Use of Serum Cholesterol/Triglyceride Ratio to Discern for Which Individuals the Friedewald Formula Can Be Used Confidently

Matilde González Gª-Estrada, Carmen R. Rodríguez Ferrer, Inaki Recalde Astarloa, and Enrique Montalvo Lahera

The values of low-density lipoprotein cholesterol obtained according to the Friedewald formula (Clin Chem 1972; 18:499–502), or by the De Long transformation (J Am Med Assoc 1986;256:2372–7), were compared with the values obtained when the individual cholesterol/triglyceride ratio of very-low-density lipoprotein was used for estimating the contribution of this lipoprotein to the total cholesterol. We found that these formulas gave the greatest errors for individuals with a low serum cholesterol/triglyceride ratio. We propose criteria for deciding when the numerically calculated value of low-density cholesterol is appropriate, and when it is not.

The knowledge of the low-density lipoprotein (LDL) cholesterol value is of interest in the differential diagnosis of hyperlipoproteinemias and can be a decisive factor in deciding which individuals need to be treated. Analytical difficulties in measuring this analyte directly have led to methods of estimating its concentration by numerical calculation, the formula of Friedewald et al. (1) being the first and most extensively used. More recently, the calculation proposed by De Long et al. (2) has gained advocates. Both formulas assume that the cholesterol/triglyceride ratio of very-low-density lipoprotein, (C/TG)VLDL, is roughly constant, and that all triglycerides (TG) are from VLDL, such that LDL-C can be calculated by using the formula LDL-C = TG – HDL-C – (TG × f), where f is a factor that represents the (C/TG)VLDL value and HDL is high-density lipoprotein. f is estimated by Friedewald et al. to be 0.45 when the cholesterol and triglycerides concentrations are expressed in mmol/L, or 0.02 when in mg/L; for De Long et al., f = 0.37 (for mmol/L) or 0.016 (for mg/L).

However, (C/TG)VLDL is not a constant (3), and doubts have emerged about the applicability of the Friedewald formula for this discrimination. Thus, several studies (4, 5) have been undertaken to determine in which cases this indirect form of obtaining the LDL-C value is applicable. For example, Rao et al. (4) report that, in individuals with high concentrations of serum triglycerides and cholesterol, the Friedewald formula underestimates the true LDL-C value. In addition, Marsal et al. (5) have demonstrated that progressive increases in serum triglycerides are accompanied with a progressive increase in the proportion of individuals in whom the estimated LDL-C value is significantly erroneous. In these works, it is evident that the accuracy in the applicability of the Friedewald formula varies with the concentration of serum triglycerides, and a threshold for concentrations of serum triglycerides above which the Friedewald formula should not be used is recommended (1). These reports emphasize that, for some groups of individuals, their serum lipid profile is such that the calculated LDL-C value is erroneous. We have therefore focused on searching for criteria that will permit us to make more accurate decisions about individuals.

Materials and Methods

We studied 62 apparently healthy subjects and 33 patients diagnosed as having vascular disease. Subjects fasted for at least 12 h before blood sampling. Blood samples were adjusted to contain 1 mg of EDTA (sodium salt) per milliliter and were immediately placed on ice. After obtaining plasma by centrifugation (600 × g for 15 min at room temperature), we overlayed 4 mL of plasma with 2 mL of NaCl solution (density = 1.006 kg/L) and centrifuged at 150 000 × g in a 50 Ti rotor in an L-7-55 ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA). VLDL fractions of 1.5 mL were collected by aspiration from the surface with a Pasteur pipette. Cholesterol and triglycerides in plasma and VLDL fractions were determined by enzymatic methods (cholesterol CHOD-PAP and triglycerides GPO-PAP; Boehringer Mannheim, Mannheim, F.R.G.) (6, 7), standardized with control sera (Precipath U and Precipath U) provided by the same manufacturer. HDL-C was determined after precipitation of apolipoprotein B-containing lipoproteins with phosphotungstate in the presence of magnesium ions (HDL-cholesterol precipitant; Boehringer Mannheim) (8).

Results and Discussion

The mean ratio for (C/TG)VLDL in the control group was not significantly different (P > 0.05) from that for patients. Also, the mean value for LDL-C obtained by using the true individual value for the (C/TG)VLDL ratio (LDL-C<sub>individual</sub>) was not significantly different from that obtained by using the Friedewald formula (LDL-C<sub>F</sub>) in controls or in patients (Table 1).

However, as Figure 1 shows, the (C/TG)VLDL ratio varies greatly both in controls and in patients. Therefore, although there is no difference between the mean values of LDL-C<sub>individual</sub> and of LDL-C<sub>F</sub>, any one individual obviously may show considerable differences. Moreover, if the three extreme values in controls and the one extreme value in the patients are omitted, the (C/TG)VLDL values of the controls are grouped about a mean of 0.39 (when the cholesterol and triglyceride values are expressed in mmol/L) or 0.17, when the same analytes are expressed in mg/L, and the mean for the patients is 0.40 (or 0.175, respective-

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1 Nonstandard abbreviations: C, cholesterol; TG, triglycerides; VLDL, LDL, HDL, very-low-, low-, and high-density lipoproteins, respectively; and (C/TG)VLDL, cholesterol/triglycerides ratio of VLDL.

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Table 1. (C/TG) VLDL Ratio, LDL-C<sub>individual</sub>, LDL-C<sub>phys</sub>, and Percentage of Errors Obtained with the Friedewald Formula

<table>
<thead>
<tr>
<th></th>
<th>(C/TG) VLDL&lt;sup&gt;a&lt;/sup&gt;</th>
<th>LDL-C&lt;sub&gt;individual&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>LDL-C&lt;sub&gt;phys&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</th>
<th>% errors&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.44 (0.04)</td>
<td>2.70 (0.11)</td>
<td>2.71 (0.11)</td>
<td>18</td>
</tr>
<tr>
<td>Patients</td>
<td>0.42 (0.03)</td>
<td>4.03 (0.20)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>3.95 (0.19)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>21</td>
</tr>
</tbody>
</table>

<sup>a</sup> C/TG obtained from measured C and TG.
<sup>b</sup> Obtained by using the (C/TG) VLDL ratio for each individual.
<sup>c</sup> Obtained by using the Friedewald formula.
<sup>d</sup> Within each group.
<sup>*</sup> Significantly different from controls (P < 0.001).

Table 2. Error Distributions in Calculating LDL-C from Measured Individual C/TG Ratio

<table>
<thead>
<tr>
<th>C/TG, mmol/L</th>
<th>n</th>
<th>% error</th>
<th>Mean error, mmol/L (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3.39</td>
<td>14 (23)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50 (7/14)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.32 (125.5)</td>
</tr>
<tr>
<td>3.4-4.5</td>
<td>10 (16)</td>
<td>40 (4/10)</td>
<td>0.58 (227.0)</td>
</tr>
<tr>
<td>4.51-5.66</td>
<td>12 (19)</td>
<td>—</td>
<td>0.10 (38.0)</td>
</tr>
<tr>
<td>&gt;5.67</td>
<td>26 (42)</td>
<td>—</td>
<td>0.07 (26.0)</td>
</tr>
<tr>
<td>Totals</td>
<td>62 (100)</td>
<td>18 (11/62)</td>
<td>0.21 (83.0)</td>
</tr>
</tbody>
</table>

<sup>a</sup> % of total is listed in parentheses.
<sup>b</sup> In parentheses are the no. of individuals with error > ±10%/total no in that C/TG group.

Fig. 1. (C/TG) VLDL distribution in (a) controls and (b) patients
Cholesterol and triglycerides are expressed in mmol/L.

Likely. These results are more consistent with the factors used in the De Long calculation than with those used in the Friedewald formula. From this we conclude that the De Long calculation is more convenient than the original Friedewald formula for most cases, although the rate of errors is still high. In 18% of controls and 21% of patients, the calculated LDL-C (by both formulas) differs by ±10% or more from the value obtained from the individual (C/TG) VLDL ratio (Table 1).

Analysis of the lipid profile of the individuals shows that the greatest errors are found in those whose serum (or plasma) C/TG ratio is lower (Table 2). The proportion of patients in the lowest grouping for the serum C/TG ratio greatly exceeded the proportion of controls.

As Table 2 shows, for controls with a serum C/TG ratio (with C and TG expressed in mmol/L) > 4.5, and > 3.4 for patients, the LDL-C can be accurately calculated by using the De Long formula. At lower values for serum C/TG, there is a higher probability of significant errors.

Approximately 39% of the individuals in the control group have a high level of uncertainty, i.e., those in the two lowest strata. None of these has serum TG > 3.3 mmol/L, a threshold above which use of the Friedewald formula for calculating LDL-C is never recommended. In the patients' group, the stratum with the greatest errors certainly includes not only all those with the highest serum TG values, but also some patients with serum TG < 2.2 mmol/L, some of which show the greatest errors. Therefore, the concentration of serum TG is not a reliable criterion for deciding when the Friedewald formula can be used confidently, and when not.

In the groups with C/TG ratio < 4.5 for controls and < 3.4 for patients (the major errors), the numerical calculation of the LDL-C is not reliable. We propose a judicious approach in this case: to identify those for whom LDL-C falls unquestionably below the recommended levels of risk (desirable LDL-C < 3.4 mmol/L; borderline high-risk LDL-C 3.4-4.1; high-risk LDL-C > 4.1) (9), and to identify those who should be investigated more extensively. Conservatively, a value of total C - HDL-C (which is more than the real LDL-C) equal to or less than such a value can be disregarded. In our control group, 10 of the 24 individuals in the two lowest strata had an underestimated LDL-C value > 3.4 mmol/L (1300 mg/L); of these, five had an LDL-C value > 4.1 mmol/L (1600 mg/L), two of whom were young men whose fathers had had a myocardial infarction. In the patients' group, of the 18 individuals from the lowest stratum, only two had a value of TG - HDL-C < 4.1 mmol/L. For the other 16, we obtained a more nearly accurate value for the LDL-C, always proceeding conservatively, assuming that LDL-C is very probably greater than total C - HDL-C - (TG x 0.23), where 0.23 is the value for the (C/TG) VLDL ratio (when the cholesterol and triglycerides values are expressed in mmol/L; 0.10 when they are in mg/L). Almost all the values for the (C/TG) VLDL ratio, both in control and in patients, are greater than this. When we used this approximate value for LDL-C as f in the above formula for calculations, in only two cases was the estimated value smaller than that obtained by using the (C/TG) VLDL<sub>individual</sub> ratio. We verified that all the individuals with serum TG > 3.3 mmol/L were those in which LDL-C was more greatly underestimated.
In summary, for patients we can obtain a more approximate value for LDL-C that, when the serum concentrations of TG are >3.3 mmol/L, is almost surely overestimated. Only those with an estimated LDL-C >4.1 mmol/L, especially if their serum TG is <3.3 mmol/L, will merit additional investigation. This proposed approach is summarized in Figure 2.

![Strategy proposed to determine if further investigation is necessary among those individuals having a possibly unreliable calculated LDL-C](image)

**Fig. 2.** Strategy proposed to determine if further investigation is necessary among those individuals having a possibly unreliable calculated LDL-C.

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**References**


**CLIN. CHEM. 36/9, 1675-1678 (1990)**

**Quantitative Nephelometric Assay for Determining Myoglobin Evaluated**

**orls R. Delanghe,1 Jean-Paul Chapelie,2 and Serge C. Vanderschueren1**

A recently introduced automated nephelometric immunoassay involving shell/core particles for determination of myoglobin (Behringwerke) was evaluated with the BNA Nephelometer. Method precision was good: the intra-assay CV varied between 1.5% and 6.1%; with daily calibration, the interassay CV ranged between 1.5% and 7.5%. For usual sample dilutions, the assay response varied linearly with myoglobin concentrations up to 23.1 nmol/L. After automatic dilution by the instrument, concentrations up to 2310 nmol/L could be measured without high-dose "hook" effect. Further manual dilution allowed measurement of myoglobin concentrations up to 26 000 nmol/L. Calibration was stable for at least seven days. We detected no significant interferences from hemoglobin, heaptoglobin, bilirubin, iodine-containing contrast media, and rheumatoid factors. Treating lipemic samples with Lipoclean (Behringwerke) decreased test results. Simultaneously drawn serum and plasma samples from the same subject showed no consistent differences in myoglobin concentrations. The mean reference myoglobin concentration was 1.380 (SD 0.82) nmol/L for men and 0.878 (SD 0.45) nmol/L for women. In patients with renal insufficiency, serum creatinine values were moderately related to serum myoglobin values (r = 0.465). Although a commercial radioimmunoassay (Byk-Sangtec) and the nephelometric assay intercorrelated well (r = 0.929), values obtained by nephelometry were significantly lower (P < 0.05). By both assays, results for heart and skeletal muscle tissue extracts showed no correlation, a finding that suggests the existence of multiple forms of myoglobin in human tissues. We conclude that immunonephelometry is a rapid, practical, and reliable method for measuring myoglobin in serum.

**Addtional Keyphrases:** reference values, renal insufficiency, sex- and age-related effects

Myoglobin is a small-molecular-mass oxygen-binding protein (M, 17 700), abundant in human skeletal and...