

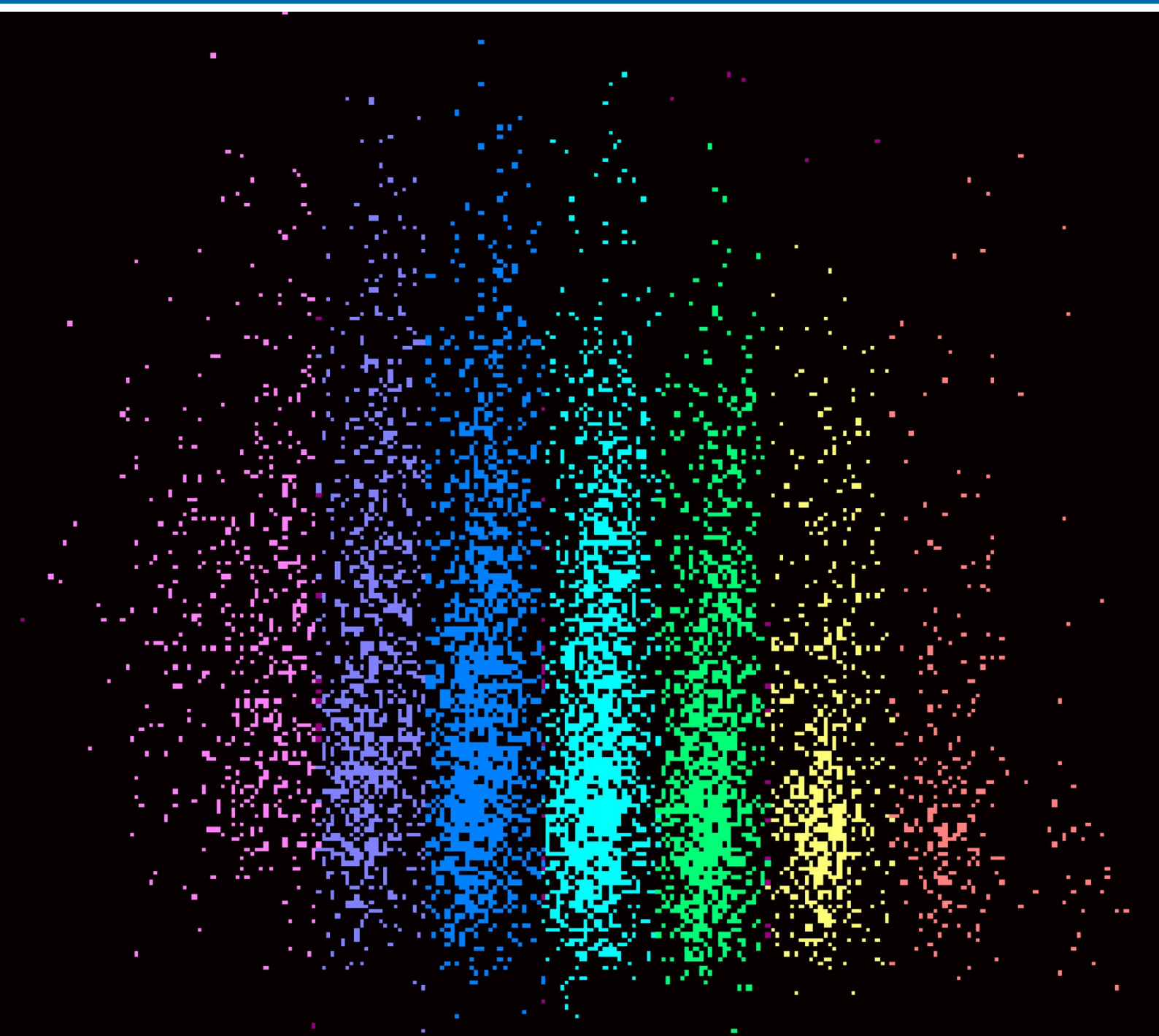
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# Lipoprotein(a) and cardiovascular risk: A roadmap for patient management

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## ABSTRACT

### Keywords

Lipoprotein(a);  
low-density lipoprotein;  
cholesterol;  
cardiovascular risk;  
atherosclerosis



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*Lipoprotein(a) [Lp(a)] is a plasma lipoprotein that shares structural elements with low-density lipoprotein (LDL), such as apolipoprotein B-100 (apoB), but differs by the presence of apolipoprotein(a) [apo(a)], which is covalently linked to apoB via a disulphide bond. In recent years, interest in Lp(a) has increased considerably, as epidemiological, genetic and biological evidence supports its causal role in cardiovascular disease. Its heterogeneous structural features, metabolic peculiarities, and ability to transport biologically active and potentially pro-atherogenic, pro-inflammatory and pro-thrombotic molecules make Lp(a) a unique lipoprotein among the apoB-containing lipoproteins. Lp(a) is now recognised as an important risk factor in cardiovascular risk assessment, as it plays a causal and independent role in the development of both atherosclerotic disease and aortic valve stenosis. Measuring Lp(a), together with other determinants of cardiovascular risk, is now recognised as essential for appropriate clinical management and the identification of new therapeutic targets. Consequently, the need to include Lp(a) in global cardiovascular risk assessment has clearly emerged, especially in individuals with a personal history of early or recurrent events, familial hypercholesterolaemia, family history of early events, or family history of high Lp(a) levels. This document, produced through the collaboration of the main Italian scientific societies in the field of cardiovascular disease management and laboratory medicine (SISA, SIC, ANMCO and SIBioC), analyses the pathogenetic role of lipoprotein(a) [Lp(a)] and the clinical significance of its measurement.*

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## Introduction

Lipoprotein(a) [Lp(a)] is a plasma lipoprotein that shares structural elements with low-density lipoprotein (LDL), such as apolipoprotein B-100 (apoB), but differs by the presence of apolipoprotein(a) [apo(a)], which is covalently linked to apoB via a disulphide bond [1]. The unique characteristics of Lp(a) confer both pro-atherogenic and pro-thrombotic properties, making it of particular clinical and scientific interest. Although its physiological function is not yet fully understood, it is hypothesised that Lp(a) may be involved in tissue repair, the transport of cholesterol and oxidised phospholipids, and the maintenance of vascular wall structural integrity [2, 3]. Plasma Lp(a) concentrations are associated, independently of LDL-cholesterol (LDL-C) levels and other traditional risk factors, with a significant increase in cardiovascular risk, particularly for atherosclerotic diseases such as early coronary artery disease, ischaemic stroke, and calcific aortic valve stenosis [4].

In recent years, interest in Lp(a) has grown considerably due to epidemiological, genetic, and biological evidence supporting its causal role in cardiovascular disease. Consequently, the need to include Lp(a) in the assessment of overall cardiovascular risk has emerged, especially in individuals with a personal history of early or recurrent events, familial hypercholesterolaemia, a family history of early events, or a family history of elevated Lp(a) levels.

This document, developed through the collaboration of leading national scientific societies (SISA, Italian Society for the Study of Atherosclerosis; SIC, Italian Society of Cardiology, ANMCO, National Association of Hospital Cardiologists; SIBioC, Italian Society of Clinical Biochemistry and Clinical Molecular Biology) addresses the role of Lp(a) in cardiovascular risk and discusses the available methods for Lp(a) assessment. It aims to provide operational guidance, supported by the most recent evidence and international guidelines, on essential aspects of clinical practice: the pathogenetic importance of Lp(a) in cardiovascular risk, the relevance of its measurement, and, above all, the correct methods for using laboratory data in risk stratification.

Through a critical and integrated analysis of current knowledge, this document provides concrete answers to fundamental questions for clinicians: when, where, and why to measure Lp(a), and how to interpret the values obtained appropriately, with the ultimate goal of improving the management of residual cardiovascular risk, which remains largely unmet in daily practice.

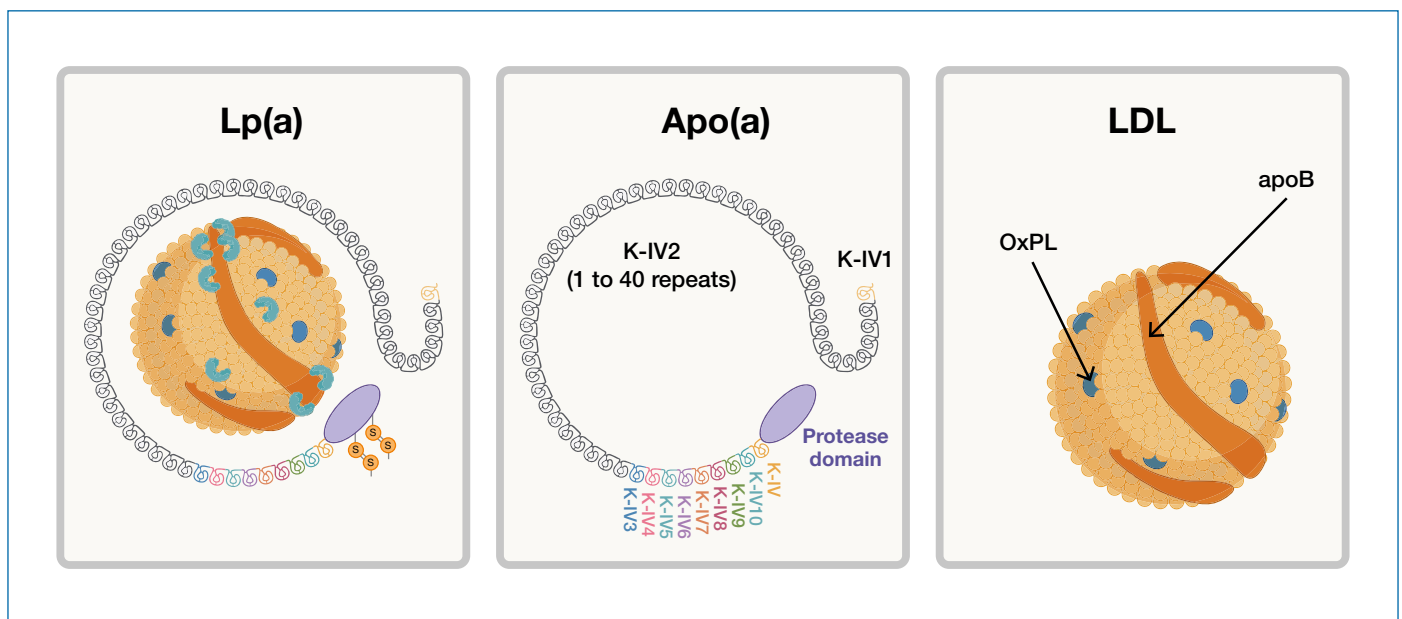
The synergy between scientific societies with different areas of expertise adds value, ensuring a comprehensive and multidisciplinary perspective, aimed at translating the best available science into practical tools for clinicians and laboratory professionals.

## Structure and metabolism of Lp(a)

Apo(a) consists of protein sequences called “kringles,” which are triple-looped structures stabilised by three internal disulphide bonds, also found in other proteins such as plasminogen. Specifically, apo(a) contains several kringle IV domains and a kringle V, followed by an inactive catalytic domain similar to a serine protease. Kringle IV domains are categorised into 10 subtypes (designated K-IV type 1 to type 10), with subtype IV-2 present in multiple copies, in a pattern that varies between individuals (**Figure 1**) [1]. Compared to plasminogen, apo(a) lacks the activation sequence and has an inactive protease domain [1]. Kringles exhibit highly specific structural properties that determine the variability of the structure and circulating levels of Lp(a).

K-IV type 2 (K-IV2) is present in the apo(a) structure in a variable number of identical copies, repeated from 2 to 40 times (**Figure 1**). The variability in the number of repeats confirms the polymorphism of the *LPA* gene that encodes apo(a) and explains the different sizes of the various apo(a) isoforms [5-7]. K-IV7 and K-IV8 contain binding sites at the lysine level, which are essential for the non-covalent interaction between apo(a) and apoB. This interaction is in addition to the disulphide bond between a cysteine present on K-IV9 and an unpaired cysteine at position 4326 of apoB [5-7] (**Figure 1**).

The kringle K-IV type 10 is structurally very similar to plasminogen K-IV and is characterised by the presence of a high-affinity lysine-bind-



**Figure 1 | Structure and main components of lipoprotein(a).** apo(a), apolipoprotein(a); apoB: apolipoprotein B; LDL, low-density lipoprotein; Lp(a), lipoprotein(a); OxPL, oxidised phospholipids.

ing site that plays a dominant role in the binding of apo(a) to fibrin and components of the vascular extracellular matrix [8, 9]. The lysine-binding site is also essential for binding to oxidised phospholipids [10] with pro-inflammatory activity [11]. Oxidised phospholipids are mainly present in the binding region between apo(a) and apoB.

At the transcriptional level, expression of the LPA gene is regulated by multiple factors. Among those that positively regulate gene transcription, the oestrogen receptor (ER- $\alpha$ ) activated by estradiol should be mentioned, as it recognises and binds to a 26-kb oestrogen-dependent enhancer region (ERE) present in the LPA gene promoter [12]. Among the negative regulatory factors, HNF1A [13], HNF3A (FOXA1), and GATA4 [14] have been identified. The farnesoid X receptor (FXR), implicated in bile metabolism, can also negatively regulate expression of the LPA gene by binding the DR-1 control element located in the -826 region of the LPA gene [15]. Factors involved in inflammatory mechanisms are also known to promote LPA expression, including interleukin 6 (IL-6), or inhibit it, such as tumour necrosis factor-alpha (TNF- $\alpha$ ) or transforming growth factor beta (TGF- $\beta$ ) [16].

Once the gene is transcribed and the protein is synthesised, apo(a) undergoes multiple post-translational modifications that regulate its secretion. N-glycosylation and protease inhibition occur during the passage of the lipoprotein containing apoB from the endoplasmic reticulum to the Golgi complex; calnexin acts as a chaperone for apo(a) in the endoplasmic reticulum and prevents its degradation. The lipidation processes of Lp(a) are common to those of all other apoB-containing lipoproteins.

During the passage from the endoplasmic reticulum to the Golgi apparatus, the structural variability of K-IV2 determines the likelihood of apo(a) proceeding through the assembly phases with the lipoprotein containing apoB and avoiding transfer to the proteasomal system [17]. A high number of K-IV2 repeats increases the molecular weight of apo(a) and its susceptibility to proteasome-mediated degradation, compared to a smaller number of repeats, which produces a protein with a lower molecular weight and less degradation [18, 19]. The regulatory mechanisms underlying apo(a) degradation have not yet been fully described, but they are crucial for understanding the impact of genetic determinism on plasma Lp(a) levels.

In circulation, like all other apoB-containing lipoproteins, Lp(a) can bind to the LDL receptor (LDLR) [20] as well as very-low-density lipoprotein receptor (VLDLR), LDL receptor-related protein 1 (LRP1), asialoglycoprotein receptor 1 (ASGR1), scavenger receptor class B type 1 (SR-B1), and the plasminogen receptor [21-24]. Furthermore, there is evidence that Lp(a) can bind to other receptors involved in inflammatory mechanisms, including Cluster of Differentiation 36 (CD36) and Toll-like receptors (TLRs) [23].

In kinetic studies using Lp(a) isolated from human donors and injected into various mouse models (which physiologically do not produce Lp(a)) knockout for LDLR or apoE, or treated with specific glycoproteins to saturate ASGR1, hepatic elimination of Lp(a) was comparable to that of wild-type models, suggesting a minimal contribution of these three pathways to the hepatic catabolism of Lp(a) [25]. In humans, studies on families with defective familial apoB or heterozygous familial hypercholesterolaemia (FH) have demonstrated that mutations in the APOB and LDLR genes can influence plasma Lp(a) concentrations [26].

Lp(a) has also been proposed as a non-competitive ligand with plasminogen for the plasminogen receptor (KT) (PlgRKT) [17, 19], which could partially explain additional catabolic pathways, independent of those of other apoB-containing lipoproteins. In any case, regardless of the receptor, the catabolic mechanism of Lp(a) and apo(a) once internalised in the liver is not yet completely clear.

## Pathophysiological role of Lp(a)

The physiological function of Lp(a) has not yet been fully clarified, but several hypotheses have been proposed [27]. It is believed that Lp(a) may contribute to tissue repair and wound healing due to its structure, which is similar to that of plasminogen. This similarity allows Lp(a) to interact with fibrin and facilitate tissue remodelling after injury [27]. Supporting this, a recent study has shown that high levels of Lp(a), associated with the presence of oxidised phospholipids, are also involved in the formation of keloids [2]. Like other lipoproteins, Lp(a) may also play a role in the transport of cholesterol and phospholipids, contributing to the repair and maintenance of blood vessel structure. Furthermore, Lp(a) may have a function in the immune system, aiding in defence against pathogens and regulating inflammatory responses [28, 29].

Beyond these potential physiological activities, Lp(a) is recognised as a significant and independent risk factor for cardiovascular diseases, including atherosclerosis and aortic valve stenosis [28, 30]. This relationship has been confirmed in epidemiological, genetic, Mendelian randomisation studies, and meta-analyses [30-32].

Due to the high homology of apo(a) with plasminogen [33-35], Lp(a) was hypothesised to play a role in platelet activation and atherothrombosis [36]. Early in vitro studies showed that Lp(a) could accelerate thrombosis and slow clot lysis [37] by inhibiting plasminogen activation [38]. However, subsequent observational and genetic studies have questioned the clinical relevance of these pro-thrombotic and anti-fibrinolytic properties [4, 39, 40]. Instead, a role for Lp(a) has emerged in the development of ischaemic cardiovascular disease and atherosclerosis. Lp(a) appears to have a greater propensity for oxidation, to transport oxidised phospholipids, and is more significantly removed through the scavenging action of macrophages compared to other apoB-containing lipoproteins [41]. It also plays a role in the inflammatory activation of monocytes that infiltrate atherosclerotic plaque [42, 43]. Finally, Lp(a) plays a crucial role in the formation and progression of calcified aortic valvular and supravalvular stenosis.

### *Pro-atherogenic mechanisms*

Lp(a) plays a crucial role in promoting atherosclerosis by transporting cholesterol esters and oxidised phospholipids. The latter are potent inflammatory molecules that stimulate endothelial cells to express adhesion molecules, such as VCAM-1 and selectins, facilitating leukocyte adhesion and migration into the vascular walls [43, 44]. The accumulation of these cells in the vascular system leads to the formation of vulnerable atherosclerotic plaques, increasing the risk of acute cardiovascular events [45]. The interaction of Lp(a) with macrophages also promotes the formation of foam cells: macrophages take up Lp(a) and release pro-inflammatory cytokines, amplifying local inflammation and lipid accumulation. This process contributes to the progression of atherosclerosis and destabilisation of plaques [4].

### *Pro-inflammatory properties*

The structure of apo(a) gives Lp(a) specific inflammatory properties (11). Despite structural similarities to plasminogen, Lp(a) can inhibit the normal clot dissolution process, generating a pro-inflammatory environment and promoting persistent vascular inflammation [46, 47]. The oxidised phospholipids carried by Lp(a) stimulate the production of reactive oxygen species (ROS) and activate cytokine-mediated inflammatory pathways, further contributing to endothelial dysfunction [46, 48].

### *Pro-thrombotic role*

Apo(a) appears to compete with plasminogen for binding to fibrin, interfering with the formation of plasmin and the process of fibrinolysis. This inhibition is thought to promote clot stability and increase the risk of thrombosis, especially in the presence of other cardiovascular risk factors [49]. Lp(a) also upregulates the expression of plasminogen activator inhibitor type 1 (PAI-1), which contributes to reduced clot degradation and promotes a pro-thrombotic environment [39].

The pathophysiological properties of Lp(a), including its pro-atherogenic, pro-inflammatory, and prothrombotic effects, make it a key risk factor for the development and progression of cardiovascular disease, a critical marker for cardiovascular risk stratification, and a potential target for future therapeutic strategies currently under development [4].

## **Role of Lp(a) as an independent, genetically determined causal risk factor for cardiovascular disease**

### *Epidemiological evidence*

Lp(a) is widely recognised as an independent, genetically determined cardiovascular risk factor. Epidemiological evidence shows that Lp(a) levels above 50 mg/dL (125 nmol/L) are associated with a significantly increased risk of cardiovascular events [31]. A meta-analysis of data from 24 cohort studies found that the rates of coronary heart disease in the upper and lower tertiles of Lp(a) were 5.6 (95% confidence interval [CI], 5.4–5.9) per 1,000 person-years and 4.4 (95% CI, 4.2–4.6) per 1,000 person-years, respectively. The adjusted hazard ratio for age and sex was 1.16 (95% CI, 1.11–1.22) for a 3.5-fold (i.e., 1 standard deviation) [50] increase in Lp(a) concentration. An increase in Lp(a) levels of 40 mg/dL (100 nmol/L) is associated with a 35% increased risk of cardiovascular events [4]. These data reinforce the importance of considering Lp(a) as a key risk factor in the assessment of cardiovascular risk.

Major international scientific societies, such as the European Atherosclerosis Society (EAS) and the American Heart Association (AHA), have recognised the threshold value of 50 mg/dL (125 nmol/L) for identifying high cardiovascular risk [4]. This value requires thorough re-evaluation in light of consolidated evidence indicating a continuous relationship between plasma concentrations of Lp(a) and the absolute risk of atherosclerotic cardiovascular disease (ASCVD). It is now clear that the cardiovascular risk associated with Lp(a) is not a threshold phenomenon, but rather a progressive gradient, in which each increase in plasma levels results in a proportional increase in risk, modulated by interaction with other traditional and non-traditional risk factors.

Although the adoption of cut-off values is useful in clinical practice to identify high-risk patients and facilitate operational decisions, it is important to emphasise that this approach does not fully capture the pathogenetic complexity of Lp(a). In particular, the use of discrete thresholds risks underestimating the overall impact of Lp(a) on cardiovascular risk when it acts in synergy with other determinants, such as hypercholesterolaemia, diabetes mellitus, arterial hypertension, chronic renal failure, and chronic inflammatory conditions.

Several studies, supported by genetic analyses and prospective meta-analyses, have shown that in individuals with Lp(a) levels above 180 mg/dL (450 nmol/L), the cumulative lifetime risk of cardiovascular events is comparable to that observed in subjects with heterozygous familial hypercholesterolaemia [51]. These data reinforce the

need to consider Lp(a) not only as an isolated risk factor, but also as a modulator and amplifier of overall risk, capable of significantly altering the individual risk profile.

In this context, there is an urgent need to move beyond a dichotomous view of the risk associated with Lp(a) and to promote the adoption of dynamic and integrated stratification models that take into account overall risk and the interaction between multiple determinants. This approach is consistent with the recommendations of the most recent European guidelines (ESC/EAS) [52, 53], which emphasise the importance of a holistic and continuous assessment of cardiovascular risk.

Promoting a more sophisticated integration of Lp(a) into risk stratification systems represents not only a significant scientific advance, but also improves the precision and effectiveness of preventive and therapeutic interventions, supporting the ultimate goal of increasingly personalised medicine based on actual risk exposure.

It is therefore important to measure Lp(a) levels as part of cardiovascular risk assessment, as elevated concentrations are associated with increased risk of atherosclerotic cardiovascular disease and calcific aortic valve stenosis (CAVS), independently of other lipid and metabolic risk factors.

Regarding CAVS, recent studies have shown that elevated Lp(a) levels predict a higher risk of CAVS and disease progression [54]. The increased risk of developing CAVS is related to the ability of Lp(a) to transport oxidised phospholipids, which contribute to valvular calcification and aortic valve inflammation [4]. In patients with elevated Lp(a), the risk of progression of valvular stenosis is higher than in those with normal levels, indicating Lp(a) as an independent predictor of CAVS and a potential target for future therapies.

### *Genetic variability of Lp(a) associated with cardiovascular risk*

Individual plasma Lp(a) concentrations are predominantly heritable. Approximately 90% of the variability in Lp(a) levels is determined by changes in the sequence of the gene encoding apo(a), and about 70% of the interindividual variability is explained by the number of KIV-2 repeats, with an inverse relationship between the size of the apo(a) isoform and plasma Lp(a) levels [4, 32, 55].

In addition to the genetic determinism dictated by KIV-2 copy number, several single-nucleotide polymorphisms (SNPs) have arisen over the course of evolution in the gene encoding apo(a), which significantly alter plasma Lp(a) levels depending on ethnicity [4, 56]. In the Dallas Heart Study, six SNPs in the gene locus encoding apo(a) (rs3798220, rs10455872, rs9457951, rs1801693, rs41272110, G+1/inKIV-8A) were sufficient to explain most of the variability in Lp(a) levels, regardless of the number of KIV-2 repeats [4, 57], with different distributions among white, black, and Hispanic individuals [57, 58].

Although some of these SNPs are in linkage disequilibrium with the copy number variant of KIV-2, SNPs have been identified that are independently associated with both high and low levels of Lp(a) [59]. Beyond the *LPA* gene locus, the *APOE*  $\epsilon$ 2 allele has been associated with lower levels of Lp(a), explaining approximately 0.5% of the variation in Lp(a) plasma levels. Recent GWAS studies have highlighted a relationship between Lp(a) levels and the *APOH36* gene, which encodes  $\beta$ 2-glycoprotein 1, is associated with PCSK9, and interacts with apo(a) KIV-2 [60].

### *Significance of Lp(a) levels as a cardiovascular risk factor*

All epidemiological and genetic studies agree that elevated Lp(a) levels increase the risk of atherosclerotic cardiovascular disease and aortic valve stenosis, similar to other apoB-containing lipoproteins [4, 39, 61–67]. However, increased plasma Lp(a) levels are more strongly associated with the risk of developing myocardial infarction than

with aortic valve stenosis or ischaemic stroke [31, 50, 68, 69]. Furthermore, gene variants that increase plasma Lp(a) levels result in a higher risk of major cardiovascular events, while gene variants that reduce plasma Lp(a) levels (such as certain forms of splicing, including 4733 G>A, KIV-2.2 -11G>A and 4925G>A, KIV-2.2 +0G>A), although less common in the population, provide significant protection and a reduced lifetime risk [70], further supporting the causality between Lp(a) levels and the pathophysiology of atherosclerotic disease.

Genetics also provides important information regarding the consideration and clinical use of Lp(a) as a risk factor. The relationship between genetically determined plasma Lp(a) levels and the risk of developing a first cardiovascular event is linear over a broad range between 50 and 300 mg/dL (125-700 nmol/L) in all primary prevention cohorts in which it has been evaluated [39, 62, 71, 72], while the association between the same levels and the risk of recurrence of a second event reaches a plateau between 150 and 300 mg/dL (125-700 nmol/L) [71].

These observations reinforce the need to consider Lp(a) levels at any level of cardiovascular risk, especially in patients already exposed to other risk factors [73], but they also highlight the importance of identifying a “threshold value”. Scientific societies have proposed a threshold value of 50 mg/dL (125 nmol/L) as indicative of an increased risk of cardiovascular events over a ten-year period [59, 65]. However, it is important to emphasise that this value, although useful in clinical practice to guide risk management, does not fully reflect the continuous nature of the risk associated with Lp(a) or the complex interaction between Lp(a) and other cardiovascular risk factors. Several studies show that risk increases progressively with rising Lp(a) levels, without a precise threshold, and that this risk is further amplified by the presence of other clinical determinants.

Therefore, measuring Lp(a) alone may not be sufficient for an accurate assessment of cardiovascular risk. It is more appropriate to adopt an integrated approach, combining the Lp(a) value with personal medical history and exposure to the main risk factors, as suggested by the most recent international guidelines [4].

## Measuring Lp(a): Methodological aspects

### *Pre-analytical phase*

**Sample collection:** Fasting is not required for Lp(a) measurement [74]. No significant changes in Lp(a) levels have been reported from 1 to 6 hours after meals [39, 75]. Lp(a) can be measured in plasma or serum, but levels of several lipids, including Lp(a), are lower in plasma than in serum. It is therefore recommended to pay attention to the biological matrix specified for Lp(a) measurement and not to use plasma and serum interchangeably.

**Centrifugation:** The whole blood sample should be centrifuged at 1500 g for 15 minutes at 4°C.

**Storage:** After centrifugation, if samples are not processed immediately, they can be stored at 2-8°C if analysed within 8 hours of collection, or at -70°C if analysed within 48 hours. Long-term storage, for periods exceeding 24 months, at -80°C or -20°C, can cause significant changes in Lp(a) levels, with an average decrease of 7% and 13%, respectively [39], especially for the smaller isoforms [76]. It is strongly recommended to thaw samples only once, as repeated thawing and freezing results in a significant decrease in Lp(a) concentration, with a greater impact on samples stored at -20°C than at -80°C.

### *Analytical phase*

The measurement of Lp(a) levels presents several critical issues, primarily due to the composition of Lp(a) itself. The highly dimensionally heterogeneous structure, the covalent association of apo(a)

with apoB, and the high homology between apo(a) and plasminogen pose challenges for the development of tests that allow accurate measurement of Lp(a).

The ideal test for determining Lp(a) should use an antibody specific for the analyte being measured, and the analyte in the sample should have the same structural characteristics as the analyte in the assay calibrator to ensure the same degree of immunoreactivity per particle.

The main aspects of measuring Lp(a) concern the sensitivity of the measurement systems to KIV-2 isoforms and the calibrators used by the assay. The use of antibodies directed against a repetitive motif of the apo(a) protein (isoform-sensitive) introduces measurement bias: serum concentrations of small isoforms with a small number of KIV-2 repeats, usually associated with high levels, are underestimated, while serum concentrations of large isoforms with a high number of KIV-2 repeats, usually associated with low levels, are overestimated. The relative bias can be considerable, with an overestimation of 25-35% in carriers of large isoforms, while for most carriers of small isoforms it is about 10%, which translates to less than 5-10 nmol/L. However, in both cases, there are exceptions where the absolute bias can be quite high. For example, a relative underestimation of Lp(a) of 30-35% has been observed for individuals with 15 and 16 KIV-2 repeats, which translates to an absolute underestimation of 35-45 nmol/L, potentially altering the risk classification of these patients.

One approach to avoid this bias involves the use of a non-commercial immunoassay developed by the Northwest Lipid Metabolism and Diabetes Research Laboratories (NLMDRL) in Seattle, which uses a specific antibody directed against a unique epitope in KIV type 9: each Lp(a) particle is recognised only once, and the ratio in nmol/L can be calculated without ambiguity. Therefore, this test is considered an established reference method [77].

Another approach that avoids the sensitivity of the test to Lp(a) isoforms uses an antibody directed against Lp(a)-apoB, since each Lp(a) molecule contains only one apoB molecule. This approach is applicable with an enzyme-linked immunosorbent assay (ELISA) in which an antibody directed against apo(a) is bound to the ELISA plate to capture the Lp(a) particle; for detection, a second antibody directed against apoB is used. Since Lp(a) contains only one apoB molecule, each Lp(a) particle is recognised only once, allowing a molar measurement of Lp(a). However, it is not possible to distinguish “free” apo(a) from apo(a) not bound to Lp(a) [78].

Another important aspect for the accuracy of Lp(a) determination is the calibrator used by different assays. If the calibrator has an incorrect value, all measured samples will be systematically overestimated or underestimated. In 1999, a primary reference standard (IFCC PRM1) was created [79], which became the basis for the secondary reference standard (WHO/IFCC SRM-2B) (80), consisting of a pool of sera from 17 donors and used for the calibration of tests by diagnostic companies. The Lp(a) concentration in SRM-2B was assigned with an ELISA method using a monoclonal antibody directed against a unique epitope located in KIV type 9, with no reactivity with the K-IV2 apo(a) region, making this method insensitive to variability in the size of apo(a). However, the impending depletion of supplies of this reference material prompted researchers to prepare an optimal and more widely available reference material using mass spectrometry [74, 81]. Currently, to calibrate different instruments, five independent standards with values ranging from low to high for the apo(a) isoforms are used, rather than serial dilutions of a single standard with elevated Lp(a) levels.

### *Lipoprotein(a) determination methods*

Lp(a) is measured primarily using immunochemical methods that detect the apo(a) component. As previously reported, a major

challenge in quantifying Lp(a) is the size polymorphism of apo(a).

The main methods for determining circulating Lp(a) levels and their characteristics are shown in **Table 1**.

Various ELISA tests for determining Lp(a) have been developed since 1985. However, the test with the best characteristics and performance appears to be the one developed by Marcovina et al. This isoform-independent ELISA test provides values in moles/L and is considered the gold standard, on which the SRM-2B reference standard was based [77].

The gold standard ELISA showed an excellent linear relationship with mass spectrometry (LC-MS/MS) in a series of 64 samples with well-characterised apo(a) isoforms, and excellent agreement with the value of the WHO/IFCC SRM-2B secondary reference material: 104.7±8.4 nmol/L compared to the assigned value of 107 nmol/L. The gold standard ELISA method is not commercially available.

Alternative methods to the gold standard ELISA are based on immunoturbidimetry and nephelometry.

Immunoturbidimetric methods use five-point calibration curves constructed with five different lyophilised standards, covering a range of apo(a) isoform values from low to high, and have demonstrated concordance with the gold standard ELISA [82].

Immunonephelometric tests use a five-point calibration curve generated from serial dilutions of a single Lp(a) standard, providing calibration values in mg/dL and nmol/L; this reduces concordance with the reference ELISA [83].

A recent study compared the performance of six commercially available tests (immunoturbidimetric and nephelometric) in 144 serum samples [84] against the previously validated reference immunoturbidimetric test. The tests used different calibrators and produced significantly different results across the clinically relevant concentration range.

Another study compared immunoturbidimetric methods based

on mass or molarity, demonstrating that these tests were interchangeable for measuring Lp(a) [85]. Latex immunoturbidimetric methods have also recently been described, providing rapid and automated results, potentially suitable for routine clinical assessment [86].

Another reference method is targeted liquid chromatography with tandem mass spectrometry (LC-MS/MS), which measures a peptide specific to the analyte of interest. This is the method of choice for biomarker standardisation, but it is not currently available in routine clinical laboratories.

In conclusion, all assay methods should express levels in nmol/L. The gold standard assay is an ELISA, which is not commercially available. Among available tests, immunoturbidimetric assays using calibration curves constructed with five different standards (covering low and high isoform values) give the best results. The extreme variability of results obtained with other commercially available methods requires further comparative studies with the gold standard and particular attention from clinicians regarding the method used in the population under study.

#### Post-analytical phase

In laboratory medicine, the post-analytical phase is extremely important as it represents the interface between the laboratory, the clinic, and the patient. It is essential to provide clinicians and patients with the tools to interpret laboratory data appropriately.

Therefore, defining reference ranges, threshold values, and biological variables that can influence the levels of a risk factor are fundamental steps for introducing it into clinical practice.

First, it is important to define the unit of measurement for Lp(a). Plasma Lp(a) concentration can be expressed in milligrams per decilitre (mg/dL), which indicates the mass of Lp(a) particles per volume, but ignores the high variability of Lp(a) mass, or in nanomoles per litre (nmol/L), which indicates the actual number of Lp(a)

**Table 1** | Main immunological methods for the determination of commercially available Lp(a) levels.

Kit Name (Manufacturer)	Single calibrator vs independent calibrators	Unit of measurement	Reference Material	Detection antibody
<b>Immunonephelometry test</b>				
Siemens N Latex	Serial dilutions of a single calibrator	10-100 (mg/dL)	Internal Standard	Rabbit polyclonal Ab
LPAX (Beckman)	5 Independent calibrators	2-128 (mg/dL)	Not specified	Rabbit polyclonal Ab
<b>Immunoturbidimetry test</b>				
Lp(a)-Latex SEIKEN (Denka Seiken)	5 Independent calibrators containing specific isoforms of apo(a)	nmol/L or 3-90 mg/dL	WHO/IFCC SRM-2B	Rabbit polyclonal Ab
TinaQuant Lipoprotein(a) Gen.2 (Roche) (Beckman)	5 Independent calibrators	7-240 nmol/L	WHO/IFCC SRM-2B	Rabbit polyclonal Ab
Lipoprotein(a) Assay (Randox)	5 Independent Calibrators Licence from Denka	nmol/L or 3-90 mg/dL	WHO/IFCC SRM-2B	Rabbit polyclonal Ab
Abbott Alinity c Lp(a) (Abbot)	5 Independent calibrators	3.1-90 mg/dL	In-house Reference Material	Rabbit polyclonal Ab
ADVIA Chemistry Lipoprotein(a) (Siemens)	5 Independent calibrators	10-85 mg/dL	In-house standards	Rabbit polyclonal Ab
Lipoprotein(a) (Mannheim Herb)	Serial dilutions of a single calibrator	6-260 nmol/L	Not specified	Rabbit polyclonal Ab
DiaSys 21 FS (DiaSys)	5 Independent calibrators	6-260 nmol/L	WHO/IFCC SRM-2B	Rabbit polyclonal Ab
Lipoprotein(a) assay (Diazyme)	5 Independent calibrators	5.4-100 mg/dL	Declaration of equivalence with Denka assay	Rabbit polyclonal Ab

particles per volume and is therefore the most appropriate unit of measurement. The conversion between these two units is nonlinear and varies depending on the composition of the particle. This discrepancy makes standardisation of levels complex and can lead to errors in interpretation if the units used are not taken into account. Therefore, it is recommended not to perform conversions between these units of measurement.

However, in clinical practice, to approximately convert mg/dL to nmol/L, a conversion factor of  $\times 2-2.5$  can be used, and to convert nmol/L to mg/dL, the value can be multiplied by 0.4 [87]. This is currently also accepted by the Consensus Statement of the European Atherosclerosis Society (EAS) [4].

Lp(a) values should be interpreted as follows:

- <75 nmol/L (< $\approx$ 30 mg/dL): low risk;
- 75–125 nmol/L ( $\approx$ 30–50 mg/dL): intermediate risk;
- >125 nmol/L ( $\approx$ 50 mg/dL): high risk.

Plasma Lp(a) concentration exhibits wide inter-individual variability in the general population, with values ranging from <1 mg/dL (2 nmol/L) to >1000 mg/dL (2000 nmol/L) [88], but low intra-individual variability. In particular, the levels observed in adulthood are reached by 2 years of age and remain stable throughout the lifespan in males, while in females they tend to increase after menopause.

Factors influencing circulating Lp(a) levels include genetics, ethnicity, kidney function, liver function, inflammatory status, and hormonal changes (**Figure 2**).

As genetics is not the only determinant of Lp(a) levels, genotyping is not entirely accurate for predicting plasma Lp(a) levels. Among non-genetic factors, different ethnic groups show significantly different Lp(a) levels, with lower concentrations in individuals from East Asia, Europe, and Southeast Asia, intermediate levels in those from South Asia, the Middle East, and Latin America, and higher levels in individuals from Africa [28, 89].

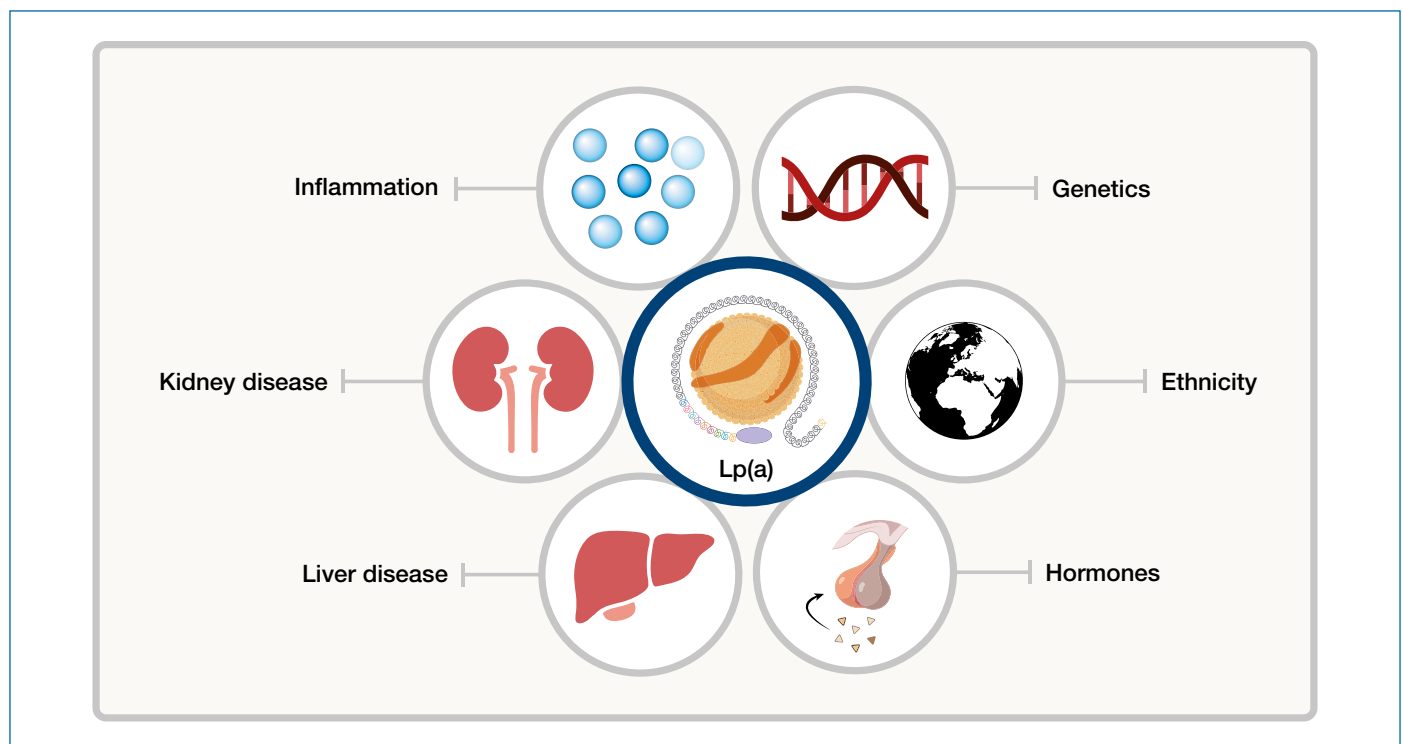
Conflicting evidence exists regarding the possible influence of age and sex on Lp(a) levels, with some studies showing higher levels in women than in men and in elderly individuals compared to younger people. However, this evidence has not been sufficiently confirmed. Some authors have suggested that diet, particularly a high intake of unsaturated fatty acids, may cause a negligible increase in Lp(a) levels, in contrast to the effect on LDL-C (90). The post-prandial state does not significantly affect Lp(a) levels.

An inverse association between circulating Lp(a) levels and renal function has been described. The kidney is involved in the catabolism of Lp(a); therefore, renal disorders, such as chronic kidney disease and nephrotic syndrome lead to increased Lp(a) levels [90]. Conversely, as Lp(a) is produced in the liver, liver damage, such as that caused by viral hepatitis, results in reduced levels.

Thyroid hormones, growth hormone, and sex hormones (oestrogen and testosterone), which are known to influence lipid metabolism, also affect Lp(a) levels. In particular, pregnancy, menopause, hypothyroidism, and low testosterone levels increase Lp(a) levels, while growth hormone strongly stimulate lipoprotein synthesis in both patients with acromegaly and those receiving replacement therapy [90].

An acute inflammatory state, such as sepsis, inflammatory bowel disease, or acute myocardial infarction, also increases Lp(a) levels due to the interaction of acute-phase reactants, such as IL-6, with binding sites in the *LPA* gene promoter [91].

It is important to note that the cholesterol present in Lp(a) can affect LDL-C measurement, especially in individuals with high Lp(a) levels. A portion of the cholesterol attributed to LDL may actually be contained in Lp(a), resulting in an overestimation of LDL-C. As an Lp(a) particle is composed of cholesterol for approximately 30% of its weight, individuals with high Lp(a) concentrations experience a significant overestimation of the LDL-C concentration. Therefore,



**Figure 2 | Factors influencing plasma Lp(a) levels.**

it is appropriate to correct LDL-C levels for Lp(a), particularly in groups such as those with high Lp(a) concentrations or those who do not respond adequately to therapy to reduce LDL-C levels [92].

Due to the lack of commercially available methods to directly measure the cholesterol content of Lp(a), the most common approach is to use the Dahlen formula, in which each individual is assigned an Lp(a) cholesterol value equivalent to 30% of the Lp(a) mass. The known LDL-C values (calculated using the Friedewald formula) are then adjusted by multiplying by the Lp(a) levels in mg/dL by a correction factor.

– *Dahlen formula:* ([LDLFriedewald] - 0.3× Lp(a) in mg/dL) [93]  
 Another formula for calculating Lp(a) levels is the Yeang formula, which uses the correction factor -0.173:

– *Yeang formula:* ([LDLFriedewald] - 0.173× Lp(a) in mg/dL) [94]

However, these formulas provide only a rough estimate of the contribution of Lp(a) to LDL-C and, therefore, their use is not recommended.

ApoB measurement has also been proposed as an indicator of all atherogenic lipoproteins, replacing LDL-C measurement [67]. Because there is a 1:1 molar relationship between apoB and each individual atherogenic lipoprotein, the relative contribution of Lp(a) to an individual's total atherogenic lipoprotein burden can be estimated by dividing apoB into Lp(a) and non-Lp(a) components. For example, apoB can be converted to molar concentration (nmol/L) using a conversion factor of 19.49; therefore, an apoB level of 100 mg/dL is equivalent to 1.949 nmol/L. By measuring Lp(a) in molar concentration, the ratio of apoB between the two lipoproteins can be determined.

However, some limitations remain: adjusting for apoB can explain almost all of the LDL-C-mediated risk but does not account for the Lp(a)-mediated risk, and measuring apoB can improve risk prediction in epidemiological studies, but it may be difficult to choose

targeted therapies without measuring all atherogenic lipoproteins.

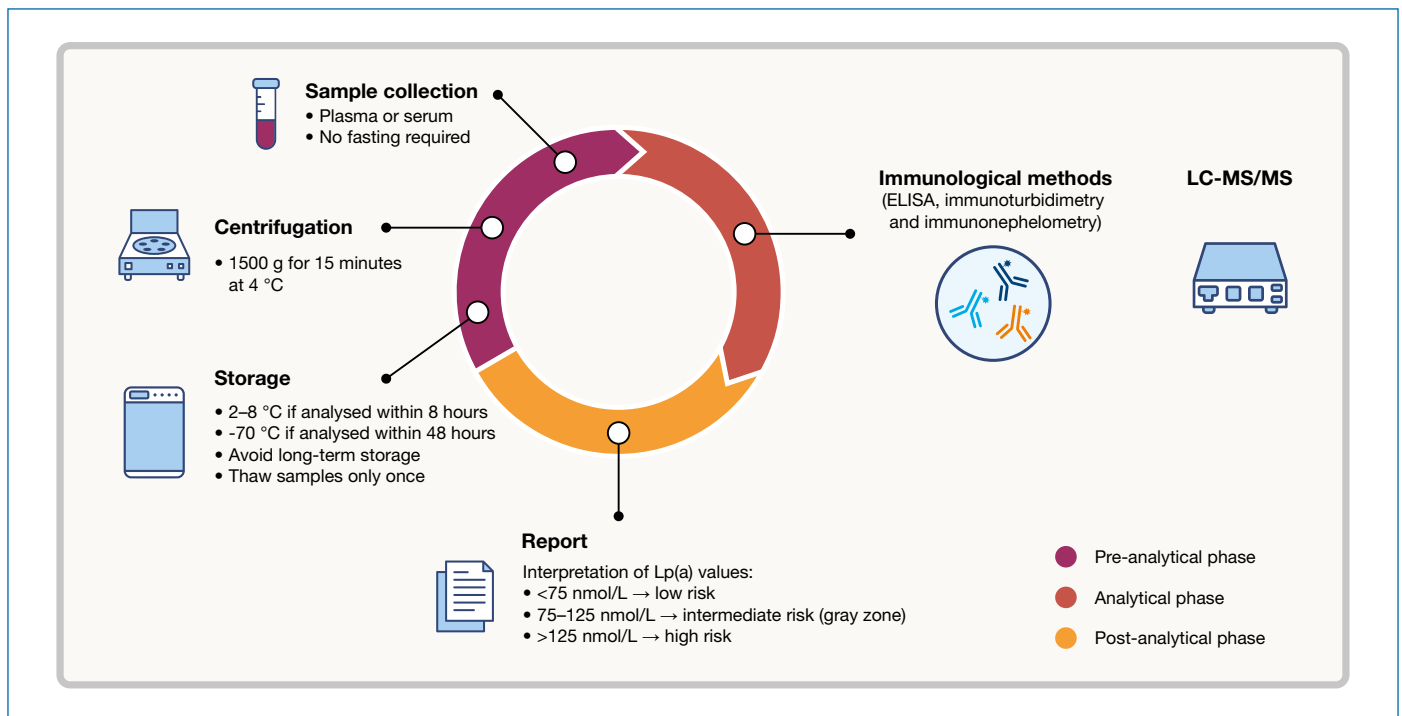
Accurate measurement of the cholesterol content of Lp(a) and its contribution to LDL-C has important implications for the risk assessment, diagnosis, and treatment of both atherosclerotic cardiovascular disease and familial hypercholesterolaemia, whether genetically confirmed or genetically negative [95]. **Figure 3** describes the steps in the analytical process.

### Clinical critical issues and practical indications for interpreting Lp(a) measurements in cardiovascular risk stratification

#### Cardiovascular risk stratification in relation to Lp(a) concentration

The relationship between Lp(a) concentration and cardiovascular risk is continuous; as Lp(a) levels increase, cardiovascular risk rises linearly. Interpreting Lp(a) values as a dichotomous variable is limiting; instead, it is appropriate to use an integrated approach that considers Lp(a) levels as a continuum, in the context of other cardiovascular risk factors such as LDL-C, hypertension, and diabetes [91]. According to this approach, the clinical decision-making process does not depend exclusively on the presence of an elevated Lp(a) value, but on the degree of increase in Lp(a) in association with other individual risk factors, thus allowing estimation of the contribution of Lp(a) to an individual's overall risk.

For example, an Lp(a) concentration of 150 mg/dL results in an almost threefold increase in cardiovascular risk, regardless of the individual's baseline risk defined by canonical risk factors [96]. In a subject with a baseline risk of 20%, an Lp(a) concentration of 150 mg/dL (300 nmol/L) leads to an estimated overall risk increase of almost 50%, while in a subject with a baseline risk of 5%, the same Lp(a) concentration results in a relatively small overall risk increase



**Figure 3 | Phases of the analytical process in the determination of Lp(a) levels and reporting.** Note that the pragmatic approach to Lp(a) level and risk must be integrated with the overall risk assessment.

(approximately 15%). Therefore, Lp(a) can be considered a risk “enhancer”.

Data on the association between Lp(a) levels and the risk of developing type 2 diabetes remain controversial. Lp(a) concentration does not appear to be associated with the risk of developing type 2 diabetes in individuals with prediabetes [97]. Post-hoc analyses of the ODYSSEY OUTCOMES trial have shown that baseline Lp(a) levels are inversely associated with the risk of developing diabetes, which, however, is not influenced by treatment with alirocumab [98].

#### *Monitoring Lp(a) levels*

Given the pathophysiological role of Lp(a), there is no category of subjects for whom its measurement is not recommended. Considering the cardiovascular risk associated with high levels of Lp(a) and their substantial stability in adulthood, guidelines recommend measuring Lp(a) at least once in each individual’s lifetime (52, 99), to identify subjects at high risk of events and optimise primary prevention strategies [100].

From this perspective, Lp(a) can also serve as a parameter for reclassifying patients at moderate cardiovascular risk [52]. Several studies have shown that adding Lp(a) improve the prediction of traditional risk scores such as the Framingham risk score, Reynolds risk score, or Pooled Cohort Equations [101-104].

However, there are certain categories of subjects in whom there is a significant probability of elevated Lp(a) levels, which should necessarily be included in risk assessment. Among these are patients with a premature cardiovascular event (before age 55 for men or 65 for women). Patients with extreme Lp(a) values ( $\geq 180$  mg/dL, 360 nmol/L) have a cardiovascular risk comparable to that of subjects with heterozygous familial hypercholesterolaemia [51], and cumulative exposure from a young age can result in a cardiovascular event in young subjects, even in the absence of other risk factors. Another group to consider is patients who experience recurrent events despite optimisation of classic cardiovascular risk factors [105].

Lp(a) is one of the risk factors that constitute the so-called “residual risk” in patients with atherosclerosis [106] and has been identified as an independent predictor of events, regardless of LDL-C levels [107-109]. Several studies have demonstrated a neutral effect or even an increase in Lp(a) levels with statin therapy [110, 111]. For this reason, in patients whose LDL-C values respond poorly to traditional lipid-lowering therapy, it is advisable to measure Lp(a), as it is possible that most of the LDL-C is incorporated into Lp(a) itself [112].

Finally, since Lp(a) is genetically determined, it is essential to perform cascade screening of first-degree relatives of patients with elevated Lp(a) levels [113].

### **Management of patients with elevated Lp(a) levels: Current pharmacological perspectives for reducing elevated Lp(a) levels**

#### *Current pharmacological perspectives for controlling Lp(a)*

Lp(a) is considered an important pharmacological target for substantially reducing cardiovascular risk. However, the most commonly used lipid-lowering therapies, statins and ezetimibe, do not significantly lower Lp(a) levels [114-116].

A reduction in Lp(a) levels has been reported with PCSK9 inhibitors, including the monoclonal antibodies evolocumab and alirocumab (20-30%) [117, 118], as well as inclisiran (PCSK9 gene silencing therapy, -18.6% and -25.6% in ORION-10 and ORION-11 trial, respectively) [119, 120]. In the FOURIER study, patients treated

with evolocumab who had Lp(a) levels  $>50$  mg/dL benefited from a greater reduction in the risk of major atherosclerotic cardiovascular events (MACE) compared to those with lower Lp(a) (-2.4% in the first group vs -1.4% in the second group) [117]. Similarly, in a sub-analysis of the ODYSSEY OUTCOMES study, the absolute reduction in the risk of MACE was 3.7% in patients with Lp(a)  $>60$  mg/dL compared to 0.5% in patients with Lp(a)  $<7$  mg/dL at baseline [121]. In this study, 25% of the observed reduction in cardiovascular events was attributed to the 20 mg/dL reduction in Lp(a) by alirocumab in patients with Lp(a)  $>60$  mg/dL at baseline [121]. This evidence confirms the need to evaluate Lp(a) as a pharmacological target.

The extent of Lp(a) reduction achieved with drugs that promote LDL-C catabolism is very similar to that observed with drugs that block the production of apoB-containing lipoproteins in the liver, including mipomersen (which inhibits apoB synthesis, reducing Lp(a) by 26%) [122] and lomitapide (which inhibits microsomal triglyceride transfer protein activity, stopping the assembly/lipidation of lipoproteins, and reduces Lp(a) by 17%) [123].

Evinacumab, which inhibits angiopoietin-like protein 3 (ANGPTL3) and thereby increases the lipolytic activity of endothelial lipases, promoting the catabolism of apoB-containing lipoproteins, is effective in reducing LDL-C levels in patients with HoFH, who do not express hepatic LDLR, but does not affect Lp(a) levels [124]. This suggests that forcing the catabolism of apoB-containing particles by an LDLR-independent mechanism does not increase Lp(a) catabolism. It is plausible that robust elimination of Lp(a) from the circulation could be achieved by exploiting catabolic pathways alternative to those of all apoB-containing lipoproteins, even when LDLR is deficient.

One clue in this regard may come from data showing that some emerging therapies for cardiovascular prevention can ensure a more robust reduction of Lp(a). For example, resmetirom, a selective agonist for the hepatic thyroid hormone receptor  $\beta$  and recently approved for the treatment of patients with chronic steatohepatitis and liver fibrosis, has demonstrated a reduction in Lp(a) of approximately 33%, compared with a more modest reduction in LDL-C (20%) [125].

The most potent inhibitors of cholesteryl ester transfer protein (CETP), involved in the exchange of cholesterol esters (CE) from apoB-containing particles to high-density lipoprotein (HDL) cholesterol, are also able to reduce Lp(a) levels. In addition to increasing HDL cholesterol (by an average of 104%) and reducing LDL-C (by an average of -41%), anacetrapib can reduce Lp(a) by -10 to -30% [126], while obicetrapib can reduce it by up to -56% [127]. The mechanism underlying this difference is unknown.

In summary, a reduction in Lp(a) levels of up to 25% can be achieved with approved LDL-C-lowering drugs. Mendelian randomisation studies suggest that a 22% reduction in cardiovascular risk (as observed, for example, following a  $\sim 40$  mg/dL or 1 nmol/L reduction in LDL-C) is achieved when Lp(a) levels are reduced by at least 100 mg/dL [73, 128]. This implies that only a large and robust reduction in Lp(a) would translate into an effective reduction in cardiovascular risk. To date, therefore, all pharmacological approaches developed specifically against Lp(a) must reduce this lipoprotein to a greater extent in order to achieve a benefit in reducing risk.

The approaches currently under development are based on antisense oligonucleotides (ASOs) (pelacarsen) and small interfering RNA (siRNAs) (olpasiran, lepodisiran, and zerlasiran), designed with GalNAc technology to selectively target the liver [114]. The mechanism of action aims to degrade apo(a) mRNA, thus reducing its hepatic synthesis. In clinical studies, these therapies are showing a reduction in Lp(a) of over 80%, sustained over time.

In 2026, the results of the Lp(a)HORIZON study for pelacarsen

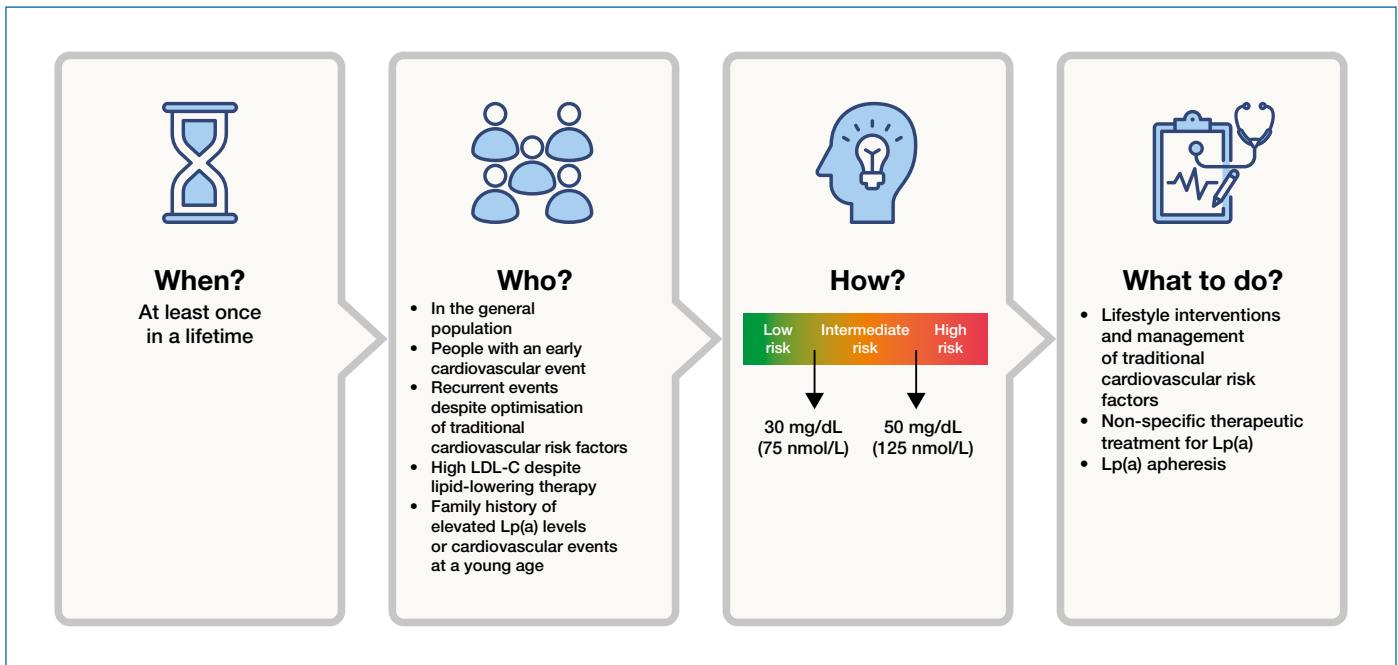


Figure 4 | Roadmap for the assessment of Lp(a) levels in clinical practice.

en (NCT04023552) [129] and the OCEAN(a) study for olpasiran (NCT05581303) on cardiovascular outcomes are expected. Both studies enrolled subjects at very high cardiovascular risk, but baseline Lp(a) levels differed (Lp(a) >70 mg/dL in the Lp(a)HORIZON study and Lp(a) ≥200 nmol/L in the OCEAN(a) study). A high intra-individual variability in serial Lp(a) measurements has been observed, suggesting the need for routine clinical assessment of Lp(a) to determine the reliability of single Lp(a) measurements.

Two additional approaches to target Lp(a) have been proposed:

- 1) an oral inhibitor of apo(a) assembly with apoB-containing lipoprotein (muvalaplin) [130], and
- 2) gene editing, either via CRISPR-Cas9 [131] or TALEN technology [132], both of which are currently under development.

#### Current management perspectives for patients with elevated Lp(a) levels

For patients at higher cardiovascular risk, with a history of recurrent cardiovascular events, even if all risk factors are already controlled, and with very high Lp(a) levels, Lp(a) plasmapheresis is currently the most effective available strategy, although it is a highly invasive procedure [4, 133, 134]. It should be emphasised that Lp(a) plasmapheresis has demonstrated significant efficacy in reducing cardiovascular events in treated patients [135, 136].

The first essential step in treating patients with elevated Lp(a) levels is to optimise lifestyle and all other cardiovascular risk factors, based on the goals recommended for the risk class determined by multiparametric assessment (Figure 4).

## Conclusions

Lp(a) is now recognised as a key risk factor in cardiovascular risk assessment, due to its causal and independent role in the development of both atherosclerotic disease and aortic valve stenosis. Its heterogeneous structural characteristics, metabolic specificities,

and ability to transport pro-atherogenic, pro-inflammatory, and pro-thrombotic components make it unique among apoB-containing particles.

Accurate measurement of Lp(a) remains challenging, mainly due to the variability of apo(a) isoforms, which can significantly influence test results. Standardisation of analytical methods and the use of molar units (nmol/L) are essential to effectively integrate this risk factor into clinical practice. It is also important to consider the contribution of Lp(a) when estimating LDL-C, particularly in patients with elevated Lp(a) levels, to avoid underestimating risk and to optimise therapeutic strategies.

Current guidelines recommend measuring Lp(a) at least once in a lifetime, highlighting its usefulness in stratifying and reclassifying cardiovascular risk, especially in cases of premature or recurrent events, or in patients with a suboptimal response to lipid-lowering therapy. Early identification of high-risk individuals is a crucial step towards personalised medicine, which also considers the residual risk that cannot be modified with conventional treatments.

Although currently available therapeutic options have a limited impact on Lp(a) levels, specific therapies are in advanced stages of development and promise significant reductions in plasma Lp(a). The expected results from large ongoing clinical trials will be crucial in clarifying the actual cardiovascular benefit of targeted Lp(a) reduction and could pave the way for new paradigms in secondary prevention.

In conclusion, Lp(a) is now not only an emerging risk factor, but a consolidated and recognised determinant of cardiovascular risk, whose integration into clinical assessment has become essential. Lp(a) measurement, if appropriately interpreted within a comprehensive approach to risk stratification, allows for more precise and personalised management of cardiovascular patients, improving the ability to identify individuals at high residual risk who cannot be fully identified with traditional factors alone.

In this process of clinical innovation, a fundamental role has been played by the relevant scientific societies, such as SISA, SIC, AN-

MCO, and SIBIOC. These societies, through careful analysis of the most up-to-date scientific evidence and a shared vision of real clinical needs, have helped promote a cultural and operational change that has led to the recognition of Lp(a) as a key element in modern cardiovascular prevention.

The resulting document therefore represents a milestone in this process, laying the foundation for the increasingly widespread dissemination of knowledge and clinical use of Lp(a), and for the development of future targeted therapeutic strategies, with a view to increasingly predictive, preventive, and personalised medicine.

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# Impact of Evinacumab on coronary plaques in patients with Homozygous Familial Hypercholesterolemia: Protocol of the “EVOLVE-HoFH” study (EVALUATION of atherOma pLaque Volume after Evinacumab in HOmozygous Familial Hypercholesterolemia)

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## ABSTRACT

### Keywords

Homozygous Familial Hypercholesterolemia; Evinacumab; Coronary Computed Tomography Angiography (CCTA); Atherosclerosis; Plaque Regression; Observational Study; Rare Disease



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**Background:** Homozygous Familial Hypercholesterolemia (HoFH) is a rare and severe genetic disorder characterized by markedly elevated low-density lipoprotein-cholesterol (LDL-C) levels from birth onwards, leading to accelerated and premature atherosclerotic cardiovascular disease (ASCVD). Evinacumab, a monoclonal antibody targeting angiopoietin-like protein 3 (ANGPTL3), has been shown to effectively reduce LDL-C in this population. However, its direct impact on coronary atherosclerotic plaque burden remains to be established. Given the rarity of the condition, randomized placebo-controlled clinical trials on major adverse cardiovascular events (MACE) are unfeasible.

**Aim and Methods:** This document describes the methodology of the EVOLVE-HoFH study, a real-world, observational, multicenter, international study (prospective and retrospective) designed to assess whether intensification of lipid-lowering therapy (LLT) with Evinacumab leads to regression or stabilization of the coronary atherosclerotic plaque burden as well as altered composition in patients with HoFH. The study will use Coronary Computed Tomography Angiography (CCTA), a validated surrogate risk marker, to compare changes in plaque volume in a group of patients initiating treatment with Evinacumab (“intensified treatment group”) with a “conventional treatment comparator group.” The primary endpoint is the difference in change in percent non-calcified plaque volume (%NCPV), a key indicator of plaque instability, between baseline and 18-24 months follow-up.

**Conclusion:** This pragmatic methodological approach is designed to overcome the barriers of research in rare diseases, allowing for the evaluation of a clinically relevant and mechanistically informative surrogate efficacy endpoint. The results will provide the first evidence of the beneficial impact of Evinacumab on coronary atherosclerosis, filling an important knowledge gap.

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## Introduction

Homozygous Familial Hypercholesterolemia (HoFH) is a rare, life-threatening genetic disorder caused by bi-allelic mutations that result in near-complete or complete loss of low-density lipoprotein receptor (LDLR) function. This elicits lifelong exposure to extremely elevated LDL cholesterol (LDL-C) levels from birth, often exceeding 13 mmol/L (500 mg/dL) [1]. As a result, patients develop widespread and severe atherosclerosis, placing them at exceptionally high risk of premature cardiovascular events such as myocardial infarction and sudden cardiac death, often already in the second or third decade of life [2, 3].

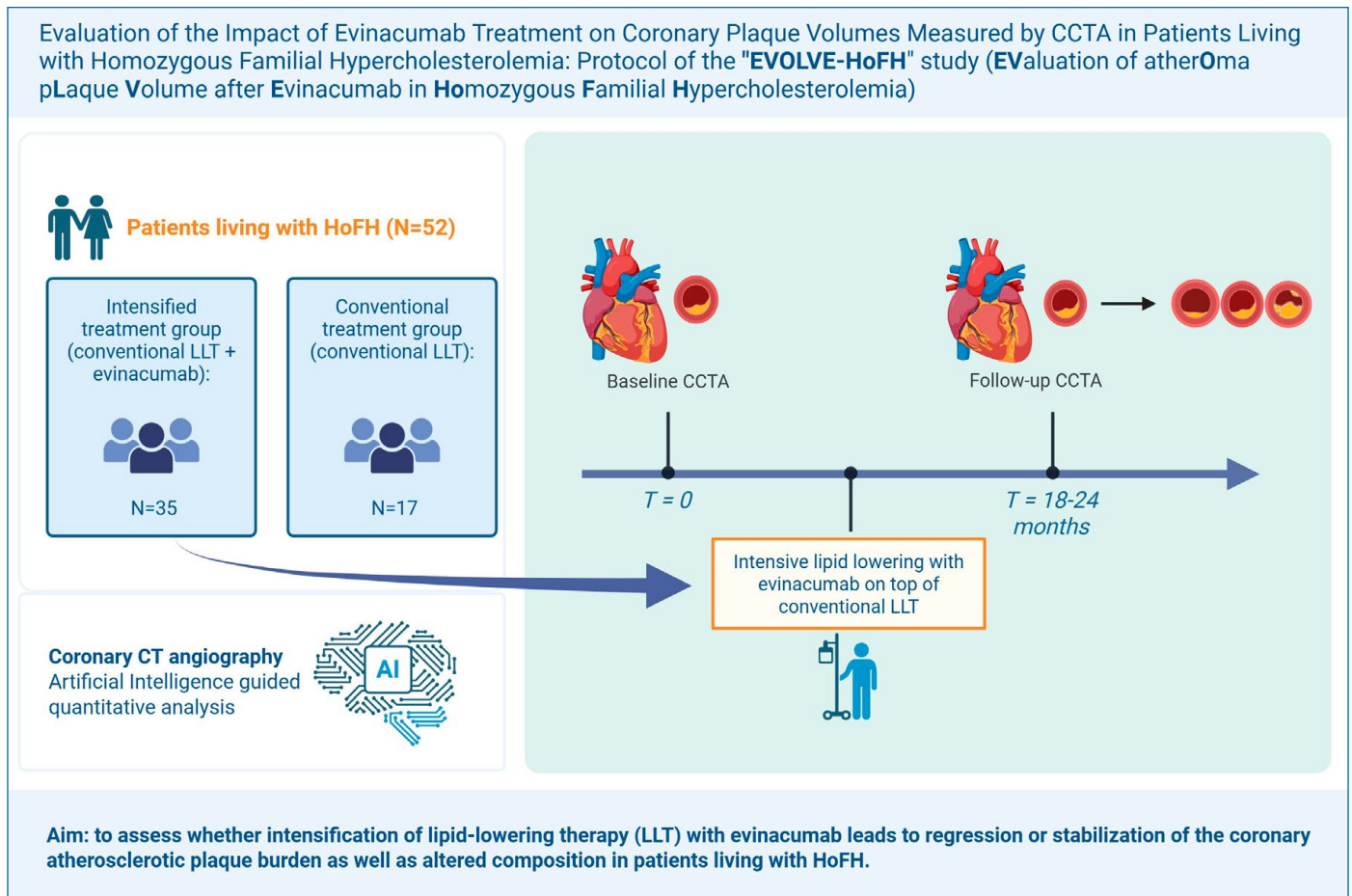
Managing HoFH presents a major clinical challenge. Conventional lipid-lowering therapies (LLTs), including statins and ezetimibe, act primarily by upregulating hepatic LDLR activity. However, in individuals with HoFH – where LDLR function is severely impaired or absent – these therapies typically yield only modest LDL-C reductions. As such, standard pharmacological approaches are often insufficient to achieve the substantial LDL-C lowering needed to reduce cardiovascular risk in this high-risk population [3].

For many years, the escape treatment for refractory elevated LDL-C levels in HoFH revolved around lipoprotein apheresis (LA); an invasive, extracorporeal procedure analogous to dialysis. It involves filtering the blood outside the body to remove LDL-C before returning the filtered blood to the patient. While LA is effective in reducing LDL-C, it is also associated with considerable drawbacks. The procedure is costly, requiring specialized equipment and trained

medical personnel. It is burdensome for patients, as it typically necessitates weekly or bi-weekly visits to a hospital or specialized clinic. Additionally, reliable venous access is crucial for the procedure, which can be challenging to maintain in some patients. The frequency and burden of LA significantly impact patients' quality of life, disrupting their daily routines, school, or work.

The therapeutic landscape for HoFH has evolved significantly with the introduction of novel lipid-lowering agents. Among these, Evinacumab has emerged as a very effective LLT. Evinacumab is a fully human monoclonal antibody that inhibits angiopoietin-like protein 3 (ANGPTL3), a key regulator of lipid metabolism [4-6]. ANGPTL3 inhibits lipoprotein lipase, an enzyme involved in the hydrolysis and clearance of triglyceride-rich lipoproteins [6, 7]. By inhibiting ANGPTL3, Evinacumab promotes lipoprotein clearance via LDL receptor-independent pathways; a relevant mechanism particularly in HoFH, where LDL receptor function is profoundly impaired or absent.

Clinical studies have demonstrated that Evinacumab can reduce LDL-C levels by approximately 50% in patients with HoFH, representing a substantial improvement compared to conventional LLTs [4-6]. These reductions can be expected to confer meaningful cardiovascular benefit. While the lipid-lowering efficacy of Evinacumab is well established, direct evidence of its impact on atherosclerotic disease progression remains to be established. Recent findings provide important mechanistic insights [8]. In this small retrospective imaging study, Evinacumab treatment in patients with HoFH appeared to be associated with reduced progression of coronary atherosclerotic



plaque, as assessed by serial coronary computed tomography angiography (CCTA).

In other patient populations with dyslipidemia, invasive intra-coronary imaging studies [9, 10] as well as non-invasive CCTA studies [11, 12] have substantiated that intensified LDL-C reduction by combining statin and PCSK9 inhibition translates into a greater regression of coronary atheroma volume coinciding with a more stable plaque phenotype. Extending this paradigm to HoFH is critical, particularly given the extreme lipid burden and the undefined optimal level of LDL-C reduction needed to effectively reduce the markedly elevated cardiovascular risk in this population. Although Schonck et al. [8] provided preliminary evidence suggesting that Evinacumab may attenuate progression of coronary atherosclerosis in HoFH patients attaining a time-weighted cumulative LDL-C levels lower than 3 mmol/L/year, validation in a larger prospective cohort, including control group, is warranted. Given the inherent challenges of conducting randomized placebo-controlled clinical trials powered for clinical outcomes in an ultra-rare disease, the use of validated surrogate endpoints such as CCTA is essential. CCTA offers a non-invasive, reproducible method to assess both total plaque burden and compositional features, thereby enabling robust evaluation of treatment effects. The EVOLVE-HoFH study was designed to build upon this initial evidence, aiming to further characterize the impact of Evinacumab on coronary plaque burden and phenotype in patients with HoFH using quantitative serial CCTA imaging.

## Methods

### *Study Design and Rationale*

EVOLVE-HoFH is a real-world, observational, multicenter, international, open-label study that combines retrospective and prospective data collection. The design is based on a comparison between an “intensified treatment group” (HoFH patients starting Evinacumab in clinical practice) and a “conventional treatment comparator group” (HoFH patients who, for reasons of access or personal choice, do not receive Evinacumab). This “real-world” approach leverages the natural variation in clinical practice across different countries, allowing for the collection of data on the drug’s effectiveness in a generalizable context.

### *Study Population*

Adolescent and adult patients (age  $\geq 12$  years) with a confirmed diagnosis (clinical or genetic) of HoFH (bi-allelic FH), according to the European Atherosclerosis Society (EAS) consensus panel criteria (1), will be enrolled. Patients must be on a stable baseline LLT for at least 30 days prior to the initiation of Evinacumab (intensified treatment group) or to a baseline CCTA (conventional treatment group), with an LDL-C level  $> 3.6$  mmol/L (140 mg/dL), indicating a high residual cardiovascular risk despite maximal LLT. The target sample size is set at approximately 52 patients (35 in the intensified treatment group and 17 in the conventional treatment group); a number considered adequate to detect a clinically significant signal in an imaging study on an ultra-rare disease.

### *Study Periods and Data Collection*

The study includes two key CCTA assessments: a baseline visit (visit 1) performed from 6 months prior to 1 month after the initiation of Evinacumab (for the treatment group) and a follow-up visit (visit 2) performed 18-24 months after the initiation of Evinacumab to assess changes over time. All clinical, laboratory, and imaging data will be collected via a centralized and secure electronic Case Report Form (eCRF).

### *Study Endpoints*

**Primary Endpoint:** The difference in the change in percent of non-calcified plaque volume (%NCPV) between baseline and follow-up, as assessed by CCTA, in HoFH patients receiving intensified lipid lowering therapy including Evinacumab versus those managed with conventional lipid-lowering therapy under routine clinical care. This endpoint was chosen because non-calcified plaque, rich in lipids and inflammatory cells, is considered the main determinant of plaque instability and cardiovascular event risk. %NCPV is sensitive to treatment-related changes in atherosclerotic burden and able to capture early plaque modifications.

**Secondary Endpoints:** These include difference in the change in total plaque volume (TPV, an indicator of overall atherosclerotic burden), calcified plaque volume (CPV, an increase of which could indicate a “healing” and stabilization process), and percent atheroma volume (PAV) between baseline and follow-up within each treatment group and between the intensified treatment group and the conventional lipid-lowering treatment group. Additional endpoints include changes in high-risk plaque features (e.g., low attenuation plaque, positive remodeling), in segment involvement score and plaque phenotype (from non-calcified to calcified) and in LDL-C levels between baseline and follow-up, both within and between treatment groups. This will provide a comprehensive evaluation of plaque morphology and composition and metabolic response.

### *CCTA Analysis and Centralization*

A methodological pillar of the study is the centralized analysis of all CCTA images. The use of validated Artificial Intelligence-based software to perform quantitative analysis of the CT-images (AI-QCT, Cleerly Inc.) provides an accurate and highly reproducible method allowing for absolute plaque quantification as well as evaluation of adverse plaque features (outward remodeling, low-attenuation plaque) [13, 14].

### *Statistical Analysis*

The primary analysis will use linear regression models to compare the change in the primary endpoint between the two groups, adjusting for potential confounding factors such as age, sex, baseline LDL-C levels, and the use of concomitant therapies (e.g., Lomitapide, LA). This statistical approach aims to isolate the specific effect of Evinacumab. Safety analyses will be descriptive. A detailed Statistical Analysis Plan (SAP) will be finalized before the database lock.

### *Ethical Considerations*

The study will adhere to the principles of the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines. Approval of the protocol by local Ethics Committees is a prerequisite for enrollment at each center. All participants must sign a written informed consent form after receiving a full and clear explanation of the objectives and procedures. Stringent measures will be implemented to protect patient data confidentiality and privacy.

## Discussion

The EVOLVE-HoFH study employs a pragmatic approach; a strategic choice necessary to balance scientific rigor with the limitations of clinical research in ultra-rare diseases. In the context of HoFH, where randomized controlled trials (RCTs) on major clinical endpoints (MACE) are ethically and logistically unrealistic, innovative methodologies like this are a necessity to advance knowledge. This study aims to bridge the critical gap between the

established biochemical efficacy of Evinacumab (LDL-C reduction) and the demonstration of a direct anatomical impact on coronary atherosclerotic plaques, as validated surrogate of cardiovascular event risk.

The strength of the present study is the shift from a biochemical endpoint to a quantitative assessment of the atherosclerotic burden. The use of CCTA, enhanced by artificial intelligence-based analysis software, allows for an objective assessment of both total plaque burden as well as the presence of adverse plaque features predisposing to a higher coronary event risk. First, Non-calcified plaque volume (NCPV) has been shown to closely associate with future risk of major adverse cardiovascular events [14]. A reduction in NCPV following therapy has therefore been proposed as valuable surrogate to estimate clinical benefits of cardiovascular interventions; a concept which is currently being validated in large prospective randomized studies (Transform trial, NCT03296813). The ability to demonstrate stabilization or regression of NCPV after intensifying LLT with Evinacumab will be of great interest to treating physicians. This aligns with the concept of imaging-based treatment optimization [15], which provides strong mechanistic evidence of the drug's anti-atherosclerotic effects. Second, enhanced assessment of coronary atherosclerosis combining additional measures of plaque composition on top of plaque volume, has been shown to provide a further refinement in MACE prediction [15]. Third, an endpoint in an ultra-rare disease such as HoFH requires optimal reproducibility in order to allow detection of benefit in a modestly-sized intervention group. Traditionally, the use of serial CCTA has been hampered by relatively poor reproducibility [16], since visual assessment is invariably affected by reader experience and interobserver variability. The introduction of AI-QCT has markedly improved reproducibility of plaque-burden evaluation and stenosis detection [17, 18], thus allowing relevant findings despite the use of relatively small patient groups.

Alongside these strengths, the design has inherent limitations that must be acknowledged. The absence of randomization introduces a risk of confounding and selection bias. It is plausible that physicians may be more inclined to prescribe an innovative treatment such as Evinacumab to patients perceived to be at higher risk (confounding by indication). Although attempts will be made to mitigate this risk through careful construction of a comparator group and the use of statistical adjustment analyses, the possibility of unmeasured residual confounders (e.g., differences in lifestyle, adherence to other therapies, genetic background) cannot be ruled out. Furthermore, the "real-world" nature of the study, while increasing its external validity and generalizability to daily clinical practice, also introduces potential heterogeneity in baseline therapies and standards of care among the various centers, making it more complex to isolate the net effect attributable to Evinacumab alone.

In summary, the EVOLVE-HoFH study has the potential to provide crucial and highly relevant clinical information. In the event of a positive result, the demonstration of a regression or stabilization of coronary plaque would represent tangible and convincing evidence of the drug's effectiveness in modifying the atherosclerotic process. This would not only strengthen the rationale for the use of Evinacumab in this population but could also pave the way for the introduction of imaging-based treatment, allowing for the personalization and optimization of long-term management of these high-risk patients. By integrating advanced imaging and a pragmatic trial design, EVOLVE-HoFH not only addresses a critical clinical gap but also sets the stage for a new era of precision care in ultra-rare cardiovascular diseases.

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The funding entity will not be involved in patient recruitment, data collection, monitoring, or data and results analysis.

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## The XIX National Congress of the Società Italiana di Terapia Clinica e Sperimentale (SITECS)

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### CONFERENCE REPORT



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The XIX National Congress of the Società Italiana di Terapia Clinica e Sperimentale (SITECS) was held in Milan on October 23-25, 2025, and, as in previous years, was organised in close collaboration with the Italian Society for the Study of Atherosclerosis (SISA), Lombardy Regional Section. In line with previous editions, the meeting brought together clinicians and researchers to discuss advances in atherosclerosis and cardiometabolic diseases, with a particular focus on translational research and innovative therapeutic strategies. In the context of the SISA Regional Meeting on October 23, 2025, the first session entitled “Beyond LDL: Ischemic cardiovascular disease”, was devoted to residual risk factors beyond low-density lipoprotein cholesterol (LDL-C). The session highlighted how inflammatory activation, thrombotic pathways and genetically determined lipoprotein abnormalities contribute to plaque destabilization and ischemic complications, and how these mechanisms are increasingly being framed as causal drivers rather than simple markers of cardiovascular risk.

In the first lecture, Giuseppe Danilo Norata discussed the role of inflammation in cardiometabolic diseases and atherosclerotic cardiovascular events, highlighting atherosclerosis as a chronic inflammatory condition driven by hypercholesterolaemia and apoB-containing lipoproteins that activate innate and adaptive immune responses in the arterial wall. He focused on high-sensitivity C-reactive protein (hsCRP) as a marker of vascular inflammation and residual inflammatory risk, noting its independent association with cardiovascular events even in patients receiving lipid-lowering therapy. The lecture addressed whether cardiovascular prevention should target LDL-C, inflammation, or both, reviewing evidence that lipid-lowering therapies variably reduce hsCRP and suggesting that combined lipid

and inflammatory modulation may offer added benefit in selected high-risk patients. Finally, Norata reviewed anti-inflammatory strategies targeting the IL-1 $\beta$ -IL-6-CRP axis and the NLRP3 inflammasome, presenting results from CANTOS, COLCOT, and LoDoCo2 and emphasized ongoing studies aimed at defining inflammation as a causal driver and therapeutic target in atherosclerotic disease.

Next, Marina Camera focused on the contribution of platelets and coagulation to cardiovascular events in patients with coronary artery disease. She highlighted that, despite optimal antiplatelet therapy, a substantial proportion of patients still experience adverse thrombotic events and that current clinical risk scores do not incorporate direct measures of platelet activation or thrombotic risk. Drawing on experimental and clinical data, she showed how platelets initiate and amplify atherothrombosis, not only by forming occlusive thrombi on disrupted plaques, but also by sustaining vascular inflammation and plaque growth through adhesion, secretion and expression of procoagulant molecules. She then discussed the search for soluble and platelet-associated biomarkers of platelet activation, with particular emphasis on tissue factor-positive platelet subsets, which in recent studies emerged as promising candidates to improve thrombotic risk stratification and may help guide more personalized antiplatelet strategies in high-risk patients. In the final talk of the morning session, Andrea Baragetti focused on lipoprotein(a) (Lp(a)) illustrating its distinctive structural and metabolic features and its strong genetic determination. He reviewed epidemiological and Mendelian randomization data showing a continuous, independent association between elevated Lp(a) levels and major cardiovascular outcomes, including myocardial infarction, stroke and aortic valve

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stenosis, and discussed how Lp(a) refines risk assessment beyond LDL-C and C-reactive protein, particularly in high-risk or genetically predisposed individuals. The lecture also covered current challenges in Lp(a) measurement, the concept of lifelong “Lp(a) burden” and the emerging pipeline of targeted Lp(a)-lowering therapies such as antisense oligonucleotides and small-interfering RNAs, which are expected to clarify whether selective Lp(a) reduction translates into additional cardiovascular benefit and to support earlier, more intensive LDL-C-lowering strategies in patients with markedly elevated Lp(a).

In the session dedicated to dyslipidaemias, Manuela Casula presented Lombardy data from the Italian LIPIGEN registry. The LIPIGEN (LIpid transPort disorder Italian GEnetic Network) was established in 2009 by the Italian Society for the Study of Atherosclerosis through its Foundation (Fondazione SISA) to promote and facilitate the clinical and genetic diagnosis of familial dyslipidaemias. She showed how regional enrolment contributes substantially to the overall cohort, providing detailed information on age at diagnosis, lipid profiles, genetic variants and treatment patterns in patients with suspected or confirmed familial hypercholesterolaemia. Emphasis was placed on the persistent diagnostic delay, the under-recognition of paediatric and young adult cases and the suboptimal achievement of LDL-C targets despite lipid-lowering therapy, underscoring the need for broader cascade screening and earlier, more intensive treatment strategies in high-risk families.

Maurizio Averna followed with an overview of current therapy for familial hypercholesterolaemia (FH) across the spectrum from heterozygous to homozygous forms and other severe LDL-C disorders. He summarised guideline-based treatment algorithms, stressing early initiation and lifelong intensification of lipid-lowering therapy using maximally tolerated statins, ezetimibe, PCSK9 inhibitors and bempedoic acid, and discussed the role of more advanced options, including lipoprotein apheresis and novel agents, in patients who remain far from LDL-C targets or present with particularly high cardiovascular risk.

Alberto Zambon then presented the recent SISA consensus document on hypertriglyceridaemias, focusing on their classification, clinical implications and management. He outlined the distinction between moderate and severe hypertriglyceridaemia, highlighting the dual concern of cardiovascular risk and acute pancreatitis, and stressed the importance of identifying secondary causes such as diabetes mellitus, obesity, alcohol intake excess and use of specific drugs. The talk summarised recommended therapeutic approaches, from lifestyle modification and optimisation of glycaemic control to the use of statins, fibrates, omega-3 fatty acids and newer agents, with an emphasis on tailoring treatment intensity to triglyceride levels and overall cardiovascular risk profile.

Next, Alberico Luigi Catapano turned to possible therapeutic approaches to reduce Lp(a) levels, framing elevated Lp(a) as a key contributor to residual cardiovascular risk in patients who are otherwise optimally treated for LDL-C. He briefly reviewed the limitations of currently available, non-specific strategies and focused on the development of targeted Lp(a)-lowering agents, in particular antisense oligonucleotides and small-interfering RNAs directed against apolipoprotein(a), which have shown profound and sustained reductions in Lp(a) levels in early-phase trials and are expected to clarify, through ongoing outcome studies, whether selective Lp(a) lowering translates into incremental cardiovascular benefit.

Chiara Pavanello then focused on lomitapide as a therapeutic option for patients with homozygous familial hypercholesterolaemia (HoFH). She described its mechanism of action as an inhibitor of microsomal triglyceride transfer protein, which reduces very-low-density lipoprotein assembly in the liver and thereby lowers

LDL-C through an LDL-receptor-independent pathway, making it particularly valuable in patients with minimal or absent LDL-receptor function. The presentation reviewed efficacy data showing substantial LDL-C reductions in HoFH, as well as key safety considerations such as hepatic steatosis and gastrointestinal adverse effects, and stressed the importance of careful patient selection, monitoring and combination with other lipid-lowering strategies in specialised centres.

Marcello Arca concluded the session with a focus on evinacumab as a treatment option for patients with HoFH. He described evinacumab as a fully human monoclonal antibody targeting angiopoietin-like protein 3 (ANGPTL3), capable of producing marked LDL-C reductions through mechanisms largely independent of LDL-receptor activity and therefore particularly effective in patients with HoFH due to LDL-receptor deficiency. The lecture summarised pivotal clinical trial data demonstrating substantial and sustained LDL-C lowering and a favourable safety profile and discussed how evinacumab can be integrated with other lipid-lowering therapies and apheresis in specialised centres to improve control of extreme LDL-C elevations and cardiovascular risk in these patients.

The second day of the conference, which was the first of the XIX National Congress of the SITECS, was opened with a session focused on proprotein convertase subtilisin/kexin type 9 (PCSK9). The opening lecture was held by Massimiliano Ruscica who has recapitulated current evidence about the main biological features and roles of PCSK9. Particularly, he has emphasized PCSK9 pivotal role in cholesterol metabolism through its control of LDLR degradation. Both genetic and pharmacological modulations of PCSK9 critically influence plasma LDL-C levels and cardiovascular (CVD) risk, establishing PCSK9 as a key biological and therapeutic target in dyslipidaemia management.

To this aim the use of anti-PCSK9 monoclonal antibodies, such as alirocumab and evolocumab, has been revealed effective in reducing circulating LDL-C levels and mitigating CVD risk as illustrated by Alessandro Maloberti. His lecture has reported an update of data obtained from clinical studies based on the application of such lipid-lowering therapies. Specifically, Alessandro Maloberti highlighted how anti-PCSK9 monoclonal antibodies can reduce the incidence of major adverse cardiovascular events (MACE) when used in appropriate patient populations, including heterozygous familial hypercholesterolemia (HeFH), atherosclerotic CVD (ASCVD) with elevated LDL-C and tolerating statin therapy, as well as in statin intolerant patients.

In addition to the use of anti-PCSK9 monoclonal antibodies, the application of small interfering RNA (siRNA) against PCSK9 has been revealed crucial to improve therapeutic adherence and long-lasting effect while reducing the injection frequency. This topic has been addressed by Gianluca Perseghin who showed some of the latest results from clinical studies about siRNA against PCSK9, namely inclisiran and solbinsiran. He has illustrated major advantages of siRNA compared to statins, as statins have to be administered to patients daily, compared to siRNA (one injection every six months), and are not supposed to be administered to statin-intolerant patients. One of the major challenges for siRNA is also to improve the long-term adherence to the treatment and cardiovascular outcomes.

A substantial resignation regarding novel drug therapies for the treatment of dyslipidaemias was thereby carried out by Alberto Corsini. Indeed, in recent years therapeutical options for the treatment of dyslipidaemias have been expanded well beyond statins. Accordingly, novel pharmacological tools are based on protein-targeting antibodies, RNA-based gene silencing, enzyme inhibitors, and emerging gene-editing technologies. While gene-editing based technologies targeting PCSK9 and ANGPTL3 are still under experimental investigation, the combination of classical lipid-lowering drugs (*i.e.* statins) with novel

pharmacological approaches, such as ezetimibe and PCSK9-based agents has allowed more precise, durable, and individualized lipid control, particularly for high-risk patients and those with inherited lipid disorders. The next frontier of therapeutic approaches for dyslipidaemias will aim at genetic risk profiling to guide therapy selection in the context of personalized medicine.

The session dedicated to PCSK9 was concluded by Marcello Arca who held a lecture on an update of the monitoring registries by AIFA (Agenzia Italiana del Farmaco). He covered updates related to real-world data collection on effectiveness, adherence, and treatment patterns for advanced lipid-lowering drugs, specifically focused on PCSK9-based agents. Accordingly, inclisiran has been recently integrated into AIFA monitoring. Moreover, AIFA has renewed the reimbursement terms for the usage of inclisiran with defined indications consistent with the registry criteria (*i.e.* primary and secondary prevention with specified LDL-C thresholds).

The following session of the SITECS congress was dedicated to risk factors involved in CVD, specifically to ASCVD. The main lecture of this session was conducted by Stefano Bellosta and followed by an open discussion about the lecture topic. He provided a detailed panel of experimental data obtained from *in vitro* evidence showing the adverse impact of cigarette smoke and new tobacco products on the cardiovascular health. Specifically, Stefano Bellosta showed the direct effects on atherosclerosis as smoke and new tobacco products were found to induce smooth muscle cell phenotypic alteration and migration, overall contributing to the formation of the atherosclerotic plaque.

The second part of the conference day was focused on a joint symposium between AMD (Associazione Medici Diabetologi), SID (Società Italiana di Diabetologia), SISA Lombardy and SITECS. This session was opened by the lecture of Matteo Conti, who focused his talk on the application of the novel drugs for the treatment of type 2 diabetes mellitus (T2DM) to prevent chronic kidney disease (CKD). Among this class of novel drugs sodium-glucose cotransporter 2 (SGLT2) inhibitors, glucagon-like peptide-1 receptor agonists (GLP-IRAs) and non-steroidal mineralocorticoid receptor antagonist (MRA) are included as they were recently approved to reduce CKD progression. Accordingly, Matteo Conti presented recently produced data from clinical trials based on the use of SGLT2 inhibitors, such as empagliflozin, dapagliflozin, and canagliflozin, which were established as first-line, disease-modifying therapies for diabetic kidney disease. Their reno-protective effects are mediated through reductions in intraglomerular pressure, albuminuria, and systemic blood pressure, and occur independently of glycaemic control. Moreover, the combination of empagliflozin with finerenone (MRA class) has shown to reduce renal and cardiac fibrosis, improving both cardiovascular and renal outcomes. On the other hand, among GLP-IRAs, semaglutide has gained approval specifically to reduce the risk of CKD progression, MACE, and albuminuria in patients with T2DM. Similarly, Conti illustrated that tirzepatide was found to exert cardio-renal protection in patients with T2DM by reducing the composite kidney endpoint (*i.e.*  $\geq 40\%$  decline in estimated glomerular filtration rate, renal death, progression to end-stage renal disease, or new-onset macroalbuminuria) following tirzepatide administration, as observed in the SURPASS-4 trial.

The effects of such novel drugs for the treatment of T2DM were further investigated in patients in CVD primary prevention, as illustrated by Cristina Mascadri. Her lecture provided real-world data from clinical trials based on the application of SGLT2 inhibitors and GLP-IRAs in patients in CVD primary prevention, highlighting the main cardiovascular outcomes. Specifically, Cristina Mascadri illustrated reduction of MACE and heart failure hospitalizations in patients following SGLT2 inhibitor-based therapy. Whereas GLP-IRAs

were found to reduce MACE, particularly the risk of stroke, heart attack, and death compared to other agents.

This session was closed by the lecture of Paolo Magni who has focused his talk on the interconnection between T2DM, triglycerides levels and CVD risk. Accordingly, Paolo Magni has shown a detailed and updated view of the existing link connecting metabolic disorders, such as T2DM and dyslipidaemias with increased CVD risk. Indeed, from epidemiological and genetic studies it has been demonstrated that hypertriglyceridemia and remnant cholesterol are independently associated with increased cardiovascular disease risk, particularly in individuals with T2DM with heightened baseline CVD risk. As a result, elevated triglycerides in T2DM represent both a marker and mediator of residual cardiovascular risk, even when low-density lipoprotein cholesterol is optimally controlled.

The third day of the conference, that is the second of the XIX National Congress of the SITECS, was opened by a session dedicated to current therapies for the treatment of dyslipidaemias. Alberico Luigi Catapano held a lecture on the update of the guidelines for the management of dyslipidaemias, released by the European Society of Cardiology (ESC) together with the European Atherosclerosis Society (EAS). The talk was focused on refining cardiovascular risk estimation, emphasizing earlier and more intensive LDL-C lowering, integrating newer therapies beyond statins, and highlighting risk modifiers like coronary imaging and Lp(a). The lecture was followed by an open discussion including experts like Francesco Bandera, Paolo Fabbrini, Aldo Pietro Maggioni.

This first session was closed by a lecture focused on the importance of omega-3 fatty acids in the prevention of dyslipidaemias, particularly hypertriglyceridemia. Indeed, their principal lipid-modifying effect rely on the reduction of plasma triglyceride levels (approximately 20-40% reduction), mainly through decreased hepatic very-low-density lipoprotein (VLDL) synthesis and enhanced clearance of triglyceride-rich lipoproteins, according to clinical studies illustrated by Aldo Pietro Maggioni.

The second session was dedicated on main therapeutic strategies to manage dyslipidaemias, including bempedoic acid and cholesteryl ester transfer protein (CETP) inhibitors. Particularly, Alberico Luigi Catapano held the lecture about the use of bempedoic acid, an oral lipid-lowering agent targeting LDL-C reduction by approximately 15–25% as monotherapy and providing additional LDL-C reduction when combined with statins or ezetimibe. As bempedoic acid is activated only in the liver and not in skeletal muscle, it is particularly useful in statin-intolerant patients. Moreover, clinical outcome data (CLEAR Outcomes) demonstrated a modest but significant reduction in cardiovascular events, supporting the role of bempedoic acid in primary and secondary cardiovascular prevention.

Ultimately, the session was closed by Laura Calabresi who provided an articulated lecture about CETP inhibitors, including mechanisms of action and latest updates about their effects and efficacy in preventing dyslipidaemias. Particularly, early agents failed due to safety concerns or lack of clinical benefit. However, newer compounds, such as obicetrapib, showed effective LDL-C lowering (*i.e.* by 30–45%) with improved safety profiles. Ongoing trials are evaluating whether these lipid changes translate into meaningful cardiovascular risk reduction. The talk was followed by an open discussion among experts attending the conference.

In the end, the conference was closed by the joint symposium between SISA Lombardia and SITECS, during which the 2025 “G. Galli” Award was presented to Clara Rossi, and young researchers, specifically Marco Bellomare, Alice Colombo, Elsa Franchi, Laura Gullà, Marta Iaia, and Arianna Moretti, were awarded for the best works presented at the conference.



## XIX SITeCS Congress 2025 Selected Abstracts

### Low density lipoprotein target achievement in very high and extreme cardiovascular risk patients during a cardiac rehabilitation program

**Marco Bellomare<sup>1</sup>, Boma Patricia Diri<sup>2</sup>, Chiara Tognola<sup>1</sup>, Atea Shkodra<sup>2</sup>, Michela Algeri<sup>1</sup>, Giulia Brioschi<sup>2</sup>, Marta Campana<sup>2</sup>, Marco Le Van<sup>2</sup>, Valentina Colombo<sup>2</sup>, Maria Catarinacci<sup>2</sup>, Filippo Brucato<sup>2</sup>, Cristina Giannattasio<sup>1,2</sup>, Alessandro Maloberti<sup>1,2</sup>**

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**Background:** Low-Density lipoprotein (LDL) cholesterol is one of the most relevant CardioVascular (CV) risk factors. Very low therapeutic targets have been set by guidelines in the secondary prevention setting in order to reduce the risk of ischemic event recurrence. However, many studies demonstrate that these targets are largely unreached in the real-life setting, particularly in the higher cardiovascular risk classes. Our aim was to evaluate LDL target achievement in very high and extreme CV risk patients during a Cardiac Rehabilitation (CR) program.

**Methods:** A total of 940 patients with recent acute coronary syndrome or a diagnosis of chronic coronary syndrome who participated in a CR program were enrolled between January 2012 and December 2023 at the Niguarda Hospital (Milan). For each patient, LDL and Lipid Lowering Therapies (LLT) were evaluated at the beginning and at the end of the CR program, together with anthropometric, clinical, biochemical, and instrumental parameters. LDL targets were considered <70 mg/dL for patients before August 2019, <55 mg/dL after 2019 and <40 mg/dL for extreme CV risk subjects.

**Results:** Mean age was 66.9±0.6 years, 82.9% of the subjects were males, and LDL cholesterol changes from 107.3±39.3 to 64.5±24.6

from the beginning to the end of CR. At CR discharge, 88% of the subjects were on high-intensity statin (atorvastatin or rosuvastatin) therapies, and 38.1% were on ezetimibe, while only 4.6% of the subjects were treated with PCSK9 inhibitors and 0.9% with bempedoic acid. 53.1% of the patients reached the LDL therapeutic target with particularly positive peaks in 2018 (72.8%, the year before the release of the latest dyslipidaemia guidelines that reduced the target) and 2022 and 2023 (78.8% and 75.7% respectively). 29.8% of the patients had extreme CV risk, they achieved the target of LDL <40 mg/dL only in 16.4%, with a higher prevalence in the latest years (32% in 2022 and 22.7% in 2023).

**Conclusions:** Our results demonstrate a higher achievement of LDL cholesterol target in a secondary prevention program when compared to previous observational studies. The longer distance from guidelines publication, together with the new pharmacological treatment, could be the reason for these positive results. However, more attention should be paid to extreme CV risk both in terms of identification and treatment.

## Integrated miRNomic and lipidomic analysis in mice for the identification of novel miRNAs involved in lipid metabolism

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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.

## Survivors of acute lymphoblastic leukemia show an increased cardio-immune-metabolic risk

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**Background and aims:** Acute lymphoblastic leukaemia (ALL) is the most common haematological cancer in children. In high-risk cases, treatment involves chemotherapy, radiation and stem cell transplantation. Despite high survival rates (up to 90%), increased cardiovascular and metabolic risk has been observed in survivors. This project aims to study post-transplant metabolic and inflammatory changes and their impact on mature immune cells derived from stem cells.

**Methods:** 14 ALL survivors who underwent transplantation (11 males, 3 females) and 14 matched sibling donors (3 males, 11 females) were selected according to defined criteria. Each recipient-donor couple were profiled for medical anamnesis and clinical assessment and were subjected to a blood collection, for immunophenotyping, transcriptomic analysis and telomere length measurement. An ultrasound examination of the supra-aortic trunks was also performed to assess intima-media thickness (IMT).

**Results:** No differences have been detected in anthropometric characteristics, however, signs of dyslipidaemia were observed in recipients (cholesterol: 157.5 SE±9.194 vs 171.9 SE±10.43; triglycerides 65.57 SE±5.216 vs 105.0 SE±11.31; hyperinsulinemia 8.457 SE±1.870

vs 15.23 SE±2.945 and an increase in the HOMA index (1.587 SE±0.3653 vs 2.981 SE±0.6225). Metabolic syndrome was diagnosed in 14.3% of recipients. Carotid IMT analysis showed accelerated thickening (0.0083 vs 0.0034). Plasma proteomic analysis revealed increased levels of pro-inflammatory proteins (CRP, SAAP) and proteins related to dyslipidaemia (APOB, APOC4-APOC2).

This metabolic and inflammatory phenotype is associated with accelerated telomere shortening in circulating immune cells (slope -0.009 vs -0.0008), a reduction in CD34+ and an increase in circulating CD19+. RNA-seq confirmed inflammation and B-cell dysfunction. Plasma IgM immunoglobulins were decreased in recipients compared to donors (58.62 SE±4.062 vs 49.23 SE±3.188), while IgG levels were comparable (350.6 SE±14.49 vs 329.7 SE±16.43).

**Conclusions:** These data suggest that TBI conditioning negatively impact on the immunometabolic profile of cALL survivors, contributing to an increased risk of long-term cardiovascular complications. Together this evidence suggests the need to optimize the clinical follow-up strategies to mitigate the increased cardiometabolic risk in cALL survivors.

## Uncovering the dual role of hepatocyte-derived Apolipoprotein E in lipoprotein metabolism

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**Background and Aims:** Apolipoprotein E (ApoE) is a key regulator of lipid metabolism, controlling transport and clearance of triglyceride-rich lipoproteins. Although ApoE is produced in several tissues, the liver, especially hepatocytes, is the main source of circulating ApoE. Wholebody ApoE knockout mice develop severe dyslipidemia, whereas mice lacking ApoE only in hepatocytes (ApoEΔHep) display only mild alterations on a standard diet, suggesting compensation from extrahepatic sources and raising questions about the specific contribution of hepatic ApoE. This work investigated the distinct role of hepatocyte-derived ApoE in hepatic lipid handling.

**Methods:** ApoEΔHep mice were generated by crossing ApoE flox/flox mice with AlbuminCre mice. Animals were kept on a standard chow diet for 12 weeks. Lipid metabolism was assessed by FPLC, Western blotting, hepatic geneexpression analysis and functional assays of lipoprotein production and clearance.

**Results:** Plasma WB confirmed that hepatocyte-derived ApoE is the main circulating source of the protein, which was absent in ApoEΔHep mice. Total plasma cholesterol was similar to controls

under both standard diet (76,19±9,99 vs. 85,64±15,20 mg/dL) and highcholesterol diet (154,7±26,45 vs. 151,4±14,35 mg/dL). However, lipoprotein profiling revealed a shift toward LDL-enriched cholesterol in ApoEΔHep mice, in contrast to the VLDL-dominant pattern of global ApoEKO mice. ApoEΔHep animals showed delayed clearance of triglycerides, consistent with impaired removal of triglyceride-rich lipoproteins, and a 46% reduction ( $p < 0.0001$ ) in VLDL production, indicating a role for hepatocyte ApoE in the synthesis and secretion of these particles. Hepatic transcript analysis pointed to deregulated lipid-metabolic pathways.

**Conclusions:** 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 contributions of ApoE to lipid metabolism and dyslipidemia.

## Evaluation of siRNA therapeutic approaches for the regulation 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®) 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® 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.

## Immune-metabolic characterization of a humanized mouse model for translational studies on cardiovascular diseases

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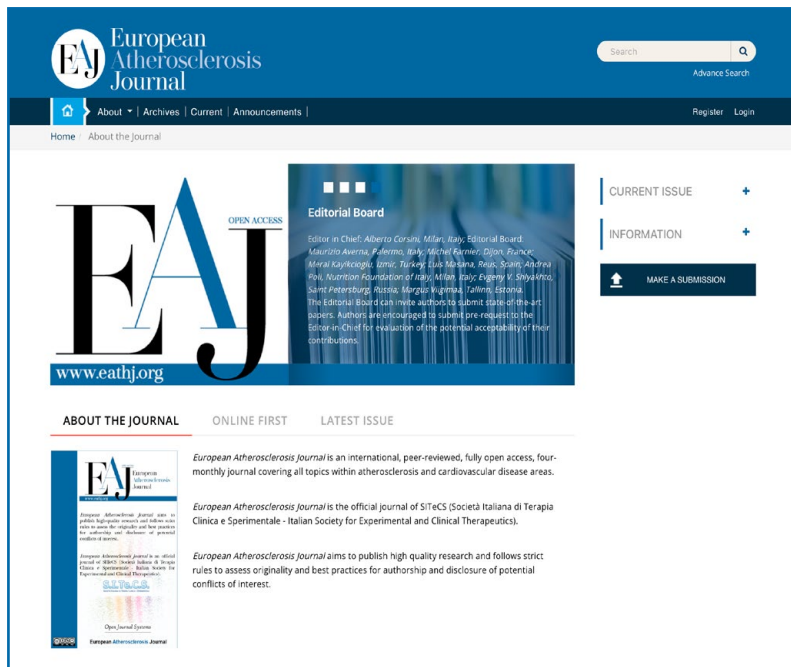
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**Background:** Atherosclerosis represents one of the main cardiovascular risk factors and is driven by high plasma cholesterol levels and an impaired immuno-inflammatory response. Despite effective lipid-lowering therapies, some patients exhibit persistent residual inflammatory risk, highlighting the need for experimental models that enable the translation of molecular mechanisms and immunomodulatory cardiovascular therapies from preclinical to clinical studies. In this context, we present the immuno-metabolic characterization of an immunodeficient mouse model with an atheroprone genetic background, whose immune system is reconstituted with human hematopoietic stem cells (CD34+).

**Metodi:** Humanized TKO-LDLr KO mice (obtained by crossing LDLr KO mice with immunodeficient Rag2-KO/IL2rg-KO/CD47-KO mice, HuTKOL) were generated by irradiating 2-3-day-old pups at low doses (250cGy), followed by intrahepatic injection of commercial human CD34+ cells or those derived from iPSCs (250,000-300,000 cells/mouse). After 12 weeks, human cell engraftment was assessed by FACS. The HuTKOL mice were then fed a high-cholesterol Western-type diet (WTD) for 12weeks to study the immuno-metabolic phenotype and atherosclerosis development.

**Risultati:** HuTKO-L mice showed good engraftment of commercial human CD34+ cells in circulation (%hCD45+/total live leukocytes: 40.97%,SE±3.46%). B-lymphocytes were the most abundant population at 8weeks (%hCD19+/hCD45+: 72.81%,SE±2.33%), but decreased progressively (%hCD19+/hCD45+: 13.85%,SE±2.98%), while T-lymphocytes showed the opposite trend, becoming the dominant population after 12 weeks of WTD (%hCD3+/hCD45+: 58.84%,SE±4.86%). Exposure to WTD induced dyslipidemia (plasma cholesterol: 1086.90mg/dl,SE±65.15) and atherosclerosis (%plaque obstruction in the aortic root: 24.79%,SE±3.17%; atherosclerotic lesion volume: 0.32mm<sup>3</sup>; %fibrosis/plaque area: 33.94%,SE±16.17%), with infiltration of human immune cells into the plaques. Furthermore, WTD feeding induced the expansion of CD4-memory T-lymphocytes and the production of IgM against atherosclerosis-associated antigens. Characterization of iPSC-derived CD34+ cells and their engraftment in TKO-L mice is currently ongoing.

**Conclusioni:** HuTKOL mice represent a tool for studying the dynamics of human adaptive immunity under dyslipidemic conditions and for testing immunomodulation strategies in cardiovascular diseases.



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