-inflammatory proteins (CRP, SAAP) identified by proteomic analysis of plasma, which also confirmed an increased abundance of proteins associated to dyslipidemia, such as APOB, APOC4-APOC2. Impaired metabolic and inflammatory phenotype was associated with an impaired immune cell response. First, recipients presented an accelerated telomere shortening in circulating immune cells (PBMC, slope -0.009 vs -0.0008) and, by flow cytometry, a decreased CD34+ and increased CD19+ frequency was reported in the circulation of recipients. ScRNA-seq on PBMC confirmed inflammation and impaired B cell functions associated to antigen presentation and metabolism. By contrast, plasma immunoglobulins IgM were diminished in recipients vs donors (58.62 mg/dl, SE±4.062 vs 49.23 mg/dl, SE±3.188), while comparable levels of IgG (350.6 mg/dl, SE±14.49 vs 329.7 mg/dl, SE±16.43) were reported.

**Conclusions:** These data suggest that TBI conditioning nega-

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tively impacts the immune-metabolic profile of cALL survivors, contributing to an increased risk of long-term cardiovascular complications. Analysis of B cell subsets is on-going to depict the functional impairment of these cells in recipients. Together this evidence suggests the need to optimize the clinical follow-up strategies to mitigate the increased cardiometabolic risk in cALL survivors.

### INCREASED PLASMA C-REACTIVE PROTEIN LEVELS PREDICTS UNFAVORABLE CARDIOVASCULAR PROGNOSIS IN SUBJECTS AT INCREASED ASCVD RISK AND IMPAIRED KIDNEY FUNCTION: LONGITUDINAL RESULTS FROM THE IMPROVE STUDY

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**Background and Aim:** Chronic Kidney Disease (CKD) is a detrimental condition that leads to an increased risk of atherosclerotic cardiovascular disease (ASCVD). Low-grade systemic inflammation, as reflected by plasma high-sensitivity C-Reactive Protein (hsCRP) levels above 2 mg/L, is considered a significant ASCVD risk modifier. The impact of elevated hsCRP (>2 mg/L) on the incidence of cardio-, cerebro-, and peripheral-vascular (ASCVD) events among patients at increased ASCVD risk and free from ASCVD at baseline has been examined across glomerular filtration rate (GFR) strata in the IMT-Progression as Predictors of VEs (IMPROVE) study.

**Methods:** Among 3,040 IMPROVE participants, plasma hsCRP levels were available for 2,072 subjects without inflammatory disease or anti-inflammatory therapy. The prevalence of high hsCRP levels across different GFR strata [group A ≥ 79 mL/min (median) and group B <79 mL/min; group A1: >90 mL/min,

group B1: 60-90 mL/min, group C1: <60 mL/min] was estimated, and the prognostic impact of high hsCRP levels on incident ASCVD events [at a median follow-up of 3.0 (short follow-up) and 8.5 years (long follow-up), respectively] was explored after correction for confounders.

**Results:** The median hsCRP levels were 1.63 mg/L (IQR 0.67-3.43) and 2.02 mg/L (0.89-3.65) in group A and B (p=0.001), respectively, and 1.46 mg/L (IQR 0.64-3.37 mg/L), 1.89 mg/L (IQR 0.81-3.51 mg/L) and 2.42 mg/L (IQR 1.04-4.25 mg/L) in groups A1, B1 and C1 (p for trend=0.001); the prevalence of hsCRP >2 mg/L in group A and B was 44.3% and 50.5% (p=0.006), and 42.2%, 48.4% and 53.3% in groups A1, B1 and C1 (p for trend=0.005). During a median follow-up of 3.0 and 8.5 years, 97 and 209 ASCVD events were recorded. Elevated hsCRP (>2 mg/L) was associated with an increased ASCVD risk (HR 2.05, 95%-CI 1.32-3.17 in the short follow-up; HR 1.59, 95%-CI 1.20-2.12, in the long follow-up), irrespective of confounders. A significant interaction has been found between hsCRP>2 mg/L and below-median GFR in predicting ASCVD risk both in the short- and long-term follow-ups (p=0.018 and p=0.029, respectively), with hsCRP>2 mg/L being associated with increased short-term and long-term ASCVD risk in group A [HR 3.05 (1.63-5.69) and HR 2.01 (1.40-3.17)] but not in group B [HR 1.28 (0.66-2.50) and HR 1.21 (0.80-1.84)]. Elevated hsCRP predicted an increased ASCVD risk in group C1 and B1 [short follow-up: HR 4.66 (1.35-16.12) and HR 1.90 (1.10-3.32), respectively] & [long follow-up: HR 2.87 (1.21-6.83) and HR 1.48 (1.04-2.10), respectively], but not in group A1 [short follow-up: HR 1.60 (0.62-4.12)] & [long follow-up: HR 1.64 (0.87-3.12)] (p for interaction=NS).

**Conclusions:** Approximately half of subjects at increased ASCVD risk with impaired kidney function have an elevated plasma hsCRP level. An increase in hsCRP level is longitudinally associated with an increased ASCVD risk in subjects with lower kidney function, in both the short-term and long-term follow-ups. Conversely, elevated hsCRP levels were not associated with an increased ASCVD risk in subjects with normal GFR. It is worthwhile exploring whether high risk inflamed CKD subjects might be a target for specific anti-inflammatory therapies in order to improve their cardiovascular prognosis.

### IMMUNE-METABOLIC CHARACTERIZATION OF A HUMANIZED MOUSE MODEL FOR TRANSLATIONAL STUDIES ON CARDIOVASCULAR DISEASES

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**Aim:** Atherosclerosis is one of the leading cause of cardiovascular diseases and it's driven by elevated plasma cholesterol levels, paralleled by an impaired immune-inflammatory response. Despite effective lipid-lowering therapies, some patients still show a residual inflammatory risk, potentially linked to immune response dysfunctions, thus fueling the interest in developing cardiovascular immunomodulatory approaches. This scenario highlights the need for experimental models that can directly translate both molecular mechanisms and therapeutic strategies of

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CVD immunomodulation from preclinical to clinical studies. Here we will present the immune-metabolic characterization of an immunodeficient mouse – on an atheroprone genetic background –, whose immune system is reconstituted with human hematopoietic stem cells (CD34).

**Methods:** Humanized TKO-LDLr KO mice (generated by crossing LDLr KO with immunodeficient Rag2-KO/IL2rg-KO/CD47-KO mice, HuTKOL) were generated after low-dose irradiation (250 cGy) of 2-3 days old pups followed by hepatic injection of commercial or iPSCs-derived CD34 (250.000-300.000 cells/mouse). After 12 weeks, human cell engraftment was evaluated by FACS. HuTKOL were then fed 12-week high-cholesterol diet (WTD) to investigate immunometabolic phenotype and atherosclerosis.

**Results:** HuTKOL presented human leukocytes (% hCD45+ cells/total number of live leukocytes: 40,97%, SE±3,46%) in circulation after engraftment with commercial hCD34+ cells, with B cells representing the most abundant population at 8 wks (% hCD19+ cells/hCD45+ cells: 72,81%, SE±2,33%), but they proportion decreased over time (% hCD19+ cells/hCD45+ cells: 13,85%, SE±2,98%). In contrast, T cells show the opposite trend (% hCD3+ cells/hCD45+ cells at 8 wks: 10,93%, SE±3,76%) being the most represented human subset after 12-wks of WTD (% hCD3+ cells/hCD45+ cells: 58,84%, SE±4,86%), similar to human lymphocyte profile. On WTD, HuTKOL developed dyslipidemia (plasma cholesterol levels: 1086,90 mg/dl, SE± 65,15) and atherosclerosis (% aortic sinus plaque occlusion: 24,79%, SE±3,17%; atherosclerotic lesion volume: 0,32 mm<sup>3</sup>; % fibrosis/plaque area: 33,94%, SE±16,17%), with human immune cell infiltration into the plaques replicating the composition of human plaques. Moreover, the feeding with high cholesterol diet induced the expansion of hCD4 memory T cells and the production of IgM against atherosclerosis-related antigens, confirming an activation of the human adaptive immune response. Characterization of iPSCs-derived CD34+ cells and their engraftment in TKO-L is ongoing.

**Conclusion:** HuTKOL mice represent a valuable platform to investigate human adaptive immunity dynamics under dyslipidemia and to test immuno-modulation for CVD. Furthermore, we envision that the use of iPSCs-derived CD34+ cells would allow to investigate the pathological and pharmacological editing of immune cells for cardiovascular disease prevention.

In obese adipose tissue, some macrophages develop “metabolic inflammation,” driven by fatty acid accumulation and disrupted lipid metabolism, whereas a newly described subset of lipid-associated macrophages (LAMs) appears protective. The mechanisms that determine whether lipid-loaded macrophages are detrimental or beneficial remain unclear. One understudied mode of adipocyte-macrophage communication is through extracellular vesicles (EVs), particularly their lipid cargo.

**Aim:** We hypothesised that adipocyte-derived EVs, and especially their lipidome, drive macrophage metabolic activation.

**Methods:** To test this hypothesis, 3T3-L1 mature adipocytes were treated for 48 h with 400-800 µM U-<sup>13</sup>C-palmitic acid (PA) to simulate an obesogenic environment. EVs isolated by ultracentrifugation were then added to bone marrow-derived macrophages (BMDMs). Adipocyte function was assessed by insulin sensitivity, lipolysis, and expression of lipid metabolism, inflammation, and ER stress markers. EVs size distribution and concentration were analysed via nanoparticles tracking analysis (NTA). In parallel, fatty acid (FA) composition and exogenous PA fate were analysed by gas chromatography coupled with mass spectrometry (GC-MS).

**Results:** PA treatment impaired adrenergic-stimulated lipolysis and increased IL-6 expression without affecting insulin signalling. FA composition of adipocytes remained largely unchanged, with compensatory upregulation of SCD-1 and active FA remodelling as assessed by tracing U-<sup>13</sup>C- PA fate. Moreover, PA treatment modulated EVs size distribution and abundance following a concentration-dependent U-shaped trend. While 400 µM PA increased the number of particles per millilitre but decreased their size, the opposite was true for 800 µM PA. EVs derived from PA-treated adipocytes upregulated markers of lipid associated macrophages. Interestingly, while EVs upregulated the lipid transporter CD36, the canonical lipid droplet marker PLIN2 was not upregulated. This result suggests that EVs promote a lipid-associated macrophage phenotype independently of conventional lipid droplet accumulation.

**Conclusion:** In conclusion, PA-loaded adipocytes actively remodel their lipid metabolism and release EVs that trigger macrophage metabolic activation, identifying EVs as potential mediators of obesity-induced inflammation.

## EXTRACELLULAR VESICLES FROM HYPERTROPHIC ADIPOCYTE DRIVE BONE-MARROW DERIVED MACROPHAGES METABOLIC ACTIVATION

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**Background:** Obesity is a chronic, low-grade inflammatory disease that increases the risk of diabetes, stroke, and heart disease. A hallmark of obesity is the infiltration and activation of adipose tissue macrophages (ATMs). While adipose tissue inflammation is clearly linked to cardiometabolic complications (CMCs), clinical trials targeting classical inflammatory pathways (e.g. IL-1β) have shown limited success, suggesting that macrophage activation in obesity differs from other inflammatory con-

## COFFEE BIOACTIVES REDUCE INFLAMMATION AND ATHEROGENESIS IN CARDIAC ADIPOCYTES AND MONOCYTES FROM CAD PATIENTS

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**Background:** Obesity induces metabolic shifts in hypertrophic epicardial and pericardial fat, promoting inflammatory and ath-

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erogenic phenotypes. These are characterized by the excessive accumulation of monocytes and macrophages, which drive a self-perpetuating cycle of cardiac adipose tissue and coronary vascular inflammation (1).

Understanding how specific dietary compounds influence dysmetabolism and inflammation in epicardial and pericardial adipose tissue may offer promising preventive strategies to mitigate coronary atherosclerosis and enhance patient outcomes. Coffee, a widely consumed beverage known for its psychoactive stimulant effects, contains alkaloids such as caffeine and trigonelline, as well as bioactive compounds like chlorogenic acids. Although coffee consumption has been linked to cardiovascular risk in a U-shaped manner, limited information exists on its impact on cardiac adipose tissue metabolism (2).

This study aimed to investigate whether, and how, coffee components modulate the pro-inflammatory and dysmetabolic characteristics of hypertrophic pericardial adipose tissue (PAT), particularly focusing on its interactions with pro-inflammatory monocytes.

**Objective:** To evaluate the effects of coffee components on PAT adipocytes and peripheral blood mononuclear cells (PBMCs) isolated from coronary artery disease (CAD) patients undergoing coronary artery bypass graft (CABG) surgery.

**Methods:** PAT and PBMC samples were collected from coronary patients undergoing surgery and processed immediately. Isolated mature adipocytes and PBMCs were morphologically and molecularly characterized. The cellular response, including the production of adipokines, cytokines, and metalloproteinases (MMPs), was evaluated through qPCR, ELISA, and zymography after 24-hour exposure to coffee components at physiological concentrations corresponding to three cups of coffee daily, with or without caffeine (complete coffee -CC- vs decaffeinated coffee -DC-).

**Results:** Exposure of PAT adipocytes to both CC and DC resulted in a downregulation of mRNA and protein expression for monocyte chemoattractant protein (MCP)-1, interleukin (IL)-6, and CXC-L10 ( $p < 0.05$ ), with a more pronounced effect observed for DC compared to CC. Similarly, exposure of PBMCs to CC and DC components significantly downregulated the mRNA and protein expression of MCP-1, IL-6, CXC-L10, MMP-9, and MMP-1 ( $p < 0.05$ ), while leaving NLRP3 inflammasome expression unaffected. The downregulation of MMP-9 expression was further confirmed by assessing MMP-9 activity via zymography. These findings were consistently reproduced in 8 CAD patients. **Conclusion(s)** Our data suggest that both CC and DC bioactive components, corresponding to a daily intake of three cups of coffee, reduce inflammatory markers in both PAT adipocytes and PBMCs isolated from CAD patients. These findings highlight the potential of coffee to alleviate the inflammatory and dysmetabolic characteristics of cardiac adipose tissue and interacting monocytes.

**Funding:** This work was carried out within the project entitled: Role of coffee circulating metabolites in the regulation of the epicardial adipose tissue (EAT) pro-atherogenic properties: a fat-depot specific approach to reduce coronary atherosclerosis (Codice progetto: 2022NZNZH8), Bando PRIN 2022.

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## CHARACTERIZATION OF THE METABOLIC RESPONSE TO AN ORAL LIPID LOAD IN SUBJECTS WITH AND WITHOUT TYPE 2 DIABETES

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**Background:** Type 2 diabetes (T2D) is a metabolic disorder characterised by impaired glucose and lipid metabolism. Individuals affected by T2D are at high cardiovascular risk, mainly driven by diabetic dyslipidaemia. Nevertheless, impaired clearance of postprandial lipemia may represent an additional cardiovascular risk factor. Thus, characterising postprandial lipemia in response to a lipid load in individuals with T2D might offer an important contribution to further explore their metabolic alterations. **Purpose:** The aim of this study is to evaluate the metabolic response to an oral lipid load in subjects with and without T2D.

**Methods/Patients:** The study included 28 patients diagnosed with T2D (HbA1c $\geq$ 6.5%) and 11 normoglycemic controls (HbA1c $<$ 5.7%), recruited as part of the LIPINFAT Diabetes Study. Anthropometric, nutritional and metabolic characterisation of study participants was performed at baseline. Thereafter they were asked to consume a high-fat meal, consisting of 179 grams of mascarpone cream cheese (equivalent to 75 grams of fat). Blood samples were collected at baseline (fasting) and at 1, 2, 3, 4 and 6 hours after the test meal (T1,T2,T3,T4,T6) to evaluate its impact on blood levels of glucose, insulin, triglycerides, total cholesterol (TC), HDL-C, as well as LDL-C, Apo-B and IL-6. Statistical analysis was performed using Microsoft Excel and Graphpad Prim using a two-way ANOVA followed by Sidak and Dunnet post-hoc tests.

**Results:** Fasting blood glucose and insulin levels were significantly higher in subjects with T2D across all timepoints. As expected, HOMA-IR and TYG-index were significantly higher in individuals with T2D confirming a higher degree of insulin resistance in this group. Following the lipid load, circulating triglycerides increased significantly in both groups and remained elevated throughout the 6 hours after the test meal. The triglyceride rise was earlier and more pronounced in T2D, with significantly higher levels vs controls at T1, T2, T3, T4. No significant changes in TC, HDL-C or LDL-C were observed in either group, likely reflecting baseline therapy differences. IL-6 rose more markedly in controls than in T2D.

**Conclusions:** The triglyceride response to a high-fat meal was earlier and more pronounced in T2D, exposing these individuals to a higher and more prolonged postprandial lipid burden. This impaired metabolic response may increase cumulative lifetime exposure to postprandial lipemia and contribute to cardiovascular risk. The blunted IL-6 response in T2D suggests that chronic low-grade inflammation may mask acute postprandial stimuli.

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**ABSTRACT**


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**TRIGLYCERIDE GLUCOSE INDEX AND MYOCARDIAL DAMAGE IN PATIENTS WITH DIFFERENT GLYCOMETABOLIC PROFILE**

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Type 2 diabetes mellitus and insulin resistance represent major cardiovascular risk factors, with subclinical myocardial damage occurring across different glycometabolic profiles. The Triglyceride-Glucose Index (TyG) has emerged as a reliable, non-insulin-based surrogate marker of insulin resistance, calculated as  $[\text{Ln}(\text{fasting triglycerides mg/dl}) \times \text{fasting glucose mg/dl}]/2$ . Unlike the gold standard hyperinsulinemic-euglycemic clamp technique, TyG offers a practical, cost-effective alternative for clinical practice. Myocardial mechano-energetic efficiency indexed (MEEi), defined as the ratio between stroke volume and heart rate normalized for left ventricular mass, represents an ultrasound-based measure of cardiac performance that can identify subclinical myocardial dysfunction before overt left ventricular ejection fraction impairment. This study aimed to evaluate the potential association between TyG and myocardial damage, estimated using MEEi, in patients with different glycometabolic profile. We enrolled 545 Caucasian patients (314 males and 231 females, mean age  $60.0 \pm 12.1$  years) naïve to drug therapy. Exclusion criteria included chronic respiratory disease, malignant disease, endocrinological pathologies, malabsorption diseases, familial dyslipidemia, cardiovascular complications, and drug therapies affecting glucose or lipid metabolism. All subjects underwent physical examination, anthropometric evaluation, and oral glucose tolerance test (OGTT) with blood samples collected at 0, 30, 60, 90, and 120 minutes. Laboratory measurements included triglycerides, cholesterol fractions, plasma glucose, insulin concentrations, and high-sensitivity C-reactive protein. Blood pressure was measured using standard protocols. Comprehensive 2D and Doppler echocardiography was performed according to American Society of Echocardiography recommendations, with MEEi calculated as stroke volume/heart rate normalized for left ventricular mass. The population was divided into quartiles according to increasing TyG values for analysis. The study population was distributed across four TyG quartiles, with 57.6% males and varying prevalences of diabetes (2.2% to 51.4% from quartile I to IV), pre-diabetes (15.4% to 55.7%), and hypertension (43.3% overall). From quartile I to IV, there was progressive worsening of the lipid profile with significant decreases in HDL cholesterol ( $p < 0.0001$ ) and increases in total cholesterol, LDL cholesterol, and triglycerides (all  $p < 0.0001$ ). Glucose metabolism deteriorated significantly across quartiles, demonstrated by increased fasting plasma glucose, 2-hour post-load glucose, fasting insulin, and HbA1c levels (all  $p < 0.0001$ ), with concomitant decreases in the Matsuda insulin sensitivity index ( $p < 0.0001$ ). Echocardiographic parameters revealed progressive cardiac structural and functional changes, including increased interventricular septal thickness, left ventricular mass, and left atrial volume index. Global longitudinal strain worsened significantly from quartile I to IV ( $p < 0.0001$ ), indicating subclinical left ventricular dysfunction.

MEEi decreased progressively across quartiles ( $p < 0.0001$ ), demonstrating reduced myocardial efficiency. Linear regression analysis revealed significant correlations between MEEi and TyG ( $r = -0.342$ ,  $p < 0.0001$ ), Matsuda index ( $r = 0.294$ ,  $p < 0.001$ ), and estimated glomerular filtration rate ( $r = 0.147$ ,  $p < 0.0001$ ). Multiple regression analysis identified TyG as the major independent predictor of MEEi, accounting for 11.7% of its variation, while the Matsuda index contributed an additional 1.6%. This study demonstrates that TyG is significantly associated with reduced MEEi in a large cohort of Caucasian patients with normal left ventricular ejection fraction across different glycometabolic profiles. The progressive deterioration of MEEi with increasing TyG values indicates the index's capability to identify subclinical myocardial damage before conventional echocardiographic parameters become abnormal.

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**RELATIONSHIP BETWEEN SMAD3 VARIANTS AND NON-AORTIC CARDIOVASCULAR FEATURES IN LOEYS DIETZ SYNDROME**

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**Background:** Loews-Dietz syndrome (LDS) is an autosomal dominant connective tissue disorder typically characterised by vascular, skeletal, craniofacial, cutaneous, and ocular abnormalities. LDS is strictly defined by the triad of hypertelorism, bifid uvula/cleft palate, tortuosity, and aortic/arterial aneurysms. LDS type 3 (LDS3) is associated with mutations in the SMAD3 gene, which codes for mothers against decapentaplegic homolog 3, a component of the transforming growth factor beta (TGF- $\beta$ ) signalling pathway. SMAD3 variants are known to exhibit hereditary thoracic aortic disease (HTAD) and significant osteoarthritis features. While aortic aneurysms and dissections are the primary concern, previous studies have reported severe non-aortic cardiovascular phenotypes, suggesting a heightened risk of ventricular arrhythmias and sudden cardiac death, which necessitates further investigation into myocardial disease in patients with SMAD3 variants. Due to limited information regarding SMAD3 genotype-phenotype correlation and variance in clinical presentation, this study investigated the main cardiovascular settings in subjects harbouring variants in SMAD3.

**Methods:** A cohort of 173 patients [mean age 46.42 years, ( $\pm 17.84$ ) years, 130 males and 43 females] attending the Regional Referral Centre for Marfan Syndrome and Related Disorders in Florence was investigated for differential diagnosis of aortopathy-related conditions and hereditary thoracic aortic disease (HTAD). Subjects underwent multidisciplinary evaluations, including cardiologic and genetic assessment. Targeted Next Generation Sequencing (NGS) of at least 97 genes was performed. All rare variants identified were classified according to standardised guidelines for variant interpretation.

## ABSTRACT

**Results:** Eight rare variants in the SMAD3 gene were identified in 7 index cases out of the 173 subjects investigated. Six out of the 7 index patients underwent thoracic aortic surgery. Importantly, four of the 7 patients also exhibited non-aortic phenotypes. Two patients (P001 and P016) presented with heart failure, which was the cause of death in P001 who showed dilated cardiomyopathy. Patient P014 experienced a brief cardiac arrest following spontaneous coronary artery dissection (SCAD). Patient P010 had an episode of hemorrhagic stroke. Segregation analysis in the relatives of the index cases identified 11 additional carriers of SMAD3 variants. Among the combined cohort of 18 subjects (7 index cases and 11 relatives), mitral valve disorders were the most frequently reported manifestation. Seven subjects had mitral valve prolapse (MVP), and three of these also had mitral annular disjunction (MAD). One patient underwent surgery for a ruptured mitral chord. In the P001 family, all the three carriers of the same pathogenic SMAD3 mutation showed dilated cardiomyopathy.

**Conclusion:** Data from this study confirm that SMAD3 genetic variants contribute to a range of phenotypic manifestations associated with Loeys-Dietz syndrome type III. Beyond aortopathy present in the majority of LDS patients with LP/P SMAD3 mutations, further non-aortic cardiovascular phenotypes may present in combination or as isolated manifestation especially at early disease onset. These non-aortic manifestations, including dilated cardiomyopathy, heart failure, spontaneous coronary artery dissection, and neurovascular events, should not be underestimated and necessitate appropriate clinical and genetic evaluation in index and familial cases. Further investigation is recommended to deepen the molecular mechanisms underlying these genotype-phenotype correlations.

**Methods:** THP-1-derived human macrophages and coronary VSMCs were incubated with doxorubicin [200 nM, 24 h] to induce a senescent phenotype. Cholesterol loading was accomplished by incubating cells with native and acetylated LDL (LDL, acLDL) [50 µg/ml, 24 h]. Intracellular cholesterol content was quantified using a fluorometric assay. Senescence-associated β-galactosidase (SA-β-Gal) expression, a hallmark of cellular senescence, and p65, an inflammatory transcription factor, were assessed by Western blot analysis. The expression of Sirtuin1, a gene encoding an enzyme with anti-senescence activity, was analyzed by RT-qPCR. The secretion of IL-6 was assessed with ELISA assay.

**Results:** In THP-1 cells, doxorubicin increased SA-β-Gal expression (+78%,  $p < 0.0001$ ) but did not affect intracellular cholesterol content. Incubation of macrophages with acLDL raised intracellular cholesterol content, as expected, (+45%,  $p < 0.01$ ) and in parallel significantly increased SA-β-Gal expression (+52%,  $p < 0.0001$ ). In parallel, cholesterol loading suppressed Sirtuin1 expression (-30% vs basal,  $p < 0.01$ ) and significantly enhanced p65 expression (+24% vs. basal  $p < 0.0001$ ), indicating NF-κB pathway activation. In VSMCs, doxorubicin enhanced SA-β-Gal expression (+63%,  $p < 0.0001$ ) and slightly but significantly increased intracellular cholesterol content (+23%,  $p < 0.05$ ). Incubation with LDL and acLDL, as expected, led to an increase in cholesterol content (30% and 24%, respectively;  $p < 0.05$ ) and, in parallel, induced SA-β-Gal expression, which was moderately elevated after LDL incubation (+45%,  $p < 0.05$ ) and markedly enhanced after acLDL incubation (+205%,  $p < 0.05$ ). Moreover, acLDL incubation significantly increased IL-6 secretion (+64%,  $p < 0.001$ ).

**Conclusions:** These findings reveal a strong association between cholesterol accumulation and senescence in macrophages and coronary smooth muscle cells (VSMCs), highlighting a link between derangement in cholesterol homeostasis, inflammation, and vascular aging. Therefore, targeting pathways involved in cholesterol homeostasis could be a promising strategy for preventing or slowing age-related vascular diseases, such as atherosclerosis. Future studies should evaluate whether pharmacological interventions aim at restoring cholesterol homeostasis, can effectively improve the negative effects of vascular aging and improve cardiovascular outcomes.

## RELATIONSHIP BETWEEN CHOLESTEROL ACCUMULATION AND CELLULAR SENESCENCE IN AN IN VITRO MODEL OF VASCULAR CELLS

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**Background and Goals:** Aging is one of the major risk factors for the onset of various chronic diseases, including atherosclerosis. Age-related diseases are associated with the accumulation of senescent cells in tissues, which may accelerate disease progression through the release of cytokines and other pro-inflammatory factors. Recent studies suggest a close relationship between cholesterol homeostasis and the process of cellular senescence: cholesterol homeostasis appears to be altered in senescent cells, and cholesterol itself may impact key processes involved in cellular senescence. However, the reciprocal relationship between these two aspects remains only partially understood. The aim of this study was to investigate the interplay between cellular senescence and cholesterol homeostasis, particularly in the context of atherosclerosis, using an in vitro model of vascular cells, namely human macrophages and smooth muscle cells (VSMCs), the main cellular components involved in the development of atherosclerotic plaques, as a result of cholesterol accumulation.

## FAMILIAL HYPERCHOLESTEROLEMIA BEYOND LDLR GENE: ROLE OF THE 12-SNPS LDL-C POLYGENIC RISK SCORE

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**Background:** Familial Hypercholesterolemia (FH) represents an autosomal disorder due to pathogenic variants in LDLR, APOB or PCSK9, and in LDLRAP1. However, it still represents an underdiagnosed and undertreated clinical condition today. Many definite FH (20-40%) or possible/probable/definite FH

## ABSTRACT

subjects (about 60%) did not demonstrate functional mutations in major candidate genes. Over the past 10 years, extensive genome-wide association studies have provided insight into the role of polymorphisms in influencing LDL-C levels, mainly responsible for the increased risk of cardiovascular disease, starting with Talmud and colleagues in 2013: in mutation-negative patients, clinical phenotype can be associated with an accumulation of common small-effect LDL-C-raising alleles using a 12-single nucleotide polymorphisms (SNPs) score. Aim of this study was to evaluate Talmud genetic score in patients with possible/probable/definite FH with and without variants in LDLR.

**Method:** We analyzed 177 patients with clinically possible/probable/definite FH using Dutch Lipid Clinic Network (DLCN) Score diagnostic criteria. A targeted 57 gene panel, including LDLR gene and Talmud SNPs, was evaluated through a high throughput sequencing technology, using MiSeq Illumina technology. The classification of pathogenicity of LDLR variants was evaluated according to the American College of Medical Genetics (ACMG) guidelines. For statistical analysis we used SPSS v.28 software.

**Results:** Among 177 patients analyzed, 60 had an uncertain significance (VUS)/likely pathogenic (LP)/pathogenic (P) variant in LDLR and 117 were mutation-negative or carried only benign (B)/likely benign (LB) variants in this gene. Significantly higher Talmud score median value in patients LDLR-negative with respect to LDLR-positive was observed [median (interquartile range): 1.02 (0.90-1.11) vs 0.97 (0.85-1.06),  $p=0.037$ ]. Moreover, when stratifying both groups according to a Talmud score cut-off of 0.93, corresponding to the 5th decile of risk (calculated by ROC curve analysis), 32 out of 60 LDLR-positive patients had a Talmud score  $\geq 0.93$  (53.3%), whereas 82 out of 117 LDLR-negative patients had a Talmud score  $\geq 0.93$  (70.1%), reaching statistical significance ( $p=0.0276$ ). In LDLR-negative group, when comparing patients with a Talmud score  $\geq 0.93$  and patients below this cut-off value, we observed significantly higher median levels of LDL-C in patients with a higher decile risk of Talmud score with respect to the others [211.0 (184.5-252.5) vs 194.0 (158.0-215.0);  $p=0.014$ ]; we also observed a significantly higher prevalence of cardiac events (STEMI, NSTEMI, unstable angina, hypertensive cardiopathy) in the first group (11/82 patients with Talmud score  $\geq 0.93$  presenting cardiac events vs 0/35 patients with Talmud score  $< 0.93$ ;  $p=0.0228$ ).

**Conclusion:** These results confirm and expand the contribution of common variants evaluated by the Talmud polygenic risk score in modulating the lipid profile and cardiovascular risk of FH patients, and support the potential utility of the polygenic score in routine clinical practice, especially in patients without a causative mutation in LDLR. Due to the emerging data in literature on further genetic variants associated to lipid profile deregulation and FH clinical manifestations, these data support also the need to expand to a higher number of genetic variants the evaluation in order to implement new and more predictive polygenic risk scores.

## INCLISIRAN REAL WORLD DATA: EFFICACY AND SAFETY

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**Background:** Hypercholesterolemia is one of the most important cardiovascular risk factors. ESC/EAS guidelines recommend stringent LDL-C targets ( $< 55$  mg/dL in high-risk patients). Inclisiran, a small interfering RNA targeting PCSK9, reduces LDL cholesterol with a twice-yearly dosing regimen. Real-world data in Italy remain limited and are essential to define patient profiles for a better patients tailored therapy.

**Purpose of the study:** Our study was aimed to evaluate the response to lipid-lowering therapy (LLT) with inclisiran in patients belonging to different cardiovascular risk classes and to relate it to background therapy.

**Materials and methods:** Retrospective and descriptive analysis of 180 patients treated with Inclisiran in our center, recruited since March 2023. Demographic, clinical, and laboratory data were collected. Patients with available baseline LDL-C and at least one follow-up measurement (3, 9, or 15 months) were included. Primary outcomes were percentage change in LDL-C from baseline and the proportion of patients achieving LDL-C targets, also analyzed in relation to clinical and therapeutic variables. Paired t-tests were performed.

**Results:** Mean age was 64.7 (10.8) years; 62.0% were male. ASCVD prevalence was 44.6%, diabetes 28.4%, FH 12%. Around 70% of patient have an very High CV risk, 23% High CV risk, 3% very very High, 2% moderate and low risk. Mean baseline LDL-C was 122.6 (60.0) mg/dL. Most patients were on background statin and ezetimibe therapy. The safety profile was favorable, with no significant discontinuations due to adverse events. Overall, 51.7% (93/180) of patients achieved LDL-C targets, more frequently among those with higher baseline LDL-C and in optimized combination therapy (rosuvastatin and ezetimibe).

- At 3 months (n=91):  $\Delta -65.4$  mg/dL [95% CI  $-77.0$ ;  $-53.7$ ],  $-45.2\%$  [95% CI  $-52.3$ ;  $-38.0$ ],  $p < 0.001$
- At 9 months (n=66):  $\Delta -45.7$  mg/dL [ $-56.8$ ;  $-34.6$ ],  $-33.7\%$  [ $-42.2$ ;  $-25.2$ ],  $p < 0.001$
- At 15 months (n=37):  $\Delta -50.7$  mg/dL [ $-68.5$ ;  $-32.8$ ],  $-29.1\%$  [ $-45.4$ ;  $-12.9$ ],  $p < 0.001$
- At last available follow-up (n=147):  $\Delta -49.0$  mg/dL [ $-59.9$ ;  $-38.1$ ],  $-30.9\%$  [ $-41.8$ ;  $-20.1$ ],  $p < 0.001$

**Conclusions:** In our experience Inclisiran produced a consistent and sustained LDL-C reduction up to 15 months, confirming the efficacy observed in clinical trials also in routine practice. Approximately half of patients achieved guideline-recommended LDL-C targets, with particularly favorable rates in those on combination lipid-lowering therapy. Adherence to therapy was close to 100%, and no serious adverse events leading to treatment discontinuation were observed.

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 ABSTRACT
 

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## INTERMITTENT FASTING AND CONTINUOUS CALORIC RESTRICTION SHOW SIMILAR EFFECTS ON CHOLESTEROL DISTRIBUTION IN LDL SUBFRACTIONS

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**Background:** Small dense LDL (sdLDL) are highly atherogenic because they oxidize easily and penetrate the vascular intima. Diet strongly influences the lipid profile, including sdLDL levels, but it is unclear whether continuous calorie restriction (CCR) and intermittent fasting (IF) differ in their effects on LDL subfractions.

**Aim:** The aim of the study was to evaluate the effect of IF, compared to CCR, on the distribution of cholesterol in circulating lipoproteins, with particular focus on LDL subfractions.

**Methods and Patients:** The InterFast study is a randomised, controlled, open-label, parallel-group clinical trial. A total of 24 overweight or obese individuals were randomly assigned to two different diets: CCR or IF. Participants in the IF group consumed 25% of their daily energy requirements for two consecutive days and 100% for the remaining five days of the week, whereas subjects in the CCR group consumed 75% of their energy requirements daily. Blood samples were collected at baseline (T0) and after six months of dietary intervention (T6). The FDA-approved Lipoprint electrophoretic system was used to analyze the distribution of serum cholesterol among VLDL, 3 subtypes of IDL, 7 subtypes of LDL, and HDL lipoproteins. LDL subfractions were further grouped into large, buoyant particles (lLDL) and small, dense particles (sdLDL). Comparisons between the CCR and IF groups were performed using Student's t-test for independent data, while Student's t-test for paired data was used to compare T0 and T6 within each group. Statistical significance was set at  $p < 0.05$ .

**Results:** At baseline, demographic, anthropometric and metabolic parameters did not differ between groups. After six months, both diets led to significant weight loss as well as a reduction in body fat and waist circumference, but neither diet produced significant changes in the overall lipid profile. No differences in cholesterol distribution were observed between diets, although CCR reduced cholesterol in IDL-1 and IDL-3 at T6. This occurred in the absence of changes to LDL particle size. To compare the impact of the two diets, the percentage change of the variables ( $\Delta$ ) between T6 and T0 was calculated. No statistically significant differences were observed in key parameters, such as body weight  $\Delta$ , lLDL, and sdLDL cholesterol  $\Delta$ , between the two groups, with the only exception of a greater reduction in ApoB  $\Delta$  levels in the IF group compared with the CCR group.

**Conclusions:** Both diets improved body weight and composition without affecting lipid metabolism, except for lower ApoB in IF. LDL size and cholesterol distribution in lLDL and sdLDL remained unchanged, suggesting IF is comparable to CCR as a caloric restriction strategy. Long-term studies are needed to clarify their cardiovascular impact.

## UNCOVERING THE DUAL ROLE OF HEPATOCYTE-DERIVED APOLIPOPROTEIN E IN LIPOPROTEIN METABOLISM

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**Background and Aim:** Apolipoprotein E (ApoE) is a key regulator of lipid metabolism, mediating the transport and clearance of triglyceride-rich lipoproteins through interactions with LDL receptor family members. While ApoE is expressed in multiple tissues, the liver is the main source of circulating ApoE, positioning hepatocytes as central players in systemic lipid homeostasis. Although global ApoE knockout models have revealed its systemic functions, previous studies have shown that hepatocyte-specific ApoE knockout mice exhibit only mild dyslipidemia under normal dietary conditions (Wagner et al., 2015). This raises the hypothesis that other cellular sources of ApoE may compensate its hepatic deficiency and questions the specific contribution of hepatocyte-derived ApoE in hepatic lipid metabolism. This prompted us to investigate its liver-specific role to elucidate the molecular and cellular mechanisms underlying hepatic lipid metabolism.

**Methods:** Hepatocyte-specific ApoE knockout (ApoE Hep-KO) mice were generated using the Cre-loxP recombination system by crossing ApoE flox/flox mice with Albumin-Cre transgenic mice. Mice were maintained on a standard diet for 12 weeks. To assess their metabolic profile, poloxamer assay, oral lipid tolerance test (OLTT), fast protein liquid chromatography (FPLC), western blot (WB) and gene expression analyses were performed.

**Results:** We confirmed that hepatocyte-derived ApoE is the main circulating source of the protein, as WB analysis on plasma showed a complete absence of ApoE in ApoE Hep-KO mice. Despite this, total plasma cholesterol levels were comparable between ApoE Hep-KO and control mice under both chow ( $76,19 \pm 9,99$  vs.  $85,64 \pm 15,20$  mg/dL) and high cholesterol diet ( $154,7 \pm 26,45$  vs.  $151,4 \pm 14,35$  mg/dL). Lipoprotein profiling by FPLC revealed a redistribution of plasma cholesterol, with a prominent LDL peak in ApoE Hep-KO mice, in contrast to the VLDL-dominant profile typically observed in full ApoE knockout models. Yet, ApoE Hep-KO mice showed delayed triglyceride clearance following OLTT, consistent with impaired clearance of triglyceride-rich lipoproteins. To explain the unexpected lipid profile, we assessed VLDL production using the poloxamer 407 assay, which showed a 46% reduction ( $p < 0.0001$ ) in VLDL-triglyceride secretion in ApoE Hep-KO mice, suggesting a role for hepatocyte-derived ApoE in the synthesis and secretion of triglyceride-rich lipoproteins. Additionally, liver gene expression analysis revealed a general trend toward dysregulation of pathways involved in lipid metabolism, supporting a potential broader role for hepatocyte-derived ApoE in maintaining lipid homeostasis.

**Conclusion:** This work highlights a dual role of hepatocyte-derived ApoE in both the production and clearance of TG-rich lipoproteins, pointing to distinct function of ApoE at systemic versus hepatocellular level. Ongoing molecular analyses aim to further dissect the intracellular mechanisms of lipoprotein synthesis and clarify the hepatocyte-specific contribution of ApoE to lipid metabolism and dyslipidemia.

## ABSTRACT

**ANALYSIS OF HDL FUNCTION IN HEALTHY, PRE-DIABETIC AND DIABETIC WOMEN**

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High-density lipoproteins (HDL) play an antiatherogenic role by mediating reverse cholesterol transport (RCT), acting as an antioxidant, and providing anti-inflammatory and endothelial cell protection. In prediabetes and diabetes, the alteration of HDL functionality is paralleled by an atherogenic serum lipid profile characterized by high levels of triglycerides and low levels of HDL cholesterol (HDL-C). The assessment of HDL-C is used in clinical practice for the assessment of its atheroprotective capacity. However, low or high HDL-C levels alone fail to accurately reflect the functional status of these particles.

It is now widely acknowledged that HDL-C levels alone may not serve as a reliable predictive biomarker for cardiovascular disease risk in either healthy individuals or those with comorbidities. In contrast, the functional properties of HDL particles appear to play a more critical pathophysiological role in maintaining arterial health and hold greater promise as prognostic indicators. Therefore, the aim of this study was to compare the antioxidant and anti-inflammatory functionality of HDL between patients with prediabetes and diabetes and control subjects. For this pilot study, the enrolled women (n=207) were divided into three groups: healthy (n=114), pre-diabetic (n=50), and diabetic (n=43) based on fasting blood glucose and glycated haemoglobin (Hb1Ac) values. Clinical data and fasting serum samples were collected from each patient. Routine clinical chemistry (lipid profile analysis, blood sugar, glycated haemoglobin, and, where possible, for the calculation of HOMA-IR and TyG index) was performed. The oxidative status of HDL particles and their functionality were evaluated through different enzymatic markers (PON-1, GpX3, MPO, LCAT) and by the quantification of oxidized HDL.

Diabetic women were older and had higher BMI than the other groups, confirming the role of these risk factors in the disease. The circulating lipid profile was confirmed to be impaired in diabetics, with alterations in total cholesterol, LDL-C as well as HDL-C and triglycerides. Insulin resistance indices (HOMA-IR and TyG) were also significantly higher in subjects with altered glycaemic homeostasis. Regarding the functional markers of HDL, in patients with defective glucose metabolism, there was a reduction in the antioxidant activity of PON-1 and an increase in the activity of LCAT. In parallel, an increase in the pro-oxidant activity of myeloperoxidase (MPO) was detected, which, in turn, contributed to the deterioration of HDL. On the contrary, no significant differences were observed between the groups in terms of GpX3 and oxHDL.

In conclusion, the transition from a healthy state to prediabetes and diabetes, leads to a deterioration in HDL quality. This confirms that HDL functionality might be a more relevant indicator compared to HDL-C concentration alone, to assess cardiovascular risk.

**USE OF STATINS FOR PRIMARY PREVENTION IN FRAIL GERIATRIC PATIENTS: RESULTS FROM THE START REGISTRY**

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**Background and Aims:** The effectiveness of lipid-lowering therapies (LLTs) for primary cardiovascular prevention in older adults remains a subject of ongoing debate. In individuals aged 75 years and above, the strength of evidence supporting their benefit is considerably weaker. A central concern is the potential for disutility, defined as the burden or negative impact on quality of life associated with treatment, independent of direct adverse drug reactions. Determining the threshold at which the risk of disutility outweighs potential clinical benefits is essential. Current clinical guidelines emphasize the importance of considering frailty when assessing whether to initiate statin therapy in older populations. However, overestimating the influence of age or isolated frailty indicators – without a comprehensive assessment of frailty syndrome – may contribute to the underuse of LLTs in the elderly. This study aims to assess the impact of statin therapy on cardiovascular outcomes in a subgroup of patients aged  $\geq 75$  years from the nationwide START registry, with no prior cardiovascular events and at least one indicator of frailty.

**Methods:** This is a post-hoc analysis of the START registry, a multicenter, observational cohort study enrolling adult patients initiating anticoagulation therapy across Italy until December 2023. From this dataset, we selected individuals aged  $\geq 75$  years without a history of cardiovascular events and with at least one frailty indicator. Frailty was defined by the presence of conditions such as dementia, immobilization syndrome, wheelchair use, fall risk, social isolation, or lack of family support.

**Results:** Among 10,369 patients with atrial fibrillation enrolled in the START registry, 1,651 (15.9%) met the inclusion criteria; of these, 442 were receiving statins. Over a median follow-up of 20.2 months (IQR 12.2–37.6), 188 deaths (41 cardiovascular-related) and 211 cardiovascular events (CVEs) were recorded. The overall CVE incidence rate was 5.6 per 100 person-years (95% CI: 4.9–6.5). Statin use was associated with a significantly reduced risk of CVEs (adjusted Hazard Ratio [aHR] 0.226, 95% CI: 0.070–0.734;  $p=0.013$ ). These findings were consistent in sensitivity analyses among patients aged  $\geq 85$  years. Multivariable stepwise Cox analysis, stratified by follow-up duration, indicated that the protective effect of LLTs became evident only after 6 months (aHR 0.330, 95% CI: 0.131–0.833;  $p=0.019$ ) and 12 months (aHR 0.115, 95% CI: 0.016–0.846;  $p=0.034$ ), but not at 3 months.

**Conclusions:** Our findings support the clinical value of statin therapy in elderly patients (aged  $\geq 75$  and  $\geq 85$  years) with at least one frailty indicator. The lack of a significant reduction in CVE risk within the first 6 months of follow-up suggests a possible life expectancy threshold below which continuation of statin therapy may no longer be beneficial.

## ABSTRACT

## EVALUATION OF GLYCEMIC STATUS AND SUBCLINICAL ATHEROSCLEROSIS IN FAMILIAL HYPERCHOLESTEROLEMIA SUBJECTS WITH OR WITHOUT LDL RECEPTOR MUTATION

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**Background and Aim:** Familial hypercholesterolemia (FH) is a genetic condition characterized by elevated LDL-C and increased cardiovascular risk. Beyond LDL-C levels, the impact of genotype on glucose homeostasis has not been well evaluated. We aimed to evaluate the impact of genotype on glycemic status and on atherosclerotic injury in FH subjects.

**Methods:** We conducted a cross-sectional study on 322 FH subjects not on lipid-lowering therapy (LLT) and without history of cardiovascular disease. Biochemical and genetic analyses as well as vascular profile assessment were obtained from all subjects. The study population was divided into two groups according to genotype: LDL receptor (LDLR) group and non-LDLR (NLDLR) group.

**Results:** The LDLR group exhibited a higher prevalence of low glycemic status (LGS) than the NLDLR group (44.1% vs. 26%,  $p < 0.01$ ), whereas a high glycemic status (HGS) was more prevalent in the NLDLR group compared with LDLR group (74% vs. 55.9%,  $p < 0.01$ ). The NLDLR group exhibited a higher prevalence of peripheral atherosclerotic plaques than the LDLR group (93.4% vs. 73%,  $p < 0.05$ ), while coronary artery calcification (CAC) presence was more prevalent in the LDLR group compared with the NLDLR group (74.7% vs. 48%,  $p < 0.01$ ). In a secondary analysis the study population was stratified into three groups based on LDLR genotype: NLDLR, LDLR defective, LDLR null groups. The prevalence of LGS progressively increased from the NLDLR to the LDLR null group, while HGS showed an inverse trend ( $p$  for trend  $< 0.05$ ). Peripheral atherosclerotic plaque prevalence decreased from the NLDLR to the LDLR null group ( $p$  for trend  $< 0.05$ ), while CAC prevalence increased progressively in the three groups ( $p$  for trend  $< 0.01$ ). Logistic regression analysis showed that FH groups with an LDLR mutation were inversely associated with HGS ( $p$  for both  $< 0.01$ ) and the LDLR null group exhibited the strongest association.

**Conclusions:** FH subjects with NLDLR mutations exhibited a worse glycemic profile, while null LDLR mutations showed the strongest inverse association with HGS. The integrations of genetic, lipid and glucose data could be useful to better identify the metabolic profile and the atherosclerosis distribution in FH subjects.

## BEYOND LDL LOWERING: EFFECTS OF PCSK9 INHIBITORS ON HOMOCYSTEINE AND VASCULAR BIOMARKERS IN FAMILIAL HYPERCHOLESTEROLEMIA

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**Background:** PCSK9 inhibitors (PCSK9i) provide robust LDL-C reduction in familial hypercholesterolemia (FH). Their effects on homocysteine (Hcy), vascular biomarkers, and metabolic pathways relevant to methylation remain incompletely characterized.

**Methods:** We enrolled FH consecutive patients treated with Evolocumab for 12 weeks on top of standard therapy. Lipid profile, plasma Hcy, oxidative-stress and endothelial biomarkers (isoprostanes, DH series, ADMA, SDMA, arginine bioavailability) were measured at baseline and follow-up. Paired t-tests quantified within-patient changes; multivariate linear regression identified independent predictors of  $\Delta$ Hcy. Metabolomic signals were examined for pathway coherence with Hcy metabolism.

**Results:** We included 25 FH patients. LDL-C decreased by 49% (from  $201 \pm 70$  to  $103 \pm 58$  mg/dL), total cholesterol by 35%, and Lp(a) by 17%. Plasma Hcy fell by 29% (from  $12.6 \pm 6.7$  to  $8.9 \pm 2.4$   $\mu$ mol/L,  $p < 0.001$ ). Oxidative stress improved: isoprostanes  $-15.3\%$  ( $p = 0.017$ ) and DH2  $-17.2\%$  ( $p = 0.045$ ); DH1 showed a trend ( $-11.7\%$ ,  $p = 0.072$ ). Endothelial markers showed non-significant mean changes (ADMA  $+5.3\%$ ,  $p = 0.37$ ; SDMA  $-9.1\%$ ,  $p = 0.21$ ; arginine bioavailability  $-8.2\%$ ,  $p = 0.13$ ). Correlation analyses linked larger  $\Delta$ Hcy reductions with greater improvements in LDL particle indices and Lp(a). In multivariate analysis, the change in LDL score ( $\Delta$ LDL score) emerged as the strongest independent predictor of  $\Delta$ Hcy ( $\beta -0.028$ ,  $p < 0.001$ ) while SDMA, ADMA, arginine bioavailability, isoprostanes, and  $\Delta$ Lp(a) contributed as additional, smaller predictors. Metabolomic readouts indicated lower plasma choline and modulation of the beta-ine-dependent remethylation pathway of Hcy, providing a mechanistic link between lipid changes, methylation flux, and Hcy reduction.

**Conclusions:** Beyond LDL-C lowering, PCSK9i therapy is associated with a 29% reduction in Hcy and measurable improvements in oxidative stress.  $\Delta$ LDL score is the key predictor of Hcy change, supporting a tight relationship between LDL particle characteristics and homocysteine metabolism. The concurrent metabolomic signature (choline decrease; betaine-pathway engagement) reinforces a pleiotropic vascular benefit of PCSK9i in FH.

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## ABSTRACT

**CEREBROSPINAL FLUID AND PLASMA HDL (DYS)FUNCTION IN MULTIPLE SCLEROSIS**

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**Background:** Multiple sclerosis (MS) is a multifactorial neurodegenerative disease in which cholesterol plays a key role. Cerebral cholesterol transport is essential to provide neurons with cholesterol to maintain their physiological functions, and it is mediated by lipoproteins similar to plasma HDL, identified in human cerebrospinal fluid (CSF).

Their main function is to promote the first step of the brain cholesterol transport, namely cholesterol efflux from astrocytes through the major efflux transporters ABCA1 and ABCG1. Dysregulation of cholesterol transport in the central nervous system has been associated with neurodegenerative disorders; however, whether HDL dysfunctions occur in MS has not yet been clarified. Moreover, HDLs exert anti-inflammatory activity, another protective function that may be critical for protection against various diseases, including MS.

This function could be altered by the presence of Serum Amyloid A (SAA), an acute phase protein associated with HDLs in inflammatory conditions. In addition, another important factor implicated in the regulation of both peripheral and cerebral lipid metabolism is the protein PCSK9, which seems to be involved in neurodegenerative disorders, such as Alzheimer's disease, as we previously demonstrated.

This study aimed at identifying possible disturbances in HDL function in MS by measuring CSF and serum HDL cholesterol efflux capacity (CSF and serum HDL-CEC), as well as HDL inflammatory phenotype and PCSK9 levels in MS subjects compared to a control group.

**Methods:** We conducted an observational in vitro study measuring HDL-CEC of CSF and serum from 45 MS patients and 14 age- and sex-matched controls. CSF and serum HDL-CEC were evaluated in cerebral and peripheral cell models using a standardized radioisotopic technique. PCSK9 and SAA levels were measured in CSF and serum by ELISA assays.

**Results:** CSF HDL-CEC from astrocytes was significantly lower in MS patients compared to control subjects (-32%,  $p=0.0003$ ), with a specific impairment of the ABCG1-mediated efflux (-18%,  $p=0.0196$ ). No significant differences were observed between the groups for serum HDL-CEC mediated by ABCA1 and ABCG1.

Stratification of MS subjects by severity of pathology based on the presence of oligoclonal bands (OCB), pointed out a significantly lower CSF HDL-CEC from astrocytes (-21%,  $p=0.0374$ ) and a lower serum HDL-CEC specifically mediated by ABCA1 (-27%,  $p=0.0086$ ) in MS OCB+ subjects. Interestingly, we observed significantly higher PCSK9 levels in CSF of MS subjects compared to controls ( $p=0.0158$ ), while no differences were observed for PCSK9 levels in the serum of the two groups. Moreover, by evaluating SAA levels in serum, we found significantly higher SAA levels in MS serum compared to the control group ( $p=0.0002$ ). SAA quantification in CSF is still ongoing.

**Conclusions:** Overall, our results show that CSF HDL-CEC, the first step of cerebral cholesterol transport, is dysfunctional in MS, suggesting cerebral HDL as a potential pharmacological target. In addition, the observation that serum HDL-CEC mediated by ABCA1 is lower in MS subjects OCB+ may put the premises to study serum HDL-CEC as a potential peripheral biomarker of the disease.

## ABSTRACT

## POSTER

### RUNT-RELATED TRANSCRIPTION FACTOR 1 (RUNX1) IN ATHEROSCLEROSIS

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**Background:** During atherosclerosis, Vascular smooth muscle cells (VSMCs) shift from a contractile to a synthetic state, transiently stabilizing plaques before fueling rupture through macrophage- and osteochondrogenic-like fates. Similar plasticity is also critical in other cardiovascular conditions, like cardiac fibrosis. This plasticity is orchestrated by a multitude of transcription factors, but this project aims at focusing on the central role of Runt-related transcription factor 1 (Runx1). Runx1 has been so far only studied in the context of hematopoiesis, where it up-regulate the transcription of multiple genes to favor proliferation.

**Aim:** We intend to investigate if (i) Runx1 is over-expressed under atherogenic conditions in VSMCs and (ii) if ablating Runx1 in VMSCs limits the progression of atherosclerosis.

**Methods:** We leverage in vitro models to study if RUNX1 is over-expressed in atherogenic conditions. Therefore, primary human aortic VSMCs are stimulated with PDGF- $\beta$  to then study its over-expression and that of main inflammatory genes (COL1A1, COL3A1, MKI67, TBX18). A siRNA directed against RUNX1 is used to test if this modulates the expression of these genes as well. Similar experiments are performed on primary VSMCs isolated from the aortas of LDLR<sup>-/-</sup> mice models of atherosclerosis. In vivo models include the use of a VSMCs-specific, tamoxifen induced, Runx1-null mice, on the LDLR<sup>-/-</sup> background (Myh11-CreERT2<sup>+</sup>; Runx1<sup>flox/flox</sup>;LDLR<sup>-/-</sup>, "Runx1-SMC-iKO"). These mice are treated with atherogenic diet (0,15% cholesterol) by 8 to 16 weeks. We next plan to characterize the extent of the aortic atherosclerosis, and the lipid content. Given the role of Runx1 in haematopoiesis, we also plan to transfer its role on the immune adaptations to atherosclerosis and acute cardiovascular conditions, by providing a comprehensive immune phenotypic characterization of the aorta, the blood and main hematopoietic organs in Runx1-SMC-iKO and in a model undergoing TAC (Transverse aortic constriction) and in which RUNX1 was ablated in cardiac fibroblasts.

**Results:** So far, we collected initial data on the human VSMCs lineage. Indeed, Runx1 expression increased, showing a two-fold rise after 6 hours. A slight increase in COL1A1 and TBX18 expression was also detectable at 6 hours and persisted at 12 hours, whereas COL3A1 and MKI67 levels remained largely unchanged. Same experiments on the LDLR<sup>-/-</sup> is underway. Meanwhile, the Runx1-SMC-iKO is generated and we are monitoring the progression of atherosclerosis. Finally, in the fibroblast-specific RUNX1-knockout model, circulating immunophenotyping revealed a significant decrease in myeloid cells and a significant increase in lymphoid cells, independent of TAC surgery. Bone marrow showed no changes in progenitors or overall immunophenotype. In the spleen, TAC itself was associated with a non-significant rise in myeloid cells and a significant drop in lymphoid cells, paralleled by increased spleen weight.

**Conclusions:** This very preliminary data of the ongoing project aim at exploring the role of Runx1 in atherosclerosis and cardiovascular conditions, to support its potential as a therapeutic target.

### POSTPRANDIAL VLDL AND ENDOTHELIAL INFLAMMATORY ACTIVATION: IN VITRO STUDIES

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**Introduction:** Elevated plasma triglyceride (TG) levels during the postprandial state are an independent risk factor for cardiovascular disease (CVD). In this condition, triglyceride-rich lipoproteins (TRLs), including VLDL and remnants VLDL, accumulate in circulation, promoting endothelial dysfunction. Clinical evidence indicates that these lipoproteins trigger low-grade chronic inflammation, promoting atherogenesis. However, the molecular mechanisms underlying this process remain unclear. The aim of this study was to characterize the molecular responses induced by postprandial VLDL in human endothelial cells and to identify potential early biomarkers.

**Methods:** VLDLs were isolated from plasma collected four hours after an oral fat load (OFTL) in normolipidemic subjects by iodixanol 8% density-gradient ultracentrifugation. Lipoprotein fractions were standardized at triglyceride concentrations of 10, 25, and 100  $\mu$ g/mL. Human endothelial cells (HUVEC) were pre-incubated for two hours in serum-free medium and subsequently exposed to VLDL for four hours. Gene expression of IL-6, MCP-1, ICAM-1, P-selectin, ELAM-1, and VCAM-1 was assessed by RT-qPCR and normalized to RPL13A. Lipopolysaccharide (LPS, 30 ng/mL) was used as a positive control to confirm cellular responsiveness.

**Results:** VLDL induced a dose-dependent endothelial inflammatory response (n=5). At 100  $\mu$ g/mL TG, a significant increase was observed for IL-6 (1.45 $\pm$ 0.54 vs 1.02 $\pm$ 0.02; p=0.03) and MCP-1 (1.95 $\pm$ 0.81 vs 1.02 $\pm$ 0.02; p=0.02). Adhesion molecules were also up-regulated: ICAM-1 (1.86 $\pm$ 1.01 vs 1.00 $\pm$ 0.05; p=0.02), P-selectin (2.07 $\pm$ 0.59 vs 1.12 $\pm$ 0.13; p=0.02), and ELAM-1 (1.63 $\pm$ 0.59 vs 1.01 $\pm$ 0.01; p=0.02). By contrast, VCAM-1 showed no significant variation (1.19 $\pm$ 0.71 vs 1.00 $\pm$ 0.06). As expected, LPS exposure strongly activated all analyzed genes (e.g., IL-6: 3.00 $\pm$ 2.72 vs 1.02 $\pm$ 0.02; MCP-1: 2.93 $\pm$ 3.14 vs 1.02 $\pm$ 0.02).

**Conclusions:** Postprandial VLDL isolated without KBr induced endothelial inflammation, characterized by the expression of cytokines such as MCP-1 and IL-6, and adhesion molecules including ICAM-1, ELAM-1, and P-selectin, but not VCAM-1. These preliminary results support existing evidence and propose a useful experimental approach to investigate the molecular mechanisms through which postprandial VLDL modulate endothelial function.

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**ABSTRACT**


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**GRAPE POMACE POLYPHENOLS  
ATTENUATE SENESCENCE  
AND PRESERVE THE DIFFERENTIATION  
CAPACITY OF CARDIAC ADIPOSE  
TISSUE-DERIVED MESENCHYMAL  
STEM CELLS FROM ELDERLY PATIENTS  
WITH CARDIOVASCULAR DISEASE**

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Aging and age-related coronary artery disease (CAD) are closely associated with impaired tissue regeneration and reduced stem cell function, with important implications for cardiac repair. Cardiac adipose tissue-derived stem cells (ADSCs) represent a promising source for regenerative therapies due to their proximity to the heart and their multipotent characteristics. However, in elderly patients, stem cell functionality may be compromised by a shift toward adipogenic differentiation and increased cellular senescence, associated with oxidative stress and mitochondrial dysfunction (1-2). Polyphenol-rich natural compounds have shown potential in counteracting oxidative stress and cellular senescence (3). The aim of this study was to analyze the stemness and differentiation potential of cardiac ADSCs isolated from elderly CAD patients and to investigate the effects of a grape pomace polyphenolic extract (GPE), a winemaking byproduct rich in bioactive nutraceutical compounds. ADSCs were freshly isolated from the stromal vascular fraction of pericardial adipose tissue fragments obtained from elderly patients (n=8) undergoing cardiac surgery and phenotypically characterized by flow cytometry. Using multiple assays, we evaluated the effects of GPE on ADSC viability, proliferation, and cellular senescence, as well as their ability to differentiate into various lineages, including adipocytes, osteoblasts, and endothelial cells. Flow cytometry analyses identified ADSCs as mesenchymal stem cells, characterized by the expression of stemness markers CD73, CD90, and CD105, and the absence of the hematopoietic marker CD45. ADSCs from elderly CAD patients retained the ability to proliferate and differentiate into adipocytes, osteoblasts, and endothelial cells under appropriate induction conditions. GPE treatment suppressed adipogenic differentiation of ADSCs, as demonstrated by the Oil Red O assay, by reducing the expression of adipogenic mediators (PPAR $\gamma$ , FABP4, and CD36). In contrast, GPE promoted osteogenic differentiation, as evidenced by the Alizarin Red S assay, by inducing the expression of osteogenic mediators (RUNX2, COL1A1 and osteocalcin), without influencing endothelial differentiation. Senescence analysis of ADSCs from elderly CAD patients, based on  $\beta$ -galactosidase positivity, revealed the presence of senescent cells, whose number decreased following GPE treatment. Mechanistically, GPE effects were associated with reduced reactive oxygen species levels and improved mitochondrial function. In conclusion, our results demonstrate that GPE acts as a func-

tional ingredient that counteracts aging and preserves the regenerative capacity of ADSCs without promoting excessive fat accumulation, which could impair their functionality and therapeutic potential in the treatment of cardiovascular diseases.

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**NOVEL METHOD FOR DETERMINATION  
OF PLASMA TRIMETHYLAMINE N-OXIDE  
(TMAO) BY LIQUID CHROMATOGRAPHY  
TANDEM MASS SPECTROMETRY**

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**Background:** Trimethylamine N-oxide (TMAO) is produced by the intestinal bacteria from phosphatidylcholine. Carnitine and phosphatidylcholine from dietary animal proteins are converted by the intestinal bacteria to trimethylamine (TMA), which is absorbed by enterocytes, metabolized by hepatic flavin-containing monooxygenase (FMO) enzymes and oxidized by the liver to TMAO. Increased concentrations of plasma TMAO causes atherosclerosis in animal models and markedly increases the risk of stroke, myocardial infarction and cardiovascular mortality, particularly in persons with renal impairment. The microbiome-liver-kidney axis regulates TMAO production and accumulation. Currently, studies on cardiovascular diseases suggest that TMAO is associated with oxidative stress, whereas there is a lack of evidence for the involvement of oxidative stress mediated by TMAO in the pathogenesis of MAFLD. Elevated plasma TMAO concentrations likely reflect a specific metabolic pattern characterized by low HDL and phospholipids in addition to hypomethylation. The aim of this study is the development of a rapid and sensitive LC-MS/MS method for the quantitative determination of TMAO in order to design, successively, a study of TMAO alterations in plasma of selected groups of patients with neurological, metabolic diseases and diabetes.

**Methods:** Plasma samples were extracted with a precipitating solution containing 2H9-TMAO. Three microliters of supernatant were injected and analyzed by LC-MS/MS in positive ionisation mode. Optimal chromatographic separation was achieved using a C18 column with gradient elution in 5 minutes. Standard curves were linear from 0.10 to 200  $\mu$ mol/L. Limit of detection (LOD) and limit of quantitation (LOQ) were respectively 0.005 and 0.10  $\mu$ mol/L. Intra and inter-assay CVs for were both <8%. Recovery experiments adding 10 and 50  $\mu$ mol/L were 102.95% and 96.45%, respectively. The method was applied to 100 plasma controls from healthy subjects to obtain control values.

**Conclusion:** A rapid and high sensitive LC-MS/MS method was developed and validated for determination of TMAO in plasma samples. This method will be useful to study TMAO alterations and to understand its use as good predictive and prognostic marker for vascular damage in paediatric inherited or acquired diseases.

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**ABSTRACT**


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**PROFILING THE *IN VIVO* PHARMACOKINETICS, BIODISTRIBUTION AND SAFETY OF A NEW CLASS OF PCSK9 INHIBITORS**

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**Aim:** This study aims to determine the *in vivo* safety of newly synthesized, small-molecule PCSK9 inhibitors. PCSK9 controls peripheral and central cholesterol levels, and it plays a crucial role in hypercholesterolemia, being a well-established pharmacological target to treat this pathology, while a possible involvement in the aetiopathogenesis of AD has been postulated. Currently, the available PCSK9i are expensive biotechnological drugs and only subcutaneously administered. Based on these premises, orally bioavailable small-molecule may be a valuable addition to existing treatments.

**Methods:** After preliminary *in vitro* screening of 30 compounds in hepatic and neuronal cells, 4 compounds were selected to test tolerability and bioavailability *in vivo* in wild-type mice (C57BL/6J male/female) at 12.5mg/kg, 25mg/kg, 50mg/kg, and 100mg/kg for 5 days. MR-532 and MR-533 were administered subcutaneously, while MR-3 and MR-644 both subcutaneously and orally. Body weight and phenotype analysis were conducted daily to assess tolerability and macroscopic toxicity. After sacrifice, hepatotoxicity (histological analysis and ALT activity) and biodistribution (LC-MS/MS) were evaluated.

**Results:** All compounds and doses were well tolerated (no change in weight, food intake, coat condition, or lethargy). No inflammation or cell death was detected in liver sections for all compounds after 100 mg/kg treatment, and MR-532 and MR-533 didn't show elevated levels of ALT activity compared to vehicle (66mU±55, 76mU±127, and 130mU±203, respectively).

Following subcutaneous administration, all compounds (MR-532/MR-533/MR-3/MR-644) were detected at all doses in plasma (261-318nM; 159-192nM; 742-1700nM; 618-1467nM), liver (522-1063 pmol/g; 2824-3135pmol/g; 13707-20744pmol/g; 1885-3198 pmol/g) and brain (513-779pmol/g; 457-380pmol/g; 509-1340 pmol/g; 426-812pmol/g). No dose-dependent trends were observed. Interestingly, MR-3 and MR-644 exhibited higher oral bioavailability with plasma (1243-7051nM; 3531-7781nM), liver (16771-112249pmol/g; 16394-57642pmol/g), and brain (1002-8499 pmol/g; 1241-7763pmol/g) levels, showing a dose-dependence.

**Conclusion:** All compounds were well tolerated and successfully reached plasma, liver, and brain. Oral administration enhanced their availability in all districts. Further analysis are ongoing to determine the full pharmacokinetic profile of these molecules. Additional studies will be needed to assess their efficacy in cardiovascular and neurodegenerative diseases.

**EFFECT OF DIETARY PUFAs AND ANTIOXIDANTS OF HDL FUNCTIONALITY**

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High-density lipoproteins (HDLs) are widely recognized for their atheroprotective properties, primarily attributed to their antioxidant and anti-inflammatory functions. These protective activities are largely mediated by HDL-associated proteins, notably Paraoxonase-1 and glutathione peroxidase 3 (GPX3), which exhibit strong antioxidant effects; lipoprotein-associated phospholipase A2 (Lp-PLA2), a pro-inflammatory enzyme; myeloperoxidase (MPO), which promotes oxidative and inflammatory responses, and lecithin cholesterol acyltransferase (LCAT), a key enzyme in reverse cholesterol transport that esterifies free cholesterol on the HDL surface, facilitating its maturation and contributing to antioxidant defense.

Despite growing interest in identifying agents capable of enhancing HDL functionality, both dietary and synthetic modulators remain poorly characterized. To address this gap, we conducted a novel investigation into how the intake of saturated and unsaturated fatty acids, along with dietary antioxidants, influences key HDL-associated proteins in women. Our findings revealed that MPO activity was inversely associated with total polyunsaturated fatty acid (PUFA) intake, including both omega-3 and omega-6 fatty acids ( $p < 0.05$ ), as well as polyphenols ( $p < 0.001$ ) and overall antioxidant capacity ( $p < 0.05$ ). Similarly, Lp-PLA2 levels declined with increased antioxidant consumption ( $p < 0.05$ ). In contrast, GPX3 activity, a protective HDL enzyme, rose in response to higher omega-3 and antioxidant intake. Furthermore, a composite HDL antioxidant/anti-inflammatory score, integrating all measured proteins, showed a positive correlation with total PUFA ( $\beta = 0.289$ ,  $p < 0.001$ ), omega-6 ( $\beta = 0.276$ ,  $p < 0.001$ ), omega-3 ( $\beta = 0.201$ ,  $p < 0.01$ ), polyphenols ( $\beta = 0.212$ ,  $p < 0.05$ ), and total antioxidant intake ( $\beta = 0.220$ ,  $p < 0.05$ ). Importantly, all these associations remained significant after adjustment for age, menopause, obesity and comorbidities. Finally, women with high HDL antioxidant/anti-inflammatory score consumed significantly more Omega-3, Omega-6, polyphenols, and total antioxidants, an increase that ranged between 15 and 20% compared to those with low score. Overall, these results suggest that increased dietary intake of PUFAs, particularly omega-6 fatty acids, and antioxidants may enhance the atheroprotective functionality of HDL particles

## ABSTRACT

## SUSTAINABLE MALUS DOMESTICA ‘CRIPPS PINK’ APPLES EXTRACT IMPROVES METABOLIC ALTERATIONS IN A CELL-BASED MODEL OF METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE (MASLD): IMPLICATIONS FOR NUTRACEUTICAL STRATEGIES IN CARDIOMETABOLIC HEALTH

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is a progressive global health concern affecting approximately 30% of adults. It is intimately associated with obesity, type 2 diabetes mellitus (T2DM), and atherosclerotic cardiovascular disease (ASCVD), which remains the leading cause of global mortality. Shared pathogenic mechanisms, including insulin resistance, low grade-chronic inflammation, and oxidative stress, highlight the urgent need for effective and sustainable treatment strategies. Despite the progressive nature of MASLD, which can evolve into steatohepatitis, cirrhosis, and hepatocellular carcinoma, there are currently no pharmacological treatments specifically approved for its management. Lifestyle modification remains the only effective approach, emphasizing the urgent need for sustainable therapeutic strategies. Nutraceuticals derived from agro-food waste represent a promising avenue, combining health benefits with sustainability principles aligned with the Circular Economy, One Health, and the Agenda 2030 promoted by the United Nations. This study details the systematic development and validation of a functional nutraceutical derived from the agro-food waste of *Malus domestica* ‘Cripps Pink’ apples, a variety selected for its high flavonoid concentration and traceable, sustainable supply chain. By exploiting an eco-friendly enzymatic extraction protocol, the resulting extract was chemically and functionally characterized. Its therapeutic potential was assessed in an *in vitro* steatotic-like hepatocyte model (HepG2-OA), generated by treating HepG2 cells with 100  $\mu$ M oleic acid for 7 days, characterized and validated with different agro-food waste products. The extract demonstrated good cytocompatibility and led to significant biological benefits. Treatment with the extract significantly reduced intracellular lipid accumulation, as evidenced by Oil Red O staining and quantitative spectrophotometry, and substantially attenuated reactive oxygen species (ROS) production. Parallel gene expression analyses revealed the downregulation of key lipogenic markers such as DGAT1 and FASN, as well as a reduction in oxidative stress-related transcripts including SOD, NRF2, and CAT. The extract also improved glucose uptake, pointing to a restoration of metabolic balance. These findings collectively demonstrate that bioactive compounds from enzymatically processed *Malus domestica* by-products can restore metabolic balance in steatotic-like hepatocytes. The results highlight the potential of sustainable nutraceuticals for MASLD and ASCVD management, supporting the integration of agro-food waste valorization into circular and responsible health innovation.

## INCLISIRAN TREATMENT IN PERITONEAL DIALYSIS: INSIGHTS FROM A CASE SERIES

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**Introduction:** Inclisiran is a small interfering RNA that inhibits proprotein convertase subtilisin/kexin type 9 (PCSK9) synthesis, lowering low-density lipoprotein cholesterol (LDL-C). In ORION trials, its efficacy and safety were consistent across different degrees of renal impairment, and dose adjustment was not required. However, the influence of peritoneal dialysis on inclisiran pharmacokinetics has not been evaluated, and only limited data exist regarding the use of inclisiran in patients receiving peritoneal dialysis.

**Case report:** We present the cases of 3 patients (2 female, 1 male) with non-familial hypercholesterolemia and Lp(a) hyperlipoproteinemia, who failed to achieve LDL-C target despite a maximum tolerated lipid-lowering therapy and were treated with inclisiran. All patients had chronic kidney disease stage 5 on peritoneal dialysis due to different etiologies (post-streptococcal glomerulonephritis, arterial hypertension/diabetes mellitus, bilateral vesicoureteral reflux). Medical history included established atherosclerotic cardiovascular disease (ASCVD) in two patients. Common comorbidities included carotid artery stenosis, arterial hypertension, and smoking habit. Two patients were receiving atorvastatin 80 mg, one was on simvastatin 20 mg, and all were concurrently treated with ezetimibe. One patient self-reduced the atorvastatin dose to 20 mg during follow-up. A baseline lipid profile was obtained prior to inclisiran administration and repeated at 1 and 3 months. After a single subcutaneous injection, mean LDL-C levels significantly decreased from 80 $\pm$ 11 mg/dl to 51 $\pm$ 19 mg/dL (-36%) at 1 month and 28 $\pm$ 15 mg/dL (-40%) at 3 months. Considerable interindividual variability in LDL-C response was observed, potentially influenced by background statin intensity. ApoB concentrations decreased by 25% at 1 month and 35% at 3 months. PCSK9 levels significantly reduced from 423 $\pm$ 145 ng/ml to 150 $\pm$ 52 ng/ml (-65%) at 1 month and 152 $\pm$ 33 ng/ml (-64%) at 3 months. Mean baseline Lp(a) levels were 125 $\pm$ 33 mg/dl, and reductions were variable, with one patient achieving a decrease of up to 26%. No adverse effects were reported during follow-up. One patient underwent a new elective percutaneous transluminal angioplasty.

**Conclusion:** Patients on peritoneal dialysis exhibit a distinct atherogenic lipid profile and face unmet needs in the management of dyslipidemia. This case series suggests that inclisiran may represent an effective and safe lipid-lowering therapy in very high cardiovascular risk patients undergoing peritoneal dialysis. Patients should be encouraged to adhere to the maximum tolerated statin therapy during follow-up. Larger and longer-term studies are warranted.

## ABSTRACT

## SIRNA VERSUS MABS FOR PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) INHIBITION: REAL WORLD EVIDENCE SINGLE-CENTER STUDY ON LIPID PROFILE IN FAMILIAL HYPERCHOLESTEROLEMIA PATIENTS

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**Background:** PCSK9 inhibitor (PCSK9i) mAbs showed a dramatic effect on low-density lipoprotein cholesterol (LDL-C) reduction. A double-stranded small-interfering RNA (siRNA) therapeutic agent that suppresses PCSK9 translation (inclisiran) has been recently licensed. RCTs showed that inclisiran administered every 6 months is associated to a similar LDL-C reduction compared to every 14 days PCSK9i mAb treatment. The aim of our study is to compare in real world evidence lipid lowering effect of siRNA versus mAbs.

**Methods:** We performed a prospective cohort study. Familial hypercholesterolemia (FH) patients with a stable treatment (>6 month) with PCSK9i mAbs were included. Group1 was switched to treatment with PCSK9i siRNA (inclisiran) and Group2 continued the treatment with PCSK9i mAbs (Alirocumab or Evolocumab). Lipid profile [total cholesterol (TC) and LDL-C] and % of LDL-C target achievement were evaluated at the baseline (T0) and at 6-months follow-up (T1).

**Results:** 52 FH patients were enrolled. Group1 (n=26) 56% males, average age of 56.1±14.8years; 50% in secondary prevention. At T0, TC was 179.6±67.5mg/dl, LDL-C was 105.7±62.2mg/dl. At T1, TC was 175.4±53.1 mg/dl and LDL-C value was 97.6±47.9 mg/dl. Group2 (n=26) 42% males, average age of 61.5±12.4years; 46% in secondary prevention. At T0, TC was 139.8±54.8 mg/dl, LDL-C was 65.8±51.0mg/dl. At T1, TC was 137.6±35.0mg/dl and LDL-C was 63.1±32.2mg/dl. No significant difference between T1-T0 LDL-C Δ mean % reduction was found comparing the 2 groups (10% for Group1 vs 35% for Group2 p=0,260). No significant difference in % of target achievement at T1 was found (31% for Group 1 and 50% for Group 2; OR: 95%CI:36.4-61.9, p=0.131).

**Conclusions:** Our preliminary results showed that no significant difference in efficacy were found between treatment with PCSK9i mAbs and siRNA, confirming siRNA a promising option mainly in low-adherent patients.

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## REAL-WORLD ADHERENCE TO BEMPEDOIC ACID IN A COHORT OF PATIENTS FROM ATS VAL PADANA

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**Background:** Despite the consistent emphasis in guidelines on lowering LDL-C to reduce cardiovascular (CV) risk, real-world data show a low prevalence of LDL-C target achievement in high and very-high-risk patients. A key barrier is poor adherence to lipid-lowering therapy (LLT), especially statins, often due to muscle-related side effects. Bempedoic Acid (BA) is a novel oral drug shown to reduce LDL-C and CV events. Unlike statins, BA has no action on skeletal muscle and may therefore improve adherence. However, real-life data on its use and adherence are lacking.

**Aim:** To evaluate one-year adherence to BA in clinical practice.  
**Methods:** This retrospective observational study used administrative data from ATS Val Padana, including pharmaceutical records, hospital discharge data, outpatient services, and the chronic disease exemption registry. We analyzed demographic characteristics and CV prevention status (primary vs secondary) of patients prescribed BA. One-year therapeutic adherence was assessed using the Medication Possession Rate (MPR), and high adherence was defined as MPR ≥80%. Adherence was compared between BA and statin users.

**Results:** Between January 2023 and August 31, 2024, 878 patients received BA (394 females, 484males). The most represented age group was 65–79 years (487 patients; 55.5%). Secondary prevention accounted for 594 cases (67.7%), of whom 249 had diabetes. After one year, 82.6% of BA users showed high adherence, compared to 36.9% of statin users. The difference in adherence was statistically significant (p<0.0001).

**Conclusions:** In this real-world cohort, BA users demonstrated significantly higher one-year adherence than statin users. BA may represent a valuable option in patients with low statin adherence or intolerance. Further studies are needed to evaluate the long-term clinical impact.

## ABSTRACT

## MONOCENTRIC REAL-WORLD OBSERVATIONAL STUDY ON THE EFFICACY AND TOLERABILITY OF BEMPEDOIC ACID THERAPY

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**Background:** LDL cholesterol (LDL-C) plays a causal role in the genesis of atherosclerotic disease and cardiovascular (CV) events. Therefore, several drugs and therapeutic strategies have been developed to reduce LDL-C levels but, nevertheless, achievement of the therapeutic target (TT) and retention in therapy remain low. In this context, bempedoic acid (BA) appears to be a good therapeutic option for reducing LDL-C, achieving TT, and overcoming statin intolerance.

**Aim:** The objective of our study was to observe 1) the use of BA in real-life as a means of achieving the LDL-C target in poly-pharmacotherapy when other treatment strategies aren't possible; 2) adherence and retention in therapy; 3) tolerability.

**Methods:** Patients prescribed with BA at our centre were monitored from March 2023 (start of availability BA in Italy) to June 2025 according to the Italian regulatory (AIFA) prescribing criteria. Data were collected from 215 patients (M 34.88%, F 65.12%, mean age 66.9 years, weight 71.79 kg, BMI 27.91, WC 98.39 cm). Cardiovascular risk (CVR) was calculated based on SCORE2, SCORE2-OP, and SCORE2-DIABETES charts and the physician's clinical assessment, then patients were divided into: low CVR 8.37%, moderate 21.86%, high 46.51%, and very high 23.26%. Background therapies at baseline, 1-3-months, and 6-12-24-months, as well as LDL-C and non-HDL-C target achievement at baseline, 1-3 and 6-12-24-months, were analysed. Adverse events and/or reasons for discontinuation were recorded.

**Results:** At baseline, 68 patients (31.63% of the whole 215 sample) were not taking any therapy, 65 (30.23%) were on monotherapy, and 82 (38.14%) were on poly-pharmacotherapy. We analysed data of 174 patients returned for follow-up after 1-3 months. Among these, 140 (82.8%) continued therapy: 29.29% were on BA monotherapy and 70.71% on combination therapy with 2 drugs or more (40% BA+ezetimibe; 3.57% BA+statin; 24% BA+ezetimibe+statin; 3.57 BA+other drugs). The remaining 34 patients (17.2%) discontinued therapy: 9 (5.2%) due to drug-related adverse effects, while 25 patients (14.4%) for administrative reasons, not-related to the drug and didn't return at follow-up visits. Out of these latter, 19 (75%) were lost in the first year of observation, and only 6 (25%) in the second year. LDL-C and non-HDL-C target were assessed: at baseline, only 5.58% of patients had already achieved the LDL-C target (9.77% for non-HDL-C) but needed to modify the therapy due to poor tolerability, while at the 1-3-month follow-up, the LDL-C target was achieved by 35.7% (43.57% for non-HDL-C). Sub-analyses were also performed to assess target achievement based on risk classes. Nineteen patients (11%) reported Adverse Effects: 8 (42%) musculoskeletal, 3 (15.8%) gastrointestinal disorders, 3 (15.8%) elevated uric acid, and 5 (26.3%) other disorders (impotence, headache, fatigue); only 9 patients (5.2%) discontinued therapy due to AEs. These results were substantially confirmed in the 12-24-month follow-up visits although not reported due to the progressive reduc-

tion in the observed sample size and the lack of significance of the data.

**Conclusions:** BA has proven to be an effective, fast-acting, safe, and well-tolerated drug, resulting in improved patients' compliance, including those with the multi-pharmacotherapies required to achieve the therapeutic targets.

## REAL-WORLD OUTCOMES WITH BEMPEDOIC ACID: LIPID-LOWERING EFFICACY AND ADVERSE EVENT PROFILE

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**Introduction:** In the 2019 Clear Harmony study, 2,200 patients were enrolled and received 180 mg of bempedoic acid in a single daily dose versus placebo for 52 weeks. They have shown that the most common adverse effects include increased liver enzyme levels, renal events, and hyperuricemia with a higher incidence of gout. In particular, the incidence of hyperuricemia was reported at 10.9% in the bempedoic acid group compared to 5.6% in the placebo group, while gout was observed in 3.1% versus 2.1%, respectively. Myalgias, often associated with statin use, were similar or slightly less frequent in the bempedoic acid group compared to placebo (5.6% vs 6.8%). Moreover, treatment discontinuation due to adverse events was higher in the bempedoic acid group (around 11%) compared to placebo (7–8%). **Methods:** We retrospectively analyzed 100 consecutive patients with hypercholesterolemia followed at our lipid clinic in treatment with bempedoic acid 180mg in a single daily dose.

**Results:** In our real-world cohort of 100 patients, all experienced an approximate 30% reduction in LDL-C levels. Twenty-eight patients developed hyperuricemia and started allopurinol, which resulted in subsequent normalization of uric acid levels. Three patients reported myalgias without elevation of muscle enzymes. Notably, no cases of gout were observed. A distinctive finding was that 14 patients reported intense pruritus during the first two weeks of therapy, in the absence of elevated uric acid or other laboratory abnormalities. The pruritus resolved spontaneously after approximately two months of continued treatment.

**Conclusion:** Overall, both trial data and clinical experience confirm that bempedoic acid is effective and generally well tolerated, with an acceptable safety profile. Monitoring of uric acid, liver function, and possible transient dermatologic symptoms is recommended during therapy.

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 ABSTRACT
 

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**LONG TERM EFFICACY AND SAFETY OF BEMPEDOIC ACID IN A COHORT OF DYSLIPIDEMIC PATIENTS**

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**Background and Aim:** Bempedoic acid inhibits ATP citrate lyase, resulting in reduced intrahepatic cholesterol production, increased hepatic LDL receptor expression, and enhanced clearance of LDL cholesterol. The aim of this study is to evaluate the efficacy and safety of bempedoic acid in a cohort of real life dyslipidemic patients followed at the Outpatient Clinic for Dyslipidemia (Varese, Como) over a 6-month follow-up.

**Methods:** In this observational, ambispective, cohort study, 96 patients were recruited as follows: Group 1: 18 with bempedoic acid, with or without a statin; Group 2: 63 with fixed-dose bempedoic acid plus ezetimibe, with or without a statin; Group 3: 5 with bempedoic acid plus a PCSK9 inhibitor (PCSK9i), with or without a statin; Group 4: 10 with fixed-dose bempedoic acid plus ezetimibe and a PCSK9i, with or without a statin. The primary endpoint is the change in plasma LDL-c levels. Secondary endpoints include changes in plasma total cholesterol, HDL-c, triglycerides and non-HDL-c; the percentage of patients achieving the therapeutic LDL-c target at the end of follow-up; the prevalence of adverse events related to bempedoic acid use, including increases in uric acid, plasma creatinine and hepatic cytolysis markers (AST, ALT).

**Results:** Bempedoic acid reduced LDL-c levels from baseline by 23% ( $p=0.008$ ); bempedoic acid/ezetimibe by 18.1% ( $p<0.001$ ) and bempedoic acid plus PCSK9i by 51.9% ( $p=0.042$ ). For secondary endpoints, total cholesterol decreased by 18.1% ( $p=0.001$ ) with bempedoic acid; by 8.8% ( $p<0.001$ ) with bempedoic acid/ezetimibe and by 37.3% ( $p=0.043$ ) with bempedoic acid plus PCSK9i. Regarding HDL-c, reductions were 7.8% ( $p=0.008$ ) with bempedoic acid; 15.5% ( $p=0.006$ ) with bempedoic acid/ezetimibe and 15.3% ( $p=0.043$ ) with bempedoic acid plus PCSK9i. For non-HDL cholesterol, reductions were 22% ( $p=0.003$ ) with bempedoic acid; 11.6% ( $p<0.001$ ) with bempedoic acid/ezetimibe and 47.3% ( $p=0.043$ ) with bempedoic acid plus PCSK9i. No statistically significant changes were observed in triglyceride levels ( $p=0.679$ ) with bempedoic acid therapy, nor with bempedoic acid/ezetimibe. Safety analysis showed a slight, non-significant increase in hepatic cytolysis markers. A statistically significant increase in plasma uric acid was observed ( $p=0.0004$ ), with mean levels rising from 4.92 at baseline to 5.72 after 6 months. Plasma creatinine changes were not significant ( $p=0.06$ ), with mean values increasing from 0.92 at baseline to 1.00 after 6 months. Among patients excluded from the primary endpoint evaluation, 19 discontinued treatments due to adverse events. Overall, 13.8% of patients prescribed bempedoic acid stopped therapy because of adverse reactions.

**Conclusion:** The results of bempedoic acid treatment are encouraging, both in terms of clinical efficacy and medium- to long-term safety. Bempedoic acid can therefore be considered a valid therapeutic alternative for hypercholesterolemic patients with high LDL-c levels who fail to achieve target levels and/or are intolerant to first-line therapies.

**REAL-LIFE CLINICAL EFFICACY OF PCSK9 INHIBITORS IN THE MANAGEMENT OF DYSLIPIDEMIA**

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**Background and Objectives:** This single-center retrospective study analyzes the efficacy of PCSK9 inhibitors (Evolocumab and Alirocumab) in patients with dyslipidemia over a two-year period. The primary objective is to evaluate the reduction in plasma LDL cholesterol levels. Secondary objectives include the percentage of patients who achieve the recommended LDL and non-HDL cholesterol targets, the effect on lipoprotein(a) levels, and a comparison between Evolocumab and Alirocumab, also considering the different dosages of Alirocumab.

**Methods:** Data from 155 patients with lipid profile abnormalities were analyzed. Biochemical parameters were monitored before and after administration of PCSK9 inhibitors in four serial checks over a two-year period.

**Results:** The study showed a significant reduction in LDL and non-HDL cholesterol levels after starting PCSK9 inhibitors therapy. A variable decrease in lipoprotein(a) levels was found in different patients. The median reduction in LDL cholesterol after starting PCSK9 inhibitor therapy was 62.6%. No significant differences in efficacy were observed between Evolocumab and Alirocumab. The 150 mg dosage of Alirocumab was more effective than the 75 mg dosage. After stratification of patients according to cardiovascular risk, 60.6% achieved the recommended plasma LDL-C targets. 63.5% of patients achieved plasma non-HDL cholesterol targets.

**Conclusions:** PCSK9 inhibitors are effective in reducing plasma LDL and non-HDL cholesterol levels, with a variable effect on lipoprotein(a) in different patients. These results support their use in clinical practice for the management of dyslipidemia.

## ABSTRACT

**THE EFFICACY OF COMBINATED THERAPY WITH STATIN AND PCSK-9I IN TARGET LDL-C PATIENT WITH HIGH LEVEL OF LP(a)**

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**Background:** For patients with a recent acute coronary event, a target LDL-C level of less than 55 mg/dL is recommended. If this target is reached with an optimized statine therapy, is important to consider the residual risk associated with Lp(a) levels.

**Methods:** We evaluated 42 patients attending our clinic, all of whom received optimized statin therapy with LDL-C levels < 55 mg/dL. 21 of these patients received statin therapy alone, while the remaining 21 patients received statin therapy in combination with Alirocumab.

**Results:** In the first group, Two patients had a new coronary event in one year (9,52%) and in the second group two patients had a new coronary event in the same time. In all cases, the patients had a high level of Lp(a). So we evaluated 12 patients with Lp(a) levels > 50 mg/dl but LDL - C < 55 mg/dl with optimized statin therapy. In a group of six patient we associated statin and Alirocumab, in the second group we have administrated only statin therapy.

At one-year follow-up, no patients in the first group had experienced a new coronary event, while in the second group, only one patient (16.7%) had experienced a new acute coronary event.

**Conclusion:** In patient with recent acute coronary event with LDL-C < 55 mg/dl in optimized statine therapy, the association with pcsk-9i, provides incremental clinical benefit above all when Lp(a) concentration is at least midly elevated.

**GENETICS AS A MOTIVATOR: ENHANCING TREATMENT UPTAKE IN FH THROUGH MOLECULAR DIAGNOSIS**

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Familial hypercholesterolemia (FH), if promptly recognized and treated, represents a preventable cause of cardiovascular disease and premature death, with management strategies strongly supported by evidence-based guidelines. However, misconceptions and fears surrounding statin therapy remain common and often contribute to underutilization, particularly in pediatric patients. We present clinical, biochemical, and molecular data from 382 patients diagnosed in our Rare Diseases and Medical Genetics Unit, with a focus on the relationship between awareness of genetic confirmation and adherence to therapy.

As expected, patients carrying pathogenic LDLR variants displayed significantly more severe dyslipidemia than those with inconclusive genetic results. Importantly, therapeutic uptake was higher in the pathogenic group, with 87% initiating statin-based therapy compared to 50% in the inconclusive group. These findings suggest that a confirmed genetic diagnosis may play a key role in improving treatment adherence in FH.

## ABSTRACT

## HIGH CARDIOVASCULAR RISK AND POOR RISK FACTOR CONTROL IN PATIENTS LIVING WITH COPD: REAL-WORLD EVIDENCE

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**Background:** Chronic obstructive pulmonary disease (COPD) is a major global health burden and is frequently associated with cardiovascular (CV) comorbidities. Although COPD patients experience higher CV morbidity and mortality, the disease itself is not explicitly included in traditional CV risk prediction algorithms. Shared risk factors, such as smoking habit, hypertension, diabetes mellitus (DM), and dyslipidemia, as well as disease-specific mechanisms – including chronic hypoxia, oxidative stress, vascular remodeling – may contribute to the elevated CV burden in COPD. This study aimed to assess CV risk in COPD patients, based on SCORE2/SCORE2-OP risk charts (using the validated HUMTELEMED web-application), and to identify clinical and biochemical variables associated with higher CV risk.

**Methods:** We conducted an observational study including 198 consecutive COPD patients referred to the Respiratory Medicine Service and the ESH Hypertension Excellence Centre of IRCCS INRCA (Ancona, Italy), between January 2013 and May 2024. Inclusion criteria were confirmed diagnosis of COPD and availability of parameters required for CV risk calculation. CV risk was estimated by the www.humtelem.it validated web-app, according to SCORE2/SCORE2-OP charts and the latest 2021 ESC guidelines on the prevention of CV disease.

**Results:** Characteristics of the study population (65.7% males): mean age of 72.2±9.4 years, mean BMI 28.9±5.3 kg/m<sup>2</sup>. The study population showed a high prevalence of comorbidities: current or former smokers (77.8%), hypertension (97.5%), DM (25.8%), dyslipidemia (80.8%), known heart failure (12.1%), atherosclerotic cardiovascular disease (ASCVD, 36.4%), and chronic kidney disease (CKD, 17.7%). Mean CAT score was 16.0±5.6, and mMRC scores indicated moderate-to-severe dyspnea in most patients. According to SCORE2/SCORE2-OP, 10.6% of participants were classified at high risk and 89.4% at very high risk, while none were at low-to-moderate CV risk. Factors associated with very high risk included older age (70.3±8.8 vs 60.5±6.2 years, <0.001), presence of ASCVD (40.7% vs 0%, <0.001), peripheral artery disease (72.3% vs 4.8%, <0.001), DM (28.8% vs 0%, p=0.004), and CKD (19.8% vs 0%, p=0.025). Higher systolic BP, lower total cholesterol, and lower non-HDL cholesterol were also associated with a very high risk. Symptom severity correlated with risk stratification: patients with CAT≥17 or mMRC score of 3 were consistently at very high risk (<0.05). Alarmingly, 76.3% of participants failed to achieve BP targets, and 86.9% were not at LDL cholesterol goal according to ESC 2021 guidelines, with no significant difference between high and very high risk groups.

**Conclusions:** The vast majority of COPD patients in this cohort exhibited an elevated CV risk profile, mainly driven by advanced age, cardiometabolic comorbidities, and atherosclerotic burden. Symptom severity appeared to be associated with increased risk, suggesting a possible interplay between respiratory impairment and CV disease. Despite their elevated risk, most participants did not meet recommended BP and serum lipid targets, underscoring a substantial gap in preventive care. These findings highlight the urgent need for comprehensive, integrated CV risk assessment and aggressive management strategies in COPD populations.

## GENETIC ASSESSMENT AND CLINICAL CORRELATES IN SEVERE HYPERTRIGLYCERIDEMIA: A SYSTEMATIC REVIEW

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**Background:** Severe hypertriglyceridemia (SHTG) is linked to acute pancreatitis, metabolic dysfunction, and increased cardiovascular risk. Its genetic basis range to biallelic variants causing familial chylomicronemia syndrome (FCS) to polygenic/multifactorial chylomicronemia syndrome (MCS).

**Methods:** We systematically reviewed scientific literature up to 2025. Eligible studies re-reported genetic data, clinical features, or treatment outcomes in adults with TG ≥500 mg/dL. Data were synthesized for genotype, polygenic risk score (PRS), TG levels, metabolic comorbidities, hepatic steatosis, pancreatitis and therapeutic response.

**Results:** Eleven studies (n=3389) were included. FCS due to biallelic LPL, APOC2, GPIIIBP1, or LMF1 variants accounted for <5% of cases, showing extreme TG levels (>2800 mg/dL) and pancreatitis prevalence (>70%). APOA5, APOC3, and APOB variants were as-associated with intermediate TG elevations, mostly related to MAFLD, and variable therapy response. Polygenic hypertriglyceridemia represented ~70–80% of cases, with TG ≈2200 mg/dL and pancreatitis prevalence 15–20%, determined by metabolic triggers. MAFLD was mostly present in >70% of polygenic cases, supporting a "two-hit" model in which hepatic overproduction of TG-rich lipoproteins amplifies TG excess. Interventional trials demonstrated TG reductions with APOC3 antisense therapy (70–80%) and ANGPTL3 inhibition (50–55%), while GLP-1RA significantly reduced hepatic fat (30–35%) and resolved NASH in 59% of patients.

**Conclusions:** SHTG shows a genotype-phenotype gradient: FCS is linked to recurrent acute pancreatitis, whereas MCS is tightly associated with MAFLD and metabolic dysfunction. It supports a precision-medicine approach, where genetic testing and PRS can lead to APOC3/ANGPTL3-targeted TG-lowering therapy for FCS and combination strategies for MCS with MAFLD to reduce pancreatitis recurrence and liver disease.

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## ABSTRACT

## BEMPEDOIC ACID±EZETIMIBE ALONE OR IN COMBINATION: A COMPARATIVE STUDY ON LIPID CONTROL AND METABOLIC SAFETY

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**Background:** Bempedoic acid, with or without ezetimibe, has emerged as an effective lipid-lowering therapy, especially in patients with statin intolerance. The aim of this study was to compare the efficacy and metabolic impact of bempedoic acid±ezetimibe alone versus its combination with other lipid-lowering agents, including statins, PCSK9 inhibitors, and omega-3 fatty acids.

**Methods:** We conducted a prospective observational study involving 52 dyslipidemic patients (21 males, 31 females; mean age: 59 years). Genetic testing revealed heterozygous LDL receptor mutations in 15 patients, polygenic hypercholesterolemia in 18, and no specific mutations in the remaining 19. Comorbidities included hypertension (36 patients), atherosclerosis (18), type 2 diabetes<sup>7</sup>, and active smoking (16). Patients were divided into two treatment groups: Group A (bempedoic acid±ezetimibe) and Group B (same treatment plus other lipid-lowering agents). Blood samples were collected at baseline and after 6 months to assess total cholesterol, LDL-C, HDL-C, triglycerides, AST, ALT, creatinine, urea, and uric acid.

**Results:** After 6 months, Group A showed an 18.5% reduction in total cholesterol (from 221 to 180 mg/dL) and a 28.9% decrease in LDL-C (from 142 to 101 mg/dL). HDL-C decreased slightly by 8.5%, while triglycerides increased by 16.5%. Mild increases were observed in liver enzymes (ALT +38.5%, AST +30.8%), urea slightly decreased by 2.4% (from 42 to 41 mg/dL), whereas creatinine increased by 12.2% (from 0.9 to 1.01 mg/dL), and uric acid (+17.9%). In Group B, the lipid-lowering effect was more pronounced, with a 30.3% reduction in total cholesterol (from 201 to 140 mg/dL) and a 50% drop in LDL-C (from 124 to 62 mg/dL). However, HDL-C decreased more significantly (-17%), and triglycerides increased by 14.6%. Liver enzymes showed a more modest rise (ALT +8.3%, AST +21.7%), creatinine remained stable, but urea increased by 21.6%, and uric acid by 8.6%.

**Conclusions:** Both treatment strategies effectively reduced LDL-C levels, with the combination therapy (Group B) showing superior efficacy. However, it also led to a greater reduction in HDL-C and a modest increase in triglycerides. The rise in transaminases was more notable in Group A, while Group B showed a greater impact on renal function markers, particularly urea. These findings highlight the importance of personalized therapy and regular monitoring of liver and renal parameters when using combination lipid-lowering regimens. Larger studies are needed to validate these preliminary results and better define the risk-benefit profile of such therapeutic strategies.

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## PCSK9 INHIBITORS COMPARED: AN OBSERVATIONAL STUDY ON MoAB, siRNA, AND SWITCHING STRATEGY

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**Introduction:** Hyperlipidemia is a major cardiovascular risk factor, particularly in patients with genetic predisposition or comorbidities. Inhibitors of proprotein convertase subtilisin/kexin type 9 (PCSK9), administered either as monoclonal antibodies (MoAb) such as alirocumab and evolocumab, or as small interfering RNA (siRNA) such as inclisiran, have emerged as effective therapies to reduce LDL cholesterol (LDL-C). However, the comparative efficacy between these two approaches and the impact of a sequential strategy (from MoAb to siRNA) remain unclear. Objective: To compare the lipid-lowering efficacy and hepatic safety of three PCSK9-inhibitor-based strategies:

- 1) initial therapy with MoAb,
- 2) initial therapy with siRNA, and (3) therapeutic switch from MoAb to siRNA.

**Methods:** This retrospective observational study included 25 adult patients (mean age: 57 years; 10 males, 15 females) with hyperlipidemia and different genetic backgrounds: 17 were heterozygous for LDL receptor mutations, 4 had a polygenic variant, and 4 showed no detectable mutations.

**Comorbidities included:** 3 cases of acute myocardial infarction, 2 strokes, 5 hepatic steatosis, 2 diabetes mellitus, 12 hypertension, 10 diffuse atheromatosis, and 5 active smokers. All patients were on combination therapy: 20 with ezetimibe, 15 with bempedoic acid, 10 with statins, and 6 with omega-3. Patients were divided as follows:

- Group A (n=7): continuous treatment with MoAb for 6 months;
- Group B (n=9): continuous treatment with siRNA for 6 months;
- Group A→B (n=9): switch from MoAb to siRNA; for these patients, baseline was defined as the time of switching, excluding any prior data under MoAb therapy.

**Results:** siRNA therapy showed the greatest efficacy in reducing lipid levels: LDL -53% and total cholesterol -35.9%. In comparison, MoAb-treated patients had lower reductions (LDL -42.7%, total cholesterol -28%). Surprisingly, the A→B switch group showed increased LDL (+16.5%) and total cholesterol (+13%). Transaminase levels remained stable in the MoAb group, with a slight increase in the siRNA group (ALT +6 U/L; AST +7 U/L). Triglyceride levels increased in all groups.

**Conclusions:** siRNA demonstrated superior efficacy over MoAb in lowering LDL-C and total cholesterol, albeit with a slight increase in liver enzymes, warranting clinical monitoring. The therapeutic switch from MoAb to siRNA did not yield additional lipid-lowering benefits and, in fact, was associated with a deterioration in lipid profile, suggesting potential pharmacological resistance or desensitization. A personalized approach, based on genetic background and comorbidities, remains essential in selecting the most effective lipid-lowering strategy.

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 ABSTRACT
 

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**BEMPEDOIC ACID: EFFICACY AND TOLERABILITY IN A REAL-WORLD EXPERIENCE**

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**Background:** Statins are the first-line treatment for hypercholesterolemia, but approximately 10% of patients develop muscle-related side effects that limit their use. Bempedoic acid is a liver-activated prodrug that inhibits ATP-citrate lyase, a key enzyme in the cholesterol biosynthesis pathway upstream of HMG-CoA reductase. This inhibition leads to a reduction in intrahepatic cholesterol synthesis, an increased expression of LDL receptors, and enhanced clearance of LDL cholesterol from plasma.

**Materials and Methods:** This was a monocentric retrospective observational study conducted on 252 patients with dyslipidemia, including 218 with non-familial hypercholesterolemia and 34 with heterozygous familial hypercholesterolemia. 87% of the patients were in primary prevention and 13% of them were in secondary prevention. Patients were treated with bempedoic acid either as monotherapy (Nilemdo, 53%) or in combination with ezetimibe (Nustendi, 47%). Inclusion in the study required absence of contraindications to treatment (eGFR <30 mL/min/1.73m<sup>2</sup> or uric acid >6 mg/dL). The 10 year cardiovascular risk was estimated using the SCORE algorithm, and patients were classified based on LDL-C targets recommended by the Italian AIFA note 13. Follow-up included visits at 3, 9, and 21 months with monitoring of lipid and safety parameters.

**Results:** Treatment reduced LDL-C levels by an average of 30.48%, with greater efficacy observed for Nustendi (-39%) compared to Nilemdo (-29%). The individual target was achieved in 76% of patients, although this percentage was lower when considering the new targets set by the ESC/EAS guidelines (54%). Only 5% experienced mild adverse effects, which rarely required treatment modifications. There were transient and non-clinically significant increases in serum creatinine (+0.04 mg/dL) and uric acid (+0.86 mg/dL), consistent with the drug's mechanism of action involving inhibition of the renal OAT2 transporter.

**Conclusions:** Bempedoic acid proved to be effective and well tolerated in real-life clinical practice, especially in patients with intolerance or insufficient response to statins. Among the two bempedoic acid-based drugs evaluated, Nustendi showed greater efficacy than Nilemdo, with similar safety. The drug represents an important therapeutic option for cardiovascular risk control in patients at moderate or high risk. The study is still ongoing and will provide further long-term data.

**REAL-WORLD USE OF OMEGA-3 MIXTURES IN ITALY: EVIDENCE FROM ATS VALPADANA**

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**Aims:** Cardiovascular diseases (CVD) are the leading cause of death in Europe. Despite achieving very low LDL-C levels, patients with atherosclerotic CVD (ASCVD) remain at high residual risk (RR), to which hypertriglyceridemia contributes. Omega-3 formulations (EPA/DHA) have been widely prescribed, although only pure EPA has demonstrated cardiovascular benefit. This study aimed to assess the real-world use of omega-3-containing therapies in the ATS Valpadana population.

**Methods and Results:** We conducted a retrospective observational analysis of 669,292 beneficiaries of ATS Valpadana between 2022 and 2024. Among them, 154,335 (23.1%) received lipid-lowering drugs and 12,286 (1.2% of the total population; 7.9% of lipid-lowering drug users) were prescribed omega-3 formulations. Omega-3 users were predominantly male (65.4%), aged 45–79 years (79.7%), and more than half (54.4%) were in secondary cardiovascular prevention (CVP). Notably, 38.2% of omega-3 users did not receive any other lipid-lowering therapy. In secondary CVP, one in four patients received omega-3 monotherapy, while 16% were treated with the combination of high-intensity statin, ezetimibe, and omega-3. According to current EAS/ESC guidelines, this subgroup would be eligible for pure EPA (Icosapent ethyl), the only omega-3 therapy with proven cardiovascular benefit.

**Conclusion:** In this large, real-world cohort, omega-3 therapies were frequently prescribed outside guideline recommendations, particularly as monotherapy in very high CV risk patients without adequate LDL-C-lowering therapy. Importantly, a significant subgroup (16%) of secondary CVP patients treated with high-intensity statin, ezetimibe, and omega-3 would be candidates for pure EPA, underscoring the need to align clinical practice with evidence-based cardiovascular prevention strategies.

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**ABSTRACT**


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**NEUTROPHIL-GELATINASE ASSOCIATED LIPOCALIN (NGAL) AS BIOMARKER OF KIDNEY PERFUSION IN LIPOPROTEIN APHERESIS**

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**Background:** Lipoprotein apheresis (LA) represents a supportive tool for inherited dyslipidemias resistant/intolerant to lipid lowering drugs. High plasma levels of atherogenic lipoproteins may be a sufficient trigger to upregulate endothelial adhesiveness, a phenomenon that can be acutely “restored”, by LA. For this reason, atherosclerotic inherited dyslipidemias under LA treatment represent a good clinical model to study the effects of atherogenic lipoproteins modulation on endothelial function and microcirculation in different organs. Urinary neutrophil gelatinase-associated lipocalin (Ur-NGAL) is an ion-transporting agent, produced in the distal nephron, its synthesis is up-regulated in response to kidney injury. Nowadays is considered a “renal troponin” and is also a powerful marker of renal function and kidney disease progression.

Aim of this pilot study is to evaluate the Ur-NGAL as new biomarker of kidney perfusion and renal endothelial restoration in patients with inherited dyslipidemias on chronic LA.

**Methods:** We evaluated 28 patients (mean age 60±9 years, male 68%) with inherited dyslipidemias on maximally tolerated lipid lowering therapy and chronic LA. The measurement of Ur-NGAL (NGAL Test™ - BioPorto Diagnostics, Gentofte - Denmark) were performed in spot urine samples collected before and after LA treatment. Lipoprotein apheresis was performed by dextran-sulphate absorption from plasma (Liposorber®-LA MA-03 systems; Kaneka, Osaka, Japan; 15/28 patients), heparin-induced LDL precipitation apheresis (HELP®, Plasmal Futura®; B. Braun, Melsungen, Germany; 11/28 patients) or immunoadsorption (TheraSorb™ LDL pro Adsorber, Miltenyi biotec, Bergisch Gladbach, Germany; 2/28 patient) in agreement with guidelines and manufacturer’s instructions.

**Results:** In parallel with the expected improvement in the lipid profile (LDL cholesterol – 72%, Lp(a) -79%; p<0.001), LA treatment showed a concomitant significant reduction in Ur-NGAL on spot urine samples collected after treatment (10.5±7.2 ng/mL vs 18.3±14.8 ng/mL; p 0.006). All patients included in this study had a baseline Ur-NGAL in normal range (<50 ng/mL). In addition, the two mains used LA systems showed, in absence to baseline value difference (Liposorber®-LA 11.2±12.7 ng/mL vs. HELP® 11.3±11.2 ng/mL; p 0.965), a comparable Ur-NGAL reduction (Liposorber®-LA 6.5±12.6 ng/mL vs. HELP® 6.0±6.6 ng/mL; p 0.875).

**Conclusion:** The goal of lipid lowering therapy is to improve arterial homeostasis and organ perfusion, thus LA, by acutely modulating blood cholesterol levels, may improve impaired endothelial and microcirculation function in peripheral vascular beds and myocardium. As known Ur-NGAL concentrations rapidly response to kidney homeostasis because NGAL is readily filtered in the glomerulus, readily reabsorbed in the proximal tubular segments and up-regulated in the distal parts of the nephron. Renal endothelial dysfunction is related to impaired function of the cells lining the blood vessels within the kidneys. Furthermore, nitric oxide (NO) is a key mediator of coronary and kidney vasodilation. LA, in addition to reduces endothelial expression of VCAM-1, may modulate the NO/endothelin-1 balance towards vasodilation. All these aspects are able to improve renal perfusion leading to Ur-NGAL reduction. This preliminary data,

thanks to the favorable Ur-NGAL reduction by LA, could offers new horizons on the effect of the lowering atherogenic lipoproteins in the improvement of renal function, presenting insights into new potential pathways for assessing the microcirculatory system.

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**DIRECT ORAL ANTICOAGULANTS USE AND CARDIAC EVENTS IN PATIENTS WITH ATRIAL FIBRILLATION: A SYSTEMATIC REVIEW AND NETWORK META-ANALYSIS**

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**Aims:** Direct oral anticoagulants (DOACs) reduce thromboembolism in atrial fibrillation (AF), but their effect on cardiac outcomes is still unclear. For this reason, we investigated the risk of cardiac events in atrial fibrillation patients.

**Methods:** We performed a systematic review and network meta-analysis including AF patients on DOACs/vitamin K antagonists (VKAs). Endpoints were major adverse cardiac event (MACE) and myocardial infarction (MI). Rankograms and SUCRA were performed. Subgroup analysis included age (</≥ 75 years) and length of follow-up (</≥ 12 months).

**Results:** 44 studies with 1,807,703 patients were included (62.6% on DOACs and 45.3% women). Compared to VKA, apixaban (hazard ratio [HR] 0.73, 95% credible interval [95%CI] 0.61–0.91), dabigatran (HR 0.83, 95%CI 0.70-1.00), edoxaban (HR 0.87, 95%CI 0.68-0.99) and rivaroxaban (HR 0.85, 95%CI 0.73-1.00) associated with lower MACE risk. Analysis of SUCRA showed apixaban as first choice for preventing MACE overall and in patients treated for ≥12 months. The MI risk (39 studies with 1,392,113 patients) was lower in all DOACs compared to VKA. SUCRA showed edoxaban as first choice for preventing MI overall. In patients aged ≥75 years a lower MI risk was found for apixaban (HR 0.86, 95%CI 0.70–1.00), and rivaroxaban (HR 0.83, 95%CI 0.70-0.98), while in those aged <75 years, a lower MI risk for dabigatran (HR 0.82, 95%CI 0.68-0.99) and edoxaban (HR 0.60, 95%CI 0.41-0.85) was noted. Heterogeneity was low in all analyses. Network meta-analysis showed no differences among DOACs.

**Conclusions:** In conclusion, DOACs use was associated with a lower risk of MACE/MI in AF. The effect of DOACs on cardiovascular risk differs according to aging. PROSPERO. CRD42023407778

## ABSTRACT

## SGLT2-INHIBITORS AND GLP1-RECEPTOR AGONISTS AFFECT 3-MONTH PLASMA NT-proBNP LEVELS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS WITHOUT HEART FAILURE

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**Aims:** We investigated the effect of a short-term treatment with sodium-glucose cotransporter-2 inhibitors (SGLT2i) and glucagon-like peptide-1 receptor agonists (GLP1-RA) on N-terminal pro-brain natriuretic peptide (NT-proBNP) levels in asymptomatic T2DM patients without known heart failure, but with elevated blood pressure and heart stress (HS), a subclinical cardiac organ damage.

**Materials and Methods:** Multicenter observational longitudinal "real-life" study on 100 consecutive patients. Plasma NT-proBNP was assessed at baseline, before starting SGLT2i/GLP1-RA and after a 3-month follow-up. For the analyses, NT-proBNP levels were adjusted for estimated glomerular filtration rate (eGFR) in SGLT2i group and for body mass index (BMI) in GLP1-RA group.

**Results:** Mean age: 73.3±7.7 years. Dyslipidemia was present in 92.9% of patients, and 80.8% were on lipid-lowering therapy (mean LDL cholesterol=73.0±29.0 mg/dl). Baseline median NT-proBNP: 192.5 (114.0-966.0) pg/mL, without difference according to history of dyslipidemia (p=0.387). At follow-up, we found a slight reduction in adjusted NT-proBNP in the SGLT2i group (n° 57 patients) (-2.3%; p=0.049). Adjusted NT-proBNP showed a statistically significant decrease (-6.5%; p=0.002) within the entire GLP1-RA group (n° 43 patients), with a significantly greater reduction in patients with higher baseline NT-proBNP (-13.3%; p<0.001). In both subgroups, patients aged <70 years showed a greater reduction in NTproBNP.

**Conclusions:** In asymptomatic patients with T2DM and HS, SGLT2i and especially GLP1-RA led to a slight but significant improvement in cardiac wall stress, as evidenced by the NT-proBNP decrease.

## LIPOPROTEIN(a) AS AN INDEPENDENT RISK FACTOR FOR CARDIOVASCULAR AND THROMBOEMBOLIC EVENTS: EVIDENCE FROM A RETROSPECTIVE COHORT OF 155 PATIENTS

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**Background:** Lipoprotein(a) [Lp(a)] has emerged as a genetically determined, independent cardiovascular risk factor, exerting both pro-atherogenic and pro-thrombotic effects. However, the threshold of clinical relevance and its prognostic role in unselected clinical populations remain debated.

**Purpose of the Study:** To investigate whether elevated lipoprotein(a) [Lp(a)] levels represent an independent risk factor for cardiovascular, cerebrovascular, and thromboembolic events in a retrospectively analyzed, unselected clinical cohort, and to assess the potential prognostic value of different Lp(a) stratification (<30, 30–50, >50 mg/dL).

**Materials and Methods:** We retrospectively analyzed 155 unselected patients (mean age 49.8 years; 55.5% male) referred to a tertiary lipid clinic between 2015 and 2025. Clinical data, cardiovascular risk factors, laboratory values, and imaging findings were collected, with a mean follow-up duration of 5 years. Patients were stratified according to Lp(a) concentration: Low (<30 mg/dL, n=60), Moderate (30-50 mg/dL, n=22), and High (>50 mg/dL, n=73). Baseline clinical status (primary vs. secondary prevention) was recorded, and cardiovascular, cerebrovascular, and thromboembolic events occurring during follow-up were assessed.

**Results:** The mean Lp(a) concentration was 76.2 mg/dL. 21% already have developed a CV events. A total of 32 patients (20.6%) developed new clinical events during follow-up: 24 cardiovascular (15.5%), 5 cerebrovascular (3.2%), and 3 thromboembolic (1.9%). Patients with Lp(a) ≥30 mg/dL showed a higher incidence of cardiovascular events compared with those <30 mg/dL (19 vs. 5 events). Notably, all thromboembolic events occurred in the High Lp(a) group (>50 mg/dL). In contrast, cerebrovascular events did not correlate with Lp(a) levels, being more frequent in the Low Lp(a) group. Importantly, traditional risk factors (age, BMI, hypertension, dyslipidemia, diabetes, smoking) were similarly distributed across groups, suggesting an independent effect of Lp(a).

**Conclusions:** Elevated Lp(a) was associated with an increased incidence of cardiovascular and thromboembolic events, independently of conventional risk factors, supporting its role as a residual risk factor. The observation that even values between 30–50 mg/dL were associated with adverse outcomes suggests that current thresholds (>50 mg/dL) may underestimate risk. These findings reinforce the need for systematic Lp(a) measurement and the development of targeted therapies.

## ABSTRACT

## EVALUATING THE METABOLIC RESPONSE TO AN ORAL LIPID LOAD IN HETEROZYGOUS FAMILIAL HYPOBETALIPOPROTEINAEMIA: A CASE REPORT

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**Background:** Familial hypobetalipoproteinaemia (FHBL) is a rare codominant genetic disorder caused by impaired apolipoprotein B (APOB) metabolism. Patients affected by FHBL have a distinctive lipid profile characterized by reduced levels of circulating total cholesterol (TC), LDL-C and triglycerides (TG). This is coupled with an increased susceptibility to hepatic lipid accumulation, leading to liver steatosis and fibrosis. Despite these defects in lipid metabolism, it remains to elucidate how individuals suffering from FHBL respond to an oral fat load.

**Aim:** This study aims at characterizing the metabolic response to an oral lipid load in a young patient with heterozygous FHBL.

**Methods:** A 9 year old male presented to our outpatient setting with a circulating lipid profile suggestive for FHBL. Genetic confirmation of FHBL was obtained using targeted gene sequencing via NGS (Ion GeneStudio™, S5 System). Lipid load was performed by administering a high-fat meal (179 g of mascarpone cream cheese). Blood samples were obtained before (T0) and after 1, 2, 3, 4 and 6 hours after the consumption of the high-fat meal (T1, T2, T3, T4, T6) to evaluate the variations in the metabolic parameters of interest. The latter included TC, LDL-C, HDL-C, TG, APOB100, APOA1, insulin and glucose.

**Results:** Gene sequencing was positive for the presence of the heterozygous variant c.2608C>T, p.Q870\* (exon 18) in the APOB gene, diagnostic for heterozygous FHBL. Despite the already low circulating lipid levels, the oral fat load induced a decrease in TC (76,40 mg/dl->67,60 mg/dl), HDL-C (49,04 mg/dl->39,08 mg/dl), LDL-C (33,05 mg/dl->28,83 mg/dl) and apolipoproteins A (125,00 mg/dl->107,00 mg/dl) and B (21,00 mg/dl->17,00 mg/dl) at T3 compared to baseline. TG levels gradually increased during the six hours following the consumption of the test meal (27,35 mg/dl->60,90 mg/dl). Glucose decreased progressively, reaching a minimum at T1, then rising and stabilizing around T2, whereas insulinemia showed an opposite pattern, with a peak during the first hour of analysis.

**Conclusions:** To our knowledge, this is the first characterization of the metabolic response to a lipid load in a subject with FHBL and therefore susceptible to multiple interpretations. Despite the progressive increase in TG suggests the production of chylomicrons after the lipid meal, the reduction in TC and LDL-C at T3 differs from the expected response in a healthy subject. This could imply an alteration in the maturation of VLDL cholesterol which may be further impaired by the chylomicrons remnants reaching the liver. However, this hypothesis remains to be confirmed with further studies being warranted to gain a better understanding of the pathophysiological mechanisms underpinning the metabolic response to a lipid load in FBHL subjects.

## LEUKOCYTE TELOMERE LENGTH IN HYPERCHOLESTEROLEMIC PATIENTS: DIFFERENTIAL DYNAMICS BETWEEN CLINICAL AND GENETIC FAMILIAL HYPERCHOLESTEROLEMIA

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**Introduction:** Aging has been recognized as an important risk factor for many pathologies, such as cardiovascular disease (CVD). Recently, clonal hematopoiesis of indeterminate potential (CHIP), an age-related phenomenon, has been reported as an independent risk factor for atherosclerotic CVD, heart failure and thrombosis. The CHIP is characterized by hematopoietic stem cells (HSCs) that incur somatic mutations in driver genes, like DNA methyltransferase 3a (DNMT3A), ten-eleven translocation-2 (TET2), additional sex combs-like 1 (ASXL1) and regulatory tyrosine kinase JAK2, in individuals without diagnosed hematologic disorder. Moreover, in humans, decrease in leukocytes telomeres length (LTL) associates with common CV factors, the onset and progression of cardiometabolic diseases, including diabetes, metabolic syndrome, chronic kidney disease and coronary heart disease, and has been associated with CHIP.

**Methods:** A total of 95 hypercholesterolaemic subjects (older than 50 years) from the LIPIGEN Registry (Milan) underwent clinical and biochemical evaluation, including Dutch Lipid Clinic Network Score assessment and documentation of cardiovascular history. Genetic testing distinguished patients with heterozygous familial hypercholesterolaemia (He-FH) from those with only a clinical diagnosis (CD-FH). LTL was compared with 487 population-based controls (older than 50 years) from the PLIC study. Genomic DNA was extracted from leukocytes, and LTL was determined by a validated qPCR assay calculating the telomere-to-single copy ratio (T/S). Each sample was analyzed in triplicate on two separate days (inter-assay CV 6.4%), normalized to internal controls, and re-tested for extreme values, with amplicon specificity confirmed by melting curve analysis. All assays were performed by blinded operators using a Bio-Rad® CFX Connect system.

**Results:** LTL is found to be significantly increased in CD-FH individuals (2,21±1,36) compared with PLIC controls (1,25±0,97) (p<0,0001; Mann-Whitney test). Although He-FH individuals (1,43±0,94) show a similar trend toward longer telomeres relative to PLIC controls (1,25±0,97), this difference does not reach statistical significance (p=0,1035). Notably, when directly compared with CD-FH individuals (2,21±1,36), He-FH individuals (1,43±0,94) present a significantly reduced TL (p=0,0007; Mann-Whitney test).

**Conclusions:** Our findings indicate that LTL is significantly increased in hypercholesterolaemic CD-FH subjects as compared with PLIC controls, while He-FH patients display a less pronounced effect. The differential telomere dynamics observed between CD-FH and He-FH individuals suggest distinct underlying biological mechanisms, possibly reflecting heterogeneous cardiovascular risk profiles. These results highlight the relevance of telomere biology in hypercholesterolaemic conditions and require further investigation into its potential correlation with CHIP in subjects with FH.

## ABSTRACT

## COLONIC (POLY)PHENOL METABOLITES AS PROMISING TOOLS TO CONTROL INFLAMMATION AND PREVENT CARDIOVASCULAR DISEASE

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**Aim:** Inflammation is a crucial biological process for the repair of injured tissues, but its persistence can lead to chronic conditions associated with numerous diseases, such as atherosclerosis. The development of anti-inflammatory agents, including those derived from natural sources, represents a strategic goal in pharmacological research aimed at creating functional products for use during the early stages of chronic inflammatory diseases. Based on these premises, this study focused on the *in vitro* analysis of the anti-inflammatory activity of a series of chiral phenyl- $\gamma$ -valerolactones, the main colonic metabolites of flavan-3-ols, a widely consumed class of dietary flavonoids.

**Methods:** Human dermal fibroblasts were treated with 10 different phenyl- $\gamma$ -valerolactones at a concentration of 1  $\mu$ M for a total of 48 hours. The tested compounds belong to the class of chiral (poly)hydroxyphenyl- $\gamma$ -valerolactones, including pure enantiomers and several methylated or sulphated conjugates at the phenol moieties. The concentration used was chosen as it is representative of plasma levels detected following dietary intake and metabolism. In the first 24 hours, the cells were exposed to compounds under basal conditions; in the following 24 hours, the treatment was repeated both in the presence and absence of lipopolysaccharide (LPS, 1  $\mu$ g/ml) to induce an inflammatory response. Evaluation of cytotoxicity was conducted by MTT and lactate dehydrogenase (LDH) assays. The anti-inflammatory effect was determined by quantifying the secretion of the pro-inflammatory cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8) using ELISA. Data normalization was performed by expressing measured cytokine over protein concentration in each well (bicinchoninic acid method). To investigate the underlying mechanism of action, NF- $\kappa$ B activation was evaluated by determining p65 expression through western blot analysis, using  $\beta$ -actin as a loading control. Protein levels were expressed as the p65/ $\beta$ -actin ratio. A 24-hour pharmacokinetic study was performed to evaluate the biotransformations of the incubated compounds and to characterise their metabolic products, monitoring 31 distinct phenyl- $\gamma$ -valerolactones.

**Results:** None of the compounds induced cytotoxic effects. (4R)-5-(4'-hydroxyphenyl)- $\gamma$ -valerolactone (R-CC01) reduced IL-6 secretion by 76% ( $p=0.0001$ ) and IL-8 secretion by 70% ( $p=0.0046$ ). Its enantiomer (4S)-5-(4'-hydroxyphenyl)- $\gamma$ -valerolactone (S-CC01) inhibited IL-6 by 89% ( $p=0.0001$ ) and IL-8 by 86% ( $p=0.0015$ ). (4R)-5-(3',4'-dihydroxyphenyl)- $\gamma$ -valerolactone (R-CC02) showed an 83% reduction in both IL-6 ( $p=0.0001$ ) and IL-8 ( $p=0.0019$ ). (4S)-5-(3'-hydroxy-4'-methoxyphenyl)- $\gamma$ -valerolactone (S-CC03) resulted in a 90% reduction in IL-6 ( $p=0.001$ ) and an 87% reduction in IL-8 ( $p=0.0014$ ). (4R)-5-(3'-hydroxy-4'-methoxyphenyl)- $\gamma$ -valerolactone (R-CC03) inhibited IL-6 by 78% ( $p=0.0001$ ) and IL-8 by 71% ( $p=0.0032$ ). Western blot analysis confirmed a reduction in NF- $\kappa$ B activation: compared to LPS-stimulated cells, p65 levels decreased by 37% with R-CC01 ( $p=0.310$ ), 61% with R-CC02 ( $p=0.0022$ ), and 73% with R-CC03 ( $p=0.0008$ ). The kinetic study revealed distinct metabolic patterns: R-CC01 remained unmodified, whereas R-CC02 and R-CC03 generated sulphate metabolites.

**Conclusions:** Four phenyl- $\gamma$ -valerolactones significantly reduced proinflammatory cytokine secretion in LPS-activated human fibro-

blasts, at least partly through inhibition of NF- $\kappa$ B activation, with comparable activity between enantiomers and independently of substituents. However, additional pathways are likely to be involved and deserve further exploration. If these effects are confirmed *in vitro*, *in vivo*, and in clinical studies, colonic phenyl- $\gamma$ -valerolactones could be included into personalized dietary strategies and novel nutraceutical or pharmacological interventions to counteract chronic inflammation and reduce cardiovascular risk.

## EFFECT OF GENDER AND GENOTYPE ON SPHINGOLIPID METABOLISM: A STUDY IN C57BL/6J AND PCSK9-KO MICE

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**Background and Aim:** Sphingolipids, a lipid class which includes ceramides as the central sphingolipid species, are constituents of cell membranes and circulate in plasma lipoproteins. Alterations of plasma ceramide levels or composition have been associated with an increased incidence of dysmetabolic conditions and atherosclerotic cardiovascular disease. This study was aimed at investigating, in mouse models, whether Pcsk9 deletion was able to affect ceramide homeostasis.

**Methods:** C57BL/6J mice and Proprotein convertase subtilisin/kexin 9 gene knock-out (Pcsk9-KO) mice in the C57BL/6J background were enrolled. From 6 to 22 weeks of age, mice were fed either a standard rodent diet (SD) containing 3% fat or a Western-type diet (WD) containing 0.2% cholesterol and 21% fat. Each experimental group was composed of both males ( $n=3-8$ ) and females ( $n=3-8$ ). At the end of the experimental period, blood was collected from fasted mice. Mice were then sacrificed, and the livers were harvested to evaluate the expression of genes involved in ceramide synthesis/metabolism by quantitative PCR (qPCR). Lipidomic analysis was performed on plasma and liver samples.

**Results:** On SD, plasma levels of 18:1 ceramides and glycosphingolipids (GlcGalCer, LacCer) were higher in C57BL/6J mice compared with Pcsk9-KO mice of both sexes. In both genotypes, and particularly in male mice, WD caused an increase of ceramide plasma levels, which however remained lower in Pcsk9-KO compared to C57BL/6J mice. Differently from plasma, sphingolipid levels in the liver were comparable between the two genotypes on SD and showed similar increases after WD. The evaluation of gender differences within each genotype showed that the levels of several sphingolipid classes (GlcGalCer, LacCer, Gb3, SM) in the liver were higher in C57BL/6 females than in males, both on SD and WD. The expression of several genes encoding for enzymes involved in the ceramide biosynthetic pathways was analysed. The results obtained indicated that, for some of the genes, there was no shared expression profile among groups. Of note, a strong upregulation of the ceramide *de novo* synthesis was observed in WD-fed Pcsk9-KO females.

**Conclusions:** In spite of a differently regulated hepatic expression of genes involved in ceramide homeostasis, sphingolipid levels in liver were similar between Pcsk9-KO and C57BL/6J mice, when the same gender and dietary condition were compared. Further analyses will be carried out to extend the evaluation of gene expression to the biosynthesis of ceramide-derived glycosphingolipids, such as GlcGalCer and LacCer.

## ABSTRACT

**IMPACT OF OPTIC ATROPHY PROTEIN 1 ON LIPID METABOLISM ON DIET INDUCED OBESITY**

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**Background/Aim:** Mitochondria are dynamic organelles, composed of distinct outer (OMM) and inner (IMM) membranes with different functions and compositions, that maintain cellular homeostasis through a continuous balance of biogenesis, fusion, fission, and mitophagy. A crucial protein for the fusion of IMM is Optic atrophy Protein 1 (OPA1). Modulating OPA1 expression influences cellular metabolism and anabolic processes; based on this, we investigated whether enhancing mitochondrial plasticity through OPA1 overexpression could impact the cardiac environment in a condition of metabolic syndrome.

**Methods:** OPA1-overexpressing transgenic mice (OPA1-Tg) and wild-type (WT) controls were fed a high-fat diet (45% kcal from lipids) for 20 weeks. To assess the impact of OPA1 overexpression on systemic metabolism, mice underwent indirect calorimetry; additionally, histological analyses were performed on paraffin-embedded tissues.

**Results:** OPA1 overexpression significantly reduced weight gain, mainly due to decreased adipose tissue, especially subcutaneous (SCAT) and pericardial fat. It was also associated with increased hepatic lipoprotein production and enhanced peripheral catabolism of circulating lipoproteins. Moreover, the liver of OPA1-Tg mice was characterized by lower accumulation of lipid droplets compared to WT littermates, suggesting a protective role of OPA1 against steatosis progression. In the heart, OPA1 overexpression led to higher triglyceride accumulation, suggesting an enhanced cardiac metabolic capacity. Morphological analyses of various heart sections revealed a significant reduction in the lateral wall thickness and a reduced width of cardiomyocytes in OPA1-Tg mice. The observed changes in cardiac wall thickness may have implications for cardiac function and efficiency, as alterations in myocardial structure can impact contractility and overall cardiac performance.

**Conclusions:** Overall, our findings suggest that OPA1 overexpression modulates hepatic cellular metabolism by promoting enhanced lipid handling and utilization, which may, in turn, influence the metabolism of extrahepatic tissues such as the heart. These mechanisms likely result from complex mitochondrial interactions affecting cellular lipid metabolism.

**TNF- $\alpha$ , BUT NOT PALMITIC ACID, PROMOTES SIMPSON-GOLABI-BEHMEL SYNDROME (SGBS) ADIPOCYTE METABOLIC DYSFUNCTION**

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**Background:** Chronic low-grade inflammation, together with the overconsumption of long-chain saturated fatty acids such as palmitic acid (PA), has been implicated in the development of insulin resistance and related metabolic impairments. However, the individual contribution of saturated fatty acid overload and metabolic inflammation on adipocyte dysfunction remains elusive.

**Aim:** The aim of the study was to compare the effects of PA and TNF- $\alpha$  in terms of their impact on insulin signalling, inflammatory pathways, expression of adipokines and other genes involved in lipid metabolism in Simpson-Golabi-Behmel Syndrome (SGBS) adipocytes.

**Methods:** SGBS adipocytes were treated with either 1000  $\mu$ M PA or 10 ng/mL TNF- $\alpha$  for 48 hours and 24 hours, respectively. Insulin signalling was assessed by evaluation of protein kinase B (Akt) and Akt substrate of 160 KDa (AS160) phosphorylation using Western blot. Nuclear factor- $\kappa$ B (NF- $\kappa$ B) pro-inflammatory pathway activation was evaluated by quantifying the abundance of its inhibitor (IKB $\alpha$ ). Inflammation was further characterised by assessing the expression of interleukin 6 (IL-6), interleukin 1-beta (IL-1 $\beta$ ) and monocyte chemoattractant protein-1 (MCP-1) by Real-Time qPCR. Finally, the expression of peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ), fatty acid binding protein 4 (FABP4), serine palmitoyltransferase long chain subunit 1 (SPTLC1), ceramide synthase 6 (CERS6) and adiponectin was quantified always by RT-qPCR. Data are expressed as mean  $\pm$  standard error (SEM). Differences between groups was evaluated using Student's t-test, with  $p < 0,05$  considered as statistically significant.

**Results:** PA overload was insufficient to cause adipocyte dysfunction in this model. Indeed, PA did not significantly impair insulin-stimulated phosphorylation of Akt and AS160 nor trigger the activation of pro-inflammatory responses or affect the expression of adipokines or genes involved in lipid metabolism. On the contrary adipocyte exposure to TNF- $\alpha$  led to a significant reduction in Akt and AS160 phosphorylation, indicating an impaired insulin signalling. This effect was associated with the activation of the NF $\kappa$ B pro-inflammatory inflammatory pathway marked by a decrease in the abundance of IKB $\alpha$ . This was further confirmed by the transcriptional upregulation of NF $\kappa$ B targets, namely IL-6 and MCP-1. Additionally, TNF- $\alpha$  significantly downregulated the adipogenic regulator PPAR $\gamma$ , the fatty acid binding protein FABP4, and the insulin-sensitizing adipokine adiponectin. While expression of SPTLC1 (the first rate-limiting enzyme of the de novo ceramide synthesis) remained unchanged at both gene expression and protein level in response to TNF- $\alpha$ , this cytokine significantly upregulated the expression of CERS6.  
**Conclusions:** In this model, PA alone was insufficient to induce metabolic inflammation or insulin resistance. By contrast, TNF- $\alpha$  impairs insulin signalling, while suppressing the expression of the master adipogenic regulator PPAR $\gamma$ , the fatty acid transporter FABP4, and the insulin-sensitizing adipokine adiponectin. These findings highlight the role of inflammatory cytokines, rather than nutrient excess per se, in driving adipocyte dysfunction.

## ABSTRACT

**ANALYSIS BY NEXT GENERATION SEQUENCING OF A CLINICAL CASE WITH LECITHIN CHOLESTEROL ACYLTRANSFERASE DEFICIENCY (LCAT)**

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**Introduction:** Lecithin cholesterol acyltransferase (LCAT) is a key enzyme responsible for the esterification of plasma cholesterol, a process essential for the maturation of high-density lipoprotein (HDL) particles. The LCAT gene is located on the long arm of chromosome 16 (16q22), and over forty mutations have been identified that correlate with enzyme deficiency. LCAT deficiency manifests in two clinically distinct forms: the severe Familial LCAT Deficiency (FLD) and the milder Fish-Eye Disease (FED). In FLD, LCAT activity is completely absent, leading to negligible cholesterol ester levels, whereas in FED, the enzyme loses its ability to esterify cholesterol in HDL but retains partial activity in apolipoprotein B (APOB)-containing lipoproteins. Affected individuals are typically homozygous or compound heterozygous for LCAT mutations and present with low HDL-C, ApoA-I, and LDL-C levels, alongside normal to elevated triglycerides.

**Materials and Methods:** We report a 48-year-old patient with a history of moderate-to-severe hypertriglyceridemia (500–1000 mg/dL) and a family history of type 2 diabetes mellitus. At the first visit at our lipid clinic, the patient's lipid profile showed: total cholesterol 214 mg/dL, triglycerides 1184 mg/dL and HDL-C 19.2 mg/dL. To investigate the genetic basis of the lipid abnormalities, we performed targeted Next Generation Sequencing (NGS) analysis covering coding regions and intron-exon boundaries of genes involved in the metabolism of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and HDL-C.

**Results and Conclusion:** NGS did not identify pathogenic variants in candidate genes commonly associated with hypertriglyceridemia. However, a heterozygous nonsense variant in the LCAT gene (c.321C>A; p.Tyr107Ter) was detected. This variant introduces a premature stop codon, predicted to result in an abnormal protein product. The variant is documented in population databases as pathogenic and has been associated with LCAT deficiency. The presence of a single heterozygous pathogenic variant in LCAT may contribute to qualitative and quantitative alterations in plasma lipoproteins, potentially explaining the patient's dyslipidemia. This case highlights the importance of genetic testing for LCAT variants in patients with unexplained HDL deficiency and severe hypertriglyceridemia.

**IDENTIFICATION OF EXON 3-6 DUPLICATION IN LDLR GENE IN A LARGE SICILIAN FAMILY AFFECTED BY FAMILIAL HYPERCHOLESTEROLEMIA**

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**Background:** Familial Hypercholesterolemia (FH) is an autosomal dominant disorder characterized by high plasma low-density lipoprotein cholesterol (LDL-C) levels and increased risk of premature coronary artery disease. FH is caused by mutations in three main candidate genes involved in LDL metabolism: LDLR, APOB and PCSK9. Genetic screening is commonly performed by Sanger sequencing or Next-Generation Sequencing (NGS). While most LDLR variants are point mutations (90-95%), 5-10% are large rearrangements detectable only with techniques such as Southern blotting or Multiplex Ligation-dependent Probe Amplification (MLPA).

**Materials and Methods:** We present a 44-year-old man with severe hypercholesterolemia (TC 394 mg/dL, LDL-C 332 mg/dL), corneal arcus, family history of hypercholesterolemia, cardiovascular events and aortic stenosis. Secondary causes of dyslipidemia were excluded. The Dutch Lipid Clinic Network (DLCN) score was 14 consistent with definite FH. Genetic testing performed with Sanger sequencing of LDLR, APOB, and PCSK9 revealed no pathogenic variants, prompting MLPA analysis.

**Results and Conclusion:** MLPA identified a heterozygous duplication of LDLR exons 3-6, confirmed in multiple relatives (father, sister, two uncles, and two cousins), consistent with autosomal dominant inheritance. This large rearrangement, designated FH-Caltanissetta, was identified for the first time in a Sicilian family from Caltanissetta, representing a novel Sicilian family cluster. Functional prediction indicated a 7-kb duplicated segment corresponding to c.(190+1\_191-1)\_(940+1\_941-1)dup. In patients with a clinical FH phenotype and negative standard sequencing, MLPA is crucial for detecting CNVs. This approach enables definitive genetic diagnosis, informs cascade screening, and may guide eligibility for novel targeted lipid-lowering therapies. Early identification of such rearrangements is essential for personalized management and prevention of premature cardiovascular events.

## ABSTRACT

## SET UP OF A NOVEL APPROACH TO QUANTIFY TRIMETHYLAMINE N-OXIDE, TMAO, INTEGRATED IN A MACHINE LEARNING-BASED MODEL TO PREDICT PERSONALIZED CVD RISK PROFILES IN A SUB-COHORT OF THE PLIC STUDY

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Cardiovascular diseases (CVDs) represent the leading cause of mortality worldwide. Novel factors were recently observed to contribute to CVDs. Among them, trimethylamine N-oxide (TMAO) was found as an independent predictor of CVDs. Historically believed a waste metabolite product, TMAO was observed to accumulate in subjects with renal impairments. Increased circulating TMAO (cTMAO) was correlated with increased risk to develop CVDs. Currently, pathophysiological mechanisms linking TMAO to CVDs, particularly, to atherosclerosis CVD (ASCVD) are under investigation. Moreover, growing technological advances have led to the development of a wide variety of validated methods to measure cTMAO. Nevertheless, standardization of such approaches still represents an unmet need and is central in the clinics for establishing robust TMAO ranges to improve data interpretation in clinical research. In addition, CVD risk prediction is being improved by machine-learning (ML)-based approaches to address the multifactorial origin of CVDs. Compared to traditional statistical methods, ML approaches can indeed capture complex and non-linear relationships among different factors (e.g., clinical, biochemical, imaging, or omics data) into a single predictive model. Specifically, supervised ML algorithms can identify mostly relevant predictors without strong prior assumptions, providing higher discriminative power.

Therefore, we aimed at validating a robust and highly reproducible analytical method to quantify cTMAO and integrate multiple clinical and biochemical factors, including cTMAO, into a ML-based predictive model, using a sub-cohort of subjects in CVD primary prevention of the PLIC study.

To this aim, 327 plasma samples from a PLIC study subcohort were tested to measure cTMAO with a novel stable isotope high performance liquid chromatography (HPLC)-tandem mass spectrometry (MS/MS) method. Clinical and biochemical measurements from these subjects were integrated into a ML-based model (forward feature selection coupled to supervised logistic regression algorithm) to predict CVD risk. HPLC-MS/MS showed high linearity ( $r^2$ : 0.99997). cTMAO median (Q1-Q3) value was 258 (176-417) (ng/mL). cTMAO contributed to the prediction of CVD risk with an accuracy of 0.687 in the ML-based predictive model. LDL-C and common carotid intima media thickness (C-IMT) were revealed among stronger predictors (accuracy: 0.724; area under the curve: 0.743; CI lower-upper: 0.689-0.79). It is concluded that this HPLC-MS/MS method enabled rapid and accurate quantification of cTMAO from plasma samples. ML-based predictive model revealed TMAO as a weak

er predictor of CVD risk in the sub-cohort of patients in CVD primary prevention from the PLIC study. Further studies in subjects with more severe CVD risk/disease will extend these observations.

## ACID SPHINGOMYELINASE DEFICIENCY: CASE REPORT AND DIAGNOSTIC VALUE OF LYSO-SM

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**Introduction:** Acid sphingomyelinase deficiency (ASMD) is an autosomal recessive lysosomal storage disorder (LSD) associated with biallelic pathogenic variants in the sphingomyelin phosphodiesterase 1 (SMPD1) gene.

Sphingomyelin accumulates in multiple organs including spleen, liver, lung, bone marrow, lymph nodes, and in the most severe form, in the central nervous system and peripheral nerves.

Clinical manifestations range from rapidly progressive and fatal infantile neurovisceral disease, to less slowly progressing chronic neurovisceral and visceral forms associated with significant morbidity and reduced life expectancy due to respiratory or liver disease.

The wide variability in age of onset and clinical phenotype makes diagnosis challenging.

**Material and Methods:** Here we describe the case of a 44-year-old patient with splenomegaly and moderate thrombocytopenia. Plasma levels of sphingomyelin (Lyso-SM) analysed by liquid chromatography-mass spectrometry (LC-MS) were elevated. Molecular genetic analysis was performed to identify mutations in the SMPD1 gene using direct automatic sequencing with the Sanger method.

**Results and Conclusion:** Genetic testing identified two variants in SMPD1 gene in heterozygosity. The missense variant c.1427G>A (p.Arg476Gln) is predicted to have a deleterious effect on the protein. The frameshift variant c.706\_707delinsT (p.Pro236CysfsTer20) causes the formation of a premature stop codon, resulting in a truncated, non functional protein.

Lyso-SM quantification is a valuable tool for the diagnosis of ASMD, particularly in differentiating it from other lysosomal storage disorders such as Gaucher disease, Niemann-Pick type C, lysosomal acid lipase deficiency, malignant hematologic disorders, and primary liver diseases. This method is also useful for neonatal screening. Currently, no disease-specific treatment is available for ASMD. Management focuses on monitoring symptoms and multisystem involvement. Recommended interventions aim to reduce morbidity, prevent complications, and improve quality of life. Careful management, from childhood to adulthood, involves the support of an interdisciplinary clinical team.

## ABSTRACT

### LYSOSOMAL ACID LIPASE DEFICIENCY: LIVER INSTRUMENTAL ASSESSMENT IN FIVE PATIENTS WITH CHOLESTEROL ESTER STORAGE DISEASE

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**Background:** Lysosomal acid lipase deficiency (LAL-D) is a rare autosomal recessive disorder related to LIPA gene variants, presenting with two clinical phenotypes: Wolman Disease (WD), which manifests early with severe presentation, and Cholesteryl Ester Storage Disease (CESD), with a milder and variable course mainly affecting lipid profile and liver function. Magnetic resonance imaging (MRI) and liver transient elastography (TE) are commonly employed to evaluate steatosis/fibrosis stage during follow-up, as alternatives to the more invasive liver biopsy, although there is no clear consensus on their use in LAL-D patients. We reported the results of MRI and TE assessment in patients with CESD.

**Methods:** We retrospectively analyzed data collected from 5 CESD (2 children and 3 adults) treated with ezetimibe (combined with a statin in two cases), including biochemical analysis, LAL enzyme activity, LIPA gene variants and liver steatosis/fibrosis assessments by MRI and TE. Abdominal MRI was carried out on a 1.5-T scanner with a 32-channel phased-array coil, including multiplanar T2w imaging; the steatosis protocol was based on liver signal intensity (SI) measured by T1-weighted in-phase and out-of-phase sequences, while fibrosis was evaluated calculating T1 native and apparent diffusion coefficient (APC). TE with liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) was performed using FibroScan®.

**Results:** Ezetimibe treatment led to LDL-C and ALT improvement in 4/5 CESD patients (LDL-C 19%, ALT 21.6% mean decreases) without progression of liver fibrosis in the mid- to long-term follow-up (3-19 years). During follow-up, TE stiffness in 2/5 patients showed improvement in fibrosis stage, while no progression was detected in 3/5 patients. At the last evaluation, stiffness by TE and APC by MRI were consistent with absent or mild fibrosis, while T1 native by RM indicated higher levels of fibrosis. We observed a marked discrepancy in steatosis evaluation: MRI indicated lower levels of steatosis (30–14–15–29–21.7%) compared to CAP results (367–313–261–400–355), which suggested severe steatosis.

**Conclusion:** Improvement or no progression of liver fibrosis, under lipid lowering treatment, was evidenced by MRI and TE, in mild forms of CESD. Steatosis results were conflicting between MRI and TE. Discrepancies between T1 native and APC by MR were also detected. Further studies are needed to clarify the role of MRI and TE in steatosis assessment in LAL-D. Better MRI protocols aimed at steatosis quantification (e.g. proton density fat fraction) as well as fibrosis staging (e.g. magnetic resonance elastography) may be implemented into clinical practice in order to allow for more precise assessment of liver disease severity.

### BEMPEDOIC ACID AND FENOFIBRATE ASSOCIATION: A CASE OF SEVERE HIGH-DENSITY LIPOPROTEIN CHOLESTEROL REDUCTION

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**Background:** Low high-density lipoprotein cholesterol (HDL-C) plasma concentrations is a strong independent predictor of coronary heart disease. Very low HDL-C levels, defined as <20 mg/dL, are uncommon and related to primary genetic disorder, as Tangier disease or LCAT deficiency, with mutations involving the main steps in HDL metabolism. Secondary forms of severe HDL-C deficiency are less well-described, frequently associated with hypertriglyceridemia, obesity, diabetes, male gender, sedentary lifestyle and cigarette smoking.

**Case Presentation:** Here we describe the case of a 55 years-old male patient affected by heterozygous familial hypercholesterolemia with history of previous multiple percutaneous coronary interventions, statin intolerance and obesity (BMI 31 kg/mq). In 2016, to improve the patient's low-density lipoprotein cholesterol (LDL-C) evolocumab was added to the back-bone lipid therapy with ezetimibe and fenofibrate. This lipid lowering therapy (LLT) maintained LDL-C between 70 and 100 mg/dl. In 2023, when bempedoic acid (BA) became available in own Country, we add this drug to existing LLT achieving LDL-C concentrations below 55 mg/dl. However, after the first six months of follow-up we recorded an unexpected significant HDL-C reduction (~50%). This trend, remained unchanged in the following six months, reach a nadir of 5 mg/dl (Apo A1 15.7 mg/dl). These very low HDL-C levels are similar to those observed in subjects with Tangier's disease, as well as the presence of numerous stomatocytes at the blood smear, indicating abnormalities in the composition of the erythrocyte membrane. We investigated and excluded possible secondary causes of stomatocytosis and/or severe HDL reduction as obstructive liver disease, paraproteinemia, diabetes, nephritic syndrome, alcohol abuse or HIV infection. Between the secondary causes, we also considered drugs interactions since it is known that interaction between fibrates and thiazide diuretics or PPAR $\gamma$  agonist is indicated as cause of severe and transient HDL-C reduction. Following this hypothesis, we discontinued fenofibrate recording a rapid and complete recovery in HDL-C and Apo A1 levels, as well as the disappearance of stomatocytes. These observations prompted us to a critical and retrospective analysis of our previously published studies, discovering three other cases of the same transient HDL-C reduction in patients with fenofibrate and BA co-administration (before BA 47 [43-49] mg/dl vs. on BA 19 [8-30] mg/dl). Even in these patients, enrolled in the CERTI (Costo Efficacia Regione Toscana Inibitori PCSK9) registry, the addition of BA was aimed at improving LDL-C levels despite concomitant LLT with PCSK9i and fenofibrate.

**Conclusion:** During the BA registration trial, no safety alert regarding the fenofibrate co-administration was reported, but it must be considered that only 3.6% of enrolled patient received it. The mechanism of physio-pathological interaction between fenofibrate ad BA is still unknown. Nowadays, we can only record evidence of a cross reaction between these drugs with a reversibility of HDL-C's reduction: in all patients fenofibrate discontinuation restore HDL concentrations. We believe it is important to investigate in this direction since such a severe HDL reduction can translate into a significant increase in cardiovascular risk as occurred in Tangier disease.

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**ABSTRACT**


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**WHOLE BLOOD HYPERCOAGULABLE PROFILE IN A PATIENT WITH MARKEDLY ELEVATED LIPOPROTEIN(a) PLASMA LEVELS AND THROMBOTIC COMPLICATIONS: A CASE REPORT**

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**Background:** Lipoprotein(a) [Lp(a)] is a well-established risk factor for atherosclerotic cardiovascular disease (ASCVD). Structurally, Lp(a) resembles an apoB-containing lipoprotein (i.e. Low-density Lipoprotein, LDL) contributing to endothelial dysfunction, plaque formation, and progression of atherosclerosis. Its distinctive component, apolipoprotein(a), is structurally homologous to plasminogen, but lacks fibrinolytic activity, thereby conferring on Lp(a) a potential prothrombotic effect through interference with endogenous fibrinolysis. The role of Lp(a) in thrombosis remains is only partially understood, and mechanistic data linking Lp(a) to coagulation and platelet function in vivo are still limited.

**Case report:** We present the case of a 64-year-old woman with arterial hypertension, type 2 diabetes complicated by chronic kidney disease. She was referred for evaluation of extensive ASCVD in the setting of markedly elevated plasma Lp(a) levels (925 nmol/L). Her history was characterized by recurrent major events despite maximally tolerated lipid-lowering (LLT) and antiplatelet therapy: NSTEMI in February 2024 treated with triple coronary artery bypass, followed six months later by acute ischemia of the left lower limb requiring revascularization. Bilateral moderate renal artery stenosis was also documented during her follow-up. Her best lipid profile achieved under LLT was LDL-C 102 mg/dL, triglycerides 154 mg/dL and apolipoprotein B 1.01 g/L, considering her intolerance to high-dose statins and the use of atorvastatin 20 mg plus ezetimibe. Other thrombophilic disorders were excluded. Lipoprotein apheresis was attempted but discontinued after a vasovagal syncope during the first session, upon the patient's request. Therapy was shifted to a PCSK9 inhibitor combined with aspirin 165 mg daily. The patient died one week later from acute myocardial infarction.

**Aim:** To explore whether, in the context of markedly elevated Lp(a), laboratory findings could indicate enhanced thrombin generation and suggest a contribution of Lp(a) to coagulation.

**Methods:** Venous blood was collected, processed to platelet-poor plasma, and stored at -80 °C. Platelet function was assessed by PFA-200, ROTEM®, Multiplate® impedance aggregometry, and light transmission aggregometry. Thrombin generation was measured by calibrated automated thrombography with and without thrombomodulin. Extracellular vesicles (EVs) were isolated and characterised by flow cytometry.

**Results:** ROTEM® showed hypercoagulability with reduced clot formation time (EXTEM 43 s; INTEM 48 s) and elevated maximum clot firmness (EXTEM 76 mm; INTEM 76 mm; FIBTEM 28 mm). Whole-blood thrombin generation revealed increased endogenous thrombin potential (ETP 2116.5 nM·min), elevated peak (260.5 nM), and reduced thrombomodulin-mediated inhibition (35.1% vs. 46.6% in controls). Platelet testing indicated modest ASA effect (ASPI 47 U), residual ADP-induced aggregation (68.8-81.1%), collagen response (50.2%), and reduced epinephrine response (36.8%). Platelet immunophenotyping showed nor-

mal expression of major receptors and preserved activation. PPP thrombin generation unexpectedly showed a hypocoagulable profile. EV analysis revealed generally reduced platelet-, endothelial-, angiogenesis-related, and inflammatory subpopulations. **Conclusion:** This case suggests that Lp(a) may contribute not only to atherosclerosis progression but also to haemostatic dysfunction via multiple prothrombotic mechanisms. Comprehensive haemostatic assays, including viscoelastic testing, platelet aggregometry, and thrombin generation, may provide insights for risk stratification in patients with high Lp(a) and recurrent events, supporting identification of those who could benefit from tailored antithrombotic strategies. Further prospective studies are warranted.

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**A CASE OF ATHEROSCLEROTIC RENOVASCULAR HYPERTENSION: WHEN CT ANGIOGRAPHY “WHEN CT ANGIOGRAPHY DOES NOT TELL THE TRUTH”**

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A patient (71 years old), former smoker, referred to our Hypertension Centre for arterial hypertension resistant to therapy (nebivolol/hydrochlorothiazide, spironolactone, amlodipine, torasemide). In her medical history: peripheral artery disease and arterial hypertension known for about three years and never perfectly controlled. For investigating a secondary hypertension, the following exams were performed: color Doppler ultrasound of the renal arteries, abdominal CT angiography and renalscintigraphy, which showed a non-hemodynamically significant stenosis of the right renal artery and reduction in the size of the ipsilateral kidney in absence of atheromatous lesions of the left renal artery and morphological and perfusion deficits of the left kidney. After optimizing the anti-hypertensive therapy, also including irbesartan 300 mg, 24h mean blood pressure (BP) values of 152/75 mmHg were obtained. Given the poor BP control and a slight increase in creatinine (from 1.10 to 1.40 mg/dL), arteriography was performed which showed the complete occlusion of the right renal artery and a critical stenosis of the left renal artery, not revealed on previous CT angiography. Bilateral angioplasty and stenting were performed, with polyuria in the following days and consensual lowering of BP, as in volume-dependent hypertension. Therefore, anti-hypertensive therapy was reduced, maintaining a good BP control (24h mean BP: 127/65 mmHg). At the one-month follow-up, the following were repeated: color Doppler ultrasound with patency of renal arteries, bilateral preserved Doppler spectra with slightly reduced PSV on right kidney than contralateral one (50 vs 40 cm/sec), and renal scintigraphy showing a GFR of 11 mL/min for the right kidney and 29.9 mL/min for the left kidney. Lab exams show stable renal function despite the procedure (creatinine 1.3 mg/dL). This clinical case highlights how CT angiography may not detect the real vascular damage and therefore benefits of revascularization, especially in the case of a doubtful clinical picture.

## ABSTRACT

**IN UNO OMNIA: (IT IS ONLY MATHEMATICS, BUT WE LIKE IT!)**

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**Background:** Familial hypercholesterolemia (FH) is a genetic disorder characterized by markedly elevated plasma LDL cholesterol (LDL-C) and a high-risk of premature cardiovascular (CV) events. The heterozygous form is relatively common, while the more severe variants, such as homozygosity or double/compound heterozygosity, lead to phenotypes refractory to conventional lipid-lowering therapies. Clinical management of these cases requires a personalized approach, including genetic testing, conventional and novel pharmacological options and in some cases even extracorporeal strategies.

**Case report:** A 77-year-old woman, non-smoker, with a strong family history of FH (brother and son) and premature myocardial infarction (brother with 2 AMIs at ages 27 and 38). She was diagnosed with Fredrikson type IIa Hypercholesterolemia at a young age. From 2005 to 2012 (lack of previous data), laboratory data consistently showed extremely high total cholesterol levels (over 500 mg/dl) and LDL-C 337-435 mg/dl despite multiple attempts based on treatment possibility during time (resins, high-dose statins, ezetimibe and combinations such as simvastatin 40mg + ezetimibe), all discontinued due to poor efficacy or referred statin intolerance (myalgias without CPK elevation). The first time we visited the patients, in 2012, she was 64yo and presented with tendon xanthomatosis and carotid atheromasia: clinical assessment yielded a Dutch Lipid Score of 21, diagnostic of definite FH. Given the failure of conventional therapy and the very-high CV risk, our patient was referred for apheresis treatment and underwent genetic testing (LIPIGEN = 2 LDLR gene variants, compound HeFH). In the following years, she took maximal LLT with Rosuvastatin 40 mg/day, ezetimibe 10 mg/day, LDL apheresis every 7-21 days and lomitapide 5-10mg (not tolerated) with only partial benefit. Even when she stopped Lomitapide and we added on therapy evolocumab 145 mg/mo (2014, fase II RCT), LDL-C levels remained above target (minimum 132 mg/dl) while, in the meantime, her clinical history was complicated by CV events: Left carotid TEA (2013), TAVI and coronary stenting in 2023, Right TEA (2024) with residual bilateral stenosis about 40%. Thus, today she is a compound HeFH in secondary CV prevention with extreme CV risk (LDL <40 mg/dL according to ESC/EAS update 2025). In 2025, evinacumab (15 mg/kg every 28 days) was introduced and added on ongoing maximal tolerated therapy, with five infusions performed in a day-hospital setting. The treatment was well tolerated, with no adverse events or relevant laboratory abnormalities. Serial lipid profiles showed a marked and sustained reduction in LDL-C, with levels consistently around 40 mg/dl (-69%), thereby achieving the targets recommended by the 2025 ESC/EAS guidelines update. After the 5th infusion, a home-based infusion protocol was successfully activated, managed by a dedicated medical-nursing team, with excellent adherence and patient satisfaction.

**Discussion:** This case illustrates the clinical evolution of a pa-

tient with genetically and clinically documented compound heterozygous FH, refractory to conventional therapies targeted to LDLR and only partially responsive to apheresis and lomitapide, poorly tolerated. The introduction of evinacumab (not acting on LDLR) enabled optimal lipid control and paved the way for innovative models of home-based management and better compliance to the complex poli-pharmacological treatment.

**EFFECTIVENESS IN REDUCING HOMOCYSTEINE LEVELS IN A GROUP OF PATIENTS: REAL-LIFE DATA**

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Hyperhomocysteinaemia (HHcy) is a pathological condition characterised by elevated plasma homocysteine (PH) levels (>10.1 µmol/L in females and >14 µmol/L in males – WHO). HHcy is implicated in the development of various pathological conditions, including cardiovascular and cerebrovascular diseases (CVD). A reduction in PH levels is desirable in order to lower the associated risk; however, there is limited data in the literature regarding the post-marketing effects of nutraceuticals on PH level reduction. We retrospectively evaluated the ability to reduce PH levels in a consecutive series of 80 patients (mean age 65.58±13.69; males 62.15±12.59; females 66.35±13.35) affected by HHcy, following six months of treatment with a nutraceutical compound [400 mcg (6S)-5-methyltetrahydrofolic acid, 3 mg pyridoxine, 5 mcg methylcobalamin, 2.4 mg riboflavin, 12.5 mg zinc, 250 mg betaine]. The table shows the characteristics of the patients and the changes observed after six months of therapy:

	All patients	Woman before treatment	Man before treatment	All patient after treatment	Woman after treatment	Man after treatment	All patient at goal # (%)
#	80	43	37				51 (63.75)
IMC (kg/m <sup>2</sup> )	26.07±69	25.12±11	27.17	27.47±44	25.89±17	28.11±12	
Kidney disease #	17	6	11				13 (56.52)
PH (%)	23.38±22.09	20.13±6.57	27.03±11.2	15.03±4 (-65.22)	12.8±2.1 (-60)	17.7±18 (-62.96)	

The use of combination therapy with the nutraceutical resulted in a very good reduction in homocysteine levels among the 80 patients with HHcy (-65.22%), with reductions observed in both females (-60%) and males (-62.96%). A total of 63.75% of treated patients achieved the therapeutic target set by the WHO. Patients with renal disease derived less benefit, with 56% reaching the therapeutic goal. None of the treated patients showed a significant increase in blood levels of vitamin B12 or folate. Although the number of treated patients was relatively small, it can be concluded that the treatment was effective in the majority of cases. However, in patients who did not achieve the therapeutic target, it may be necessary to increase the folic acid dosage to 800 mcg.

## ABSTRACT

## TREATMENT OF POLYVASCULAR ATHEROSCLEROSIS IN A PATIENT WITH ACQUIRED HEMOPHILIA A

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The management of atherosclerosis treatment in a patient with acquired Hemophilia A can be particularly complex, as the two conditions benefit from the use of medications with opposing effects. This is a rare bleeding disorder caused by the development of autoantibodies against factor VIII, leading to a prolonged activated partial thromboplastin time (aPTT) and, consequently, a greater susceptibility to bleeding events. Unfortunately, it is most often diagnosed in elderly patients, who frequently suffer from significant cardiovascular comorbidities. The standard therapeutic protocol, which includes high-dose corticosteroids, may significantly worsen pre-existing comorbidities, particularly blood pressure and glycemic control. Moreover, the bleeding nature of the disease severely limits the use of antiplatelet agents during the acute phase. Consider the case of our 81-year-old patient, with a history of hypertension and familial combined hyperlipidemia, who was already being treated with acetylsalicylic acid (ASA), which was subsequently discontinued upon hospital admission due to an acute bleeding event. The patient was admitted for severe posterior epistaxis and extensive subcutaneous hematoma. Coagulation tests revealed an isolated prolongation of activated partial thromboplastin time (aPTT), and the mixing test was non-corrective, suggesting the presence of a coagulation inhibitor. The diagnosis was subsequently confirmed by a factor VIII activity level of 3%, although without inhibitor titration. Immunosuppressive therapy was initiated with prednisone at a dose of 50 mg/day and rituximab at 3 mg/kg every 14 days, preceded by pre-medication with intravenous corticosteroids, until a plasma factor VIII level greater than 20% was achieved. At the same time, a Doppler ultrasound of the supra-aortic trunks revealed diffuse atherosclerosis, predominantly involving the carotid arteries, with bilateral bifurcation stenosis (60% on the right, 50% on the left), left subclavian artery stenosis (associated with a significant blood pressure difference between the two arms), and aorto-iliac involvement. Despite the severe atherosclerotic burden, the acute-phase bleeding coagulopathy prevented the use of antiplatelet agents for primary cardiovascular prevention, making clinical management particularly challenging. Antiplatelet therapy was introduced only later, once factor VIII levels exceeded 20%, thereby reducing the risk of acute bleeding. While awaiting optimization of antiplatelet therapy, the only therapeutic option to reduce cardiovascular risk was the introduction of high-dose statins (atorvastatin 40 mg), aiming to achieve an LDL target of less than 55 mg/dL, in accordance with the latest ESC guidelines for high cardiovascular risk patients. In conclusion, during the acute phase, lipid-lowering treatment takes precedence over antiplatelet therapy, which is temporarily contraindicated due to the risk of bleeding. Specifically, statins not only reduce lipid levels but also contribute to vascular remodeling and atherosclerotic plaque stabilization, making them the cornerstone of cardiovascular risk management in these complex patients.

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## IMPACT OF MEDITERRANEAN DIET ADHERENCE ON CAROTID INTIMA-MEDIA THICKNESS: EVIDENCE FROM VASCULAR ULTRASOUND ASSESSMENT

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**Background:** Coronary heart disease (CHD) is driven primarily by atherosclerosis, of which carotid intima-media thickness (CCA-IMT) is a validated marker. Although clinical trials suggest that the Mediterranean diet (assessed through the MEDAS score) can mitigate IMT progression, less is known about its effects in everyday populations. Traditional risk factors alone fail to explain all CCA-IMT variation, underscoring the importance of exploring nutritional influences.

**Methods:** Fifty-five participants underwent complete nutritional evaluation and carotid ultrasound assessment. Spearman's correlation was used to explore associations between IMT and MEDAS score. Individuals were categorized into two groups (IMT $\geq$ 0.9 mm vs. IMT<0.9 mm) to examine differences in diet, body composition, and laboratory markers.

**Results:** An inverse correlation was identified between IMT and MEDAS score ( $\rho=-0.88$ ,  $p<0.001$ ). Cardiovascular risk indicators (Total cholesterol/HDL ratio, LDL/HDL ratio, and Atherogenic Index of Plasma [AIP]) varied significantly between groups. Participants with lower IMT had significantly higher MEDAS scores ( $p=0.009$ ). Dietary differences included greater consumption of fresh cheese in the low-IMT group ( $p=0.029$  weekly;  $p=0.032$  monthly). A difference in left ventricular diastolic function was also observed ( $p=0.004$ ).

**Conclusions:** Adherence to the Mediterranean diet was strongly associated with more favorable carotid IMT values and an improved cardiovascular risk profile. These findings highlight the value of ultrasound as a non-invasive tool for detecting early atherosclerosis and reinforce the relevance of dietary strategies in cardiovascular prevention.

## ABSTRACT

## PERFORMANCE OF CARDIOVASCULAR RISK SCORES FOR DETECTING SUBCLINICAL CARDIOVASCULAR DAMAGE IN EARLY ARTHRITIS

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**Introduction:** Patients with rheumatoid arthritis (RA) and psoriatic arthritis (PsA) carry a higher cardiovascular (CV) risk than the general population, up to 30% of which could be attributable to disease-specific mechanisms. Early identification of subclinical CV involvement is crucial, but current risk calculators were developed for the general population and may not adequately capture inflammation-driven damage.

**Methods:** We enrolled 106 (age 46 [IQR 3] years, 76% females) newly diagnosed, treatment-naïve RA (n=66) and PsA (n=40) patients with no traditional CV risk factors, together with 43 matched healthy controls. All participants underwent carotid ultrasound for intima-media thickness (cIMT) evaluation and pulse wave velocity (PWV) estimation, and speckle-tracking echocardiography for global longitudinal strain (GLS). Cardiovascular risk was estimated by using SCORE2, Framingham Risk Score (FRS), Progetto Cuore, Reynolds Risk Score (RRS), and the Expanded Risk Score for RA (ERS-RA). Associations with subclinical CV damage were assessed through regression and ROC curve analyses.

**Results:** Compared with controls, patients with early arthritis showed significantly increased cIMT (p=0.011), higher PWV (p=0.047), and impaired GLS (p<0.001). Subclinical alterations were present in 53%, 43%, and 59% of patients for cIMT, PWV, and GLS, respectively, despite the absence of overt CV risk factors. Traditional scores showed modest associations, mainly with cIMT, while their predictive accuracy for PWV and GLS was limited. In contrast, ERS-RA demonstrated consistent associations across vascular and myocardial surrogates and superior discriminatory ability in ROC analyses, particularly for cIMT (AUC 0.770) and GLS (AUC 0.786). Finally, DAS28 was independently associated with both cIMT and GLS (p<0.001) and achieved good discriminatory ability in ROC analyses (AUC 0.689 for cIMT and 0.722 for GLS).

**Conclusions:** Subclinical cardiovascular injury is detectable at the very onset of RA and PsA, even in patients without traditional risk factors, and remains largely unrecognized by conventional risk calculators. ERS-RA performed better but requires recalibration to capture early disease-related risk. These findings em-

phasize the need for tailored strategies integrating vascular and myocardial markers to improve CV risk stratification in inflammatory arthritis. **Key words:** cardiovascular risk scores, arthritis, myocardial dysfunction, subclinical atherosclerosis, ERS-RA.

## PLEIOTROPIC EFFECTS OF ORAL ANTICOAGULANT THERAPY: IS THERE A DIFFERENCE BETWEEN VKAS AND DOACS?

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**Background:** Atrial fibrillation (AF) is one of the most common heart rhythm disorders encountered in clinical practice. Emerging evidence suggests a significant role of inflammation in the pathogenesis of AF, but certain questions still remain unanswered, in particular whether AF-related inflammation is a cause or a consequence of the arrhythmia, and whether inflammation reflects underlying disease or AF itself. At the current state of the art, scientific evidence on the role of oral anticoagulants (OAC) in modulating pro-inflammatory cytokines implicated in the pathogenesis of AF remains scarce. The aim of our study was to evaluate, in a population of AF patients undergoing OAC, the different roles of anticoagulant therapy [Vitamin K antagonists (VKAs) and direct oral anti-coagulants (DOACs)] in modulating the levels of inflammatory biomarkers in AF.

**Methods:** The Strat-AF study is an observational, prospective, single center, hospital-based study enrolling elderly patients with AF. Results refer to 170 subjects with complete clinical and biohumoral assessment.

**Results:** At multivariate logistic regression analysis, adjusted for several covariates, VKA treatment was an independent protective predictor for having a high grade of inflammation not balanced by anti-inflammatory cytokine levels [OR = 0.26 (0.10-0.69), p = 0.007].

**Conclusions:** These results from the Strat-AF study are "generators of hypotheses" and provide preliminary evidence for the differential effects of VKAs and DOACs on inflammatory biomarkers (e.g., IL-6, TNF- $\alpha$ ) in AF patients. These findings suggest that inflammatory biomarkers could enhance stroke risk prediction models, potentially improving a tailored AF management.

## ABSTRACT

## SUBCLINICAL ATHEROSCLEROSIS IN LOW-RISK INDIVIDUALS: TIME TO RETHINK CARDIOVASCULAR RISK ASSESSMENT

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**Background and Aim:** Current guidelines recommend cardiovascular (CV) risk estimation to identify individuals at elevated risk of atherosclerotic CV event, who may benefit from interventions to lower LDL-C. They also emphasize the need for more aggressive LDL-C reduction in higher-risk patients while low-risk patients could be undertreated due to underestimation of risk modifiers (i.e. subclinical ASCVD and many others). This study aimed to evaluate the possibility of identifying patients classified as low CV risk despite early signs of ASCVD.

**Materials and methods:** Patients aged 40 to 69 years without known CV disease, who underwent extracranial carotid Doppler ultrasonography, performed by the same experienced operator using the same machine and the same plaque evaluation criteria at the Pierangeli Clinic in Pescara between December 2022 and December 2024, were enrolled. Low, moderate, or high CV risk was estimated using SCORE2 according to ESC guidelines.

**Results:** A total of 850 participants (mean age 57.06±6.7 years; 50.4% men; 20.9% smokers) were enrolled. At baseline, mean systolic blood pressure (SBP) was 123.09±10.46 mmHg, total cholesterol (TC) 228.37±39.08 mg/dL, high-density lipoprotein cholesterol (HDL-C) 59.58±15.60 mg/dL, and non-HDL-C cholesterol (non-HDL-C) 168.79±37.69 mg/dL. Carotid atherosclerotic plaques were detected in 370 patients (43.53%). Based on SCORE2, 407 subjects (47.88%) were classified as low risk, 377 (44.35%) as moderate risk, and 66 (7.76%) as high risk. Significant differences between the three groups were found in mean age, SBP, non-HDL-C, percentage of carotid stenosis, with values progressively increasing from low-to-high-risk categories, and in mean HDL-C (all p<0.001). Analysis of the low-risk subgroup (mean age 54.32±5.68 years; 77% female; 7.9% smokers) showed the presence of atheromatous plaques in 30.5% of patients (61.29% with single plaque, 30.64% two plaques, 6.45% three and 1.61% four plaques), with a mean stenosis of 15.81±10.44% (range: 10-70%). Specifically, 67.7% of patients had 10% carotid stenosis, 15.3% had 20%, and 15.8% had ≥30%, reaching 60-70% in some cases. No significant differences were found between patients with and without carotid plaques in terms of TC, HDL-C, non-HDL-C. No significant associations were observed between the presence of plaques and either smoking status or gender. No significant differences were observed in mean levels of TC, HDL-C, or non-HDL-C stratifying by number of plaques and no association emerged with gender or smoking status. In a logistic regression model including age, SBP, TC, HDL-C, and non-HDL-C, only age (p=0.006, OR:1.060, CI 95%:1.017-1.105) and SBP (p=0.002, OR:1.038, CI 95%:1.014-1.062) were significantly associated with the presence of carotid atherosclerosis.

**Conclusions:** Among low-risk patients, defined by current guidelines as candidates for a less aggressive therapeutic approach (LDL-C target <116 mg/dL), nearly one-third already exhibit target-organ damage (carotid atheromatous disease).

The absence of significant differences in TC, HDL-C, non-HDL-C, between patients with and without carotid plaques supports the need for improved CV risk stratification tools beyond standard lipid profile.

## BEMPEDOIC ACID SIGNIFICANTLY REDUCES LDL - C AND TOTAL CHOLESTEROL IN REAL - WORLD CLINICAL PRACTICE

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**Background:** Bempedoic acid is a first-in-class ATP citrate lyase inhibitor approved for the management of hypercholesterolemia, particularly in patients intolerant or inadequately controlled with statins and ezetimibe. Despite its increasing clinical use, real-world Italian data remain limited.

**Methods:** We retrospectively analyzed 70 consecutive patients with hypercholesterolemia followed at our lipid clinic. Demographic, clinical, and therapeutic characteristics were collected, including genetic status and prior lipid-lowering therapies. Lipid parameters were assessed at baseline and after initiation of bempedoic acid. Paired Student's t tests were used to evaluate changes in lipid levels, with p<0.05 considered statistically significant.

**Results:** The cohort included 70 patients (mean age 59.5 ± 12.7 years, 57% women), of whom 42 received bempedoic acid. Baseline mean LDL-C was 131.8 mg/dl, and mean total cholesterol was 209.1 mg/dl. At follow-up, patients treated with bempedoic acid achieved a mean LDL-C of 74.8 mg/dl, corresponding to a mean absolute reduction of 54.3 mg/dl (p<0.01). Total cholesterol decreased from 209.1 to 150.5 mg/dl, with a mean absolute reduction of 55.0 mg/dl (p<0.01). The effect was consistent across both sexes and in subjects with genetically confirmed and polygenic hypercholesterolemia. No significant adverse events leading to treatment discontinuation were reported.

**Conclusions:** In this real-world Italian cohort, bempedoic acid was associated with a robust and statistically significant reduction in LDL-C and total cholesterol levels. These findings confirm its role as an effective therapeutic option in patients with hypercholesterolemia not adequately controlled with standard therapy and provide additional evidence supporting its integration into clinical practice.

## ABSTRACT

## EARLY EFFECT OF SUPPLEMENTATION WITH ESSENTIAL AMINO ACIDS ON CARDIAC PERFORMANCE IN ELDERLY PATIENTS WITH HEART FAILURE AND SARCOPENIA

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Heart failure (HF) is a prevalent and complex clinical syndrome, particularly in the elderly, often associated with comorbidities that negatively impact quality of life and prognosis. One of the most significant comorbidities is sarcopenia, defined as the progressive loss of skeletal muscle mass, strength, and function. Sarcopenia contributes to reduced physical capacity and worse clinical outcomes in HF patients. The link between HF and sarcopenia is driven by complex interactions, including chronic low-grade inflammation, oxidative stress, and a disrupted anabolic-catabolic protein balance. Treatments to prevent sarcopenia in HF patients are urgently needed. Previous research has indicated that supplementation with essential amino acids (EAAs) may improve muscle strength and mass in individuals with chronic conditions like HF. This study's objective was to evaluate the early effects of EAA supplementation on cardiac and muscular performance in elderly patients with chronic HF with reduced ejection fraction (HFrEF) and sarcopenia. This single-center, observational study included 60 elderly Caucasian outpatients (mean age 80.1±1.1 years, 47 men and 13 women) diagnosed with HFrEF and sarcopenia. All patients were already on optimal medical therapy for HFrEF at the maximum tolerated dose, with 100% of the cohort receiving sacubitril/valsartan (ARNI) and sodium-glucose co-transporter 2 inhibitors (SGLT2i). Patients were supplemented with 5.5g of a free-form EAAs mixture twice daily for six months. At baseline and follow-up, a complete physical exam was performed to measure anthropometric and hemodynamic parameters. Sarcopenia was evaluated using handgrip strength, gait speed, the SARC-F questionnaire, and the Short Physical Performance Battery (SPPB). Cardiac function was assessed via echocardiography, including left ventricular ejection fraction (LVEF) and Global Longitudinal Strain (GLS). The study also measured laboratory parameters, including biomarkers of oxidative stress (Nox-2, 8-Isoprostane) and platelet activation (sP-Selectin, Gp-VI), as well as metabolic markers like the HOMA index. After 6 months of supplementation with EAAs, we observed significant improvements in the parameters of sarcopenia, such as handgrip ( $p < 0.0001$ ) and gait speed ( $p < 0.0001$ ). In addition, there was a significant improvement in glycol-metabolic parameters, and in inflammatory index as high sensitivity C-reactive protein (hs-CRP). In accordance with these results, significant decreases were observed in circulating levels of oxidative stress biomarkers Nox-2 ( $p < 0.001$ ) and 8-Isoprostane ( $p < 0.001$ ), and platelet aggregation biomarkers such as sP-Selectin ( $p < 0.001$ ) and Gp-VI ( $p < 0.001$ ). Of particular interest, after 6 months' follow-up, there was a significant improvement in LVEF and global longitudinal strain (GLS). This study demonstrates that adding targeted nutritional intervention with EAAs to the standard therapy for HFrEF and sarcopenia significantly improves clinical symptoms, muscle strength, gait speed, and car-

diac function. These findings highlight that EAA supplementation is a viable therapeutic strategy to address the complex relationship between cardiac dysfunction and skeletal muscle wasting in elderly HF patients, offering hope for better functional outcomes and an improved quality of life.

## EVOLUTION OF NUTRACEUTICAL PRODUCTS: FROM MONACOLINE TO A NEW ALTERNATIVE FORMULATION AND EFFECTS ON LIPID LEVELS, LDL SIZE AND LDL-OXIDATION

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**Background:** LDL cholesterol (LDL-C) is a major cardiovascular risk factor, and guidelines recommend lifestyle modification for at least 6 months in low-moderate risk subjects before drug therapy. The latest updates emphasize that only nutraceuticals with proven efficacy and safety should be used. Safety concerns regarding red yeast rice (monacolin K) have led to stricter European regulations (EU 2022/860), limiting its content to <3% in commercial products, and a potential market withdrawal has been hypothesized. This scenario has driven the search for alternative multi-target nutraceutical formulations.

**Aim:** To evaluate the efficacy and safety of evolving nutraceutical combinations, comparing traditional monacolin K-based formulations with a novel composition devoid of red yeast rice.

**Methods:** Open-label studies were conducted in subjects with polygenic hypercholesterolemia or diet-resistant hypercholesterolemia, at low-moderate cardiovascular risk (SCORE <5%). The latest formulation (NUT4) includes two daily capsules with phytosterols (800 mg/day), EPA+DHA (206/104 mg), coenzyme Q10, vitamin E, and vitamin D3, initially combined with 2.6 mg monacolin K and subsequently tested without it. Primary endpoints were changes in TC, LDL-C, HDL-C, and triglycerides; secondary endpoints included hsCRP, LDL size, and LDL oxidation.

**Results:** Preliminary data showed significant reductions in TC (-10%) and LDL-C (-17%), with a modest HDL-C increase (+4%) and triglyceride reduction. Safety was generally good, with transient, asymptomatic CPK elevations in a minority of subjects. Compared with earlier formulations, the LDL-C reduction was slightly attenuated after monacolin K dose reduction, but favorable effects on LDL size and oxidizability were observed with NUT4 formulation with Monacolin K.

**Conclusions:** Nutraceutical combinations can improve lipid profile and LDL quality. Given safety concerns on red yeast rice, future strategies should focus on synergistic, multi-target formulations combining phytosterols, omega-3 fatty acids, and antioxidant compounds, with the potential to complement lifestyle changes and pharmacotherapy.

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**ABSTRACT**


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**IMPACT OF EVINACUMAB TREATMENT ON CORONARY PLAQUES IN PATIENTS WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: THE “EVOLVE-HOFH” STUDY**

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Homozygous Familial Hypercholesterolemia (HoFH) is an ultra-rare genetic disorder characterized by extremely elevated LDL cholesterol from birth, leading to early and aggressive atherosclerosis and premature cardiovascular events. Conventional lipid-lowering therapies provide limited benefit, as their mechanism relies largely on LDL receptor activity, which is severely impaired or absent in HoFH. Lipoprotein apheresis has long been the mainstay rescue therapy but is invasive and only partially effective. The advent of novel agents, such as Lomitapide, a microsomal triglyceride transfer protein inhibitor, and Evinacumab, a monoclonal antibody targeting ANGPTL3, has shifted the therapeutic landscape. Evinacumab lowers LDL-C through LDL receptor-independent pathways, making it relevant in HoFH. Clinical trials have demonstrated significant LDL-C reductions, but the effect of Evinacumab on coronary atherosclerosis remains poorly characterized. The EVOLVE-HoFH study was designed as a pragmatic, real-world, observational, multicenter, and international protocol to evaluate the impact of Evinacumab (15 mg/kg intravenous once per month) on coronary atherosclerotic plaques in HoFH patients. The trial is both retrospective and prospective. Eligible participants are adolescents and adults (≥12 years) with a clinical or genetic diagnosis of HoFH. Patients must be on a stable LLT dose for at least 30 days prior to enrollment, with an LDL-C level >140 mg/dL, indicating a high cardiovascular risk despite maximal LLT. The study compares patients initiating Evinacumab (intensified group) with those receiving conventional therapy (comparator group). The primary endpoint is the change in the percentage of non-calcified plaque volume (%NCPV), assessed by serial Coronary Computed Tomography Angiography (CCTA), between baseline and after at least 18 months of treatment. Secondary endpoints include changes in total plaque volume, calcified plaque volume, percent atheroma volume and high-risk plaque features. CCTA images are blinded and analyzed centrally to ensure reproducibility. Statistical analyses will compare plaque changes between groups using linear regression adjusted for key confounders such as baseline LDL-C and concomitant LLT. The rationale behind the study lies in the association between %NCPV and major adverse cardiovascular events. Demonstrating that Evinacumab stabilizes or regresses non-calcified plaque would provide evidence linking LDL-C lowering to direct coronary benefits. By employing imaging-based surrogate markers, EVOLVE-HoFH provides

a robust and ethical approach to assess efficacy, given the limitations of conducting large randomized outcome trials in ultra-rare diseases, where sample sizes are small and conventional clinical endpoints unfeasible. EVOLVE-HoFH represents a new methodological approach that bridges the gap between biochemical efficacy and structural cardiovascular outcomes in HoFH. The use of CCTA ensures reliable quantification even in modest sample sizes, a critical advantage in ultra-rare disease research. While the absence of randomization introduces potential confounding, careful comparator group selection and statistical adjustment aim to mitigate bias. A positive outcome would provide the first direct evidence for Evinacumab anti-atherosclerotic effects in HoFH, strengthen the rationale for its clinical use, and potentially introduce imaging-based endpoints as tools for treatment optimization, paving the way for precision, imaging-guided management strategies in high-risk, underserved populations.

**EFFECT OF INCLISIRAN ON LIPID AND MECHANICAL VASCULAR PROFILES IN FAMILIAL HYPERCHOLESTEROLEMIA SUBJECTS: RESULTS FROM A SINGLE LIPID CENTER REAL-WORLD EXPERIENCE**

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**Background and Aims:** Familial hypercholesterolemia (FH) is characterized by elevated LDL-C and an increased risk of premature cardiovascular events. Inclisiran is a small interfering RNA that inhibits hepatic PCSK9 synthesis and promotes LDL-C clearance by enhancing LDLR expression on hepatocytes. This study aimed to evaluate the efficacy of six-months add-on inclisiran on lipid profile and PWV in FH; furthermore, we investigated the association between LDL-C reduction and PWV variation. **Methods:** This prospective observational study involved 78 genetically confirmed FH subjects with an LDL-C off-target despite high-intensity statins plus ezetimibe. All subjects obtained biochemical analysis and PWV evaluation at baseline and after six months add-on inclisiran.

**Results:** After six months add-on inclisiran, 41% of subjects achieved LDL-C targets. Significant reductions of LDL-C (41.5%, p<0.001), ApoB (33.7%, p<0.01), Non-HDL-C (35.9%, p<0.001), and Lp(a) (18%, p<0.01) were observed, while PWV improved by 14.4% (p<0.001). In a secondary analysis, the Primary prevention group showed a higher prevalence of subjects on LDL-C target than the Secondary prevention group (59% vs 23.1%, p<0.001). Both groups exhibited significant improvements of lipid profile and PWV ( $\Delta$  -14.1%, p<0.01 and  $\Delta$  -14.6%, p<0.001, respectively). Linear regression showed a significant association between  $\Delta$ PWV and  $\Delta$ LDL-C in the whole study population as well as in the Primary and Secondary prevention groups (p for all <0.001). **Conclusion:** Inclisiran significantly improved lipid profile and PWV in FH subjects.  $\Delta$ PWV was significantly associated with  $\Delta$ LDL-C.

## ABSTRACT

**EFFECT OF LOMITAPIDE ON MAJOR ADVERSE CARDIOVASCULAR EVENTS IN HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: PROTOCOL OF THE LILITH STUDY**

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**Background:** Homozygous Familial Hypercholesterolemia (HoFH) is a rare genetic disorder characterized by extremely elevated levels of LDL cholesterol from birth, leading to premature and severe cardiovascular diseases. Lomitapide, a microsomal triglyceride transfer protein (MTP) inhibitor, is an effective treatment to reduce LDL cholesterol in HoFH patients. However, data on its impact on major adverse cardiovascular events (MACE) are limited, and randomized controlled trials are not feasible due to the rarity of the condition and ethical constraints. **Objectives and Methods:** The LILITH is a multicenter, observational study that compares the incidence of MACE in adult HoFH patients during the three years before and the first three years of lomitapide treatment. Collected clinical data include MACE, lipid profiles, liver function, safety outcomes, and concomitant therapies. The primary objective of the study is to compare the incidence of MACE before and after treatment. Secondary objectives include the incidence of 3-point MACE (cardiovascular death, nonfatal myocardial infarction, and ischemic stroke) and 4-point MACE (including coronary revascularization), as well as changes in lipid profiles and liver function tests. Exploratory objectives include the analysis of additional biomarkers (ApoB, Lp(a), hsCRP), hepatic steatosis, liver stiffness, and treatment adherence.

**Discussion:** The LILITH study employs a multicenter, observational design with an intra-patient comparison to evaluate the long-term impact of lomitapide on MACE in HoFH patients. This approach was chosen as a feasible alternative to randomized controlled trials, which are not practical due to the rarity of the disease. The intra-patient comparison controls for stable confounders such as genetics and comorbidities. However, the design has limitations, including the risk of time-dependent confounding due to changes in therapy or lifestyle, and potential biases in retrospective data collection, mitigated by a centralized adjudication of MACE events. The combination of retrospective and prospective data collection offers a balance between the ability to assess outcomes relatively quickly and the opportunity to collect more detailed and potentially higher-quality prospective data.

**Conclusions:** The LILITH study will provide crucial data on the effectiveness of lomitapide in reducing cardiovascular events in HoFH patients, offering valuable insights for the treatment and management of this rare and severe condition.

LILITH Study - Principal investigators:

<http://www.sisa.it/index.php?class=Comp&className=Content&op=Show&param=cid,1606,preview,0>

**REAL-WORLD EFFICACY OF INCLISIRAN IN VENETO REGION AT 15 MONTHS: THE INCLIVEN MULTICENTER REGISTRY**

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**Background:** Inclisiran is a long-acting small interfering RNA (siRNA) that prevents the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9), thus lowering low-density lipoprotein cholesterol (LDL-C). In randomized clinical trials, inclisiran demonstrated a reduction in LDL-C levels by approximately 50%. Only limited data regarding its use in real-world settings are available in the literature.

**Methods:** In this observational multicenter registry, we included 240 high and very high cardiovascular risk patients from 12 lipid clinics in the Veneto region of Italy who were unable to achieve LDL-C target despite a maximum tolerated lipid-lowering therapy (LLT) and started inclisiran therapy between October 2022 and February 2024. Primary endpoints were changes in LDL-C concentrations at 3, 9 and 15 months of follow-up after inclisiran administration. Secondary endpoints were percentage of patients reaching LDL-C targets, and efficacy of inclisiran according to background therapy and clinical characteristics of the population.

**Results:** Mean LDL-C levels at baseline were 119.5±50.2 mg/dl, then significantly fell to 55.5±39.7 mg/dl (p<0.001) at 3 months, to 59.8±37.6 mg/dl (p<0.001) at 9 months, and to 61.2±37.2 mg/dl (p<0.001) at 15 months. The percentage of patients achieving LDL-C targets according to ESC/EAS guidelines was 64.7% at 3 months, 60.1% at 9 months, and 56.6% at 15 months. At 15 months, we observed greater LDL-C reduction in male patients compared to female patients (57.9±36.5 vs 71.6±36.8 mg/dl; p=0.022), in patients with diabetes mellitus compared to those without diabetes (50.3±30.1 vs 65.0±38.4 mg/dl; p=0.015), and in patients on statin therapy compared to statin intolerant (52.8±29.8 vs 80.1±44.1 mg/dl; p<0.001). We did not observe significant differences according to BMI.

**Conclusion:** This real-world multicenter registry supports the role of inclisiran as an effective and well-tolerated option for managing hypercholesterolemia in high and very high cardiovascular risk patients.

## ABSTRACT

## LONG TERM EFFICACY AND SAFETY OF PCSK9 INHIBITORS IN A COHORT OF DYSLIPIDEMIC PATIENTS

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**Background and Aim:** This observational, ambispective, single-center cohort study aims to evaluate the efficacy and safety of PCSK9 inhibitors (alirocumab, evolocumab and inclisiran) in a real-life cohort of hypercholesterolemic patients followed at the Outpatient Clinic for Dyslipidemia (Varese, Como) in the period February 2016-July 2024. Primary endpoint: change in plasma LDL-c levels, secondary endpoints: changes in total cholesterol, HDL-c and TG values, change in liver cytolysis indices and muscle cytolysis indices and prevalence of adverse events.

**Methods:** Inclusion criteria: patients in primary or secondary prevention, aged less than or equal to 80 years, diagnosed with heterozygous familial hypercholesterolemia and LDL-c levels greater than or equal to 130 mg/dL who have not reached the therapeutic target with high-potency statin at the maximum tolerated dose in combination with ezetimibe for at least six months or with demonstrated intolerance to statins and/or ezetimibe. Each patient was assigned to following therapeutic protocols:

- Alirocumab 75 mg 1 subcutaneous injection/15 days.
- Alirocumab 150 mg 1 subcutaneous injection/15 days.
- Evolocumab 140 mg 1 subcutaneous injection/15 days.
- Inclisiran 284 mg 1 injection subcutaneously at the start of treatment, after 3 months and every 6 months thereafter.

Of the 90 patients recruited, 8 were assigned to alicumab 75 mg, 43 to alicumab 150 mg, 25 to evolocumab 140 mg and 14 to inclisiran 284 mg. For each patient demographic, clinical and laboratory data were collected at baseline before PCSK9i therapy and at 6 months and 12 months follow-up for alicumab and evolocumab, and at 1, 3 and 9 months follow-up for inclisiran therapy.

**Results:** With regard to the primary endpoint of the study PCSK9i therapy reduced LDL-c in all groups under study. Therapy with alicumab 75 mg and 150 mg led to a reduction of 56.5% and 61.9% respectively ( $p=0.016$ ;  $<0.001$ ). In the group treated with evolocumab 140 mg there was a reduction of 51.9% ( $p<0.001$ ). In the group treated with Inclisiran the reduction was 22% ( $p=0.004$ ). As regards total cholesterol levels in the group treated with alicumab 75 mg and 150 mg there was a reduction of 29.2% and 39.4% respectively ( $p=0.016$  and  $<0.001$ , respectively). In the group treated with evolocumab 140 mg there was a reduction of 32.4% ( $p<0.001$ ). In the Inclisiran group, the reduction was 17.6% ( $p=0.008$ ). A non-significant variations in plasma HDL-c and triglyceridemia were detected in the entire cohort treated with PCSK9i. No statistically significant changes in laboratory indices of the safety profile (hepatic and muscle cytolysis) during treatment were recorded. During the entire follow-up, two minor adverse events (2.2%) potentially related to PCSK9i therapy were detected: a skin reaction at the injection site and an episode of flu-like syndrome.

**Conclusion:** The results obtained with PCSK9i treatment in a real-life context are encouraging both in terms of clinical efficacy and safety in medium-long term treatment. Alirocumab, evo-

locumab and inclisiran can be considered a valid therapeutic choice for hypercholesterolemic patients with high LDL-c levels who fail to reach the lipid target and/or who are intolerant to the first lines of therapy.

## BEMPEDOIC ACID IN REAL-WORLD PRACTICE: LDL-C REDUCTION ACROSS GENETIC AND POLYGENIC HYPERCHOLESTEROLEMIA. A RETROSPECTIVE STUDY

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**Background:** Bempedoic acid, an ATP citrate lyase inhibitor, is indicated for patients with hypercholesterolemia who are statin-intolerant or inadequately controlled by standard therapy. Although randomized trials demonstrated efficacy, evidence from real-world genetically characterized cohorts remains limited.

**Methods:** We retrospectively analyzed a cohort of patients followed at our lipid clinic that underwent to genetic test and lipid profile assessment at baseline and follow-up. Paired Student's t tests evaluated intra-group changes; ANOVA compared genetic subgroups.

**Results:** The cohort included 70 patients (59.5±12.7 years, 57% women), of whom 42 received bempedoic acid. 64% had hypertension, 13% type 2 diabetes, 29% were current smokers, and 59% had hepatic steatosis. Family history of cardiovascular disease was reported in 84% of patients. Overall, the population can be classified as high to very-high risk according to ESC/EAS criteria. Genetic testing identified polygenic hypercholesterolemia in 44%, LDLR variants in 14%, APOB variants in 1%, negative testing in 14%; 21% had no available genetic data. Chronic kidney disease was present in 5.7% of patients, while 11.4% were in secondary prevention. Evidence of vascular organ damage was documented in nearly half of the cohort, with 34% showing overt atherosclerosis, 19% subclinical carotid IMT, and 7% polyvascular involvement. At baseline, mean LDL-C was 131.8 mg/dl and total cholesterol 209.1 mg/dl. At follow-up, LDL-C decreased to 74.8 mg/dl ( $\Delta=-54.3$  mg/dl,  $p<0.001$ ) and total cholesterol to 150.5 mg/dl ( $\Delta=-55.0$  mg/dl,  $p<0.001$ ). Reductions were significant in both monogenic (LDLR) and polygenic subgroups, with mean LDL-C percentage changes of -45.8% and -42.9%, respectively. ANOVA showed no significant differences in LDL-C reduction across subgroups ( $p=0.797$ ), suggesting consistent treatment effect irrespective of genetic status.

**Conclusions:** In this real-world Italian cohort, bempedoic acid achieved robust LDL-C and total cholesterol reductions across genetic subgroups. Beyond lipid lowering, these improvements may contribute to mitigating organ damage associated with dyslipidemia, including atherosclerosis, diabetes-related vascular injury, and chronic kidney disease. Our findings align with the 2025 ESC/EAS consensus, which emphasizes aggressive lipid lowering and early treatment intensification, and support bempedoic acid as a valuable tool in both primary and secondary cardiovascular prevention.

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