In terms of measuring number of small LDL particles, what are considered very high, high, and normal ranges? Does having a high number of small LDL particles put the patient at further risk for developing heart disease?

Dyslipidemia, or an abnormal serum lipid profile, is a major risk factor in the development of coronary heart disease (CHD).[1] Dyslipidemia may be characterized as abnormalities in levels of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TGs), or lipoprotein(a).[2] The National Lipid Association (NLA) asserts that an elevation in atherogenic cholesterol, namely LDL-C and non-high-density lipoprotein cholesterol (non-HDL-C), is the root cause of atherosclerosis, the underlying process contributing to most clinical atherosclerotic cardiovascular disease (ASCVD) events.[3]

A typical fasting lipid profile is comprised of the following components: total cholesterol (TC), HDL-C, LDL-C, and TGs.[1] LDL-C is usually calculated (with the Friedewald formula, depicted in Figure 1), because direct measurement, by centrifugation, is labor intensive. In the equation, TC and HDL-C are measured directly, and very low-density lipoprotein cholesterol (VLDL-C) is approximated using the measured TG level divided by 5.[4,5]

Figure 1. Friedewald equation.[1,5]

$$LDL\ cholesterol = total\ serum\ cholesterol - HDL\ cholesterol - \frac{TGs}{5}$$

HDL=high-density lipoprotein; LDL=low-density lipoprotein; TGs=triglycerides
Formula only valid if TGs <400 mg/dL

Circulating lipoprotein particles vary in size and density, and measurement or calculation of LDL-C levels does not in and of itself represent the number or size of LDL particles.[2] In fact, LDL-C is a measure of cholesterol concentration in LDL, not of LDL particle concentration.[6] This is important to note, as small LDL particles may carry less cholesterol than large LDL particles, but they may have greater atherogenic potential.[4] Also, an increased number of particles (or total LDL particle concentration) may carry a stronger association with cardiovascular disease, compared to a lower number of LDL particles.[2] Per Ivanova et al, LDL-C is defined as lipoprotein fraction with a density ranging from 1.006 to 1.063 g/mL.[7] This range includes intermediate-density lipoprotein (IDL) and VLDL. Various techniques can be used to analyze LDL-C; these techniques can separate LDL particles based on their density or size (e.g., diameter). Some of the most commonly used methods include ultracentrifugation, gradient gel electrophoresis (GGE), and nuclear magnetic resonance (NMR) spectroscopy. These techniques are outlined in Table 1. Additional methods of LDL fraction analysis include high-performance liquid chromatography (HPLC) with gel filtration, dynamic light scattering, ion mobility analysis, and homogenous assay analysis.

Table 1. Selected techniques for analyzing LDL-C.[2,7-12]

Method	Description	Subclasses/phenotypes	Comments
Ultracentrifugation	LDL particles are separated into subclasses based on their flotation rate	3 or 4 subclasses (densities): • I: large (1.025-1.034 g/mL) • II: intermediate (1.034-1.044 g/mL) • III/IV: small/very small (1.044-1.060 g/mL) III and IV referred to as sdLDL 2 phenotypes: • Pattern A: predominance (>50%) of LDL I and II • Pattern B: predominance of LDL III and IV	There are different ultracentrifugation methods (e.g., different types of gradients, such as iodixanol or salt); different methods result in slight variations in density of the separated LDL
Gradient gel electrophoresis	LDL particles are separated into subclasses by their electrophoretic mobility, determined by the size and shape of the lipoprotein	4 subclasses (peak diameters): • I: large (26.0-28.5 nm) • II: intermediate (25.5-26.4 nm) • III: small (24.2-25.5 nm) • IV: very small (22.0-24.1 nm) 2 phenotypes: • Pattern A (large and intermediate; diameter >25.5 nm) • Pattern B (small and very small; diameter ≤25.5 nm)	Particle size (assessed by gradient gel electrophoresis) and density (assessed by ultracentrifugation) are not identical, though there is a strong correlation between these parameters
Nuclear magnetic resonance spectroscopy	Reports total concentration or blood levels of the individual LDL subclasses and derives the average LDL particle size	15 subclasses, based on diameter and lipid composition (the 2 are independently measured) sdLDL defined as particle sizes 18.0-20.5 nm "High risk" value classified as >1600 nmoles/L; optimal value classified as <1000 nmoles/L[a]	Quantification is not based on cholesterol content and therefore provides a direct measure of lipoprotein particle concentrations

[a]As per Schaefer et al, referring to risk for cardiovascular disease; references 9-12
LDL=low-density lipoprotein; sdLDL=small dense low-density lipoprotein

Importantly, there is no gold standard method for LDL particle analysis.[6,7,9] Ivanova et al assert that there are no studies directly comparing the available methods.[7] As alluded to in the table, different methods of LDL subclass analysis yield different results, and significant variation may be observed even within 1 method. For example, the results of particle size measurement by NMR spectroscopy may differ significantly from the results obtained by GGE in the same patients; thus, the results cannot be directly compared.

While there are several practice guidelines for the management of dyslipidemia, most are silent on the issue of advanced lipid screening, including LDL particle analysis.[13-15] The American College of Cardiology Foundation (ACCF) and American Heart Association (AHA) recommend against advanced lipid testing in their 2010 guideline for the assessment

of cardiovascular risk in asymptomatic adults.[16] More recently, in the American College of Cardiology (ACC) 2013 guideline on management of blood cholesterol, the organization states that more data are needed to determine the utility of LDL particles for guiding treatment decisions.[15] The NLA 2015 guideline suggests that LDL particle concentration can be considered as an alternate target, particularly when non-HDL-C and LDL-C goals have been attained, but they recognize the variability in results across the available analytic techniques.[3] Accordingly, they do not recommend treatment goals for LDL particle concentration.

As mentioned previously, it is thought that small LDL particles may have greater atherogenic potential than large LDL particles, and an increase in total LDL particle concentration may carry a strong association with cardiovascular disease.[4,7] Increased atherogenicity has been linked to biochemical and biophysical properties of the particles.[7] Small particles may penetrate arterial walls more easily than large particles; once penetrated, the particles serve as a source of cholesterol and lipid storage. Additionally, small particles may have a longer circulation time than large particles, due to reduced clearance by LDL receptors in the liver with increased LDL receptor-independent binding in the arterial wall.[4] Other proposed mechanisms of increased atherogenicity of small LDL particles include enhanced oxidative susceptibility and endothelial dysfunction (independent of the concentrations of other lipids).

Multiple sources have noted that the predominance of sdLDL (i.e., phenotype pattern B) and elevated sdLDL-C, as well as elevated LDL particle concentration, are associated with cardiovascular disease risk.[1,4,6,7] Several studies have been conducted evaluating these parameters as markers of cardiovascular disease risk, including case-control and cohort studies.[10-12,17-22] Of note, these studies employed different techniques for analyzing LDL fractions, precluding comparison or conclusions regarding threshold levels and associated risks for cardiovascular disease.

In summary, small LDL particle size and elevated LDL particle concentration have been associated with an increase in cardiovascular risk.[4,7] However, techniques for LDL particle analysis are varied and there is no gold standard approach, and many of these measurements are not readily available in clinical practice.[1,6,7,9] Guidelines from the ACCF/AHA do not recommend routine advanced lipid testing.[16] The NLA suggests that LDL particle concentration may be monitored in patients who have attained target LDL-C and non-HDL-C levels; however, they do not recommend specific targets.[3] Further, prospective data comparing the available techniques and evaluating their performance for predicting ASCVD risk are necessary to elucidate the utility of LDL fraction analyses and their clinical application.

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