Clinica Chimica Acta 444 (2015) 137–142

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Comparison of equations for the calculation of LDL-cholesterol in hospitalized patients

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ARTICLE INFO

Article history:
Received 6 January 2015
Received in revised form 29 January 2015
Accepted 30 January 2015
Available online 7 February 2015

Keywords:
LDL-cholesterol
Friedewald equation
Hospitalized patients

ABSTRACT

Background: The Friedewald equation is widely used to calculate LDL-C for cardiovascular risk prediction but is less accurate with comorbidities and extreme lipid values. Several novel formulae have been reported to outperform the Friedewald formula.
Methods: We examined 14,219 lipid profiles and evaluated four formulae (Friedewald, Chen, de Cordova, Hattori) and compared these to direct measurement of LDL-C across various triglyceride (TG), total cholesterol (TC) and HDL-cholesterol (HDL-C) ranges using Beckman reagents and instruments. Linear regression and ROC analysis were performed.
Results: The de Cordova formula showed a high correlation with directly measured LDL-C ($r = 0.90$, $P < 0.001$), comparable to the Friedewald calculated values for directly measured LDL-C ($r = 0.95$, $P < 0.001$). The de Cordova formula was favorable in some ranges of HDL, TC and the lowest TG range ($r = 0.97$, $P < 0.001$) but performed least well in comparison with the three other LDL-C calculations (AUC = 0.8331), demonstrating inconsistent bias. The Chen formula performed better than Friedewald (AUC = 0.9049). The Hattori formula outperformed all formulae including Friedewald over various ranges of lipid values (AUC = 0.9097).
Conclusions: We observe favorable correlations of the de Cordova formula with Friedewald at low TG values. However, the Hattori formula appears to be best for application in hospitalized patients, even at extreme lipid values.

1. Introduction

LDL-cholesterol (LDL-C) is used for cardiovascular disease (CVD) risk assessment [1,2]. The gold standard for measurement of LDL-C is by ultracentrifugation and beta-quantification [3]. This is expensive and inconvenient for the routine laboratory. Other methods include direct measurement of LDL using a homogeneous assay, but this is too expensive for use in most laboratories. Furthermore, direct methods show poor performance with high triglyceride (TG) levels [4–6]. An earlier review comparing direct measurement of LDL-C vs calculation of LDL recommended the use of direct LDL measurements in hypertriglyceridemic patients [6]. However, a recent study comparing eight direct measurements of LDL-C and HDL-C failed to show improved CVD risk classification of most direct methods over calculated LDL-C [4].

The first formula to calculate LDL-C was developed over 40 years ago by Friedewald [7]. The formula requires fasting plasma high density lipoprotein-cholesterol (HDL-C), total cholesterol (TC), and TG, and is calculated as LDL-C = TC − HDL − (TG / 5) for mg/dl (2.2 in mmol/l). This formula is less accurate in extremes of TG or TC values [7–10] or in patients with co-morbidities (eg. renal failure or diabetes) [2,11], but is widely used. Several other formulae have been developed, but these did not perform better than Friedewald's calculation [12–14] or had varying results in different population groups [10,15–19] and including those considering TG ratios [20,21]. In the latest study validating a novel formula in comparison with Friedewald's calculation and the LDL-C reference method in 23,055 patients, the benefits over Friedewald were not considered substantial enough to replace its use in clinical practice [22], demonstrating positive bias at low levels of LDL (<1.81 mmol/l). The previously published formula by de Cordova et al. [23] has been reported to outperform several of the earlier LDL-C formulae, including Friedewald's formula, over a wide-range of lipid levels using the equation LDL-C = 0.7516 (TC − HDL-C) in 10,664 Brazilian patients, including those with co-morbidities. However, this formula also showed bias at low levels of LDL-C in a subsequent study of 576 healthy subjects in South Africa [24].

As difficulties with LDL measurements prevail, a search for new formulae and emerging cardiovascular risk markers to improve accurate CVD prediction is ongoing. We validated the application of four

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http://dx.doi.org/10.1016/j.cca.2015.01.037
0009-8981/

formulae (Friedewald, de Cordova, Chen, Hattori) to calculate LDL-C in our population of hospitalized patients. We compared the formulae to the direct measurement of LDL-C, using the largest sample size to date, where multiple formulae are compared.

2. Methods

2.1. Study population

This was a retrospective evaluation of lipid profiles in 14, 219 patients in South Africa, from 1 January 2013 to 30 June 2013, using a database from the National Health Laboratory Services, the largest provider of laboratory services in South Africa. The laboratory is accredited by the South African National Accreditation System (SANAS), and serves a large tertiary academic hospital and surrounding clinics. The laboratory participates in the EQA program, the Thistle Lipid Programme. Procedures followed were approved by the Faculty of Health Sciences Research Ethics Committee of the University of Pretoria in accordance with the Helsinki Declaration.

Blood samples were collected into serum separator tubes to determine LDL-C, HDL-C, TG and TC. Samples were centrifuged after collection and analyzed immediately. Patient details were anonymized, with only patient age and gender reported.

Measurements of LDL-C, HDL-C, TC and TG were performed using reagents by Beckman Coulter, according to the specification of the manufacturers using the Beckman DXC automated analyser (Brea, CA, USA).

The direct LDL-C method is a homogeneous assay without the need for any pretreatment or centrifugation steps and based on the Daiichi two-phase method [25]. The coefficient of variation (CV) of LDL-C using the homogenous method was 4.5% for level 1 and 4.0% for level 3.

The HDL-C measurement was performed using a homogenous, colorimetric, enzymatic method. The CVs of the HDL at levels 1 and 3 respectively were 6.3% and 4.3%. Total cholesterol measurement involved a colorimetric, enzymatic, timed-endpoint method; the CVs of the TC at levels 1 and 3 was 3.4% and 4.6% respectively. Triglyceride measurement used a sequence of three coupled enzymatic steps to form a red quinoneimine dye. The CVs of the TG measurements at levels 1 and 3 was 4.3% and 3.9% respectively. The performance standards in terms of the CVs for the lipid analysis were all within the acceptable CV for Beckman DxC800.

2.2. Data analysis

Microsoft Excel was used to capture the data, according to the different lipid levels and for the calculation of LDL-C. STATA was used to perform the statistical analysis, which included a descriptive statistics summary. Pearson's correlation was performed for directly measured LDL-C and non-HDL-C, as well as between the four formulae and directly measured LDL-C values obtained from the laboratory measurements. The root mean square error (rMSE) was calculated as a measure of accuracy in the differences between values predicted by an estimator and values observed from those being estimated to compare the formulae across various lipid ranges. Bland–Altman plots were used to evaluate the agreement between the four formulae and the directly measured LDL-C. ROC curve analysis was used to compare the performance of the different formulae considering the area under the curve (AUC). The coefficient of concordance was used to assess the relative performance of the different methods relative to the direct LDL-C measurement.

3. Results

A total of 14,219 lipid profiles were identified, of which 39% were male and 61% were female. Patient-specific data about the presence/absence of disease, treatments and ethnicity was not available. The average age was 52 years with a range of directly measured LDL-C from 10.81–712.74 mg/dl, mean 111.97 mg/dl [0.28–18.46 mmol/l (mean 2.9 mmol/l ± 1.15 Standard deviation (SD)]; for HDL-C from 4.63–400.39 mg/dl, mean 44.02 mg/dl [0.12–10.37 mmol/l (mean 1.14 mmol/l ± 0.39 SD)]; for TC from 9.28–1184.84 mg/dl, mean 184.45 mg/dl [0.24–30.64 mmol/l (mean 4.77 mmol/l ± 1.47 SD)], and 9.74–5837.91 mg/dl, mean 162.10 mg/dl [0.11–65.91 mmol/l (mean 1.83 mmol/l ± 1.90 SD)] for TG. The mean (SD) calculated LDL-C values are shown in Table 1.

Using Pearson's analysis, we show high correlations between the four formulae and directly measured LDL-C using the Daiichi two-phase method (Table 1 and Supplementary Fig. 1). The de Cordova formula, although highly correlated with directly measured LDL-C ($r = 0.90$, $P < 0.001$), was lower than the correlation observed with the other three formulae. The Friedewald formula had a higher correlation ($r = 0.9518$, $P < 0.001$) than the Chen formula ($r = 0.9498$, $P < 0.001$) but was lower than the correlation observed with the Hattori formula ($r = 0.9626$, $P < 0.001$) (Fig. 1).

We also examined correlations between directly measured LDL-C and non-HDL-C (TC–HDL-C), LDL-C and TG, LDL-C and HDL-C/TG ratio, LDL-C and TC/HDL-C ratio, LDL-C and HDL-C/LDL-C ratio, and LDL-C and LDL-C/non-HDL-C ratio. Strong correlations were observed between LDL-C and non-HDL-C ($r = 0.93$) and TC and non-HDL-C ($r = 0.964$).

Using a ROC curve (Fig. 2), the Hattori formula was shown to perform the best with an AUC of 0.9097, followed by the Chen (AUC = 0.9049), the Friedewald (AUC = 0.9018) and the de Cordova (AUC = 0.8331) formulae. Sensitivities and specificities are shown in Table 2, and are based on an LDL cut-off of 2.5 mmol/l.

Table 3 demonstrates the rMSE of the four different formulae across different levels of HDL-C, TG and TC. The de Cordova formula was the least accurate at low HDL levels with a rMSE of 559 but at high HDL-C performed better (a rMSE of 102.7) than the Friedewald and Chen formulae with a rMSE of 130.2 and 106, respectively. The Hattori formula outperformed the other equations across all HDL-C and TG ranges, and TC ranges 73.10–218.87 mg/dl (1.89–5.66 mmol/l). At TG < 187 mg/dl (<2.11 mmol/l), the Hattori formula had a rMSE from 55.6 up to 85.9 with a rMSE of 280 for TG >187 mg/dl (>2.11 mmol/l), compared to a rMSE of >400 for the other three formulae. At the high end of TG ranges [>187 mg/dl (>2.11 mmol/l)], the de Cordova showed the lowest accuracy (rMSE 479.6), followed by the Chen formula (a rMSE of 433.9) then the Friedewald formula (a rMSE of 418.5). At the lowest end of TG levels [17.71–90.35 mg/dl (0.20–1.02 mmol/l)], the de Cordova formula had the highest accuracy with a rMSE of 54.2; the Friedewald formula had the lowest accuracy with a rMSE of 74.8. The Friedewald formula had the highest accuracy at the high end of TC ranges [250.20–522.82 mg/dl (6.47–13.52 mmol/l)], with a rMSE of 120. At the different TC ranges, the rMSE for the Friedewald formula was from 92.5 up to 316.9, compared with 130.1–272 for the Chen formula, 163.1–323.7 with de Cordova and 82–185.6 with the Hattori formula.

Fig. 3 shows Bland–Altman difference plots of the directly measured LDL-C and the LDL-C derived from the four formulae. The mean bias for the Friedewald formula was 4.10 ± 27.84 mg/dl (0.106 ± 0.72 mmol/l), 6.73 ± 37.51 mg/dl (0.174 ± 0.97 mmol/l) using the de Cordova formula, −6.57 ± 27.84 mg/dl (−0.17 ± 0.72 mmol/l) for the Chen formula, and 1.39 ± 23.98 mg/dl (0.036 ± 0.62 mmol/l) for the Hattori formula.

4. Discussion

LDL-C concentrations are a primary target of diagnosis and treatment of patients with hyperlipidemia defined by The National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III [1,2]. LDL-C monitoring remains significant in the management of CVD risk despite the revised AHA practice guidelines which no longer support the use of a LDL target [26]. One of the most common problems in the laboratory is to accurately estimate LDL-C. This has important implications on CVD classification, and if done incorrectly can adversely