Quantifying atherogenic lipoproteins for lipid-lowering strategies: consensus-based recommendations from EAS and EFLM.

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Abbreviations:

apoA-I, apolipoprotein A-I

apoB, apolipoprotein B

ASCVD, atherosclerotic cardiovascular disease

FH, familial hypercholesterolemia

HDL(C), high-density lipoprotein (cholesterol)

IDL, intermediate-density lipoprotein

KIV-2, kringle IV type 2

LDL(C), low-density lipoprotein (cholesterol)

cLDLC, calculated LDL cholesterol

dLDLC, direct LDL cholesterol (homogenous assay)

LC-MS/MS, liquid chromatography tandem-mass spectrometry

Lp(a), lipoprotein(a)

NMR, nuclear magnetic resonance

PCSK9, proprotein convertase subtilisin/kexin type 9

Remnant-C, remnant lipoprotein cholesterol

SCORE, systematic coronary risk evaluation

TC, total cholesterol

TG, triglycerides

VLDL(C), very low-density lipoprotein (cholesterol)

Abstract

The joint consensus panel of the *European Atherosclerosis Society (*EAS) and the *European Federation of Clinical Chemistry and Laboratory Medicine* (EFLM) recently addressed present and future challenges in the laboratory diagnostics of atherogenic lipoproteins. Total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, and calculated non-HDL cholesterol (=LDL + remnant cholesterol) constitute the primary lipid panel for estimating risk of atherosclerotic cardiovascular disease (ASCVD) and can be measured in the nonfasting state. LDL cholesterol is the primary target of lipid-lowering therapies. For on-treatment follow-up, LDL cholesterol shall be measured or calculated by the same method to attenuate errors in treatment decisions due to marked between-method variations. Lipoprotein(a)-cholesterol is part of LDL cholesterol and should be estimated at least once in all patients at risk of ASCVD, especially in those whose LDL cholesterol decline poorly upon statin treatment. Residual risk of ASCVD even under optimal LDL-lowering treatment should be also assessed by non-HDL cholesterol or apolipoprotein B, especially in patients with mild-to-moderate hypertriglyceridemia (2-10 mmol/L). Non-HDL cholesterol includes the assessment of remnant lipoproteins and shall be reported in all standard lipid panels. Additional apolipoprotein B measurement can detect elevated LDL particle numbers often unidentified on the basis of LDL cholesterol alone. Reference ranges of lipids, lipoproteins, and apolipoproteins are reported for European men and women aged 20-100 years. However, laboratories shall flag abnormal lipid values with reference to therapeutic decision thresholds.

Introduction

In the new era of very low LDL-cholesterol (LDLC) concentrations, achievable with more intensive and novel lipid-lowering therapies, increasing attention is being focused on the assessment of lipid-related residual risk of atherosclerotic cardiovascular disease (ASCVD) using additional biomarkers beyond LDLC [1].

An important prerequisite to address present and future challenges of ASCVD prevention is the harmonisation of serum or plasma lipid and lipoprotein profiles produced by established and emerging laboratory tests and techniques. To that end, the multidisciplinary consensus panel of the *European Atherosclerosis Society (*EAS) and the *European Federation of Clinical Chemistry and Laboratory Medicine* (EFLM) recently published recommendations on the quantification of atherogenic lipoproteins in nonfasting and fasting blood samples [1,2]. This article summarizes the consensus-based recommendations of this expert panel which aimed to provide appropriate guidance on the pre-analytical, analytical, and post-analytical phases of laboratory testing of atherogenic lipoproteins.

The key recommendations are given in Table 1. Based on the Copenhagen General Population Study [3], reference nonfasting concentrations for lipids and (apo)lipoproteins are reported for 54,129 European women and 42,126 European men aged 20-100 years and not on lipid-lowering therapy in Tables 2-3.

I. Which atherogenic lipoproteins should be measured?

LDL particles

Assessment of LDLC is a key component of the management of risk of ASCVD [4-6]. Circulating LDL particles are highly atherogenic and there is a direct, graded relationship between LDLC concentration and the incidence of ASCVD observed in randomized controlled trials, prospective epidemiological cohort studies, and Mendelian randomization studies [7,8].

Despite the overwhelming evidence that LDLC-targeted therapies effectively reduce ASCVD in the population, many individuals experience ASCVD-related events or progression of atherosclerosis despite not having elevated LDLC or even concentrations <1.8 mmol/L [9]. This residual risk indicates that a focus solely on the measurement of LDLC (the cholesterol content of LDL) is not an optimal strategy for all patients, in part explained by accumulating evidence that the number of LDL particles (LDLP) measured by lipoprotein subfractionation techniques is more strongly causally related to ASCVD than the cholesterol content of the particles [9-10].

All LDL particles are atherogenic, but their concentration is not always reflected by LDLC measurement because the cholesterol content in the particles can vary widely between individuals [10]. Small lipid-depleted LDL subfractions contain less cholesterol than larger ones. They are typically predominant in patients with moderately elevated triglyceride (TG) concentrations or related conditions, such as diabetes and the metabolic syndrome, without necessarily having high LDLC concentration [11]. These compacted LDL particles are the products of intravascular remodeling of larger, TG-rich very low-density lipoprotein (VLDL) particles [11]. Concomitantly, smaller high-density lipoprotein (HDL) particles are formed in this pathway, as typically manifested in the atherogenic dyslipidemic triad involving hypertriglyceridemia, increased small dense LDLP, and low HDL-cholesterol (HDLC) concentration.

While earlier studies emphasized the atherogenicity of small LDL particles, it is now recognized that all LDL particles are atherogenic, regardless of size [10]. Thus, the primary focus of treatment should remain the reduction of the number (concentration) of LDL particles, without efforts to

distinguish between large and small LDL subfractions [2]. Measurement of apolipoprotein B (apoB), the major protein component of LDL, or advanced measurement of LDLP (not yet widely available) can also be used to assess the number of LDL particles [10,12].

Remnant particles

Postprandial accumulation of TG-rich remnant particles in blood is an important factor in atherogenesis [13,14]. These lipoproteins contain a higher load of cholesterol that is not accounted for in typical fasting lipid profiles. Nonfasting lipid profiles, therefore, can potentially be more relevant to the estimation of an individual's cardiovascular risk than fasting lipids since in real life the postprandial state predominates most of our 24-h cycle [15,16].

TG-rich chylomicrons secreted from the intestine, and VLDL secreted from the liver, are rapidly depleted of part of their TG content and acquire cholesteryl esters from HDL in the circulation [13,14]. These cholesterol-enriched remnant particles may enter the arterial intima and contribute to atherosclerosis, whereas nascent chylomicrons and very large VLDL particles do not cross the endothelial layer [17]. Mendelian randomisation studies suggest that life-long high plasma concentrations of TG-rich lipoproteins or their remnants are causally associated with increased risk of ASCVD and all-cause mortality [17,18].

Direct "homogeneous" assays have been developed to specifically measure cholesterol in remnant particles (Remnant-C) and some have revealed significant associations of Remnant-C with ASCVD [19,20]. An alternative is to calculate Remnant-C as total cholesterol (TC) – HDLC – LDLC, because Remnant-C corresponds to all cholesterol not found in LDL and HDL, that is, in all VLDL and intermediate-density lipoproteins (IDL). In the nonfasting state a relatively small amount of cholesterol can also be found in chylomicron remnants. Because both newly secreted chylomicrons and VLDL rapidly undergo lipolysis, any residual circulating chylomicrons and VLDL can be considered remnants [17]. Direct LDLC measurement should preferably be used in the calculation of Remnant-C; otherwise Remnant-C simply equals TG/2.2 (in mmol/L) when Friedewald-calculated LDLC is used, i.e. TC – HDLC – (TC – HDLC – TG/2.2), and it does not add clinical information beyond TG concentration [1]; however, it focuses the attention on the cholesterol content of remnants rather than the TG content.

Remnant-C also contributes to non-HDLC which is calculated as TC – HDLC [1]. This term is independent of the Friedewald term and therefore not correlated as tight with TG concentrations as calculated Remnant-C, and thus represents an additional clinically valuable marker. Remnant-C, measured or calculated, differs from non-HDLC in that non-HDLC contains Remnant-C plus LDLC and does not differentiate between these two causal risk factors [1].

Lipoprotein(a) particles

Lipoprotein(a) [Lp(a)] is an LDL-like particle with one molecule of apoB to which an additional apolipoprotein, apo(a), is attached. This apolipoprotein shows considerable size polymorphism originating from a variable number of kringle IV type 2 (KIV-2) repeats of apo(a) [21,22]. This size polymorphism is the most important determinant of the hepatic production rate of Lp(a): plasma Lp(a) concentrations and number of KIV-2 repeats are inversely correlated, which results in marked genetic variation of Lp(a) concentrations [21,22]. Elevated baseline and on-statin treatment Lp(a) concentration above the 80th percentile of the general population (50 mg/dL) is a strong genetic risk factor for cardiovascular disease independent of LDLC [23,24]. This has also been recognized by the recent introduction of International Classification of Diseases (ICD)-10 codes E78.41 for elevated Lp(a) concentration and Z83.430 for family history of elevated Lp(a) concentration, in response to the US National Heart, Lung, and Blood Institute (NHLBI) recommendation [25]. High Lp(a) concentration through corresponding low number of KIV-2 repeats are also associated with high risk of mortality in the general population [3].

One of the major differences between Lp(a) and LDL particles is that LDLs are effectively lowered by statins, whereas Lp(a) is typically resistant to this treatment [24]. In statin-treated patients the Lp(a)-associated risk for ASCVD becomes an even better predictor for residual risk as soon as the LDL-associated risk is decreased by statin therapy [24]. Although proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors and other novel therapies reduce Lp(a) and may contribute to reduction in ASCVD [26,27], it is yet unknown whether Lp(a) lowering per se contributes to the clinical benefit of these novel therapies [25]. Although recent studies with PCSK9 inhibitors were not designed to target patients with high Lp(a) concentrations, they nevertheless revealed that patients with higher baseline

Lp(a) concentration experienced greater absolute reductions in Lp(a) and tended to derive greater clinical benefit from PCSK9 inhibition [27]. Drugs on the horizon that specifically target Lp(a) with an Lp(a)-lowering potential of 80% and more will have to prove the benefit of an isolated lowering of Lp(a) on ASCVD outcomes [28].

Recommendation – I.

- Comprehensive testing of atherogenic lipoproteins should use a biomarker, or a panel of multiple markers, to assess the risk associated not only with LDL particles, but also remnant particles and, in selected cases, Lp(a) particles.
- Recommendations for selection of atherogenic lipoprotein tests in different clinical settings are summarized in Table 4.

II. What is the standard lipid profile?

The traditional lipid profile of TC, TG, HDLC, and LDLC remains the primary approach for diagnosis and ASCVD risk classification [29]. A cost-efficient approach is to employ measurements of three markers (TC, TG, HDLC) and from these calculate LDLC and non-HDLC. Remnant-C can also be calculated if directly measured LDLC is used in the equation.

ApoB measurement is not usually part of the standard lipid profile and ASCVD risk estimation models. Monogenic disorders such as familial hypercholesterolemia (FH) can be easily recognized from the standard lipid panel without the need to measure apoB [30,31]. In patients with mild-to-moderate hypertriglyceridemia, defined as 2-10 mmol/L [32], elevated baseline and on-treatment apoB helps identify the atherogenic dyslipidemia related to remnant lipoproteins combined with small dense LDL particles that is not reflected by LDLC and non-HDLC.

Lp(a) should be measured at least once in all patients at moderate, high, or very high cardiovascular risk, with premature ASCVD, with FH, with a family history of premature ASCVD and/or elevated Lp(a), or with recurrent ASCVD despite statin treatment [22]; Lp(a) measurement may also be considered in those with aortic valve stenosis. However, Lp(a) measurement should not be

included in repeated lipid profile measurements within the same patient, as Lp(a) concentrations exhibit little variation over a lifetime. Exceptions from this rule are transition to menopause, pregnancy, oral contraceptive use, renal impairment, or when specific Lp(a)-lowering treatment is administered [21]. Lp(a) concentrations do not change in response to normal food intake and are minimally increased in inflammation, although the influence of acute illness or acute phase reactions on Lp(a) concentration is discussed controversially [33].

Recommendation – II.

- The 'standard lipid profile' used for cardiovascular risk prediction includes TC, TG, HDLC, LDLC, non-HDLC, and optionally – if directly measured LDLC is used – calculated Remnant-C. A 'minimal lipid profile' including only TC and TG can be considered in countries where costs are a major issue such as developing countries [34].
- An 'expanded lipid profile' including Lp(a) or apoB should be used in selected cases (Table 4) [34]. 'Advanced lipid profiles' such as lipoprotein subclasses and apolipoprotein profiles have been introduced in some laboratories but their added value in a clinical setting still needs to be validated [1].

III. When to use fasting and nonfasting blood samples?

Fasting blood samples have previously been the standard for measuring TG, because the fasting state reduces variability of TG concentrations and allows for a slightly more standardized LDLC estimation with the Friedewald equation; however, a fasting sample does not capture the average atherogenic lipid profile seen in the patient over a 24-hour period [16]. In consequence, extended (8-12hrs) fasting is no longer routinely required for the determination of a lipid profile [2]. Findings from population studies showed that despite minor postprandial increases in TG and Remnant-C, quantitative changes in other lipids, lipoproteins, and apolipoproteins appear to be negligible in response to the habitual meal intake for most individuals [2]. For patients, laboratories, and clinicians alike, nonfasting lipid profiles represent an operational simplication without significant negative implications for prognostic,

diagnostic, and therapeutic options for ASCVD prevention [16]. Regardless, it may remain prudent to counsel the patients to avoid an extremely high-fat meal in the preceding 12 hours [35].

Nonfasting lipid profiles are now endorsed by several guidelines including those in Europe, the UK, Canada, Brazil, and the US [4-6,34,36]. Nonfasting and fasting measurements of the lipid profiles must be viewed as complementary and not mutually exclusive. Fasting is certainly not critical for first-time screening and general risk estimation, or to diagnose an isolated hypercholesterolemia such as FH or elevated Lp(a) without concomitant high TG [2]. Fasting is an option when nonfasting TG are >4.5 mmol/L, a concentration seen in ~3-5% of nonfasting individuals in the general population (Tables 2-3) [17]; however, this is not a requirement and a random nonfasting blood sample will still best capture the average TG concentration in a given patient. Fasting may also be recommended for starting medications that cause severe hypertriglyceridemia (e.g., isotretinoin) in genetically predisposed individuals, for patients recovering from hypertriglyceridemic pancreatitis, and when additional laboratory tests that require fasting or morning samples (e.g., fasting glucose) are requested. The EFLM Preanalytical Phase Working Group recently produced guidance on how to standardize fasting blood sampling if needed [35,37].

Recommendation – III.

- Fasting is not routinely required for the determination of a lipid profile.
- In patients in whom an initial nonfasting lipid profile reveals a TG concentration >4.5 mmol/L, a repeat lipid profile in the fasting state could be performed to assess fasting TG concentration; however, this is not a requirement.

IV. Are LDLC measurements or calculations reliable?

Operational definition of LDL

Beta-quantification, the Centers of Disease Control (CDC;USA) Reference Method for LDLC, combines ultracentrifugation to remove VLDL and chylomicrons and heparin-Mn^{2+} precipitation to

separate LDL particles, including Lp(a), from HDL [38]. With beta-quantification, the lipoprotein fraction in the density range of 1.006-1.063 g/mL is defined as LDL, and the fraction in the density range of 1.063-1.21 g/mL is defined as HDL [38]. However, it is not widely recognized that the LDLC fraction on beta-quantification also contains the cholesterol from IDL with density 1.006-1.019 g/mL and Lp(a) with density 1.04-1.13 g/mL. LDLC assays that attempt to specifically measure cholesterol in LDL may, therefore, show discordant results compared to the reference method [1].

Direct LDLC and HDLC assays

The "homogeneous" or "direct" LDLC (dLDLC) and HDLC (dHDLC) assays have largely replaced the older ultracentrifugation and precipitation techniques, particularly for HDLC, but these measurements cannot be generally assumed to provide the same clinical information [38]. The total error of measurement combines systematic bias (deviation from "true" value) and random imprecision. Despite improved analytical precision due to automation, data indicate that results can vary significantly between dLDLC and dHDLC assays from different manufacturers [39]. Most discrepancies – with marked biases between assays and the CDC Reference Methods – are observed in samples from patients with hypertriglyceridemia >2 mmol/L, mixed dyslipidemia, or other conditions involving altered lipoprotein composition and remodelling, such as diabetes and chronic kidney disease [39]. Direct measurements of dLDLC and dHDLC in normolipidemic samples usually meet the total error goals of ≤12% and ≤13%, respectively, but total error ranged from -26% to +32% for dLDLC and -20% to +36% for dHDLC in a comprehensive study of different assays in dyslipidemic samples [40]. Most discordances in dyslipidemic samples are observed at lower concentration ranges of LDLC (<1.8 mmol/L) and HDLC (<1.0 mmol/L) [40]. These errors result in misclassifications with respect to ASCVD risk assessment depending on the type of assay, as observed in accuracy-based external quality assessment (EQA) surveys of hypertriglyceridemic samples organized across different laboratories [41]. The biases noted in dHDLC measurements affect the calculations of LDLC and non-HDLC, since HDLC is used in the calculations [41].

The biases noted in dLDLC and dHDLC assays when analyzing dyslipidemic samples suggest that non-specific cross-reaction takes place, reflecting difficulties in selectively measuring cholesterol

in LDL or HDL particles in diseased subjects when atypical lipoproteins are present. The different manufacturers' direct methods do not measure the same LDL and HDL subfractions [38,39]. This non-selectivity error is of major concern in the contemporary treatment era in which low LDLC concentrations <1.8 mmol/L are increasingly seen with highly efficacious LDL-lowering therapies, and in which moderate hypertriglyceridemia >2 mmol/L – seen in ~25% of individuals in the general population (Tables 2-3) [17] – is potentially a greater problem due to the increasing prevalence of obesity, metabolic syndrome and diabetes mellitus [11].

Calculated LDLC

LDLC calculated with the Friedewald formula, cLDLC = TC – HDLC – VLDL cholesterol (VLDLC), has its limitations. The equation uses a fixed TG:cholesterol ratio (TG/2.2 in mmol/L or TG/5 in mg/dL) to estimate VLDLC and assumes lack of chylomicrons which are more TG-rich than VLDL [38]. Because the TG:cholesterol ratio in TG-rich lipoproteins progressively increases as hypertriglyceridemia becomes more severe, the equation overestimates VLDLC and therefore underestimates LDLC at high TG concentrations [1]. The equation is erroneous when TG concentration is >4.5 mmol/L and fasting blood samples should be used in this condition [2]; at TG ≤4.5 mmol/L nonfasting and fasting lipid profiles can be used alike for calculation of LDLC.

Inaccurate VLDLC estimation at high TG introduces a more significant underestimation in the calculation of cLDLC at very low LDLC concentration ranges, in which VLDLC constitutes a relatively larger fraction of cholesterol [42]. This underestimation of LDLC may translate to treatment group misclassification with regard to the guideline-recommended decision threshold (1.8 mmol/L) for patients at very high cardiovascular risk (Table 5), which may erroneously exclude such patients for initiation or intensification of lipid-lowering therapy [42]. A modified equation, Modified-cLDLC = TC – HDLC – TG/adjustable factor, which adjusts the TG/VLDLC ratio dynamically for concentrations of TG and non-HDLC has been shown to improve the accuracy of cLDLC at various conditions including low LDLC in nonfasting samples [43,44].

Other observations suggest no substantial advantage of using dLDLC assays compared with Friedewald-cLDLC [1]. Although population-based analytical correlation between dLDLC and cLDLC

is good in both fasting and nonfasting populations [2], often in individuals dLDLC may not agree with cLDLC and translate to discrepant risk classification, as observed in 20% of individuals in the Women's Health Study [45]. It should be noted, however, that most clinical trials demonstrating the evidence base for clinical benefit of LDLC lowering have used the Friedewald-calculated cLDLC; thus it could be argued that dLDLC rather than cLDLC is the method that misclassifies risk [1].

Effect of Lp(a)-cholesterol on LDLC

The Friedewald equation and also most dLDLC assays include the cholesterol content of Lp(a) [46]. Considering that an Lp(a) particle is composed of about 30-45% of cholesterol by weight, a significant overestimation of LDLC concentration occurs in subjects with high and very high Lp(a) concentrations [46]; for example, Lp(a)-corrected LDLC is only ~55-70 mg/dL in a person with LDLC concentration of 100 mg/dL and an Lp(a) concentration of 100 mg/dL.

Lp(a)-corrected LDLC should be calculated at least once in patients with suspected high Lp(a), particularly in African-Americans, in patients with nephrotic syndrome, in those undergoing peritoneal dialysis, and in any patient who does not respond sufficiently to statin therapy [1]. If a high Lp(a) concentration is indeed the cause for an apparent non-response or low response of LDLC, then it might not be useful to increase the dosage of statin under such conditions [1]. The correction is as follows with Lp(a) values reported in mg/dL:

Lp(a)-corrected LDLC (mg/dL) = LDLC (mg/dL) – [Lp(a) (mg/dL) x 0.30]

Lp(a)-corrected LDLC (mmol/L) = LDLC (mmol/L) – [Lp(a) (mg/dL) x 0.0078]

At this point of time, we do not provide alternative formulas for Lp(a) reported in nmol/L (despite a proposed rough estimate of 2-2.5x conversion factor from mg/dL to nmol/L) [25]. It has to be recognized that a simple conversion of Lp(a) from mg/dL to nmol/L or vice versa has its limitations since probably most of the available immunoassays cannot measure Lp(a) in strict molar terms due to the size heterogeneity of apo(a) isoforms and the high probability that antibodies against apo(a) recognize a repetitive KIV-2 epitope of apo(a) [47]. This will be addressed in detail in a future joint EAS-EFLM consensus initiative on Lp(a) measurement issues.

LDLC test errors: are they clinically relevant?

The ranges of uncertainty across different LDLC methods are not negligible [1]. Between-laboratory variation of a certain patient's measured or calculated LDLC can range widely over the guideline-driven critical values that determine the decision of therapeutic intervention, i.e. from 1.8 mmol/L to 3.0 mmol/L (Table 5) [1]. Depending on the methods used, different treatment decisions may be taken, or confusion may arise if the patient's samples for monitoring are sent to different laboratories using different methods or when a laboratory changes the method. Not uncommonly, changes in a patient's LDLC test result over time are within the range of uncertainty of laboratory method variation and may not be due to therapeutic intervention [1].

These issues are, however, less relevant for the monitoring of the patient by the same laboratory and method over time. In this situation, the non-specificity bias remains constant over time and only the assay imprecision (random error) and lot-to-lot variation are relevant, which may not be important given that clinicians are not aiming to achieve the LDLC targets exactly but often concentrations below it. In the present era, the percentage of LDLC reduction is more important than achieving specific targets, and indeed recent guidelines suggest that achievement of a >50% reduction in high- and very high-risk patients is paramount irrespective of baseline LDLC concentration [4].

The risk that errors in LDLC measurement or calculation affect the clinical decision is further attenuated by the recommendation that decision to initiate a treatment, or adjusting or shifting to another treatment, should not be taken on one LDLC test, but rather after multiple repeated testing (at least two times) to allow averaging for intra-individual (biological) variation [39]. The EFLM European Biological Variation Study (EuBIVAS) group recently revised biological variation data [48].

Recommendation – IV.

- Follow-up of on-treatment lipid profiles in a patient should ideally be undertaken with the same method (and preferably in the same laboratory) to minimize the misclassification of ASCVD risk.

- Clinicians should be notified by the laboratory when the test changes from one method to another, e.g. through newsletters. This will enhance clinicians' awareness of changes in methods as a potential cause of implausible test results**.** Assay methods and their limitations should always be described in any publication of clinical trials or epidemiological studies.
- Values close to therapeutic decision thresholds should ideally be confirmed by repeated measurement(s) (≥ 2) by the same method and then averaged. Often the repeated test value is lower due to improved diet after the first test if the patient is told that LDLC is elevated; in this case the second value should be accepted for decision making.

V. Are other measurements of atherogenic lipoproteins reliable?

Non-HDL cholesterol

Calculated by subtracting HDLC from TC, non-HDLC represents the cholesterol in all particles causing cardiovascular disease, i.e., LDL, VLDL, IDL, and Lp(a); in the nonfasting state this additionally includes the cholesterol in chylomicrons and their remnant particles. Non-HDLC provides a more comprehensive risk assessment than LDLC in certain individuals with hypertriglyceridemia because it adds VLDLC (= Remnant-C) to LDLC and, therefore, takes into account the atherogenic potential of remnant lipoproteins [1,17]. However, non–HDLC cannot substitute for Remnant-C because it does not differentiate between LDLC and Remnant-C. Some individuals with high Remnant-C have low LDLC and thus will have relatively low non-HDLC, and if interpreting non-HDLC instead of Remnant-C, the high Remnant-C will be masked in these individuals [29].

Like LDLC, non-HDLC is treatable with existing lipid-lowering agents and there is a direct, consistent relationship between the magnitude of non-HDLC lowering and cardiovascular risk reduction observed in meta-analyses and trials with statins and other lipid-lowering agents [49-50]. Guideline-recommended therapeutic goals for non-HDLC are arbitrarily set typically at 0.8 mmol/L higher than LDLC goals (Table 5); this value is based on the assumption that the "optimal" VLDLC (= Remnant-

C) concentration associated with the fasting TG threshold 1.7 mmol/L is 0.8 mmol/L, as estimated by the Friedewald formula (TG/2.2) [1,2].

Non-HDLC can be obtained in the nonfasting state and does not require TG to be less than 4.5 mmol/L [2]. Therefore, under conditions of high TG when cLDLC and also dLDLC is likely to be inaccurate, non-HDLC is a useful alternative to LDLC for therapeutic follow-up because it can be calculated from the standard lipid profile without extra measurement. While non-HDLC is not dependent on TG variability, dHDLC measurement errors in hypertriglyceridemic samples still affect the calculation of non-HDLC and most assays limit the measurement of dHDLC to TG<10 mmol/L. However, non-HDLC yields more accurate cardiovascular risk classification than either Friedewald-cLDLC or dLDLC, and also more consistent risk scores using different manufacturers' assays for HDLC in the calculation [51]. When compared to Modified-cLDLC, non-HDLC translates to only modest improvement for risk classification that could change clinical management (in ~2% of individuals in a general population), although discordance of Modified-cLDLC and, as a consequence, risk underestimation is still common (80-90%) in patients with TG>4.5 mmol/L [52].

Apolipoprotein B

ApoB is a clearly defined measurand. It is the structural protein for all non-HDL lipoproteins and exists as 2 isoforms: apoB100, the major isoform in VLDL, IDL, LDL, and Lp(a), and apoB48 in chylomicrons and chylomicron remnants [12]. Since each atherogenic particle contains one molecule of apoB, concentrations of apoB are therefore considered to be a direct measure of the total number of lipoproteins causing cardiovascular disease, that is, LDL, remnants, and Lp(a) [12].

ApoB can be measured in the nonfasting state because even at peak postprandial concentrations, the number of chylomicron-apoB48 particles in healthy individuals is usually <1% and the number of VLDL-apoB100 particles is <10% of the number of LDL-apoB100 particles [12]. Thus, even with cross-reactivity of antibodies to apoB48 in nonfasting samples, apoB immunoassays are essentially an estimate of LDL particle number if TG and Lp(a) concentrations are low [1]. Far higher than the maximum allowed TG concentration of 4.5 mmol/L for cLDLC, most apoB immunoassays allow TG up to at least 10 mmol/L without significant interference in turbidimetric or nephelometric measurements; a TG

concentration above this limit in nonfasting blood samples is only seen in ~0.1% of individuals in the general population [17].

Simple apoB testing by automated immunoassays obviates the need for lipoprotein subfractionation techniques such as nuclear magnetic resonance (NMR) spectroscopy or ion mobility to quantify LDLP [53,54]. Although apoB has been shown to be at least equivalent to LDLP in predicting cardiovascular risk, apoB cannot substitute for NMR- or ion mobility-based particle size measurements and does not differentiate between LDL and VLDL particle numbers [53,54]. A major impediment to LDLP testing is its limited availability in the clinical laboratories, higher cost, and lack of standardization [55], although it does provide additional information on other lipoproteins VLDL and HDL, beyond LDLP.

Common calibration with the International Federation of Clinical Chemistry (IFCC)/WHO SP3 reference material has reduced between-laboratory variability of apoB immunoassays from >19% to ~7-9%, although concerns about the comparability of immunoassay results with apoB100 derived from NMR and other methods still exist [56,57]. Liquid chromatography tandem-mass spectrometry (LC-MS/MS)-based quantification of apolipoproteins has the potential to further improve apoB100 standardization and between-method comparability [57]. Another advantage of LC-MS/MS is that it enables the simultaneous (multiplexed) measurement of multiple apolipoproteins in a single run of the assay, thus making it possible to achieve a complete apolipoprotein profile in the patient, including HDL- and VLDL-associated apolipoproteins such as apoA-I, apoC-I, apoC-II, apoC-III, and apoE for comprehensive characterization of dyslipidemias [58].

Recommendation – V.

- ApoB measurement has the potential to meet analytical performance criteria including accuracy, standardization across laboratories, unambiguous definition of the measurand, and unequivocal test results in both normo- and dyslipidemic samples – important prerequisites for medical use of a test which can not be met with LDLC and non-HDLC measurements or calculations. Global standardization initiatives and diagnostic test manufacturers should therefore further optimize and harmonize apoB assays.

- Like non-HDLC, apoB can always be measured in the nonfasting state regardless of biological TG variability.

VI. Can apoB measurement replace the standard lipid profile for monitoring of lipid-lowering therapies?

Although the analytical performance of apoB measurement is superior to measurements or calculations of LDLC and even non-HDLC, the test in question has not yet been completely validated according to key criteria defined by the EFLM Test Evaluation Working Group [59] – analytical performance, clinical performance, clinical effectiveness, and cost-effectiveness – to become a medically useful test (Table 6).

Meta-analyses of population studies [60-62] and statin trials [63-65] suggest that the clinical performance of apoB and non-HDLC, although superior to LDLC in some studies, is on average comparable to LDLC to predict risk of fatal or nonfatal ASCVD on the population level. Risk associations were at least as strong in nonfasting study populations than in those who did fast [61].

However, in ~25% of individuals in whom apoB is discordantly high despite "optimal" LDLC, cardiovascular risk tracks with apoB, suggesting that replacing LDLC by apoB would identify more individuals who could benefit from lipid-lowering therapies [63] – this implies an overall better clinical (diagnostic) performance compared to LDLC in particular among these "discordant" individuals. The substantial residual risk that persists in LDLC-targeted therapies even at LDLC <1.8 mmol/L has fueled the debate about considering apoB-targeted therapies using only one single measurement of apoB. The use of apoB or non-HDLC would be cost-effective if these tests provide information to guide therapy to prevent more ASCVD events in the population, and reduce the healthcare costs to a greater extent than standard therapy guided by LDLC, yet the evidence base for cost-effectiveness of this approach in outcome studies is still incomplete (Table 6).

Recommendation – VI.

- At this point of time, there is insufficient evidence from outcome studies to support the option to replace the standard lipid profile (with calculation of cLDLC and non-HDLC) by single measurement of apoB to guide lipid-lowering therapies.
- The clinical effectiveness of LDLC-guided management of cardiovascular risk is most strongly evidence-based [7,8]. All guidelines concur that LDLC remains the primary target of lipid-lowering strategies to prevent ASCVD [4-6]. Lowering LDLC to concentrations below a target of 1.8 mmol/L (or by ≥50% if this target cannot be attained) is of critical importance in subjects at high or very high cardiovascular risk [4-6].

VII. Should non-HDLC or apoB be used as additional tests to LDLC in lipid-lowering strategies?

Non-HDL cholesterol

Data from concordance/discordance analyses suggest that calculation of non-HDLC is at least equally good at predicting ASCVD compared with measurement or calculation of LDLC in the overall population and statin-treated patients; it may also be superior to LDLC if discordantly high, especially at normal or low LDLC concentrations and in individuals with hypertriglyceridemia because it includes VLDLC (=Remnant-C) [66-68]. LDLC does not provide incremental risk prediction of ASCVD relative to non-HDLC [66-68]. The only concern is the selection of the threshold concentration in some studies: it may be the more sensitive threshold for non-HDLC as compared to LDLC rather than the biomarker which makes the difference. Guideline-based non-HDLC thresholds have been arbitrarily defined by consensus of expert groups, based on the assumption that a normal VLDLC concentration exists when TG are <1.7 mmol/L, which is <0.8 mmol/L as estimated by the Friedewald formula [1,2]. Lowering non-HDLC thresholds leads to upward reclassification of patients (if the goal is to reduce undertreatment), higher thresholds lead to downward reclassification (if the goal is to reduce

overtreatment). Threshold values need to be validated in diagnostic performance studies, to evaluate which values most accurately classify patients within risk categories (net reclassification score).

For the present purposes, the combination of non-HDLC with Modified-cLDLC data can be considered as an appropriate strategy to guide therapy [1]. This may compensate for the under- or overestimation of LDLC in terms of clinical decision making, given the uncertainty of the measurement or calculation when LDLC lowering approaches 1.8 mmol/L and at high TG>4.5 mmol/L. However, the compromised accuracy of dHDLC assays in samples with hypertriglyceridemia reduces the benefit in reporting non-HDLC in some individuals in whom an apoB or LDLP measurement may be clinically useful.

Apolipoprotein B

Data from concordance/discordance analyses in large study populations, such as in the Women's Health Study and the Health Professionals Follow-up Study, reveal that the addition of apoB to LDLC and even to non-HDLC has the potential to improve risk prediction by identifying more high-risk individuals [69-71], consistent with the notion that risk of ASCVD is more directly related to the number of apoB-containing particles (reflected by apoB measurement) than to the cholesterol content of lipoproteins [10]. The implication of discordant LDLC vs. apoB (one normal, the other high) is most evident in patients with predominant small, cholesterol-depleted LDL particles who present with "normal" concentrations of TC and LDLC – a profile that is especially prevalent among individuals with the metabolic syndrome or diabetes and in those taking medications, such as statins and anti-PCSK9, that reduce LDLC to a greater extent than apoB [72-74]. This necessarily results in on-treatment LDL and VLDL particle numbers that are higher than would be anticipated from the concurrent LDLC follow-up measurement and may explain part of residual risk among statin-treated patients [54,75]. In patients with low LDLC concentrations, in particular those with metabolic risk factors, apoB (or LDLP) measurement as a "risk enhancing factor" could be useful [6].

Secondary treatment target: non-HDLC or apoB?

Several guidelines propose using non-HDLC or apoB as a secondary treatment target in the management of high-risk or very high-risk patients with mild-to-moderate hypertriglyceridemia (2-10 mmol/L), including patients with diabetes [4-6]. If the primary target LDLC is at goal, but non-HDLC or apoB are still high, attainment of all three targets will require intensified lipid-lowering therapy, lifestyle (re)inforcement, and/or additional TG-lowering drugs (e.g., fibrate or omega-3 fatty acids) [4]. Addition of PCSK9 inhibition to statin therapy allows more patients to achieve non-HDLC and apoB goals and lower risk of ASCVD, with no attenuation of benefit at lower concentrations [73].

Which to choose as secondary target: non-HDLC or apoB? Although apoB demonstrates competitive clinical performance compared with non-HDLC, there is no evidence yet of significant population health-economic benefit of intensifying pharmacological intervention aiming to further reduce apoB at very low concentrations of LDLC [1]. For now and until this issue is clarified, non-HDLC is the best choice, as it can be used without the additional expense of extra measurement.

Recommendation – VII.

- Use of non-HDLC or apoB should be considered as an index of the efficacy of treatment targeted at LDLC. For the present purpose, every lipid profile report should automatically add non-HDLC. To improve patient comfort and compliance, there are practical advantages of this approach for follow-up without the need to fast and without regard to TG [2].
- When cLDLC estimation is limited in the range of TG>4.5 mmol/L, use of non-HDLC calculation can be considered instead of additional dLDLC measurement to evaluate therapeutic response.

VIII. How to report the atherogenic lipid profiles?

Threshold values

We recommend that laboratory reports should flag abnormal concentrations based on threshold values defined by guidelines, i.e., decision threshold to trigger therapy or to identify increased risk of ASCVD (Table 7). For nonfasting samples, laboratories should flag abnormal TG concentrations as ≥2 mmol/L according to the Women's Health Study, which found that this threshold was optimal for ASCVD prediction [76]. The threshold for fasting TG at 1.7 mmol/L is 0.3 mmol/L lower than for nonfasting TG, corresponding to the mean maximal increase in TG following habitual food intake [2]. Nonfasting state-adjusted threshold for Remnant-C and, consequently, also non-HDLC are 0.1 mmol/L higher than fasting values [2]. In case the patient's postprandial time in the preceding 12 hours is unknown on sample reception in the laboratory, it may remain prudent to apply the lower fasting thresholds to draw attention to a potential cardiovascular risk.

For LDLC, the decision threshold for initiation of therapeutic intervention varies with the individual's risk score (Table 5) [4-6]. This personalized reporting of optimal thresholds is difficult to implement in laboratory reports because usually the clinical conditions and risk factors of the individual patients are not known to the laboratory personnel. We therefore propose a simplified flagging based on concentration threshold for moderate risk only, i.e. 3 mmol/L for LDLC, which may be complemented by more detailed information on risk-stratified thresholds in footnotes on the laboratory report or by references to web-based information [2].

Given the uncertainty of measurements and calculations of LDLC across different methods and laboratories, in hypertriglyceridemic patients the decision thresholds may not always be considered universally applicable [1]. Guideline-recommended LDLC values are based on observations with Friedewald-cLDLC using the older HDLC precipitation methods which differ from dHDLC assays used nowadays. New generation assays should ideally be validated for clinical performance to discriminate between high- and low-risk patients, e.g. by reporting risk reclassification in observational studies using the net reclassification index.

Usually, in laboratory reports, results of most tests are flagged if they are below or above the age- and sex-specific reference interval (2.5th to 97.5th percentiles). Because of the widespread unhealthy lifestyle, in most populations the upper reference limits of TC, LDLC, and TG are very high (Tables 2-3) and far above the thresholds of increased ASCVD risk (Figure 1). Therefore, flagging of lipid profiles in adults should not be based on reference intervals [2]. In a pediatric setting, reporting of age- and gender-specific reference ranges can be relevant for early identification of children with hyperlipidemia associated with premature atherosclerosis, especially FH [31]. Reference ranges in children and adolescents are available from recent population-based cohort studies and databases [77-79].

Alert values

Extremely abnormal test results beyond the reference range deserve special attention and reactions from the laboratory [2]. They should be flagged with special alert notifications to quickly initiate further diagnostic and possibly therapeutic actions by the clinician, preferably with direct referral to a specialist lipid clinic (Table 7). For example, patients with severe hypertriglyceridemia ≥10 mmol/L and chylomicronemia syndrome have high risk of acute pancreatitis but usually do not develop premature atherosclerosis, probably because chylomicrons and large VLDLs do not traverse the vascular endothelial barrier [32]. Lp(a) above the 97.5th percentile (>120 mg/dL depending on the assay) should be noted because of very high risk for myocardial infarction and aortic valve stenosis [2]. Any LDLC >5 mmol/L in adults or >4 mmol/L in children should trigger investigations to rule-out FH and, if diagnosis of FH is confirmed in the index case, cascade family screening [30,31]. In patients with mixed hyperlipidemias, routine genetic testing is not warranted, but a high non-HDLC/apoB ratio >5 mmol/g may be considered as alert value to further investigate for the rare but highly atherogenic familial dysbetalipoproteinemia (accumulation of β-VLDL caused by *APOE* gene mutation) – non-HDLC/apoB ratio is high because the cholesterol-enriched remnant lipoproteins contain a low proportion of apoB relative to cholesterol [80]. Reflective testing can proactively assist clinicians to rule-out common secondary causes of hyperlipidemia using additional tests, e.g., thyrotropin, haemoglobin A1c, liver enzymes and creatinine/eGFR, if not already known to the clinician at first-time screening [81].

Recommendation – VIII.

- Flagging of lipid profiles on laboratory reports should always be based on decision thresholds. In children, reporting of reference ranges is relevant.
- Extremely high concentrations should automatically trigger alerts to initiate immediate diagnostic investigations.

Conclusion and future research priorities

The consensus-based recommendations of EAS and EFLM provide guidance for the use of contemporary lipid, lipoprotein, and apolipoprotein tests to assist clinicians in their strategies to prevent ASCVD [82]. These recommendations take into account the strenghts and weaknesses of the tests in terms of key criteria to become a medically useful test, as defined by the EFLM Test Evaluation Working Group [59].

Calculation of non-HDLC and Remnant-C from the standard lipid profile, 'expanded' testing of apoB and Lp(a), and 'advanced' testing of LDLP have the potential to address clinical needs unmet with LDLC testing and they can always be used in nonfasting samples. The research priority is to investigate whether the diagnostic information provided by 'expanded' or 'advanced' lipid profiles can sufficiently change clinical management to reduce the risk (and cost) of ASCVD to a greater extent than the standard LDLC-centered approach.

Diabetes and abdominal obesity, disorders that underlie the clinical expression of complex dyslipidemias without elevated LDLC, are attaining epidemic proportions [11]. Hence, emerging and advanced lipoprotein testing will likely become more and more useful in the future. This underscores the need to standardize and validate advanced lipoprotein tests, such as NMR- or ion mobility-based LDL- and VLDL-particle numbers and size [54,55], and multiplexed LC-MS/MS apolipoprotein profiles [58], which have the potential to become widely available medical tests [83]. These novel technologies provide complementary diagnostic information regarding the complex molecular basis of dyslipidemias and, as such, can be used to explore and evaluate precision medicine approaches for identifying better and individualized treatment options for patients at high risk of ASCVD [83].

Author contribution

EAS-EFLM Joint Consensus Panel members were nominated by EAS, EFLM and the co-chairs M.L. and B.G.N. to represent expertise across clinical and laboratory management and lipid research from across the World. The Panel met twice, organized and chaired by M.L. and B.G.N. . The first meeting critically reviewed the literature while the second meeting reviewed additional literature and scrutinized the first draft of the joint consensus statements.

This summary embodies the consensus-based recommendations produced by the Panel [1,2,82] and was critically reviewed by independent experts of the EFLM Task Group on Cardiac Markers, chaired by P.L., who were not present at the first Panel meetings. The recommendations should be considered as consensus-based expert opinion, despite the high-quality evidence base available from systematic reviews and meta-analyses of randomized controlled trials, Mendelian randomization studies, and prospective population-based cohort studies for most but not all recommendations. All Panel members agreed to conception and design, contributed to interpretation of available and additional novel data, and all suggested revisions for this document.

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Table 1: Key EAS/EFLM recommendations for testing of atherogenic lipoproteins

Pre-preanalytical phase (test ordering)
Comprehensive testing of atherogenic lipoproteins should include tests to asses the risk conferred by LDL particles, remnant particles and, in selected cases, Lp(a).
Preanalytical phase (test sampling)
Fasting is not routinely required for assessing the lipid profile. Consider fasting sample when nonfasting TG are >4.5 mmol/L (400 mg/dL); however, this is not a requirement. Take 2 to 3 serial blood specimens, at least 1 week apart, to allow to average for biological variation (importantly when test results are near the treatment decision thresholds).[a,]
Analytical phase (test measurement)
Follow-up of measured or calculated LDLC and non-HDLC of a patient, from baseline to on-treatment measurements, should be ideally performed with the same method (and preferably the same laboratory). [b] Clinicians should be notified when the laboratory test changes from a method to another. Lp(a)-corrected LDLC should be assessed at least once in patients with suspected or known high Lp(a), or if the patient shows a poor response to LDL-lowering therapy.
Postanalytical phase (test reporting)
Laboratories should automatically calculate and report non-HDLC on all lipid profiles; Remnant-C could also be reported. Laboratory reports should flag abnormal concentrations based on threshold values. Extremely high concentrations beyond the reference range should alert clinicians (interpretative commenting on test report).
Post-postanalytical phase (test interpretation and use)
LDLC is the primary target of lipid-lowering therapy. When LDLC goal is achieved, non-HDLC or apoB should be preferred as secondary treatment targets in patients with TG 2-10 mmol/L (175-880 mg/dL), metabolic syndrome, or type 2 diabetes.

[a] Avoid measurements within ~2 months after acute myocardial infarction, acute trauma, surgery, acute infection or inflammatory illness, or pregnancy. Patients should maintain their usual diet in the preceding 2 weeks, and avoid strenuous exercise.

[b] Remove serum or plasma from cells (centrifugation) within 3 h of blood sampling, and perform lipid measurements within 1–2 days of collection. However, before measurement specimens can safely be stored at 4°C for 3 days, at -20°C for 1 month, and at -70 °C for 1–2 years.

Table 2: Concentration distribution of nonfasting lipids, lipoproteins, and apolipoproteins in 54,129 women in the Copenhagen General Population Study not on lipid-lowering therapy.

	Percentiles									
	2.5		25		50		75		97.5	
Age group	mmol/L	mg/dL	mmol/L	mg/dL	mmol/L	mg/dL	mmol/L	mg/dL	mmol/L	mg/dL
Triglycerides										
20-39	0.45	40	0.73	65	0.98	87	1.4	121	2.8	248
40-65	0.50	44	0.84	74	1.2	103	1.7	148	3.6	317
66-100	0.59	52	0.98	87	1.4	120	1.9	170	3.8	340
Total cholesterol										
20-39	3.3	127	4.2	162	4.7	182	5.3	205	6.9	267
40-65	3.8	147	5.0	193	5.6	217	6.3	244	7.9	306
66-100	4.3	166	5.5	213	6.1	236	6.8	263	8.2	317
LDL cholesterol										
20-39	1.4	54	2.1	81	2.6	101	3.1	120	4.4	170
40-65	1.7	66	2.6	101	3.2	124	3.8	147	5.3	205
66-100	1.9	73	3.0	116	3.5	135	4.1	159	5.5	213
Remnant cholesterol										
20-39	0.19	7.4	0.33	13	0.45	17	0.62	24	1.2	48
40-65	0.21	8.1	0.38	15	0.53	20	0.76	29	1.5	60
66-100	0.26	10	0.45	17	0.61	24	0.86	33	1.6	62
Non-HDL cholesterol										
20-39	1.7	67	2.6	99	3.1	118	3.7	142	5.3	203
40-65	2.1	82	3.1	121	3.8	147	4.6	176	6.3	242
66-100	2.4	93	3.5	137	4.2	162	4.9	190	6.5	251
HDL cholesterol										
20-39	0.91	35	1.3	51	1.6	61	1.9	73	2.5	98
40-65	0.93	36	1.4	55	1.7	67	2.1	80	2.8	108
66-100	0.98	38	1.5	58	1.9	72	2.2	86	3.0	117
Lipoprotein(a)										
	nmol/L	mg/dL	nmol/L	mg/dL	nmol/L	mg/dL	nmol/L	mg/dL	nmol/L	mg/dL
20-39	1.1	1.4	5.4	4.3	15	8.5	43	22	207	97
40-65	1.6	1.5	6.8	4.9	17	9.8	60	30	242	113
66-100	1.9	1.6	7.4	5.2	19	10	64	31	250	116
Apolipoprotein B										
	g/L	mg/dL	g/L	mg/dL	g/L	mg/dL	g/L	mg/dL	g/L	mg/dL
20-39	0.51	51	0.69	69	0.82	82	0.98	98	1.47	147
40-65	0.59	59	0.83	83	1.00	100	1.21	121	1.79	179
66-100	0.67	67	0.94	94	1.11	111	1.31	131	1.87	187

Nonfasting triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), and apolipoprotein B concentrations were measured by automated assays (Thermo Scientific Konelab, Vantaa, Finland). Low-density lipoprotein cholesterol (LDLC) was calculated by the Friedewald equation when TG were ≤4mmol/L and was measured directly (Konelab) when TG were >4mmol/L. Non-HDL cholesterol was calculated as TC minus HDLC. Remnant cholesterol was calculated as TC minus LDLC minus HDLC. The first 5592 individuals included in the Copenhagen General Population Study had lipoprotein(a) total mass measured using a sensitive immunoturbidimetric assay from DiaSys (DiaSys Diagnostic Systems, Holzheim, Germany), while all remaining individuals in the study had lipoprotein(a) measurements done using the apolipoprotein(a) isoform insensitive Denka Seiken assay (Denka Seiken, Tokyo, Japan) or the Roche second generation lipoprotein(a) assay developed by Denka Seiken (Roche Diagnostics, Rotkreuz, Switzerland) [3].

Table 3: Concentration distribution of nonfasting lipids, lipoproteins, and apolipoproteins in 42,126 men in the Copenhagen General Population Study not on lipid-lowering therapy.

	Percentiles									
	2.5		25		50		75		97.5	
Age group	mmol/L	mg/dL	mmol/L	mg/dL	mmol/L	mg/dL	mmol/L	mg/dL	mmol/L	mg/dL
Triglycerides										
20-39	0.54	48	0.96	85	1.4	128	2.2	190	5.1	454
40-65	0.61	54	1.10	100	1.7	146	2.5	219	5.5	485
66-100	0.62	55	1.10	98	1.6	140	2.3	201	4.6	404
Total cholesterol										
20-39	3.3	128	4.3	166	4.9	189	5.6	217	7.2	278
40-65	3.9	151	5.0	193	5.6	217	6.3	244	7.9	305
66-100	3.8	147	5.0	193	5.6	217	6.3	244	7.6	294
LDL cholesterol										
20-39	1.5	58	2.4	93	2.9	112	3.5	135	5.0	193
40-65	1.8	70	2.8	108	3.4	131	4.0	155	5.4	209
66-100	1.8	70	2.7	104	3.3	128	3.9	151	5.0	193
Remnant cholesterol										
20-39	0.22	8.5	0.43	17	0.64	25	0.95	37	1.8	71
40-65	0.26	10	0.51	20	0.74	29	1.1	43	2.0	76
66-100	0.27	10	0.50	19	0.71	27	1.0	39	1.7	67
Non-HDL cholesterol										
20-39	2.0	76	3.0	115	3.6	140	4.4	170	6.2	238
40-65	2.4	92	3.6	137	4.3	164	5.5	213	6.6	255
66-100	2.3	89	3.4	133	4.1	158	4.8	184	6.1	237
HDL cholesterol										
20-39	0.67	26	1.0	39	1.2	85	1.5	56	2.0	76
40-65	0.72	28	1.1	42	1.3	52	1.7	64	2.4	93
66-100	0.76	29	1.2	46	1.5	56	1.8	70	2.6	101
Lipoprotein(a)										
	nmol/L	mg/dL	nmol/L	mg/dL	nmol/L	mg/dL	nmol/L	mg/dL	nmol/L	mg/dL
20-39	1.0	1.2	5.5	4.3	14	8.3	49	24	219	102
40-65	1.1	1.4	5.8	4.4	15	8.9	51	25	226	105
66-100	1.1	1.4	6.2	4.6	17	9.5	50	25	211	99
Apolipoprotein B										
	g/L	mg/dL	g/L	mg/dL	g/L	mg/dL	g/L	mg/dL	g/L	mg/dL
20-39	0.56	56	0.81	81	0.99	99	1.22	122	1.86	186
40-65	0.67	67	0.96	96	1.16	116	1.41	141	2.04	204
66-100	0.66	66	0.93	93	1.11	111	1.32	132	1.86	186

Laboratory measurements and calculations were performed as described in the footnote of Table 2 [3].

Table 4: Recommendations for the clinical indications for lipid and (apo)lipoprotein quantitation.

	ASCVD risk estimation	Dyslipidemia characterization	Treatment choice	Treatment target
Primary tests				
TC[a]	YES[a]	Optional[b]	Optional[b]	Optional[b]
HDLC[c]	YES[d]	YES	NO	NO
TG	YES	YES	YES	NO
LDLC	YES	YES	YES	YES
Remnant-C[a]	Optional[e]	Optional[e]	NO	Optional[e]
Non-HDLC[a]	YES	NO[f]	NO	YES[g]
Additional tests				
ApoB[h]	Optional[g]	YES[g]	NO	Optional[g]
Lp(a)	YES[i]	YES[i]	Not yet[j]	Not yet[j]

[a] In nonfasting samples this will also include cholesterol in chylomicrons and their remnants; however, in the majority of individuals chylomicrons are rapidy converted into remnants after delivery from lymphs to the blood stream.

[b] To be considered in a minimal lipid profile (TC and TG only) or when LDLC is not available.

[c] Or ApoA-I if available.

[d] In combination with TC, if HDLC is entered as a separate variable in the risk estimation model. Ratios of TC/HDLC, non-HDLC/HDLC, or apoB/apoA-I which reflect the balance between atherogenic and neutral lipoproteins can be considered as an alternative for risk estimation, but not for diagnosis or as treatment targets. The components of the ratio have to be managed separately.

[e] Remnant-C, calculated as TC – HDLC – LDLC, is all cholesterol found in TG-rich lipoproteins VLDL, IDL, and, in the nonfasting state, additionally chylomicron remnants. Remnant-C is included in non-HDLC, but non-HDLC does not differentiate between LDLC and Remnant-C. Remnant-C is the part of non-HDLC in addition to LDLC that needs reduction in some patients. Directly measured LDLC should preferably be used in the calculation of Remnant-C, or Remnant-C could be measured directly.

[f] Non-HDLC, calculated as TC – HDLC, is all cholesterol in atherogenic lipoproteins LDLC, remnant-C, and Lp(a)-cholesterol, and thus none of the hyperlipidemias can be characterized by non-HDLC. In combination with apoB, the non-HDLC/apoB ratio >5 mmol/g can discriminate the rare dysbetalipoproteinemia (β-VLDL accumulation) from other combined hyperlipidemias because the cholesterol-enriched remnant lipoproteins contain a low proportion of apoB relative to cholesterol.

[g] In patients with mild-to-moderate hypertriglyceridemia, 2-10 mmol/L (175-880 mg/dL).

[h] Or advanced LDLP measurement if available.

[i] In patients with premature ASCVD, family history of premature ASCVD and/or elevated Lp(a), FH, recurrent ASCVD despite optimal lipid-lowering treatment, ≥3% 10-year risk of fatal ASCVD (SCORE).

[j] Unless approved treatment is available to substantially reduce Lp(a) concentration and Lp(a)-related risk.

Table 5: Primary and secondary goals of preventive therapy according to cardiovascular mortality risk categories assessed with the SCORE system.

Risk (SCORE) [a]	LDLC mmol/L (mg/dL)	Non-HDLC [b] mmol/L (mg/dL)	ApoB [b] g/L (mg/dL)
Very high	< 1.8 (70) or ≥50% reduction in LDLC	< 2.6 (100) [c]	< 0.8 (80) [d]
High	< 2.5 (100) or ≥50% reduction in LDLC	< 3.3 (130)	< 1.0 (100)
Moderate	< 3.0 (115)	< 3.8 (145)	

[a] 10-year risk of fatal ASCVD as estimated using SCORE (Systematic COronary Risk Evaluation) [4].
Very high risk = documented cardiovascular disease, diabetes with target organ damage (e.g. proteinuria), severe chronic kidney disease (GFR <30 mL/min/1.73 m^2), SCORE ≥10%;
High risk = TC >8 mmol/L (310 mg/dL) e.g. FH, severe hypertension, uncomplicated diabetes, moderate chronic kidney disease (GFR 30-59 mL/min/1.73 m^2), SCORE ≥5% and<10%;
Moderate risk = SCORE ≥1% and<5%.
Low risk = SCORE <1%.

[b] Secondary target in patients with mild-to-moderate hypertriglyceridemia, 2-10 mmol/L (175-880 mg/dL) including those with metabolic syndrome, type 2 diabetes, or chronic kidney disease.

[c] Discordant high non-HDLC at optimal LDLC goal reflects elevated Remnant-C >0.8 mmol/L (30 mg/dL).

[d] Discordant high apoB at optimal LDLC goal reflects elevated numbers of small, cholesterol-depleted LDL particles.

To convert mmol/L to mg/dL, multiply with 38.6 for cholesterol.

Table 6: Contemporary evidence for the medical use of LDLC, non-HDLC, apoB, and LDLP based on essential test characteristics.

Test characteristics	LDLC	non-HDLC	ApoB	LDLP
Analytical performance[a]				
Precise assays	Yes	Yes	Yes	Yes
Accurate assays (method independency)	No	No	Yes	No
Nonfasting measurement possible	With TG<4.5mmol/L	Yes	Yes	Yes
Widely accessible assays	Yes	Yes	Yes	No
Reasonable operational costs	Yes	No extra measurement	Yes	Not yet
Clinical performance[b]				
Robust associations with incident ASCVD?	Yes	Yes	Yes	Yes
Novel information beyond existing markers?	(Reference)	Yes	Yes	Yes
Validated decision limits?	No	No	No	No
Clinical effectiveness[c]				
Superiority to existing tests?	(Reference)	Probably	Probably	Probably
Modifiable risk association (treatment target)?	Yes	Yes	Yes	Yes
Test-guided treatment reduces ASCVD risk?	Yes	Probably	Probably	Unknown
Cost effectiveness[d]				
Test-guided treatment saves healthcare costs?	Yes	Unknown	Unknown	Unknown

Test characteristics defined by the EFLM Test Evaluation Working Group [59]:

a. Analytical validity: ability of the test to conform to predefined quality specifications to measure the marker of interest.
b. Diagnostic or prognostic accuracy: ability of the test to consistently detect patients with a high risk for developing ASCVD.
c. Clinical utility: ability of the test to improve health outcomes of the patient under standard clinical care.
d. Health-economic advantage of introducing the test in medical practice (value for money).

Table 7: Flagging of abnormal lipid and (apo)lipoprotein concentrations based on risk prediction thresholds and of extremely high concentrations.

Parameter	Thresholds	Interpretative commenting
TG [a]	Fasting ≥1.7 mmol/L (150 mg/dL) Nonfasting ≥2 mmol/L (175 mg/dL)	>10 mmol/L (880 mg/dL): severe hypertriglyceridemia with high risk of acute pancreatitis
TC	≥5 mmol/L (190 mg/dL)	
LDLC	≥3 mmol/L (115 mg/dL)	>13 mmol/L (500 mg/dL): consider homozygous FH >5 mmol/L (190 mg/dL): consider heterozygous FH
Remnant-C	Fasting ≥0.8 mmol/L (30 mg/dL) Nonfasting ≥0.9 mmol/L (35 mg/dL)	
Non-HDLC	Fasting ≥3.8 mmol/L (145 mg/dL) Nonfasting ≥3.9 mmol/L (150 mg/dL)	Non-HDLC/apoB ratio >5 mmol/g (>2 mg/mg): consider dysbetalipoproteinemia
ApoB	≥1 g/L (100 mg/dL)	<0.1 g/L (10 mg/dL): genetic abetalipoproteinemia
HDLC	Men ≤1 mmol/L (40 mg/dL) Women ≤1.2 mmol/L (45 mg/dL)	
ApoA-I	Men ≤1.2 g/L (120 mg/dL) Women ≤ 1.4 g/l (140 mg/dL)	<0.1 g/L (10 mg/dL): genetic hypoalphalipoproteinemia
Lp(a)	≥50 mg/dL (<105 nmol/L)[b,c]	>120 mg/dL: very high risk for myocardial infarction and aortic valve stenosis

Values in mmol/L were converted to mg/dL by multiplication with 38.6 for cholesterol and 88.5 for TG, followed by rounding to the nearest 5 mg/dL.

[a] TG thresholds based on assays with correction for endogenous glycerol. The free glycerol concentration in a sample, usually 1 mg/dL, equivalent to ~10 mg/dL (0.11 mmol/L) of TG, can be ignored. Increased baseline glycerol concentrations can be found in patients with diabetes and chronic kidney disease and during intravenous lipid infusion, and TG may be wrongly flagged in these patients unless glycerol-corrected TG assay is used.

[b] Threshold value for Lp(a) should represent ≥80th percentile of the population-specific Lp(a) assay.

[c] There is no consensus on which threshold value in mmol/L to be used for Lp(a); however, for conversion of Lp(a) concentrations in mg/dL to nmol/L, 13930 individuals from the Copenhagen General Population Study had measurements in both mg/dL and nmol/L by Denka Seiken assays distributed by Roche Diagnostics (Rotkreuz, Switzerland). The correlation was done by linear regression with an R^2 value of 0.996, and the conversion was done by the following equation: Lp(a), nmol/L = 2.18*Lp(a), mg/dL–3.83 [3].

Figure 1:

Distribution of nonfasting LDLC concentration in men and women from the Copenhagen General Population Study not on lipid-lowering therapy.

Nonfasting TG, TC, and HDLC were measured by automated assays (Thermo Scientific Konelab, Vantaa, Finland). LDLC was calculated by the Friedewald equation when TG were ≤4 mmol/L and was measured directly (Konelab) when TG were >4 mmol/L [3]. Population percentages of men and women are subdivided according to LDLC values above 5 mmol/L (roughly the 95th percentile above which FH should be considered), between 3-5 mmol/L, and below 3 mmol/L (the guideline-recommended threshold). Flagging based on reference ranges instead of threshold values should be avoided, as use of reference ranges would not flag a majority of LDLC test results (~60%) associated with increased ASCVD risk. Age-stratified LDLC data can be seen in Tables 2-3.