

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

Michel R. Langlois^{1*}, Børge G. Nordestgaard², Anne Langsted², M. John Chapman³, Kristin M. Aakre⁴, Hannsjörg Baum⁵, Jan Borén⁶, Eric Bruckert⁷, Alberico Catapano⁸, Christa Cobbaert⁹, Paul Collinson¹⁰, Olivier S. Descamps¹¹, Christopher J. Duff¹², Arnold von Eckardstein¹³, Angelika Hammerer-Lercher¹⁴, Pia R. Kamstrup², Genovefa Kolovou¹⁵, Florian Kronenberg¹⁶, Samia Mora¹⁷, Kari Pulkki¹⁸, Alan T. Remaley¹⁹, Nader Rifai²⁰, Emilio Ros²¹, Sanja Stankovic²², Ana Stavljenic-Rukavina²³, Grazyna Sypniewska²⁴, Gerald F. Watts²⁵, Olov Wiklund⁶, and Päivi Laitinen²⁶ for the European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Joint Consensus Initiative.

¹Department of Laboratory Medicine, AZ St-Jan, Brugge, and University of Ghent, Belgium

²Herlev and Gentofte Hospital, Copenhagen University Hospital, University of Copenhagen, Denmark

³National Institute for Health and Medical Research (INSERM), and Endocrinology-Metabolism Service, Pitié-Salpêtrière University Hospital, Paris, France

⁴Hormone Laboratory, Haukeland University Hospital, Bergen, Norway

⁵Institute for Laboratory Medicine, Mikrobiologie und Blutdepot, Regionale Kliniken Holding RKH GmbH, Ludwigsburg, Germany

⁶Institute of Medicine, Sahlgrenska Academy at Göteborg University, and Wallenberg Laboratory for Cardiovascular and Metabolic Research, Sahlgrenska University Hospital, Gothenburg, Sweden

⁷Department of Endocrinology and prevention of cardiovascular disease, Pitié-Salpêtrière University Hospital, Paris, France

⁸Department of Pharmacological and Biomolecular Sciences, University of Milan, and IRCCS Multimedica, Milan, Italy

⁹Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, The Netherlands

¹⁰Departments of Clinical Blood Sciences and Cardiology, St George's University Hospitals NHS Foundation Trust and St George's University of London, London, UK

¹¹Department of Internal Medicine, Centres Hospitaliers Jolimont, Haine-Saint-Paul, and Department of Cardiology, UCL Cliniques Universitaires Saint-Luc, Brussels, Belgium

¹²Department of Clinical Biochemistry, University Hospitals of North Midlands NHS Trust, Stoke-on-Trent, UK

¹³Institute for Clinical Chemistry, University Hospital Zurich, Zurich, Switzerland

¹⁴Kantonsspital Aarau AG, Institute for Laboratory Medicine, Aarau, Switzerland

¹⁵Cardiology Department, Onassis Cardiac Surgery Center, Athens, Greece

¹⁶Department of Medical Genetics, Molecular and Clinical Pharmacology, Division of Genetic Epidemiology, Medical University of Innsbruck, Innsbruck, Austria

¹⁷Divisions of Preventive and Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

¹⁸Department of Clinical Chemistry, University of Turku and Turku University Hospital, Turku, Finland

¹⁹Lipoprotein Metabolism Section, Cardiovascular-Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA

²⁰Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

²¹Lipid Clinic, Department of Endocrinology and Nutrition, Institut d'Investigacions Biomèdiques August Pi Sunyer, Hospital Clínic, Barcelona and Ciber Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Spain

²²Center for Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia

²³Libertas International University, Zagreb, Croatia

²⁴Dept. of Laboratory Medicine, Collegium Medicum, NC University, Bydgoszcz, Poland

²⁵Lipid Disorders Clinic, Department of Cardiology, Royal Perth Hospital, University of Western Australia, Perth, Australia

²⁶Department of Clinical Chemistry, HUSLAB, Helsinki University Hospital, Helsinki, Finland.

***Correspondence:**

Michel R. Langlois, MD, PhD

Dept. Laboratory Medicine, AZ St-Jan, Rudderhove 10, B-8000 Brugge, Belgium.

Phone:+32-50452729.

E-mail: michel.langlois@azsintjan.be

Short title: quantifying atherogenic lipoproteins: consensus-based recommendations.

Keywords:

Atherosclerotic cardiovascular disease, LDL cholesterol, remnant cholesterol, non-HDL cholesterol, apolipoprotein B, lipoprotein(a).

Abbreviations:

apoA-I, apolipoprotein A-I

apoB, apolipoprotein B

ASCVD, atherosclerotic cardiovascular disease

FH, familial hypercholesterolemia

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

dHDL, direct HDL cholesterol (homogenous assay)

IDL, intermediate-density lipoprotein

KIV-2, kringle IV type 2

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

cLDL, calculated LDL cholesterol

dLDL, 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

RemnantC, 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 (=total – HDL 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 measured or calculated 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 lipoprotein cholesterol 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 intervals 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 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 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 according to the continuous remodeling of lipoproteins in blood [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 exchange of cholesteryl esters with TG from larger, TG-rich very low-density lipoprotein (VLDL) particles (Figure 1). Concomitantly, smaller high-density lipoprotein (HDL) particles are also formed in this pathway, as typically manifested in the atherogenic dyslipidemic triad involving hypertriglyceridemia, increased small dense LDLP, and low HDL-cholesterol (HDL-C) concentration [11].

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 [1]. 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].

The total cholesterol (TC) to HDLC ratio has been proposed as a surrogate marker of the number of LDL particles – associated with low HDLC and hence higher TC/HDLC ratio in individuals with atherogenic dyslipidemia [10]. The TC/HDLC ratio may be considered as an alternative to LDLP for risk estimation, but not for diagnosis or as therapeutic decision limit because a low ratio due to high HDLC can be misleading: this may lead to assuming a low risk even if the patient has high LDLC. The components of the ratio, TC and HDLC, have to be managed separately.

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-hour 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 (Figure 1). 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 serum 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 (RemnantC) and some have revealed significant associations of RemnantC with ASCVD [19,20]. An alternative is to calculate RemnantC as $TC - HDLC - LDLC$, because RemnantC 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 RemnantC; otherwise RemnantC 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.

RemnantC 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 RemnantC, and thus represents an additional clinically valuable marker. RemnantC, measured or calculated, differs from non-HDLC in that non-HDLC contains RemnantC plus LDLC and does not differentiate between these two causal risk factors [1]. Non-HDLC also includes the cholesterol of lipoprotein(a) [Lp(a)].

Lipoprotein(a) particles

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): serum 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 is recognized by the codes of International Classification of Diseases for elevated Lp(a) and family history of elevated Lp(a), introduced in response to the US National Heart, Lung, and Blood Institute recommendation [25]. High Lp(a) concentration through a correspondingly low number of KIV-2 repeats are also associated with a higher 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 [27,28]. 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 [29].

Recommendation – I.

- Comprehensive testing of atherogenic lipoproteins should use a biomarker, or a panel of multiple markers, to assess the risk of ASCVD 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 [30]. A cost-efficient approach is to employ measurements of three markers (TC, TG, HDLC) and from these calculate LDLC and non-HDLC. RemnantC 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 [31,32]. In patients with mild-to-moderate

hypertriglyceridemia, defined as 2-10 mmol/L [33], 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) measurement should be considered at least once in each adult person's lifetime to identify those with high inherited Lp(a) concentrations, in particular among patients 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 [34].

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 RemnantC. A 'minimal lipid profile' including only TC and TG can be considered in countries where costs are a major issue such as developing countries [35].
- An 'expanded lipid profile' including Lp(a) or apoB should be used in selected cases (Table 4) [35]. '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 (8-12 hours) 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 RemnantC, 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 simplification without significant negative implications for prognostic, diagnostic, and therapeutic options for ASCVD prevention [36]. Regardless, it may remain prudent to counsel the patients to avoid an extremely high-fat or fast-food meal (e.g. a burger, fries) in the preceding 12 hours [37].

Nonfasting lipid profiles are now endorsed by several guidelines including those in Europe, the UK, Canada, Brazil, and the US [4-6,35,38]. 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], and for the phenotypic diagnosis or therapeutic follow-up of mixed dyslipidemia or isolated hypertriglyceridemia; 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, or markers with circadian rhythm) are requested [2]. The EFLM Preanalytical Phase Working Group recently produced guidance on how to standardize fasting blood sampling if needed [37,39].

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 US Centers of Disease Control (CDC) Reference Method for LDLC, combines ultracentrifugation to remove VLDL and chylomicrons and heparin-Mn²⁺ precipitation to separate LDL particles, including Lp(a), from HDL [40]. 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 [40]. 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 [41].

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 [40,41]. 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 [41]. 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 [41]. Direct measurements of dLDLC and dHDLC in normolipidemic samples usually meet the National Cholesterol Education

Program (NCEP) total error goals of $\leq 12\%$ and $\leq 13\%$, 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 [42]. Most discordances in dyslipidemic samples are observed at lower concentration ranges of LDLC (<1.8 mmol/L) and HDLC (<1.0 mmol/L) [42]. 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 surveys of hypertriglyceridemic samples organized across different laboratories [43]. The biases noted in dHDLC measurements affect the calculations of LDLC and non-HDL, since HDLC is used in the calculations [43].

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 the cholesterol in LDL or HDL fractions when atypical lipoproteins are present. The different manufacturers' direct methods do not measure the same LDL and HDL subfractions [40,41]. This non-selectivity error is of major concern in the contemporary treatment era in which very low LDLC concentrations <1.8 mmol/L are increasingly seen with highly efficacious LDL-lowering therapies, and in which hypertriglyceridemic samples – TG >2 mmol/L seen in $\sim 25\%$ of individuals in the general population (Tables 2-3) [17] – cause a greater analytical 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 [44]. 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 [44]. The equation is increasingly inaccurate at TG concentrations from 2.3 to 4.5 mmol/l [44]. **The error is regarded as unacceptably large when TG are ≥ 4.5 mmol/L by NCEP and EAS guidelines or already at ≥ 4.0 mmol/L by national consensus in certain**

countries, 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.

At very low LDLC concentration ranges <1.8 mmol/L, in which VLDLC constitutes a relatively larger fraction of the blood cholesterol, VLDLC overestimation at high TG concentration introduces a more significant error in the calculation of cLDLC [45]. The resulting underestimation of LDLC may translate to downward misclassification when using guideline-recommended 1.8 mmol/l or 1.4 mmol/l thresholds for patients at high and very high cardiovascular risk, respectively [4]. This may erroneously exclude >20% of patients for initiation or intensification of lipid-lowering therapy [45].

A modified equation, Martin-Hopkins $cLDLC = TC - HDLC - TG/adjustable\ factor$, which adjusts the TG/VLDLC ratio dynamically for concentrations of TG and non-HDLc has been developed [46]. Rather than dividing TG by a fixed factor of 5, the Martin-Hopkins equation requires the use of a 180-cell table to match each patient's TG and non-HDLc with 1 of 180 different factors ranging from 3.1 to 9.5, to give a more personalized estimation of VLDLC in mg/dL [46]. These personalized TG/VLDLC ratios were determined by direct comparison of TG to VLDLC concentrations directly measured after lipoprotein separation by ultracentrifugation in >10⁶ US individuals from the Very Large Database of Lipids Study [46]. In this database, TG and non-HDLc are the two variables that explained most of the variance in TG/VLDLC [46].

The novel Martin-Hopkins formula improves the accuracy of cLDLC at various conditions including very low LDLC <1.8 mmol/L and in nonfasting samples [47,48]. We recommend to use it preferably in the range of TG concentrations 2.0-4.5 mmol/L wherein the Friedewald equation is less accurate [47]. However, it is not trivial to install the complex 180-cell approach of this formula into automated laboratory information systems. A smartphone application ("LDL Cholesterol Calculator") has been developed to provide immediate and automated calculation of Martin-Hopkins cLDLC by simple input of a patient's TC, HDLC, and TG data from the standard lipid profile [49].

Direct LDLC assays should always be used when TG concentration is ≥ 4.5 mmol/L, which is the limit of use of Friedewald and Martin-Hopkins equations [50], although above this concentration the direct assays may also show discordances with the CDC Reference Method and will not necessarily result in accurate LDLC measurement in every patient. Even in normotriglyceridemic samples and in

samples with moderately elevated TG concentrations 2.0–4.5 mmol/L, dLDLC may not always agree with Friedewald cLDLC and translate to discrepant risk classifications as observed in fasting as well as nonfasting individuals [51]. It should be noted that most clinical trials demonstrating the evidence base for clinical benefit of LDLC lowering have used Friedewald cLDLC; thus it cannot be excluded that dLDLC rather than cLDLC is the method that misclassifies risk [1]. However, the fact that cLDLC calculated by either Friedewald or Martin-Hopkins equation depends upon three laboratory assays, that is, TG, TC, and dHDL, means that three measurement errors are involved which inevitably introduce calculation variability (Table 5).

Effect of Lp(a)-cholesterol on LDLC

The cLDLC equations and also most dLDLC assays include the cholesterol content of Lp(a) [52]. 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 [52]; 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:

$$\text{Lp(a)-corrected LDLC (mg/dL)} = \text{LDLC (mg/dL)} - [\text{Lp(a) (mg/dL)} \times 0.30]$$

$$\text{Lp(a)-corrected LDLC (mmol/L)} = \text{LDLC (mmol/L)} - [\text{Lp(a) (mg/dL)} \times 0.0078]$$

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) [53].

Recommendation – IV.

- The Martin-Hopkins modified equation may be preferable for calculation of cLDLC, most importantly in patients with low LDLC concentration <1.8 mmol/L and/or TG concentrations 2.0–4.5 mmol/L, and in nonfasting samples [6].
- Direct LDLC assays should be used for calculation of RemnantC and for assessment of LDLC when TG concentration is ≥ 4.5 mmol/L (or ≥ 4.0 mmol/L by national consensus in certain countries). However, the direct assays will not necessarily result in more accurate LDLC assessment in every patient.
- Lp(a)-cholesterol correction of measured or calculated LDLC should be applied in patients with known or suspected high Lp(a) concentration, or if the patient shows a poor response to LDL-lowering therapy.

V. LDLC test errors: are they clinically relevant?

The ranges of uncertainty across different LDLC methods are not negligible (Table 5). 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 (Table 6). Depending on the method 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 [41]. The EFLM Biological Variation Working Group recently revised biological variation data [54,55].

Recommendation – V.

- 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 misinterpretation of treatment effect.
- 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. In meta-analyses of associations of lipid tests with outcomes, it is critical to verify the comparability of quantitative data of the assays used in each trial.
- A patient's LDLC value 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.

VI. 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, that is, LDL, VLDL, IDL, and Lp(a); in the nonfasting state this additionally includes the cholesterol in chylomicrons and their remnant particles [1]. Non-HDLC provides a more comprehensive risk assessment than LDLC in certain individuals with hypertriglyceridemia because it adds RemnantC to LDLC and, therefore, takes into account the atherogenic potential of remnant lipoproteins [17]. However, non-HDLC cannot substitute for RemnantC because it does not differentiate between RemnantC and LDLC and Lp(a)-cholesterol. Some individuals with high RemnantC have low LDLC and thus will have relatively low non-HDLC if Lp(a) is also low, and if interpreting non-HDLC instead of RemnantC, the high RemnantC will be masked in these individuals [30].

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 [56,57]. Guideline-recommended therapeutic goals for non-HDLC are arbitrarily set typically at 0.8 mmol/L (30 mg/dL) higher than LDLC goals (Table 6); this value is based on the assumption that the “optimal” VLDLC 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, **which is the limitation for calculation of cLDLC** [44]. However, 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. Despite this limitation, 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 dHDLC in the calculation [58]. **Non-HDLC shows better correlation and concordance with cLDLC when using the Martin-Hopkins formula compared to the Friedewald equation in patients with atherogenic dyslipidemia [59]. When compared to Martin-**

Hopkins 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 Martin-Hopkins cLDLC and, as a consequence, risk underestimation is still common (80-90%) in patients with TG \geq 4.5 mmol/L [60].

Apolipoprotein B

In contrast to the heterogeneous LDLc fraction, 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]. ApoB100 contains the ligand that binds to the LDL receptor. 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 quantification by automated immunoassays such as immunonephelometry and immunoturbidimetry can be easily implemented in the clinical laboratories. These immunoassays obviate the need for lipoprotein subfractionation techniques such as nuclear magnetic resonance (NMR) spectroscopy or ion mobility to quantify LDLP and have been shown to be at least equivalent to LDLP in predicting cardiovascular risk [61,62]. However, apoB cannot substitute for NMR- or ion mobility-based particle size measurements and does not differentiate between LDL and VLDL particle numbers [61,62]. A major impediment to LDLP testing is its limited availability in the clinical laboratories, higher cost, and lack of standardization [63], although it does provide additional information on other lipoproteins VLDL and HDL, beyond LDLP.

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 when cross-reactivity of immunoassays to apoB48 occurs in nonfasting measurements, apoB quantification is 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 nephelometric and turbidimetric apoB measurements are limited to TG <10 mmol/L due to interference of light scattering

or absorption caused by chylomicrons and large VLDLs; a TG concentration above this limit in nonfasting blood samples is only seen in ~0.1% of individuals in the general population [17]. Nevertheless, it is cautious to consider fasting apoB measurement when TG concentration in the nonfasting sample is ≥ 4.5 mmol/L [2].

The International Federation of Clinical Chemistry (IFCC) and World Health Organization (WHO) developed the SP3 reference material, value-assigned using immunonephelometry as interim reference method for apoB [64]. Common calibration with the IFCC/WHO SP3 reference material has reduced between-laboratory variability of apoB measurements from $>19\%$ to $<10\%$, although concerns about the variability among immunoassays and their comparability with apoB100 derived from NMR and other methods still exist [65-67]. Liquid chromatography tandem-mass spectrometry (LC-MSMS)-based quantification of apolipoproteins has the potential to further improve apoB100 standardization and between-method comparability [67]. The IFCC has therefore initiated development of LC-MSMS as candidate definitive reference method for apoB [67]. Another advantage of LC-MSMS is that it enables the simultaneous (multiplexed) measurement of multiple apolipoproteins in addition to apoB100 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 for comprehensive characterization of dyslipidemias [68]. **Although nowadays throughput of LC-MSMS is low compared to immunoassays, automated LC-MSMS systems are developed and will become available for implementation in high-throughput clinical laboratories.**

Given the improvements of standardization initiated by the IFCC [69], measurements of apolipoproteins have the potential to meet analytical performance criteria including accuracy, harmonization across laboratories, unambiguous definition of the measurand, and unequivocal test results in both normo- and dyslipidemic sera – important prerequisites for medical use of a test which can not be met with LDLC and non-HDLC measurements or calculations.

Recommendation – VI.

- **ApoB measurement is superior to LDLC and non-HDLC measurements and calculations for the assessment of exposure to atherogenic lipoprotein particle numbers in the circulation.**

- ApoB is recommended for risk assessment and may be preferred over non-HDLc, if available, in persons with mild-to-moderate hypertriglyceridemia (2-10 mmol/L), diabetes, obesity or metabolic syndrome, or very low LDLc <1.8 mmol/L [4].
- Like non-HDLc, apoB can always be measured in the nonfasting state and is not affected by biological TG variability.

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

Although the traditional lipid profile of TC, TG, HDLc, and LDLc remains essential for dyslipidemia diagnosis and ASCVD risk categorization, the position of LDLc as treatment target is challenged by the analytical performance of apoB. However, the apoB test has not yet been completely validated for this clinical purpose according to key criteria defined by the EFLM Test Evaluation Working Group [70] – clinical performance, clinical effectiveness, and cost-effectiveness – beyond analytical performance to become a medically useful test (Table 7).

Clinical performance – risk estimation

Data and meta-analyses of prospective population-based cohort studies [71-74] and statin trials [75,76] suggest that the clinical performance of apoB and non-HDLc, although superior to LDLc in some studies, is on average comparable to dLDLc, Friedewald cLDLc or Martin-Hopkins cLDLc to predict risk of fatal or nonfatal ASCVD on the population level. Risk associations are similar in nonfasting study populations than in those who did fast [72,74].

For a majority of patients in whom apoB tests correlate with LDLc, measurement of traditional lipids should suffice to estimate risk. However, in ~20% of individuals in whom apoB is discordantly high with respect to population percentiles of LDLc, cardiovascular risk tracks with apoB, suggesting that replacing LDLc by apoB would identify more individuals with increased risk of ASCVD [74] – this implies an overall better clinical performance compared to LDLc in particular among this smaller subset of individuals with discordant apoB.

Clinical effectiveness – risk reduction

The substantial residual risk that persists in LDLC-targeted therapies even at LDLC <1.8 mmol/L has fueled the debate about considering using apoB rather than LDLC as treatment target. Meta-analyses of lipid-lowering trials showed that statins and other therapies which clear apoB-containing lipoproteins by upregulating LDL receptor expression, such as PCSK9 inhibitors, reduce cardiovascular risk proportional to the decrease in apoB concentration observed in these trials [77,78]. Furthermore, Mendelian randomization studies demonstrated that the reduction in cardiovascular risk associated with genetic variation in clearance and processing of apoB-containing lipoproteins, including LDL particles as well as TG-rich VLDL particles and their remnants, was correlated with the change of concentration of these particles as measured by apoB rather than the cholesterol mass carried by those particles as measured by LDLC [79,80]. These findings suggest a potential role of apoB as therapeutic target.

Cost-effectiveness – health economic benefit

Implementation of apoB assays in follow-up of lipid-lowering therapies would impose healthcare systems and patients with yearly extra cost, although one might consider replacing the standard lipid profile (needed for calculation of LDLC and non-HDL) by single follow-up measurement of apoB in order to attenuate the rise of expenses. The suggested use of apoB would be cost-effective if these tests guide therapy to reduce the healthcare costs of ASCVD to a greater extent than standard therapy guided by LDLC, yet the evidence base of this approach is still incomplete (Table 7). To evaluate cost-effectiveness of apoB-guided treatment, randomized trials should be designed in which patients are randomized to receive the apoB test vs. the standard LDLC test and the health economic outcome(s) of identifying and treating more patients at high risk (i.e., not identified or treated when using the standard test) is assessed [1].

Recommendation – VII.

- At this point of time, there is insufficient evidence of benefit from outcome studies to support the option to replace the standard lipid profile (with calculation of cLDLC and non-HDL) by single follow-up 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 in patients at high cardiovascular risk or 1.4 mmol/L in patients at very high risk (or by $\geq 50\%$ if these targets cannot be attained) is of critical importance [4].

VIII. 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 RemnantC [81-83]. LDLc does not provide incremental risk prediction of ASCVD relative to non-HDLc [81-83]. The only concern is the threshold concentration selected 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 clinical performance studies, to evaluate which values most accurately classify patients within risk categories [70].

For the present purposes, the combination of non-HDLc with Martin-Hopkins cLDLc may be considered as an appropriate strategy to guide therapy and they can be calculated from the standard lipid profile [1]. This may compensate for the under- or overestimation of LDLc in terms of clinical decision

making, given the uncertainty of dLDLC measurements or the Friedewald calculation in dyslipidemic samples when LDLC lowering approaches 1.4 mmol/L, and non-HDLc can be used 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 [1].

Apolipoprotein B

Data from concordance/discordance analyses in large case-control and prospective cohort studies 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 [74,84-86]. This is 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 “optimal” 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 [87-89]. 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 [62,90].

In patients with a moderate estimated risk score, in particular those with additional metabolic risk factors, apoB (or LDLP) measurement as a “risk-enhancing factor” could be useful [6]. Presence of risk-enhancing factors including apoB can tip the balance toward earlier initiation of drug treatment in shared decision making between clinician and patient, especially in primary prevention if goals cannot be achieved with lifestyle advice [6].

Secondary treatment target: non-HDLc or apoB?

Guidelines propose using non-HDLc or apoB as a secondary treatment target in the management of patients with mild-to-moderate hypertriglyceridemia (2-10 mmol/L), including patients with diabetes [4,5]. 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-HDL-C and apoB goals and lower risk of ASCVD, with no attenuation of benefit at lower concentrations [88].

Which to choose as secondary target: non-HDL-C or apoB? Although apoB demonstrates competitive clinical performance compared with non-HDL-C, 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 LDL-C [1]. For now and until this issue is clarified, non-HDL-C is an acceptable choice and it can be used without the additional expense of extra measurement.

Recommendation – VIII.

- Use of non-HDL-C or apoB should be considered as an index of the efficacy of treatment targeted at LDL-C. For the present purpose, every lipid profile report should automatically add non-HDL-C. To improve patient comfort and compliance, there are practical advantages of this approach for follow-up without the need to fast [2].
- At TG concentration ≥ 4.5 mmol/L, a condition wherein use of Friedewald cLDL-C or Martin-Hopkins cLDL-C is not recommended and also dLDL-C is likely to be inaccurate, use of non-HDL-C calculation may be considered instead of dLDL-C measurement to evaluate therapeutic response.

IX. 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 8). 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 [91]. 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 thresholds for RemnantC and, consequently, also non-HDLc may be considered as an option (Table 8). 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 6) [4]. 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 the threshold for low risk only (3 mmol/L), 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 assumed to be universally applicable [1]. Guideline-recommended LDLc thresholds are based on observations with Friedewald cLDLc using the older HDLc precipitation methods which differ from dHDLc assays used nowadays. This situation is challenging for new generation assays to meet regulations for in vitro diagnostic medical devices, requiring evidence of clinical performance of the assays [70].

Reference limits

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 and far above the thresholds of increased ASCVD risk (Tables 2-3, Figure 2). Therefore, flagging of lipid profiles in adults should not be based on reference limits [2]. In a pediatric setting, reporting age- and gender-specific reference intervals is relevant for early identification of children with hyperlipidemia associated with premature atherosclerosis, especially FH [32].

Reference nonfasting concentrations in adults from the Copenhagen General Population Study are reported in Tables 2-3. Reference intervals in children and adolescents are available from recent population-based cohort studies and databases [92-94]. **Countries are encouraged to establish reference intervals in their local populations, to account for differences in lifestyle and risk factors in different European regions. Ideally, these reference intervals should be regularly updated because significant changes in population lipid concentrations occur mainly related to the substantial increase in unhealthy lifestyle and obesity and changes of analytical methods over time [95-97]. In the adoption of published reference concentrations with known biological or analytical sources of variation, the clinical laboratory should verify the reference interval with its own analytical method on 20 samples drawn from its local population under similar pre-analytical conditions [96,97]. If ≤ 2 of 20 values ($\leq 10\%$) fall outside the reference interval then the interval can be adopted [96,97]. For decision thresholds it is unnecessary to validate them in the laboratory [97].**

Alert values

Using decision thresholds for flagging will lead to many lipid profiles reported with flags, as with more than 50% of LDLC results (Figure 2). There is a risk that flagging too many results will make the physician ignore very high concentrations and may distract from the severe dyslipidemias, especially when used for screening in primary care. We have considered this by defining alarming values for extreme dyslipidemias, where we recommend special notification and reaction from the laboratory [2].

Extremely abnormal test results should be flagged with special alert notifications to quickly initiate further diagnostic and possibly therapeutic actions by the clinician (Table 8). 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 [33]. 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 [31,32]. In patients with mixed hyperlipidemias, routine genetic testing is not warranted [33]. 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 [98].

Recommendation – IX.

- Flagging of lipid profiles on laboratory reports should always be based on decision thresholds. In children, reporting of reference intervals 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 [1,2]. These recommendations take into account the strengths 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 [70].

Calculation of non-HDL-C 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 LDL-C 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 LDL-C-centered approach.

Diabetes and abdominal obesity, disorders that underlie the clinical expression of complex dyslipidemias without elevated LDL-C, 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 [62,63], and multiplexed LC-MS/MS apolipoprotein profiles [68], which have the potential to become widely available medical tests [99]. 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 [99].

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 drafts of the joint consensus statements.

This summary embodies the consensus-based recommendations produced by the Panel [1,2] 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.

A first version of the document underwent public consultation by the EFLM National Societies. Comments were received from 11 Societies and have been taken into account during the revision of this document. The revised version has been sent for final voting to all 40 National Societies.

Financial support

Supported by unrestricted educational grants to EAS and EFLM from Merck, Roche Diagnostics, and Denka Seiken. These companies were not present at the Joint Consensus Panel meetings, had no role in the design or content of the joint consensus statement and had no right to approve or disapprove of the final document.

Acknowledgements

We thank Ms. Jane Stock for coordination of the joint EAS-EFLM Panel meetings. We greatly appreciate the comments and suggestions received from EFLM National Societies and their assigned reviewers.

References

1. Langlois MR, Chapman MJ, Cobbaert C, Mora S, Remaley AT, Ros E, et al. European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Joint Consensus Initiative. Quantifying Atherogenic Lipoproteins: Current and Future Challenges in the Era of Personalized Medicine and very low concentrations of LDL Cholesterol. A Consensus Statement from EAS and EFLM. *Clin Chem* 2018;64:1006-33.
2. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, et al. European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Consensus Panel. Fasting is not routinely required for a lipid profile: Clinical and laboratory implications including flagging at desirable concentration cut-points – a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. *Eur Heart J* 2016;37:1944-58.
3. Langsted A, Kamstrup PR, Nordestgaard BG. High lipoprotein(a) and high risk of mortality. *Eur Heart J* 2019;40:2760-70.
4. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. The Task Force for the Management of Dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS). *Eur Heart J* 2019 doi: 10.1093/eurheartj/ehz455 [Epub ahead of print].
5. Anderson TJ, Grégoire J, Pearson GJ, Barry AR, Couture P, Dawes M, et al. 2016 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in the Adult. *Can J Cardiol* 2016;32:1263-82.
6. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol* 2019;73:e285-e350.
7. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J* 2017;38:2459-72.
8. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, et al. Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170 000 participants in 26 randomised trials. *Lancet* 2010;376:1670–81.
9. Mudd JO, Borlaug BA, Johnston PV, Kral BG, Rouf R, Blumenthal RS, Kwiterovich PO Jr. Beyond low-density lipoprotein cholesterol: defining the role of low-density lipoprotein heterogeneity in coronary artery disease. *J Am Coll Cardiol* 2007;50:1735-41.
10. Mora S. Advanced lipoprotein testing and subfractionation are not (yet) ready for routine clinical use. *Circulation* 2009;119:2396-404.
11. Miller M, Stone J, Ballantyne C, Bittner V, Criqui MH, Ginsberg HN, et al. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. *Circulation* 2011;123:2292-333.
12. Contois JH, McConnell JP, Sethi AA, Csako G, Devaraj S, Hoefner DM, et al. Apolipoprotein B and cardiovascular disease risk: position statement from the AACC Lipoproteins and Vascular Disease Working Group on Best Practices. *Clin Chem* 2009;55:407-19.
13. Chapman MJ, Ginsberg HN, Amarenco P, Andreotti F, Boren J, Catapano AL, et al. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. *Eur Heart J* 2011;32:1345-61.
14. Nordestgaard BG, Varbo A. Triglycerides and cardiovascular disease. *Lancet* 2014;384:626–35.
15. Mora S, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. *Circulation* 2008;118:993–1001.

16. Nordestgaard BG. A Test in Context: Lipid Profile, Fasting versus Non-Fasting. *J Am Coll Cardiol* 2017;70:1637-46.
17. Nordestgaard BG. Triglyceride-rich lipoproteins and atherosclerotic cardiovascular disease: new insights from epidemiology, genetics, and biology. *Circ Res* 2016;118:547-63.
18. Thomsen M, Varbo A, Tybjaerg-Hansen A, Nordestgaard BG. Low nonfasting triglycerides and reduced all-cause mortality: a mendelian randomization study. *Clin Chem* 2014;60:737-46.
19. Varbo A, Nordestgaard BG. Remnant lipoproteins. *Curr Opin Lipidol* 2017;28:300-7.
20. Varbo A, Freiberg JJ, Nordestgaard BG. Remnant Cholesterol and Myocardial Infarction in Normal Weight, Overweight, and Obese Individuals from the Copenhagen General Population Study. *Clin Chem* 2018;64:219-30.
21. Kronenberg F, Utermann G. Lipoprotein(a): resurrected by genetics. *J Intern Med* 2013;273:6-30.
22. Nordestgaard BG, Chapman MJ, Ray K, Boren J, Andreotti F, Watts GF, et al. Lipoprotein(a) as a cardiovascular risk factor: current status. *Eur Heart J* 2010;31:2844-53.
23. Nordestgaard BG, Langsted A. Lipoprotein(a) as a cause of cardiovascular disease: insights from epidemiology, genetics, and biology. *J Lipid Res* 2016;57:1953-75.
24. Willeit P, Ridker PM, Nestel PJ, Simes J, Tonkin AM, Pedersen TR, et al. Baseline and on-statin treatment lipoprotein(a) levels for prediction of cardiovascular events: individual patient-data meta-analysis of statin outcome trials. *Lancet* 2018;392:1311-20.
25. Tsimikas S, Fazio S, Ferdinand KC, Ginsberg HN, Koschinsky ML, Marcovina SM, et al. NHLBI Working Group Recommendations to Reduce Lipoprotein(a)-Mediated Risk of Cardiovascular Disease and Aortic Stenosis. *J Am Coll Cardiol* 2018;71:177-92.
26. Tsimikas S. A Test in Context: Lipoprotein(a): Diagnosis, Prognosis, Controversies, and Emerging Therapies. *J Am Coll Cardiol* 2017;69:692-711.
27. O'Donoghue ML, Fazio S, Guigliano RP, Stroes ESG, Kanevsky E, Gouni-Berthold I, et al. Lipoprotein(a), PCSK9 inhibition and cardiovascular risk: insights from the FOURIER Trial. *Circulation* 2019;139:1483-92.
28. Ray KK, Vallejo-Vaz AJ, Ginsberg HN, Davidson MH, Louie MJ, Bujas-Bobanovic M, et al. Lipoprotein(a) reductions from PCSK9 inhibition and major adverse cardiovascular events: Pooled analysis of alirocumab phase 3 trials. *Atherosclerosis* 2019 doi: 10.1016/j.atherosclerosis.2019.06.896. [Epub ahead of print].
29. Viney NJ, van Capelleveen JC, Geary RS, Xia S, Tami JA, Yu RZ et al. Antisense oligonucleotides targeting apolipoprotein(a) in people with raised lipoprotein(a): two randomised, double-blind, placebo-controlled, dose-ranging trials. *Lancet* 2016;388:2239-53.
30. Langlois MR, Nordestgaard BG. Which lipids should be analyzed for diagnostic workup and follow-up of patients with hyperlipidemias? *Curr Cardiol Rep* 2018;20:88.
31. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. European Atherosclerosis Society Consensus Panel. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J* 2013;34:3478-90a.
32. Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. European Atherosclerosis Society Consensus Panel. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. *Eur Heart J* 2015;36:2425-37.
33. Hegele RA, Ginsberg HN, Chapman MJ, Nordestgaard BG, Kuivenhoven JA, Averna M, et al. European Atherosclerosis Society Consensus Panel. The polygenic nature of hypertriglyceridaemia: implications for definition, diagnosis, and management. *Lancet Diabetes Endocrinol* 2014;2:655-66.
34. Langsted A, Kamstrup PR, Nordestgaard BG. Lipoprotein(a): fasting and nonfasting levels, inflammation, and cardiovascular risk. *Atherosclerosis* 2014;234:95-101.
35. Langsted A, Nordestgaard BG. Nonfasting versus fasting lipid profile for cardiovascular risk prediction. *Pathology* 2019;51:131-41.
36. Mora S, Chang CL, Moorthy MV, Sever PS. Association of Nonfasting vs Fasting Lipid Levels With Risk of Major Coronary Events in the Anglo-Scandinavian Cardiac Outcomes Trial-Lipid Lowering Arm. *JAMA Intern Med* 2019;179:898-905.

37. Simundic AM, Cornes M, Grankvist K, Lippi G, Nybo M. Standardization of collection requirements for fasting samples: for the Working Group on Preanalytical Phase (WG-PA) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM). *Clin Chim Acta* 2014;432:33-7.
38. National Institute for Health and Care Excellence (NICE). NICE Guidance. Cardiovascular Disease: Risk Assessment and Reduction, Including Lipid Modification. NICE CG181. Updated 2016. Available at <https://www.nice.org.uk/guidance/cg181>. Accessed 30 January 2019.
39. Simundic AM, Bölenius K, Cadamuro J, Church S, Cornes MP, van Dongen-Lases EC, et al. Joint EFLM-COLABIOCLI Recommendation for venous blood sampling. *Clin Chem Lab Med* 2018;56:2015-2038.
40. Ramasamy I. Update on the laboratory investigation of dyslipidemias. *Clin Chim Acta* 2018;479:103-25.
41. Contois JH, Warnick GR, Sniderman AD. Reliability of low-density lipoprotein cholesterol, non-high density lipoprotein cholesterol, and apolipoprotein B measurement. *J Clin Lipidol* 2011;5:264-72.
42. Miller WG, Myers GL, Sakurabayashi I, Bachmann LM, Caudill SP, Dziekonski A, et al. Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. *Clin Chem* 2010;56:977-86.
43. Langlois MR, Descamps OS, van der Laarse A, Weykamp C, Baum H, Pulkki K, et al. Clinical impact of direct HDLc and LDLc method bias in hypertriglyceridemia. A simulation study of the EAS-EFLM Collaborative Project Group. *Atherosclerosis* 2014;233:83-90.
44. Nauck M, Warnick GR, Rifai N. Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation. *Clin Chem* 2002;48:236-25.
45. Martin SS, Blaha MJ, Elshazly MB, Brinton EA, Toth PP, McEvoy JW, et al. Friedewald-estimated versus directly measured low-density lipoprotein cholesterol and treatment implications. *J Am Coll Cardiol* 2013;62:732-9.
46. Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, Jones SR. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *JAMA* 2013;310:2061-8.
47. Sathiyakumar V, Park J, Golozar A, Lazo M, Quispe R, Guallar E, et al. Fasting Versus Nonfasting and Low-Density Lipoprotein Cholesterol Accuracy. *Circulation* 2018;137:10-19.
48. Martin SS, Giugliano RP, Murphy SA, Wasserman SM, Stein EA, Ceška R, et al. Comparison of Low-Density Lipoprotein Cholesterol Assessment by Martin/Hopkins Estimation, Friedewald Estimation, and Preparative Ultracentrifugation: Insights From the FOURIER Trial. *JAMA Cardiol* 2018;3:749-53.
49. Johns Hopkins Medicine. Johns Hopkins medical apps. LDL Cholesterol Calculator. Available at <https://www.hopkinsmedicine.org/apps/all-apps/ldl-cholesterol-calculator>. Accessed 26 August 2019.
50. Palmer MK, Barter PJ, Lundman P, Nicholls SJ, Toth PP, Karlson BW. Comparing a novel equation for calculating low-density lipoprotein cholesterol with the Friedewald equation: A VOYAGER analysis. *Clin Biochem* 2019;64:24-9.
51. Mora S, Rifai N, Buring JE, Ridker PM. Comparison of LDL cholesterol concentrations by Friedewald calculation and direct measurement in relation to cardiovascular events in 27,331 women. *Clin Chem* 2009;55:888-94.
52. Yeang C, Witztum JL, Tsimikas S. 'LDLC' = LDL-C + Lp(a)-C: implications of achieved ultra-low LDL-C levels in the proprotein convertase subtilisin/kexin type 9 era of potent LDL-C lowering. *Curr Opin Lipidol* 2015;26:169-78.
53. Tsimikas S, Fazio S, Viney NJ, Xia S, Witztum JL, Marcovina SM. Relationship of lipoprotein(a) molar concentrations and mass according to lipoprotein(a) thresholds and apolipoprotein(a) isoform size. *J Clin Lipidol* 2018;12:1313-23.
54. Aarsand AK, Díaz-Garzón J, Fernandez-Calle P, Guerra E, Locatelli M, Bartlett WA, et al. European Federation of Clinical Chemistry and Laboratory Medicine Working Group on Biological Variation. The EuBIVAS: Within- and Between-Subject Biological Variation Data for Electrolytes,

- Lipids, Urea, Uric Acid, Total Protein, Total Bilirubin, Direct Bilirubin, and Glucose. *Clin Chem* 2018;64:1380-1393.
55. Díaz-Garzón J, Fernández-Calle P, Minchinela J, Aarsand AK, Bartlett WA, Aslan B, et al. Biological variation data for lipid cardiovascular risk assessment biomarkers. A systematic review applying the biological variation data critical appraisal checklist (BIVAC). *Clin Chim Acta* 2019;495:467-75.
 56. Robinson JG, Wang S, Smith BJ, Jacobson TA. Meta-analysis of the relationship between non-high-density lipoprotein cholesterol reduction and coronary heart disease. *J Am Coll Cardiol* 2009;53:316-22.
 57. Silverman MG, Ference BA, Im K, Wiviott SD, Giugliano RP, Grundy SM, et al. Association Between Lowering LDL-C and Cardiovascular Risk Reduction Among Different Therapeutic Interventions: A Systematic Review and Meta-analysis. *JAMA* 2016;316:1289-97.
 58. van Deventer HE, Miller WG, Myers GL, Sakurabayashi I, Bachmann LM, Caudill SP, et al. Non-HDL-cholesterol shows improved accuracy for cardiovascular risk score classification compared to direct or calculated LDL cholesterol in a dyslipidemic population. *Clin Chem* 2011;57:490-501.
 59. Mehta R, Reyes-Rodríguez E, Bello-Chavolla OY, Guerrero-Díaz AC, Vargas-Vázquez A, Cruz-Bautista I, Aguilar-Salinas CA. Performance of LDL-C calculated with Martin's formula compared to the Friedewald equation in familial combined hyperlipidemia. *Atherosclerosis* 2018;277:204-10.
 60. Sathiyakumar V, Park J, Quispe R, Elshazy MB, Michos ED, Banach M, et al. Impact of novel low-density lipoprotein-cholesterol assessment on the utility of secondary non-high-density lipoprotein-cholesterol and apolipoprotein B targets in selected worldwide dyslipidemia guidelines. *Circulation* 2018;138:244-54.
 61. Cole TG, Contois JH, Csako C, McConnell JP, Remaley AT, Devaraj S, et al. Association of apolipoprotein B and nuclear magnetic resonance spectroscopy-derived LDL particle number with outcomes in 25 clinical studies: assessment by the AACC Lipoprotein and Vascular Diseases Division Working Group on Best Practices. *Clin Chem* 2013;59:752-70.
 62. Mora S, Caulfield MP, Wohlgemuth J, Chen Z, Superko HR, Rowland CM, et al. Atherogenic Lipoprotein Subfractions Determined by Ion Mobility and First Cardiovascular Events After Random Allocation to High-Intensity Statin or Placebo. The Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) Trial. *Circulation* 2015;132:2220-2229.
 63. Clouet-Foraison N, Gaie-Levrel F, Gillery P, Delatour V. Advanced lipoprotein testing for cardiovascular diseases risk assessment: a review of the novel approaches in lipoprotein profiling. *Clin Chem Lab Med* 2017;55:1453-64.
 64. Marcovina SM, Albers JJ, Kennedy H, Mei JV, Henderson LO, Hannon WH. International Federation of Clinical Chemistry standardization project for measurements of apolipoproteins A-I and B. IV. Comparability of apolipoprotein B values by use of International Reference Material. *Clin Chem* 1994;40:586-92.
 65. Cao J, Steffen BT, Guan W, Remaley AT, McConnell JP, Palamalai V, Tsai MY. A comparison of three apolipoprotein B methods and their associations with incident coronary heart disease risk over a 12-year follow-up period: The Multi-Ethnic Study of Atherosclerosis. *J Clin Lipidol* 2018;12:300-4.
 66. Grundy SM, Vega GL, Tomassini JE, Tershakovec AM. Comparisons of apolipoprotein B levels estimated by immunoassay, nuclear magnetic resonance, vertical auto profile, and non-high-density lipoprotein cholesterol in subjects with hypertriglyceridemia (SAFARI Trial). *Am J Cardiol* 2011;108:40-6.
 67. Delatour V, Clouet-Foraison N, Gaie-Levrel F, Marcovina SM, Hoofnagle AN, Kuklennyik Z, et al. Comparability of Lipoprotein Particle Number Concentrations Across ES-DMA, NMR, LC-MS/MS, Immunonephelometry, and VAP: In Search of a Candidate Reference Measurement Procedure for apoB and non-HDL-P Standardization. *Clin Chem* 2018;64:1485-1495.
 68. van den Broek I, Romijn FP, Nouta J, van der Laarse A, Drijfhout JW, Smit NP, et al. Automated multiplex LC-MS/MS assay for quantifying serum apolipoproteins A-I, B, C-I, C-II, C-III, and E with qualitative apolipoprotein E phenotyping. *Clin Chem* 2016;62:188-197.
 69. Contois JH, Delatour V. Apolipoprotein B measurement: Need for standardization. *J Clin Lipidol* 2018;12:264-5.

70. Horvath AR, Lord SJ, St John A, Sandberg S, Cobbaert CM, Lorenz S, et al. From biomarkers to medical tests: the changing landscape of test evaluation. *Clin Chim Acta* 2014;427:49–57.
71. Sniderman AD, Williams K, Contois JH, Monroe HM, McQueen MJ, de Graaf J, Furberg CD. A meta-analysis of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B as markers of cardiovascular risk. *Circ Cardiovasc Qual Outcomes* 2011;4:337-45.
72. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, et al. Emerging Risk Factors Collaboration. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* 2009;302:1993-2000.
73. Di Angelantonio E, Gao P, Pennells L, Kaptoge S, Caslake M, Thompson A, et al. Emerging Risk Factors Collaboration. Lipid-related markers and cardiovascular disease prediction. *JAMA* 2012;307:2499-506.
74. Welsh C, Celis-Morales CA, Brown R, Mackay DF, Lewsey J, Mark PB, et al. Comparison of Conventional Lipoprotein Tests and Apolipoproteins in the Prediction of Cardiovascular Disease. Data from UK Biobank. *Circulation* 2019;140:542-52.
75. Boekholdt SM, Arsenault BJ, Mora S, Pedersen TR, LaRosa JC, Nestel PJ, et al. Association of LDL cholesterol, non-HDL cholesterol, and apolipoprotein B levels with risk of cardiovascular events among patients treated with statins: a meta-analysis. *JAMA* 2012;307:1302-9.
76. Robinson JG, Wang S, Jacobson TA. Meta-analysis of comparison of effectiveness of lowering apolipoprotein B versus low-density lipoprotein cholesterol and non high-density lipoprotein cholesterol for cardiovascular risk reduction in randomized trials. *Am J Cardiol* 2012;110:1468-76.
77. Thanassoulis G, Williams K, Ye K, Brook R, Couture P, Lawler PR, et al. Relations of change in plasma levels of LDL-C, non-HDL-C and apoB with risk reduction from statin therapy: A meta-analysis of randomized trials. *J Am Heart Assoc* 2014;3:e000759.
78. Khan SU, Khan MU, Valavoor S, Khan MS, Okunrintemi V, Mamas MA, et al. Association of lowering apolipoprotein B with cardiovascular outcomes across various lipid-lowering therapies: Systematic review and meta-analysis of trials. *Eur J Prev Cardiol* 2019 doi: 10.1177/2047487319871733 [Epub ahead of print].
79. Ference BA, Kastelein JJP, Ginsberg HN, Chapman MJ, Nicholls SJ, Ray KK, et al. Association of Genetic Variants Related to CETP Inhibitors and Statins With Lipoprotein Levels and Cardiovascular Risk. *JAMA* 2017;318:947-56.
80. Ference BA, Kastelein JJP, Ray KK, Ginsberg HN, Chapman MJ, Packard CJ, et al. Association of Triglyceride-Lowering LPL Variants and LDL-C-Lowering LDLR Variants With Risk of Coronary Heart Disease. *JAMA* 2019;321:364-73.
81. Arsenault BJ, Rana JS, Stroes ES, Després JP, Shah PK, Kastelein JJ, et al. Beyond low-density lipoprotein cholesterol: respective contributions of non-high-density lipoprotein cholesterol levels, triglycerides, and the total cholesterol/high-density lipoprotein cholesterol ratio to coronary heart disease risk in apparently healthy men and women. *J Am Coll Cardiol* 2010;55:35-41.
82. Masana L, Ibarretxe D, Heras M, Cabré A, Ferré R, Merino J, et al. Substituting non-HDL cholesterol with LDL as a guide for lipid-lowering therapy increases the number of patients with indication for therapy. *Atherosclerosis* 2013;226:471-5.
83. Elshazly MB, Martin SS, Blaha MJ, Joshi PH, Toth PP, McEvoy JW, et al. Non-high-density lipoprotein cholesterol, guideline targets, and population percentiles for secondary prevention in 1.3 million adults: the VLDL-2 study (very large database of lipids). *J Am Coll Cardiol* 2013;62:1960-5.
84. Mora S, Buring JE, Ridker PM. Discordance of low-density lipoprotein (LDL) cholesterol with alternative LDL-related measures and future coronary events. *Circulation* 2014;129:553-61.
85. Lawler PR, Akinkuolie AO, Ridker PM, Sniderman AD, Buring JE, Glynn RJ, et al. Discordance between Circulating Atherogenic Cholesterol Mass and Lipoprotein Particle Concentration in Relation to Future Coronary Events in Women. *Clin Chem* 2017;63:870-9.
86. Pischon T, Girman CJ, Sacks FM, Rifai N, Stampfer MJ, Rimm EB. Non-high-density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. *Circulation* 2005;112:3375-83.
87. Wong ND, Chuang J, Zhao Y, Rosenblit PD. Residual dyslipidemia according to low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B among

- statin-treated US adults: National Health and Nutrition Examination Survey 2009-2010. *J Clin Lipidol* 2015;9:525-32.
88. Ray KK, Ginsberg HN, Davidson MH, Pordy R, Bessac L, Minini P, Eckel RH, Cannon CP. Reductions in atherogenic lipids and major cardiovascular events: a pooled analysis of 10 ODYSSEY trials comparing alirocumab with control. *Circulation* 2016;134:1931-43.
 89. Rosenson RS, Jacobson TA, Preiss D, Djedjos CS, Dent R, Bridges I, Miller M. Efficacy and Safety of the PCSK9 Inhibitor Evolocumab in Patients with Mixed Hyperlipidemia. *Cardiovasc Drugs Ther* 2016;30:305-13.
 90. Lawler PR, Akinkuolie AO, Chu AY, Shah SH, Kraus WE, Craig D, et al. Atherogenic Lipoprotein Determinants of Cardiovascular Disease and Residual Risk Among Individuals With Low-Density Lipoprotein Cholesterol. *J Am Heart Assoc* 2017;6:e005549.
 91. White KT, Moorthy MV, Akinkuolie AO, Demler O, Ridker PM, Cook NR, Mora S. Identifying an optimal cutpoint for the diagnosis of hypertriglyceridemia in the nonfasting state. *Clin Chem* 2015;61:1156-63.
 92. Dathan-Stumpf A, Vogel M, Hiemisch A, Thiery J, Burkhardt R, Kratzsch J, Kiess W. Pediatric reference data of serum lipids and prevalence of dyslipidemia: results from a population-based cohort in Germany. *Clin Biochem* 2016;49:740-9.
 93. Balder JW, Lansberg PJ, Hof MH, Wiegman A, Hutten BA, Kuivenhoven JA. Pediatric lipid reference values in the general population: the Dutch Lifelines cohort study. *J Clin Lipidol* 2018;12:1208-16.
 94. Colantonio DA, Kyriakopoulou L, Chan MK, Daly CH, Brinc D, Venner AA et al. Closing the gaps in pediatric laboratory reference intervals: a CALIPER database of 40 biochemical markers in a healthy and multiethnic population of children. *Clin Chem* 2012;58:854-868.
 95. Ichihara K, Ozarda Y, Barth JH, Klee G, Shimizu Y, Xia L, et al. Committee on Reference Intervals and Decision Limits, International Federation of Clinical Chemistry and Laboratory Medicine and Science Committee, Asia-Pacific Federation for Clinical Biochemistry. A global multicenter study on reference values: 2. Exploration of sources of variation across the countries. *Clin Chim Acta* 2017;467:83-97.
 96. Ceriotti F, Hinzmann R, Panteghini M. Reference intervals: the way forward. *Ann Clin Biochem* 2009;46:8-17.
 97. Henny J, Vassault A, Boursier G, Vukasovic I, Mesko Brguljan P, Lohmander M, et al. Working Group Accreditation and ISO/CEN standards (WG-A/ISO) of the EFLM. Recommendation for the review of biological reference intervals in medical laboratories. *Clin Chem Lab Med* 2016;54:1893-900.
 98. Jellinger PS, Handelsman Y, Rosenblit PD, Bloomgarden ZT, Fonseca VA, Garber AJ, et al. American Association of Clinical Endocrinologists and American College of Endocrinology Guidelines for Management of Dyslipidemia and Prevention of Cardiovascular Disease. *Endocrine Practice* 2017;23(Supplement 2), 1-87.
 99. Ruhaak LR, van der Laarse A, Cobbaert CM. Apolipoprotein profiling as a personalized approach to the diagnosis and treatment of dyslipidaemia. *Ann Clin Biochem* 2019;56:338-56.

Table 1: Key EAS/EFLM recommendations for testing of atherogenic lipoproteins [1,2]

<p>Pre-preanalytical phase (test ordering)</p>
<p>Comprehensive testing of atherogenic lipoproteins should include tests to assess the risk conferred by LDL particles, remnant particles and, in selected cases, Lp(a).</p>
<p>Preanalytical phase (test sampling)</p>
<p>Fasting is not routinely required for assessing the lipid profile.</p> <p>Consider fasting sample when nonfasting TG are ≥ 4.5 mmol/L (400 mg/dL); however, this is not a requirement.</p> <p>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</p>
<p>Analytical phase (test measurement)</p>
<p>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</p> <p>Clinicians should be notified when the laboratory test changes from a method to another.</p> <p>The Martin-Hopkins equation may be preferable for calculation of LDLC in patients with low LDLC concentration < 1.8 mmol/L (70 mg/dL) and/or TG concentration 2.0–4.5 mmol/L (175–400 mg/dL), and in nonfasting samples.</p> <p>Direct LDLC assays should be used for calculation of RemnantC and for assessment of LDLC when TG concentration is ≥ 4.5 mmol/L (400 mg/dL).</p> <p>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.</p> <p>ApoB assays currently provide the most accurate measurement of overall burden of atherogenic particles in the fasting and nonfasting state.</p>
<p>Postanalytical phase (test reporting)</p>
<p>Laboratories should automatically calculate and report non-HDLc on all lipid profiles; RemnantC could also be reported.</p> <p>Laboratory reports should flag abnormal concentrations based on decision thresholds.</p> <p>Extremely high concentrations beyond the reference limits should alert clinicians (interpretative commenting on test report).</p>
<p>Post-postanalytical phase (test interpretation and use)</p>
<p>LDLC is the primary target of lipid-lowering therapy.</p> <p>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), diabetes, obesity or metabolic syndrome.</p>

^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 from cells (centrifugation) within 3 hours 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.

Age group	Percentiles									
	2.5		25		50		75		97.5	
	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 \geq 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.

Age group	Percentiles									
	2.5		25		50		75		97.5	
	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 [1,4].

	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
RemnantC ^a	Optional ^e	Optional ^e	NO	Optional ^e
Non-HDLC ^a	YES	NO ^f	NO	YES ^g
Additional tests				
ApoB ^h	YES ^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 rapidly converted into remnants (**within 5-10 minutes**) after delivery from lymph 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 RemnantC, calculated as TC – HDLC – LDLC, is all cholesterol found in TG-rich lipoproteins VLDL, IDL, and, in the nonfasting state, additionally chylomicron remnants. RemnantC is included in non-HDLC, but non-HDLC does not differentiate between LDLC and RemnantC. RemnantC 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 RemnantC, or RemnantC could be measured directly.

^f Non-HDLC, calculated as TC – HDLC, is all cholesterol in atherogenic lipoproteins: LDL, remnants, and Lp(a). None of the hyperlipidemias can be characterized by non-HDLC because the composite marker does not differentiate between the atherogenic lipoprotein-cholesterol fractions.

^g In patients with mild-to-moderate hypertriglyceridemia, 2-10 mmol/L (175-880 mg/dL), diabetes, obesity or metabolic syndrome.

^h Or advanced LDLP measurement if available.

ⁱ **At least once in each adult person's lifetime, especially in patients with premature ASCVD (men <55 years, women <60 years), family history of premature ASCVD and/or elevated Lp(a), FH, recurrent ASCVD despite optimal lipid-lowering treatment.**

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

Table 5: Example of between-laboratory uncertainty when lipids are measured by different methods in a hypertriglyceridemic patient.

Test	Assumed total error	Defined concentration in model patient		Range of uncertainty	
		mg/dL	(mmol/L)	mg/dL	(mmol/L)
TC	9% ^a	200	(5.2)	182 to 218	(4.7 to 5.7)
TG	15% ^a	250	(2.8)	212 to 288	(2.4 to 3.3)
dHDLc	-20% to +36% ^b	40	(1.0)	32 to 54	(0.8 to 1.4)
Non-HDLc	(derived from TC and dHDLc)	160	(4.1)	128 to 186	(3.3 to 4.8)
cLDLc (Friedewald)	(derived from TC, dHDLc and TG)	110	(2.8)	70 to 144	(1.8 to 3.7)
cLDLc (Martin-Hopkins)	(derived from TC, dHDLc, TG and non-HDLc)	122	(3.2)	91 to 151	(2.4 to 3.9)
dLDLc	-26% to +32% ^b	122	(3.2)	90 to 161	(2.3 to 4.2)

^a Based on National Cholesterol Education Program (NCEP) analytical performance criteria [41].

^b Total error ranges observed by Miller et al. [42] across different dLDLc and dHDLc methods in dyslipidemic samples. The total error combines systematic bias and random imprecision. The table is not relevant for the monitoring of a patient by the same laboratory/method over time. In this situation the bias remains constant and only the (inevitable) imprecision is relevant. Bias should ideally be $\leq 4\%$ for LDLc and $\leq 5\%$ for HDLc to allow the methods to meet the NCEP total error goals, $\leq 12\%$ and $\leq 13\%$, respectively.

Table 6: Primary and secondary goals of preventive therapy according to cardiovascular mortality risk categories assessed with the SCORE system [4].

Risk (SCORE) ^a	LDLC mmol/L (mg/dL)	Non-HDLC ^b mmol/L (mg/dL)	ApoB ^b g/L (mg/dL)
Very high	1.4 (55) and ≥50% reduction in LDLC	< 2.2 (85) ^c	< 0.65 (65) ^d
High	< 1.8 (70) and ≥50% reduction in LDLC	< 2.6 (100)	< 0.80 (80)
Moderate	< 2.6 (100)	< 3.3 (130)	< 1.00 (100)
Low	< 3.0 (115)		

^a 10-year risk of fatal ASCVD as estimated using SCORE (Systematic COronary Risk Evaluation) [4].

Very high risk = documented ASCVD, diabetes with target organ damage (proteinuria, retinopathy or neuropathy), or early onset type 1 diabetes of >20 years duration, severe chronic kidney disease (GFR <30 mL/min/1.73 m²), SCORE ≥10%;

High risk = TC >8 mmol/L (310 mg/dL), LDLC >5 mmol/L (190 mg/dL), FH, hypertension ≥180/110 mmHg, diabetes of ≥10 years duration without target organ damage, moderate chronic kidney disease (GFR 30-59 mL/min/1.73 m²), SCORE ≥5% and <10%;

Moderate risk = young patients with diabetes (type 1 <35 years, type 2 <50 years) of <10 years duration, 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 obesity or metabolic syndrome, diabetes, or chronic kidney disease.

^c Discordant high non-HDLC at optimal LDLC goal reflects elevated RemnantC >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 LDL and non-HDL cholesterol.

Table 7: Contemporary evidence for the medical use of LDLC, non-HDLc, apoB, and LDLP based on essential test characteristics [1].

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 thresholds?	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 [70]:

- 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 discriminate patients with increased risk from those with lower 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 8: Flagging of abnormal lipid and (apo)lipoprotein concentrations based on risk prediction thresholds and of extremely high concentrations [2].

Parameter	Thresholds	Interpretative commenting
TG ^a	Fasting ≥ 1.7 mmol/L (150 mg/dL)	>10 mmol/L (880 mg/dL): severe hypertriglyceridemia with high risk of acute pancreatitis
	Nonfasting ≥ 2 mmol/L (175 mg/dL)	
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
RemnantC	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)	
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)	<0.1 g/L (10 mg/dL): genetic hypoalphalipoproteinemia
	Women ≤ 1.4 g/l (140 mg/dL)	
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 ≥ 80 th 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: $Lp(a), \text{ nmol/L} = 2.18 * Lp(a), \text{ mg/dL} - 3.83$ [3].

Figure 1: Intravascular remodeling of lipoproteins contributing to the typical serum lipid profile of atherogenic dyslipidemia.

TG-rich chylomicrons (CM) secreted from the intestine, and VLDL secreted from the liver, are remodeled in the circulation primarily through the actions of lipoprotein lipase (LPL), hepatic lipase (HL), and cholesteryl ester transfer protein (CETP). The hydrolysis of TG by LPL, leading to liberation of free fatty acids (FFA), and the acquisition of cholesteryl esters (CE) from HDL by CETP generates smaller, cholesterol-enriched remnant particles which are depleted of part of their TG content and contributes to increased RemnantC concentrations measured in serum. Higher VLDL output resulting from FFA flux to the liver activates CETP, which results in TG enrichment of HDL and LDL through increased exchange and transfer of TG and cholesteryl ester. These cholesterol-depleted, TG-enriched LDL and HDL particles are also modified by HL, producing smaller LDL and HDL and contributing to lower serum concentrations of LDLC and HDLC, respectively, as typically manifested in the serum lipid profile of a patient with atherogenic dyslipidemia – frequently accompanying insulin resistance and metabolic syndrome with increased FFA flux to the liver.

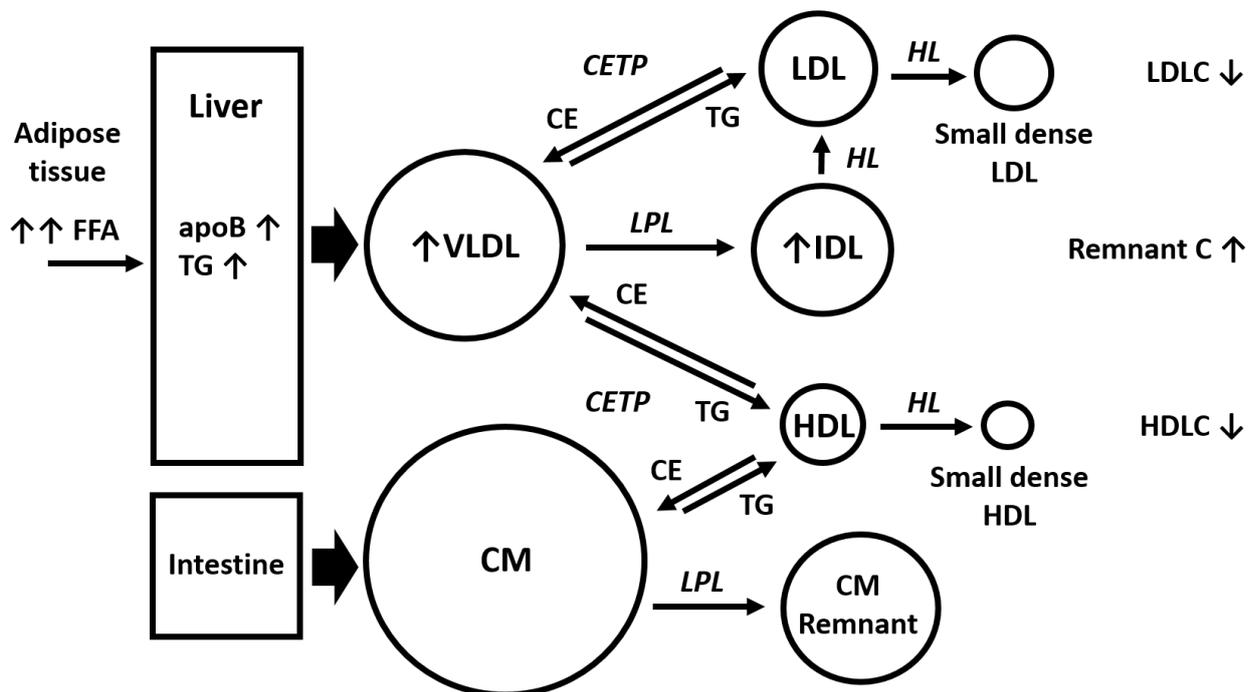


Figure 2: Distribution of nonfasting LDLC concentration in men and women from the Copenhagen General Population Study not on lipid-lowering therapy.

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 intervals instead of threshold values should be avoided, as use of reference limits 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.

