LDL Cholesterol: 'The Times They Are A-Changin'

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"LDL Cholesterol: The Times They Are A-Changin' "

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[4]Non-standard abbreviations: ASCVD, atherosclerotic cardiovascular disease; HDL-C, HDL cholesterol

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More than ever, the circulating level of LDL cholesterol (LDL-C) has become a key criterion for the assessment of cardiovascular risk worldwide, and in turn, for clinical management of such risk. This “fait accompli” in large part reflects the recognition of the causality of LDL particles in the pathophysiology of atherosclerotic cardiovascular disease (ASCVD), and of the robust body of evidence demonstrating that efficacious and sustained lowering of LDL-C levels over time confers marked reduction in both risk and cardiovascular events (1). In consequence, many national and international guidelines for ASCVD prevention now focus on LDL-C targets as a function of the level of global risk in secondary prevention patients (2).

Low-density lipoprotein-cholesterol therefore constitutes a critical component of the plasma lipid profile in the clinical laboratory, necessitating accurate, robust and reproducible methodology for its quantitation. Indeed, the direct implications of such quantitation are multiple, as it may not only be instrumental in leading the clinician to stratify a patient to a specific risk category, but may equally guide decision-making with respect to a personalised therapeutic intervention strategy (2,3).

There is an ongoing discussion as to the optimal methodology for LDL-C quantitation across a wide range of atherogenic dyslipidemias associated with elevated risk for premature ASCVD. In this regard, the reader is referred to the recent review of Langlois and colleagues for a critical appraisal of potential approaches, and equally of their respective strengths and weaknesses (3).

The Friedewald equation, which provides an indirect measure of LDL-C based on measurement of plasma total cholesterol (TC), triglyceride (TG) and high-density lipoprotein-cholesterol (HDL-C), has long been known to attain the limit of its accuracy at triglyceride levels elevated above 150 mg/dL. Indeed, many clinical laboratories do not apply this equation to calculate LDL-C when TG levels are above ~ 350 mg/dL. Furthermore, intravascular remodelling of LDL at TG levels superior to 150 mg/dL favours the emergence of metabolic constraints which drive the appearance of an LDL profile dominated by cholesterol-poor, small LDL particles, thereby leading to their underestimation. The latter comment is particularly pertinent to subjects presenting type 2 diabetes or metabolic syndrome with moderate hypertriglyceridemia. Indeed, these limitations of the Friedewald equation may well be

amplified given the trend to widespread endorsement of the use of nonfasting blood samples for lipid screening.

In 2013, Martin and collaborators (4) proposed an equation for estimation of LDL-C which featured replacement of the TG denominator of 5 in the Friedewald equation with an empirically-derived (personalised) factor selected from tables formulated from the non-HDL-C and TG values of a large cohort (n > 900,000). This approach provided a more accurate estimation of very low-density cholesterol (VLDL-C) level, and ultimately, a more accurate estimation of LDL-C than the Friedewald formulation, particularly at low LDL-C levels. Nonetheless, when TG concentrations fell within the range of 200 to 399 mg/dL, this equation misclassified a substantial proportion (59%) of patients as having LDL-C < 70 mg/dL, (despite true LDL-C levels ≥ 70 mg/dL)(5). Equally, the "Martin" equation did not perform well upon application to hypertriglyceridemic samples (Figure 2, ref. 5).

It is evident that the estimation of VLDL-C is a major determinant of the accuracy of the above methods for calculation of LDL-C. Indeed, it should be emphasised that this component represents the sum of the cholesterol content of a highly heterogeneous mixture of particles of both intestinal and hepatic origin, including chylomicrons, VLDL and their remnants, and whose relative content of cholesterol to triglyceride in the particle core varies as a function of multiple metabolic factors.

It is in light of such biological and inter- and intra-individual variability within the fraction denoted "VLDL-cholesterol" that the recent report of Sampson, Remaley and colleagues merits our full consideration (5). A substantial proportion (>20%) of the adult population in countries of the Western hemisphere presents with nonfasting triglyceride levels at or above the upper limit of the normal distribution (taken as 150 mg/dL)(5). These authors therefore employed a data set derived from a predominantly hypertriglyceridemic population which included the key lipid parameters characteristic of the CDC reference method for determination of LDL-C by β-quantification; this latter method is based on ultracentrifugal removal of d<1.006 g/mL triglyceride-rich lipoproteins (TGRL) from plasma or serum, with subsequent heparin/Mn^{2+} precipitation for determination of HDL-C. LDL-C is then calculated as infranatant cholesterol minus supernatant cholesterol (ie. HDL-C, equally determined by β-quantification). Firstly, the estimation of VLDL-C was improved with a bivariate quadratic equation, which importantly adjusts for extreme elevations in TG driven typically by elevated chylomicron content. This equation ("Equation 1") provided more accurate estimations of TG than either the formulations of Friedewald or Martin et al. (4) when compared to values obtained by β-quantification (5). Subsequently, a second equation, "Equation 2", was derived which substantially resembled the Friedewald formulation; importantly however, introduction of an intercept at the origin of the x and y

axes and fine tuning of the coefficients for each term in this equation gave LDL-C values with a superior fit to β-quantification data as compared to findings with other formulations (5). "Equation 2" was further validated using two external data sets again derived from β-quantification, one of which featured dyslipidemias frequent in the general population. Again, "Equation 2" provided superior LDL-C values to those calculated from both the Friedewald and "Martin" formulations (5).
Several additional comparisons of the three formulations were performed, one of which involved comparison with direct LDL-C assays; in this instance, "Equation 2" provided the lowest frequency of negative LDL-C results (0.01%) as compared to 0.63 and 0.08% for the Friedewald and "Martin" equations respectively (5). Finally, the misclassification rate for patients divided into distinct groups with respect to prospective LDL-C treatment was assessed; these key data revealed that "Equation 2" yielded LDL-C values which were comparable to those of β-quantification across the entire range of LDL-C risk groups.

Without doubt, the ready applicability of this newly formulated equation in clinical laboratories is a major factor in its "user friendliness". Indeed, it is based, like the Friedewald equation before it, on the classical lipid panel, and is without intellectual property limitations*. Moreover, it is valid for application to both normolipidemic as well as dyslipidemic samples (5). In this context, it is relevant that the equation of Martin et al. (4) requires selection of an optimal factor for the TG/VLDL-C ratio from a table based on TG and non-HDL-C concentrations involving 180 cells; this approach may present challenges for implementation in clinical laboratories using standard software. Equally of note is the fact that the "Martin" equation is based on data obtained for lipoprotein cholesterol concentrations subsequent to their separation by vertical spin density-gradient ultracentrifugation (VAP) rather than β-quantification; the VAP procedure is susceptible to underestimation of VLDL-C levels in hypertriglyceridemic samples due to adherence of TGRL to the walls of the vertical ultracentrifugation tubes, thereby involving partial loss of TG-rich lipoproteins (4,5).

A major contribution of "Equation 2" may derive from the added accuracy it offers for determination of low LDL-C levels. Importantly, it is now established from investigations involving low circulating LDL-C levels, be they of genetic, epidemiologic or pharmacologic origin, that such low LDL concentrations are associated with lower rates of ASCVD risk (1). Moreover, reduction in ASCVD events is typically proportional to the absolute reduction in LDL-C and to the cumulative duration of the exposure of the arterial wall to lower LDL levels (1). Such findings have prompted guideline recommendations for ever lower LDL-C targets in secondary prevention patients, an example being the 55 mg/dL target for very high-risk patients in the recent 2019 ESC/EAS Guidelines for the management of dyslipidemias (2). With the advent of combination therapies, such as PCSK9 inhibitors on a background of statins alone or

*Footnote: A spreadsheet for calculation of LDL-C using this new equation, and equally for the Friedewald and Martin equations, can be accessed at: https://doi.org/10.35092/yhjc.11903274.

combined with ezetimibe, very low LDL-C levels have become attainable and thus present more frequently to clinical chemists and clinicians alike. Such innovative therapeutic strategies are especially relevant to the residual cardiovascular risk frequently encountered on statin monotherapy, as exemplified by patients with incident coronary atheroma who respond poorly to statins and whose disease may inexorably progress.

As with any new methodological approach, do any limitations present themselves on the one hand, and does the formulation present further opportunities for identification of additional clinically-relevant information afforded by the classical lipid profile?

Firstly, it is of interest to compare the value obtained for LDL-C using Equation 2 with the the value given by TC - LDL-C - HDL-C, as in the following example for a hypertriglyceridemic profile: HDL-C, 35; TC, 259; TG, 245 and non-HDL, 224 mg/dL.

Equation 2: LDL-C, 177.3 and Equation 1: VLDL-C, 49.3 mg/dL

Interestingly, at this moderate degree of hypertriglyceridemia, the Friedewald equation yields a value for VLDL-C of 49 mg/dL.

Applying the classical equation to calculate LDL-C:

LDL-C = TC – HDL-C – VLDL-C = 259 – 35 – 49.3 = 174.7 mg/dL

The difference in LDL-C values is small (2.6 mg/dL), and represents 1.5% in this example. Such a small discrepancy would not be expected to lead to any significant mis-stratification in risk, and moreover, can be interpreted as a further validation of Equation 2. The major point to be made in this context however is that Equation 2 allows reliable calculation of LDL-C even at TG levels superior to 350 mg/dL (see figure 2 in ref (1)), in contrast to both the Friedewald and Martin formulations (1).

A further point of interest concerns estimation of LDL-C when the LDL profile is dominated by cholesterol-poor, small dense particles of elevated atherogenicity, as in the atherogenic dyslipidemia of type 2 diabetes and metabolic syndrome (1). This question could be initially evaluated by comparative analysis of LDL-C using Equation 2 and the above equation, together with non-HDL-C and apolipoprotein B, in two cohorts, one displaying diabetic dyslipidemia, the second a normolipidemic group.

One issue uniformly associated with calculated LDL-C values is the inclusion of cholesterol present in lipoprotein (a), an LDL-like lipoprotein particle whose causal role in the pathophysiology of ASCVD is

now established (1,3). Indeed, this point is of considerable relevance now that low LDL-C levels (<50 mg/dL) have become a clinical reality with the introduction of combination lipid lowering strategies.

The sole approach here is the direct assay of plasma Lp(a) mass; as ~30% of Lp(a) mass corresponds to cholesterol, Lp(a) cholesterol content can be subtracted from that of calculated LDL-cholesterol. Such direct quantitation of Lp(a) promises to be pertinent, as agents targeted to lower Lp(a) levels specifically are now in Phase 3 of development.

Finally, and as suggested by the authors, might Equation 2 provide an approach to a more precise estimation of remnant cholesterol than that derived from the relation: remnant cholesterol = TC – LDL-C – HDL-C, the latter estimating the cholesterol content of TGRL including remnants. Thus, if non-HDL-C is differentiated into cholesterol in LDL versus that in TGRL, then determination of the ratio of TG (as total)/cholesterol in this fraction might act as a guide to cholesterol load, in which case clinicians would potentially access a parameter which may be a strong predictor of CVD (1,3,5). Indeed, the ongoing development of several innovative therapeutics to lower TGRL and remnant levels, including apolipoprotein CIII and angiopoietin-like protein-3 inhibitors and omega-3 fatty acids, makes this approach of major relevance to the personalised treatment of atherogenic dyslipidemias.

Conclusion

Importantly, “Equation 2” as proposed by Remaley and collaborators for calculation of LDL-C has two major strengths: firstly, it allows accurate estimation of LDL-C over the range of TG levels up to almost 800 mg/dL, and secondly it provides reliable estimation of LDL-C at concentrations well below 50 mg/dL (5). Remarkably then, the standard laboratory lipid profile maintains its relevance to both the diagnosis and management of atherogenic dyslipidemias. Moreover, the development of “Equation 2” has highlighted the potential of this simple profile to integrate emerging insights in atherobiology and thence to translate them to cardiovascular risk estimation.

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