The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Methods of low-density lipoprotein-cholesterol measurement: analytical and clinical applications

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ARTICLE INFO

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Key words:
low-density lipoprotein-cholesterol, cardiovascular diseases, analytical and automated methods, calculation methods

ABSTRACT

Among the five major classes of lipoprotein particles, low-density lipoprotein-cholesterol (LDL-C) is the primary lipoprotein risk factor for the development of cardiovascular diseases (CVD) through the promotion of atherosclerotic pathogenesis. Therefore, it is of paramount importance to accurately measure the plasma concentration of LDL-C using an appropriate method to examine the risk of CVD and determine the efficacy of therapeutic interventions to reduce the cholesterol level and examine the risk assessment strategy. At present, there is a wide variety of methods available for LDL-C measurement. In this review, we have outlined the commonly used methods of LDL-C measurement. These methods have been classified into non-automated analytical methods, calculation methods, and automated direct measurement of LDL-C. We have also described some recently proposed promising calculation methods which are being considered for clinical adoption. This current

review could assist the clinicians to have a better understanding regarding the measurement techniques and comparative utilities of different methods of LDL-C measurement and guide them to select an appropriate method based on accuracy, turnaround time, and cost of test.

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INTRODUCTION

Lipids are essential for tissue structure and different aspects of function, including energy storage, cellular membrane formation, insulation of nerve conduction and heat dissipation, and hormone production (1). Lipids are either synthesized or absorbed from the diet and then transported to different tissues to meet their metabolic needs. Because of a relatively insoluble nature of lipids, they are transported in blood to different tissues through packaging into lipoproteins which are heterogeneous particles composed of proteins and lipids. Solubility of lipoprotein is determined by the location of proteins and polar lipids (e.g., phospholipids, sphingolipids, fatty acids (FAs) and cholesterol) at the outer surface of the particles. On the other hand, nonpolar triglycerides and cholesterol esters are retained at the core of particles (2). Based on their hydrated density and electrophoretic pattern, lipoproteins are divided into five major fractions including chylomicron, very low-density lipoprotein [VLDL], intermediate-density lipoprotein [IDL], low-density lipoprotein [LDL], high-density lipoprotein (HDL), and lipoprotein (a) [Lp (a)]. Triglycerides and cholesteryl esters-containing chylomicrons and VLDL are generated by the intestines and liver, respectively. Endothelial lipoprotein lipase activity in the capillaries of peripheral tissues converts triglycerides in lipoproteins into free FAs which is taken up by the tissues with a high demand for lipids. After losing some triglycerides, chylomicron and VLDL are converted into smaller and denser remnant lipoproteins and IDL, respectively, which are taken up and recycled by the liver. The taking up of remnant lipoproteins and IDL by the liver is mediated by the interaction between apolipoprotein E (apoE) on remnant lipoproteins/IDL and hepatic low-density lipoprotein receptor (LDLR) or LDLR-related protein. However, if remnant lipoproteins/IDL loses nearly all of their triglycerides through the lipoprotein lipase-mediated conversion of triglycerides into free FAs while they are still in circulation, they become too small to carry apoE which results in an inappropriate clearance of remnant lipoproteins/IDL from circulation by the liver. These smaller and denser lipoproteins are called LDL which is rich in cholesteryl ester/cholesterol (2).

In the clinical context, the term ‘lipids’ is kind of synonymously used for cholesterol due to the long-studied association between cholesterol and cardiovascular disease (CVD). Although a remarkable progress has been made in its treatment and prevention, CVD remains the single most common cause of death in the United States. Plasma cholesterol has an essential biological function as a structural component of cellular membranes and a precursor of many essential compounds including vitamin D, bile salts, and hormones. However, plasma cholesterol can accumulate on arterial wall leading to plaque formation which can eventually result in myocardial infarction upon rupture (3). Because LDL is the principle carrier of cholesterol in blood, low-density lipoprotein-cholesterol (LDL-C) level is used as a major risk factor for future CVD development (4). Due to the strong and positive association between LDL-C and CVD, the 2018 American Heart Association/American College of Cardiology (AHA/ACC) guideline on managing blood cholesterol emphasizes target level of LDL-C as a benchmark on directing prevention and management strategies of the Atherosclerotic CVD (5). Therefore,

a precise and accurate measurement of the concentration of circulating LDL-C using an appropriate method has become the cornerstone of cardiovascular risk assessment for the past decades. As per the recommendations of the National Cholesterol Education Program (NCEP) Working Group on Lipoprotein Measurement, methods used to measure the plasma level of LDL-C should give results equivalent to those used to establish the epidemiologic database that was used to develop the recommendations for risk evaluation (6). Currently, a variety of methods of LDL-C measurement are clinically available. In the present review, we have delineated widely used methods of LDL-C measurement which are classified into non-automated analytical methods, LDL-C calculation, and automated direct measurements including their merits and limitations.

NON-AUTOMATED ANALYTICAL METHODS

The analytical methods of LDL-C measurement are usually considered as reference methods. Commonly studied analytical methods are Beta-quantification, vertical spin density gradient ultracentrifugation-based vertical auto-profile, gradient gel electrophoresis, high-performance liquid chromatography, proton nuclear magnetic resonance spectroscopy (^{1}H-NMR), and ion mobility (Table 1). Due to their long turnaround time, non-suitability for automation, and requirement of expert technologists for operation, these non-direct methods are not used in a regular clinical laboratory setting. Rather, they are used as reference for the validation of other clinically suitable methods of LDL-C measurements using method comparison.

Beta-quantification

Beta-quantification is the precursor to the contemporary reference method adopted by NCEP in 1995. The name of the assay is originated from the location of LDL in the beta region upon electrophoretic migration of lipoprotein particles in early studies. This is the gold standard method for LDL-C measurement adopted from the National Institute Health (NIH) laboratory (6-8). An aliquot of plasma at native density of 1.006 g/mL is ultracentrifuged which separates VLDL and chylomicrons on the floating layer (d < 1.006 g/mL) over the infranatant containing IDL, LDL, Lp (a), and HDL (d > 1.006 g/mL). Concentration of cholesterol in the bottom layer fraction is measured by treating with heparin and manganese (II) chloride solution to precipitate the apoB containing particles (IDL, LDL, Lp (a)) and the precipitate is removed by centrifugation. After centrifugation, HDL is the only particle that remains in the solution. Then the concentration of HDL cholesterol is measured, and the concentration of LDL cholesterol is determined by subtracting the HDL concentration from the cholesterol concentration measured in the bottom fraction containing IDL, LDL, Lp (a), and HDL [d > 1006 g/mL cholesterol] (equation I). Concentration of VLDL-C in the discarded ultra-centrifugal supernatant fraction is measured by subtracting the cholesterol concentration measured in the bottom fraction [d > 1006 g/mL cholesterol] from the total concentration of cholesterol measured in the initial aliquot of plasma before ultracentrifugation (equation II) (9). It is preferred to calculate VLDL-C than direct measurement in the ultra-centrifugal supernatant as it is difficult to recover cholesterol in this fraction, especially at a high concentration of triglyceride.

[LDL-C] = [d > 1006 g/mL cholesterol] – [HDL-C] (I)

[VLDL-C] = [Total cholesterol] - [d > 1006 g/mL cholesterol] (II)

Because beta-quantification of LDL-C ignores the amount of IDL and Lp (a) in the precipitate, the term non-HDL-C is widely used, which reflects the cholesterol contents of all atherogenic

lipoproteins (non-HDL = TC-HDL-C) in several guidelines. One important shortcoming of beta-quantification is the heterogeneity and lack of molecular specificity as it cannot distinguish the concertation of LDL-C from the concentration of IDL-C and LP (a)-C. Both IDL and Lp (a) particles contribute to the total cholesterol measurement in the precipitated fraction. Thus, the measured concentration of LDL-C is always higher than the actual concentration, particularly in the cases of patients with CVD and the individuals who are at risk of developing CVD because of dyslipidemias. However, the elevated concentration of LDL-C measured by beta-quantification due to the inclusion of IDL-C and Lp (a)-C is considered beneficial to the clinical performance as both of IDL-C and Lp (a)-C are atherogenic in nature (9, 10). Furthermore, the NCEP Working Group

Table 1 **Merits and limitations of different analytical methods of low-density lipoprotein cholesterol measurement**

Analytical methods	Merits	Limitations
Beta-quantification	Highly standardized and high resolution	Cannot distinguish LDL-C from IDL-C and LP (a)-C Tedious and requires large amount of sample
Vertical spin density gradient ultracentrifugation-based vertical auto-profile	Separate LDL-C from IDL-C and LP (a)-C Directly measures cholesterol in different fractions of lipoproteins	Tedious and requires large amount of sample
Gradient gel electrophoresis	Can determine both particle concentration and diameter	Tedious, time consuming, and resource intensive
High-performance liquid chromatography	Can determine both particle concentration and diameter	Resource intensive and requires expertise
Proton nuclear magnetic resonance spectroscopy	High throughput quantification of particle size/concentration No preanalytical sample preparation is required	Cholesterol concentration cannot be not directly measured Requirement of costly equipment and expertise
Ion mobility	High resolution separation and measurement of particle concentration and size	Cholesterol concentration cannot be not directly measured and requires special equipment and expertise

on Cholesterol Measurement has suggested that LDL-C values should not be corrected for the presence of other atherogenic lipoproteins so that they could also be kept under consideration, not only LDL-C, during the treatment regimen (11).

Vertical spin density gradient ultracentrifugation-based vertical auto-profile

In vertical spin density gradient ultracentrifugation method, lipoprotein particles are separated and analyzed in three distinguished steps. First, a two-layer density gradient is established by dispensing saline solution in a tube and then under-layering with diluted serum. Then the tubes containing two-layer density gradients are ultracentrifuged. Second, following centrifugation, different layers of lipoproteins (HDLs being the particles of highest density are located at the bottom, VLDLs being the particles of least density are located at the top, and LDLs having the density between HDLs and VLDLs are located at the middle of the tube) are drained from the bottom of the tube and analyzed for determining cholesterol concentration using a continuous flow of cholesterol-specific enzymatic reagent through a narrow bore tubing. This instrumentation is called vertical auto-profile (VAP) analyzer. Third, as the reaction mixture flows through the tubing, absorbance of the corresponding colored-product, which is directly proportional to the cholesterol concentration of each class of lipoproteins, is measured using a spectrophotometric detector.

Because it directly measures the concentration of cholesterol in different fractions of lipoproteins, vertical spin density gradient ultracentrifugation-based VAP gives a high accuracy of LDL-C. Another important aspect of vertical spin density gradient ultracentrifugation-based VAP is that it separates IDL and Lp (a) from LDL, therefore, the cholesterol concentration of LDL particles is not confounded with that from IDL and Lp (a). Thus, VAP may represent a better reference method of LDL-C quantitation than beta-quantitation (12, 13).

Gradient gel electrophoresis

Using gradient gel electrophoresis, not only the cholesterol concentration of lipoprotein but also the size of the lipoprotein particles can be determined. In this method, serum samples are mixed with a loading solution, and then loaded to 2%-16% polyacrylamide gradient gels. To determine the size of particle, different standards having different diameters are used, such as bovine albumin, thyroglobulin, and serum VLDL. Besides, it is also important to use an internal standard, e.g., thyroglobulin is added to each serum sample. To distinguish LDL, gels are stained with Sudan Red after electrophoresis. To measure the concentration of LDL-C, gels are treated with reagent containing cholesterol ester hydrolase, cholesterol oxidase, and catalase which results in the elimination of HDL, VLDL, and chylomicrons, whereas LDL is protected. Then, the concentration of LDL-C is determined using CHOD-PAP method. To determine the diameters of LDL particles, LDL bands are compared with the calibration curve plotted on the basis of migration of standards on the gel (14).

LDL particles are heterogeneous due to their variation in size, density, and lipid composition. Gradient gel electrophoresis recognizes two different phenotypes of LDL particles. First, phenotype A comprises of large and buoyant LDL particles with a size of more than 25.5 nm. Second, phenotype B comprises of small and dense LDL with a size of 25.5 nm or less. Small and dense LDLs are considered atherogenic because they readily penetrate the arterial wall. It has been reported that the risk of coronary artery disease (CAD) is increased 2 to 3-fold in patients with short and dense LDL (15, 16). Unlike other non-automated analytical methods of LDL measurement, gradient gel electrophoresis can be

used not only to determine the concentration of LDL-C but also the diameter of lipoprotein particle.

High-performance liquid chromatography

High-performance liquid chromatography can determine the lipoprotein profile which includes both size and composition. To separate the lipoprotein particles, serum or plasma samples are pumped into tandemly connected gel permeation columns. The size of the particles is determined based on the retention times of the peaks on an observed chromatogram. To determine the lipoprotein composition, column effluent is equally split into two lines by a micro splitter. Each effluent flowing through two individual split lines are then separately used to react with cholesterol or triglyceride reagents. Then, the concentration of cholesterol or triglyceride in an individual split line representing a particular class or subclass of lipoprotein particle is determined spectrophotometrically by continuously monitoring the absorbance (17, 18). Likewise gradient gel electrophoresis, high-performance liquid chromatography can be used to determine both size and composition of lipoprotein particles. Moreover, it can separate not only the classes but also the subclasses of particles. This method can identify six LDL subfractions based on their variation in particle size (16-30 nm) (17, 18).

Proton nuclear magnetic resonance spectroscopy

Because of a number of various analytical advantages, ^{1}H-NMR spectroscopy has emerged as a valuable technique for measuring lipoproteins in plasma and serum samples. In this method, ^{1}H-NMR uses the characteristic signals generated by different lipoprotein classes of different size as the basis of their quantification. Each class of lipoproteins has Triglycerides and cholesterol esters inside the particles and has limited mobility, giving rise to the broadening of the NMR signals based on the aggregate number of methyl groups. Cholesterol esters and triglycerides which are located at the core of particles contribute 3 methyl groups each, whereas phospholipids and unesterified cholesterol which are located at the surface of the shell contribute 2 methyl groups each. The size of the lipoprotein particle determines the number of methyl groups and thereby, the methyl NMR signals. Thus, the methyl NMR signals emitted by large and less dense lipoprotein particles (e.g., VLDL and LDL) are different from the methyl NMR signals emitted by smaller and denser lipoproteins (e.g., HDL).

These signals are independent of the varying composition and relative amounts of cholesterol esters, triglycerides, and phospholipids, and degrees of unsaturation of the lipid fatty acyl chains. Therefore, the methyl NMR signal generated by a particular class of lipoproteins provides a direct measurement of the concentration of that specific class (19-21). Patients with insulin resistance, observed in metabolic syndrome and type-2 diabetes, contains a relatively higher number of particles of small dense LDL (sdLDL). Therefore, LDL particle number is a better measure of LDL as compared to the LDL-C (22). Besides LDL particle number, HDL particle number measured by NMR is also an important parameter for CVD risk assessment. In fact, as per key epidemiological and clinical trials, NMR-measured HDL particle number is a better parameter of CVD risk assessment than HDL-C (23). A limitation of ^{1}H-NMR is that it does not separate different classes of lipoprotein, and therefore it only estimates, but does not truly measure, the class-specific concentration of cholesterol (20, 21).

Ion mobility

In ion mobility, also known as gas phase differential electrical mobility, gas phase electrophoresis

is used to separate lipoproteins based on their size. First, singly charged lipoprotein particles on aerosol are generated in the electrospray chamber. The particles are then carried in the airflow to the differential mobility analyzer. As the particles pass through the differential mobility analyzer, an electric potential across the sheath flow causes the particles to drift toward a collection slit. At any given electrical potential, particles of a particular size can successfully be transported through the collection slit and finally enter a separate air stream that carries them to a particle counter. In the counter, each particle is detected by light scattering. Then, the data is analyzed to provide results on particle dry diameter, particle concentration, and mass concentrations. Ion mobility can be used for high-resolution separation and direct quantification of full spectrum of different subclasses of LDL. Because ion mobility cannot isolate different fractions of lipoprotein particles, it is not useful for measuring class-specific cholesterol concentration. LDL-C is estimated based on total cholesterol and percentage of LDL out of total lipoproteins. Furthermore, multistep preanalytical sample processing required for lipoprotein isolation and ionization is an important limitation (24, 25).

AUTOMATED DIRECT MEASUREMENT

Unlike the analytical methods, automated direct measurement methods are easily adoptable to a clinical setting. Heterogeneous selective precipitation and homogeneous methods are the two well-known types of automated direct measurement of LDL-C (Table 2).

Selective precipitation

There are several methods of LDL-C measurement based on selective precipitation. One important method is to selectively precipitate LDL-C with polyvinyl sulfate or heparin at low pH and then directly measure cholesterol concentration in the precipitate. It can also be calculated as a difference between the concentration of total cholesterol and that in the supernatant (26). A more specific method is to use polystyrene beads coated with polyclonal antibodies to apo A-I and apo E to immunoprecipitate VLDL, IDL, and HDL and then measure LDL-C is in the filtrate (27). Selective precipitation method was in good agreement with beta-quantification, but due to the requirement of a separate pretreatment step, it is now outmoded by direct homogeneous methods (27).

Homogeneous methods

Direct homogeneous methods use autoanalyzers to selectively measure the cholesterol concentration in LDL particles. Since the inception of homogeneous assays in 1998, various methods are now commercially available used by different manufacturers differing in employed detergents, modified enzymes, and other reagents allowing selective blocking and solubilization of lipoprotein classes to achieve LDL specificity. In Kyowa Medex method, LDL-C concentration is directly measured by suppressing other lipoproteins. α-cyclodextrin sulfate is used to mask cholesterol in chylomicron and VLDL and polyoxyethylene-polyoxypropylene polyether is used to block the cholesterol in HDL. Thus, cholesterol from LDL selectively produces colored product measured spectrophotometrically (28, 29). In Waki and Deika-Senken method, LDL-C is selectively masked from the initial enzymatic reaction.

All the non-LDL-C produces hydrogen peroxide which is consumed by catalase. Deprotecting reagent removes the protecting agent from LDL. Then the LDL-cholesterol generate hydrogen peroxide which eventually produces a colored complex (29-32). Similarly, in International Reagents Corp. method, first reagent containing calixarene (a detergent) forms a soluble complex with LDL. Cholesterol esters of HDL-C and

Table 2 Merits and limitations of different methods of direct measurement of low-density lipoprotein cholesterol

Direct methods	Merits	Limitations
Selective precipitation	In good agreement with beta-quantification	Requires additional pre-treatment
Homogeneous methods	Fully automated and rapid Improved precision/accuracy from automated pipetting and no sample preparation	Cost for extra reagents

VLDL-C are hydrolyzed resulting in conversion of cholesterol to cholestenone hydrazone. Second reagent, containing deoxycholate, breaks up the LDL-calixarene complex allowing LDL-C to produce cholestenone and β-NADH (29, 30). In Daiichi method, esterase and oxidase present in the reagent react with the cholesterol from non-LDL producing colorless product. On the other hand, cholesterol from LDL generates hydrogen peroxide producing colored product (29, 30).

Direct homogeneous methods are fully automated, easy to use, and cost effective. Because of being automated, they have improved precision from non-manual pipetting, and accurate control of timing and temperature (30). However, direct homogeneous measurements are not free of inaccuracies, particularly in the case of dyslipidemias. Bias between 13% and 46% has been reported between different direct homogeneous methods (7, 33). Compared to the calculation methods, direct homogeneous methods require additional steps of measurement. Therefore, homogeneous methods must demonstrate obvious advantages in performance and economics to replace calculation methods.

CALCULATION METHODS

Because inclusion of LDL-C in basic lipid panel would result in an increased turnaround time and additional cost, it is a regular practice to calculate LDL-C from the currently used components of basic lipid panel. To date, Friedewald and Martin/Hopkins are the commonly used methods for calculating LDL-C. Recently, the Sampson and extended Martin/Hopkins equations have been reported to provide more accurate calculations of LDL-C at hypertriglyceridemia (Table 3).

Friedewald equation

Friedewald et al. published a groundbreaking article in 1972 which described an equation to calculate LDL-C concentration to avoid the use of tedious ultracentrifugation-based methods (34). Considering its lower cost and shorter turnaround, Friedewald calculation has currently become the most widely used method for routine quantification of LDL-C. In this method, LDL-C is calculated by subtracting the concentration of cholesterol within all lipoproteins other than LDL from the concentration of

Table 3 Merits and limitations of different methods of calculation of low-density lipoprotein cholesterol

Calculation methods	Merits	Limitations
Friedewald equation	Good alternative of direct assay Quick turnaround and less cost	Not acceptable when triglyceride > 400 mg/dL
Martin/Hopkins calculation	Gives a more accurate calculation than Friedewald due to the use of adjustable factor	Not acceptable when triglyceride > 400 mg/dL
Sampson equation	Applicable at the triglyceride concentration up to 800 mg/dL	Not free of cholesterol contami-nation from IDL-C, and Lp (a)
Extended Martin/Hopkins calculation	Applicable at the triglyceride concentration up to 800 mg/dL Greater accuracy than the Friedewald and Sampson	Not free of cholesterol contami-nation from IDL-C, and Lp (a)

total cholesterol. In fasting condition, the major lipoprotein particles are LDL, HDL, and VLDL. Therefore, subtraction of concentration of cholesterol in HDL and VLDL from the total cholesterol concentration represents the estimate of LDL-C. But, only total cholesterol, HDL-C, and triglycerides are included in the basic lipid panel. VLDL-C is not a part of basic lipid panel, but it can be calculated from the serum concentration of triglycerides. VLDL particles carry most of the circulating triglycerides in normal fasting condition. Cholesterol within VLDL particles are reported to be contained with triglycerides in a ratio of [triglycerides]:[cholesterol] = 5 (for mg/dL unit) or 2.26 (for mmol/L) (34-36). Therefore, VLDL-C is calculated by dividing the concentration of triglycerides (mg/dL or mmol/dL) by 5 or 2.26, respectively (equation III). Then, LDL-C is estimated by subtracting the concentration of HDL-C and calculated VLDL-C from the concentration of total cholesterol (equation IV).

$$\text{VLDL-C} = {}^{[\text{Triglycerides}]}/_{5} \quad \text{(III)}$$

$$\text{LDL-C (mg/dL or mmol/L)} = [\text{Total cholesterol}] - [\text{HDL-C}] - {}^{[\text{Triglycerides}]}/_{5 \text{ or } 2.26} \quad \text{(IV)}$$

In Friedewald calculation, it is assumed that the subject is fasting, and the triglyceride concentration is less than 400 mg/dL (37). The requirement of a fasting condition significantly reduces the likelihood of triglyceride contribution by chylomicron. But in non-fasting condition, circulating triglyceride is remarkably contributed

by chylomicron, 90% of which by mass is triglyceride (38).

In that case, the actual ratio of [triglycerides]:[cholesterol] in the blood would be higher than 5. Likewise, when triglyceride concentration is higher than 400 mg/dL (e.g., in hypertriglyceridemia), the actual ratio of [triglycerides]:[cholesterol] would also be higher than 5. In such conditions, [Triglycerides]/5 is not an appropriate estimate of VLDL-C. Under those circumstances, using [Triglycerides]/5 as a fixed factor would overestimate VLDL-C and thereby, underestimate LDL-C. In fact, the Friedewald calculation of VLDL-C has been found to be most accurate in patients with a triglyceride concentration of less than 200 mg/dL (39).

Martin/Hopkins calculation

In Friedewald calculation of LDL-C, triglyceride concentration is divided by a fixed factor to determine the concentration of VLDL-C assuming an invariable ratio of triglyceride:VLDL-C. Given the inaccuracy of the fixed denominator in the case of triglycerides levels higher than mg/dL, Martin/Hopkins calculation was formulated which replaces the fixed denominator with an adjustable factor X (equation V). This adjustable factor X changes following an empirically derived 180-cells strata based on the varying concentration of triglyceride up to 400 mg/dL and non-HDL up to 220 mg/dL (40). Although the use of this adjustable factor improves the accuracy of LDL-C calculation to some extent compared to the Friedewald equation, Martin/Hopkins equation is still not a completely accurate calculation method and inapplicable in the case of triglyceride level beyond 400 mg/dL (40, 41). On top of that, Martin/Hopkins calculation formula for LDL-C is not available to users/laboratories for free.

$$\text{LDL-C} = [\text{Total cholesterol}] - [\text{HDL-C}] - \frac{[\text{Triglycerides}]}{X} \quad \text{(V)}$$

Sampson equation

Sampson LDL-C equation, also known as the National Institutes of health equation, used TG and non–HDL-C as independent variables and multiple least squares regression to develop a bivariate quadratic equation for VLDL-C (equation VI). The equation for VLDL-C was then used in a second equation for LDL-C calculation (equation VII). Sampson calculation of LDL-C is similar to Friedewald calculation in terms of subtracting VLDL-C and HDL-C from total concentration of cholesterol. Additionally, Sampson equation includes an intercept and allows the coefficients to vary for each term which made this equation a better fit with beta-quantification compared to Friedewald equation.

Whereas negative biases are sometimes observed for hypertriglyceridemic patients in the cases of other calculation methods, Sampson calculation is almost free of such negative bias. Thus, the use of continuous variables has made Sampson equation a superior method of calculation at triglyceride concentration up to 800 mg/dL (42).

$$\text{VLDL-C} = \frac{[\text{Triglycerides}]}{8.59} + \frac{[\text{Triglycerides}] \times [\text{non-HDL-C}]}{2250} - \frac{[\text{Triglycerides2}]}{16500} \quad \text{(VI)}$$

$$\text{LDL-C} = \frac{[\text{Total cholesterol}]}{0.948} - \frac{[\text{HDL-C}]}{0.971} - \left(\frac{[\text{Triglycerides}]}{8.56} + \frac{[\text{Triglycerides}] \times [\text{non-HDL-C}]}{2140} - \frac{[\text{Triglycerides}]^2}{16100}\right) - 9.44 \quad \text{(VII)}$$

Extended Martin/Hopkins calculation

Extended Martin/Hopkins calculation is a modified version of the Martin/Hopkins calculation. Likewise, Martin/Hopkins calculation, Extended Martin/Hopkins calculation uses an adaptable triglyceride to VLDL-C ratio instead of a fixed one used in Friedewald equation (equation V). Extended Martin/Hopkins equation was validated against vertical density gradient ultracentrifugation for LDL-C up to 800 mg/dL using an empirically defined table of 240/560/1040-cells of median ratios of TG:VLDL-C based on the varying number of TG and non-HDL-C strata (41). In a cross-sectional study, the extended Martin/Hopkins calculation was demonstrated to calculate LDL-C with a greater accuracy as compared with the Friedewald and Sampson equations in patients with triglyceride levels of 400 to 799 mg/dL (41).

LDL-C belongs to the category of non-HDL-C that contains all atherogenic lipoproteins, including chylomicron, VLDL, LDL, IDL and lipoprotein(a). In primary CVD prevention, a number of prospective observation studies have reported that non-HDL-C predicts risk with equal or superior performance compared to LDL-C. Since VLDL in non-HDL represents particles rich in triglyceride, which is an independent risk factor of CVD, current guidelines list non-HDL-C as secondary therapeutic target of CVD prevention in addition to LDL-C. The clinical utility of non-HDL-C is beyond the scope of this review (43-45).

CONCLUSION

Due to the long-established connection between LDL-C and CVD, it is critically important to accurately measure the concentration of LDL-C for a proper cardiovascular risk assessment and management. To date, there is a sizable preference of analytical, calculation, and direct methods for LDL-C measurement. Although each of these methods have their own merits over the others depending on the existing analytical or clinical setting, none of these methods are free from limitations in terms of operational expertise, accuracy, turnaround time, and cost. This review has briefly illustrated different methods of LDL-C measurement which gives a clearer understanding on available methods of LDL-C measurement in addition to their comparative application. Given the necessity of accuracy, shorter turnaround time, and affordability of LDL-C measurement, it demands further investigation to develop an improved alternative.

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Abbreviations

AHA/ACC: American Heart Association/American College of Cardiology; CDC: Centers for Disease Control and Prevention; CVD: cardiovascular diseases; FAs: fatty acids; HDL-C: high-density lipoprotein-cholesterol; IDL-C: intermediate-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; Lp (a): lipoprotein (a); NCEP: National Cholesterol Education Program; VLDL-C: very low-density lipoprotein-cholesterol.

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REFERENCE

1. Casares D, Escriba PV, Rossello CA. Membrane Lipid Composition: Effect on Membrane and Organelle Structure, Function and Compartmentalization and Therapeutic Avenues. Int J Mol Sci. 2019;20(9).

2. Rifai N. Lipoproteins and apolipoproteins. Composition, metabolism, and association with coronary heart disease. Arch Pathol Lab Med. 1986;110(8):694-701.

3. Linton MRF, Yancey PG, Davies SS, Jerome WG, Linton EF, Song WL, et al. The Role of Lipids and Lipoproteins in Atherosclerosis. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, et al., editors. Endotext. South Dartmouth (MA)2000.

4. Nelson RH. Hyperlipidemia as a risk factor for cardiovascular disease. Prim Care. 2013;40(1):195-211.

5. 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: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2019;73(24):3168-209.

6. Bachorik PS, Ross JW. National Cholesterol Education Program recommendations for measurement of low-density lipoprotein cholesterol: executive summary. The National Cholesterol Education Program Working Group on Lipoprotein Measurement. Clin Chem. 1995;41(10):1414-20.

7. 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(4):264-72.

8. AJKJ Hainline KLSD. Manual of laboratory operations. 1982 U.S Bethesda, Md.

9. Nakamura M, Kayamori Y, Iso H, Kitamura A, Kiyama M, Koyama I, et al. LDL cholesterol performance of beta quantification reference measurement procedure. Clin Chim Acta. 2014;431:288-93.

10. Nordestgaard BG, Tybjaerg-Hansen A. IDL, VLDL, chylomicrons and atherosclerosis. Eur J Epidemiol. 1992;8 Suppl 1:92-8.

11. Expert Panel on Detection E, Treatment of High Blood Cholesterol in A. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001;285(19):2486-97.

12. Kulkarni KR. Cholesterol profile measurement by vertical auto profile method. Clin Lab Med. 2006;26(4):787-802.

13. Kulkarni KR, Garber DW, Marcovina SM, Segrest JP. Quantification of cholesterol in all lipoprotein classes by the VAP-II method. J Lipid Res. 1994;35(1):159-68.

14. Ghassab RK, Gohari LH, Firoozray M, Yegane MN. Determination of Low Density Lipoprotein Particle Size by Polyacrylamide Gradient Gel Electrophoresis in Patients with Coronary Artery Stenosis. Laboratory Medicine. 2010;41(3):164-6.

15. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA. 1988;260(13):1917-21.

16. Sacks FM, Campos H. Clinical review 163: Cardiovascular endocrinology: Low-density lipoprotein size and cardiovascular disease: a reappraisal. J Clin Endocrinol Metab. 2003;88(10):4525-32.

17. Kanonidou C. Small dense low-density lipoprotein: Analytical review. Clinica Chimica Acta. 2021;520:172-8.

18. Mizutani H, Sako T, Arai N, Kuriyama K, Yoshimura I, Mori A, et al. Application of gel permeation HPLC for lipoprotein profiling in dogs. J Vet Med Sci. 2010;72(6):813-7.

19. 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(10):1453-64.

20. Blake GJ, Otvos JD, Rifai N, Ridker PM. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. Circulation. 2002;106(15):1930-7.

21. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. Circulation. 2009;119(7):931-9.

22. Matyus SP, Braun PJ, Wolak-Dinsmore J, Jeyarajah EJ, Shalaurova I, Xu Y, et al. NMR measurement of LDL particle number using the Vantera Clinical Analyzer. Clin Biochem. 2014;47(16-17):203-10.

23. Matyus SP, Braun PJ, Wolak-Dinsmore J, Saenger AK, Jeyarajah EJ, Shalaurova I, et al. HDL particle number measured on the Vantera(R), the first clinical NMR analyzer. Clin Biochem. 2015;48(3):148-55.

24. Kanonidou C. Small dense low-density lipoprotein: Analytical review. Clin Chim Acta. 2021;520:172-8.

25. Caulfield MP, Li S, Lee G, Blanche PJ, Salameh WA, Benner WH, et al. Direct determination of lipoprotein particle sizes and concentrations by ion mobility analysis. Clin Chem. 2008;54(8):1307-16.

26. Mulder K, van Leeuwen C, Schouten JA, van Gent CM, Snel MT, Lahey J, et al. An evaluation of three commercial methods for the determination of LDL-cholesterol. Clin Chim Acta. 1984;143(1):29-35.

27. Jialal I, Hirany SV, Devaraj S, Sherwood TA. Comparison of an immunoprecipitation method for direct measurement of LDL-cholesterol with beta-quantification (ultracentrifugation). Am J Clin Pathol. 1995;104(1):76-81.

28. Sugiuchi H, Irie T, Uji Y, Ueno T, Chaen T, Uekama K, et al. Homogeneous assay for measuring low-density lipoprotein cholesterol in serum with triblock copolymer and alpha-cyclodextrin sulfate. Clin Chem. 1998;44(3):522-31.

29. Rifai N, Horvath AR, Wittwer CT. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed: Elsevier; 2018.

30. 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(2):236-54.

31. Esteban-Salan M, Guimon-Bardesi A, de La Viuda-Unzueta JM, Azcarate-Ania MN, Pascual-Usandizaga P, Amoroto-Del-Rio E. Analytical and clinical evaluation of two homogeneous assays for LDL-cholesterol in hyperlipidemic patients. Clin Chem. 2000;46(8 Pt 1):1121-31.

32. Okada M, Matsui H, Ito Y, Fujiwara A, Inano K. Low-density lipoprotein cholesterol can be chemically measured: a new superior method. J Lab Clin Med. 1998;132(3): 195-201.

33. Miller WG, Waymack PP, Anderson FP, Ethridge SF, Jayne EC. Performance of four homogeneous direct methods for LDL-cholesterol. Clin Chem. 2002;48(3):489-98.

34. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.

35. SI Units and the Friedewald Formula. Annals of Internal Medicine. 1992;117(10):876-.

36. Samman S, Truswell AS. The Friedewald equation for the determination of low-density-lipoprotein cholesterol: a special case. Am J Clin Nutr. 1993;58(6):928-9.

37. Zhao W, Chaffin C, Desmond RA, Hodges B, Daly TM, Andrew Robinson C, et al. Overestimation of HDL-cholesterol using a homogeneous “direct” assay. J Clin Lab Anal. 2004;18(1):42-4.

38. LaRosa JC, Chambless LE, Criqui MH, Frantz ID, Glueck CJ, Heiss G, et al. Patterns of dyslipoproteinemia in selected North American populations. The Lipid Research Clinics Program Prevalence Study. Circulation. 1986;73(1 Pt 2): I12-29.

39. Warnick GR, Knopp RH, Fitzpatrick V, Branson L. Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. Clin Chem. 1990;36(1):15-9.

40. Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, et al. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. JAMA. 2013;310(19):2061-8.

41. Sajja A, Park J, Sathiyakumar V, Varghese B, Pallazola VA, Marvel FA, et al. Comparison of Methods to Estimate Low-Density Lipoprotein Cholesterol in Patients With High Triglyceride Levels. JAMA Netw Open. 2021;4(10):e2128817.

42. Sampson M, Ling C, Sun Q, Harb R, Ashmaig M, Warnick R, et al. A New Equation for Calculation of Low-Density Lipoprotein Cholesterol in Patients With Normolipidemia and/or Hypertriglyceridemia. JAMA Cardiol. 2020; 5(5):540-8.

43. 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). European Heart Journal. 2019;41(1):111-88.

44. Pearson GJ, Thanassoulis G, Anderson TJ, Barry AR, Couture P, Dayan N, et al. 2021 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in Adults. Can J Cardiol. 2021;37(8):1129-50.

45. [Detection, evaluation, and treatment of high blood cholesterol in adults]. Rev Panam Salud Publica. 2001;9(5): 338-44.