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ORIGINAL ARTICLE

Low-Density Lipoprotein Values Estimated by Friedewald Equation are Affected by Diabetes Control

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Abstract

Background: Friedewald equation (FE) is widely used to estimate the LDL-c without the use of ultra-centrifugation. However, the equation has limitations in some clinical settings.

Objective: Our goal was to investigate the potential importance of differences between FE and direct measurement of LDL-c in patients with diabetes.

Methods: We conducted a cross-sectional study among 466 patients with stable coronary disease. Total cholesterol, triglycerides, HDL-c and LDL-c were collected, and FE was calculated. Accuracy was calculated as the percentage of estimates within 30% (P30) of measured LDL. Bias was calculated as the mean difference between measured and estimated LDL-c. Agreement between methods was evaluated using Bland-Altman plots.

Results: Bias was 3.7 (p=0.005) and 1.1 mg/dl (p=0.248), and accuracy was 86% and 93% in diabetic and non-diabetic patients, respectively. Among patients with diabetes, bias was 5 mg/dl (p=0.016) and 1.93 mg/dl (p=0.179), and accuracy was 83% and 88% in subjects with Hemoglobin A1C above 8 mg/dl versus below cutoff point, respectively. Bias was similar in patients without diabetes compared to patients with diabetes and HbA1C < 8 (1.1 and 1.93 mg/dl).

Conclusion: FE is inaccurate among overall individuals with diabetes. However, when stratifying patients with diabetes into good and poor disease control, the first group behaves as if it does not have diabetes, with a good correlation between calculated and measured LDL-c. It is important to know when is it reasonable to use FE because an inaccurate estimation of LDL-c levels could result in undertreatment of dyslipidemia and predispose these patients to acute events. (Int J Cardiovasc Sci. 2016;29(5):348-354)

Keywords: Hypercholesterolemy; Cholesterol, LDL/blood; Cholesterol,VLDL/blood; Mathematics; Diabetes Mellitus.

Background

Diagnosis and management of patients with hypercholesterolemia is largely based on LDL cholesterol (LDL-c) levels, considering it the main target of cholesterol lowering therapy. In the early 70s, Friedewald and colleagues[1] created a method to estimate the LDL-c without the use of ultra-centrifugation, which was, although gold standard, an expensive and time consuming way of measuring LDL-c. This study represented a milestone in scientific literature, with its findings being used today as a reference for assessing the levels of LDL-c and therapeutic guidance. However, the correlation was not as good in situations where the relationship between VLDL and triglycerides (TG) changes, such as high TG levels (especially above 400 mg/dL) and dysbetalipoproteinemia.

In addition to these situations, more recent studies recommend caution when using Friedewald`s equation

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(FE) in certain pathological conditions that result in secondary hyperlipidemia (such as diabetes, and kidney disease, for example).[2,3] Diabetes not only has a classical pattern of dyslipidemia (increased TG, low HDL-c, and the predominance of particles of LDL-c and small dense), but it also confers a propensity for the development of premature atherosclerosis, probably due to changes in lipoprotein metabolism.[4,5] Our goal was to investigate the potential importance of differences between FE and direct measurement of LDL-c in patients with diabetes.

Methods

We conducted a cross-sectional study among patients from an outpatient clinic in a tertiary care university hospital in Southern Brazil. Between 2008 and 2013, 466 consecutive patients with CAD were enrolled at the Ischemic Heart Disease Clinic. All patients had documented CAD, which was defined by the presence of at least one of the following factors: documented history of myocardial infarction, surgical or percutaneous myocardial revascularization and lesion > 50% in at least one coronary artery assessed by angiography, or the presence of angina and positive noninvasive testing of ischemia.[6]

Blood samples were collected by venipuncture into tubes with and without EDTA and sent to the Laboratory of Clinical Pathology for total cholesterol, TG, HDL-c, direct LDL-c and other routine tests. The total cholesterol, TG and HDL-c were measured in accordance with the routine Clinical Pathology Laboratory of our hospital. The LDL-c was calculated by FE ([LDL-c] = [CT] - ([HDL-c] + [VLDL-c]), where the concentration of VLDL-c is estimated by the serum concentration of TG [VLDL-c] = [TG]/5).

The direct measurement of LDL-c was made by the LDL-c Plus kit from Roche Diagnostic, previously standardized in the same laboratory (Roche Diagnostics Brazil). The LDL-c Plus test is a homogeneous enzyme assay for quantitative determination of direct LDL-c in serum, used in automated clinical chemistry analyzers - MODULAR. For quality control purposes, we used the G Precinorm, Precipath HDL/LDL-C, and the curves provided by the device. The measuring/reference range is 3 to 550 mg/dl (0.03 to 5.5 g/l or 0.077 to 14.2 mmol/l). The determination of samples with concentrations of LDL-c > 550 mg/dl was made with the re-analysis function.

The result of measured LDL-c in direct mode was only used for research purposes. This study was approved by the Institutional Research and Ethics Committee and informed consent was obtained from all patients.

Statistical analysis

Continuous variables are expressed as mean ± 1 standard deviation (SD). Categorical variables were represented by relative and absolute frequencies. Accuracy was calculated as the percentage of estimates within 30% (P30) of measured LDL. Precision was measured as 1 SD of bias. The agreement between measured and calculated LDL-c was evaluated using Bland-Altman plots, with the calculation of agreement limits (bias ± 2 SD) and CI.[7] Bias was calculated as the mean difference between measured and estimated LDL-c. According to Bland-Altman, 100 individuals are enough to estimate bias and limits of agreement within a 95% CI of about 34% of SD.[8] All data were analyzed using SPSS (version 18.0.0; IBM Company) and P values less than 0.05 were considered significant.

Results

Overall, patients were 63.9 (± 13.3) years of age, 56.8% were women and 46.1% had diabetes. The mean directly measured LDL-c was 106.9 ± 37.1 and the mean Friedewald-estimated LDL-c was 104.7 ± 35.6. The mean total cholesterol and HDL-c were 181.1 (± 42.1) and 48.5 (± 12.3), respectively. The median triglycerides levels were 154.5. Statins were used by 76.5% of the individuals. The mean hemoglobin A1C levels among patients with diabetes were 7.8±1.8%. Complementary data are shown in table 1.

Comparing FE and directly measured LDL-c in patients with different TG levels, those with higher TG levels had greater mean differences and within-group variance (Figure 1). When we divided TG levels into quartiles (TG < 150 mg/dl, 151-200 mg/dl, 201-300 mg/dl and > 300 mg/dl), variance was wider when TG levels were higher. In patients with TG levels above 300 mg/dL, 44% had a difference between FE and directly measured LDL-c greater than 20%. Among patients with TG levels below 150 mg/dL, only 12% had the same difference.

Table 1
Demographic characteristics

Characteristics	N = 466
Age	63.9 (±13.3)
Male Sex	201 (43.2)
Diabetes	186 (46.1)
HBA1C (%)	7.85 (1.85)
HBA1C (mmol/mol)	62 (6)
Hypertension	386 (83.3)
Chronic Renal Disease	47 (10.1)
Current Smoking	45 (9.7)
Hypothyroidism	66 (14.2)
Total Cholesterol (mg/dL)	181.1 (± 42.1)
HDL Cholesterol (mg/dL)	48.5 (± 12.1)
Triglycerides (mg/dL)	154.5 (± 74.4)
< 150	262 (56.2)
150-200	99 (21.2)
201-300	78 (16.7)
> 300	27 (5.8)
Statin	354 (76.5)

Values are reported as mean ± SD, n (%). HBA1C values were only measured in diabetic patients.

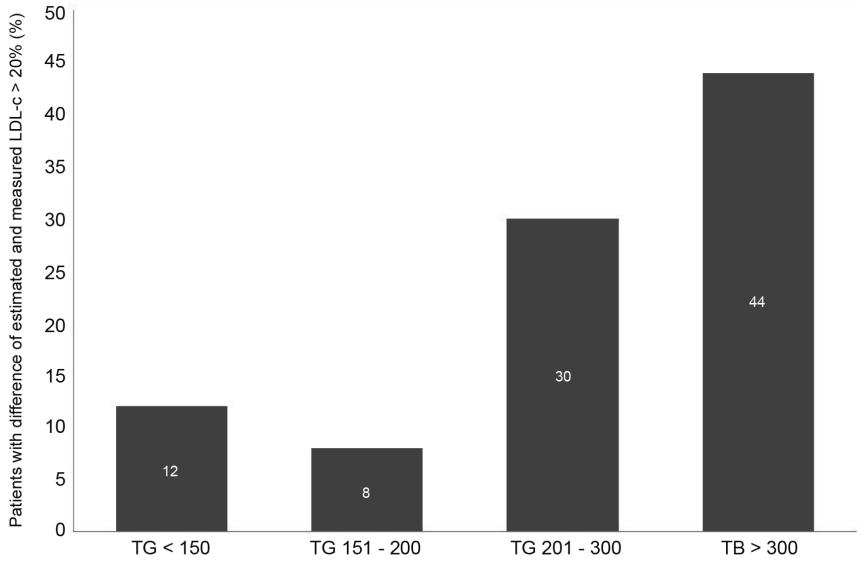

Figure 1
Percentage of patients with difference of estimated and measured LDL-c > 20%.

Among patients with diabetes, the FE systematically underestimated measured LDL-c (Figure 2). Bias was 3.7 (p=0.005) and 1.1 mg/dl (p=0.248), accuracy P30 (95% CI) was 86% and 93%, and precision was 80 and 79 in patients with or without diabetes, respectively. A non-significant p value suggests that there is no difference between tests. When we compared patients with diabetes (Figure 3), bias was 5 mg/dl (p=0.016) and 1.93 mg/dl (p=0.179), accuracy P30 (95% CI) was 83% and 88%, and precision was 78 and 77 in subjects with hemoglobin A1C above 8 mg/dl versus below cutoff point, respectively.

Bias was similar in patients without diabetes vs. patients with diabetes and HbA1C < 8% (1.1 and 1.93 mg/dl), although it was greater (5.0 mg/dl) among patients with diabetes and HbA1C >8%. There was no statistically significant difference between mean TG levels in patients with HBA1C below and above 8 mg/dl (155 and 164 mg/dl respectively, p=0.402).

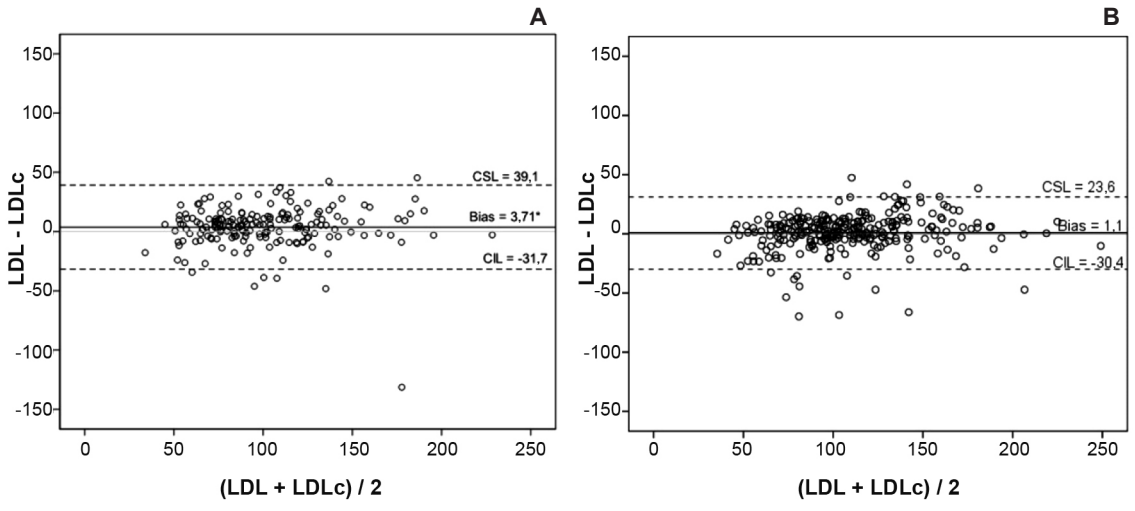

Figure 2
Bland and Altmann plots of average between measured and estimated LDL vs. difference between them in the 186 patients with diabetes (A) and 280 patients without diabetes (B) (P = 0.005 and 0.248, respectively).
Bias and concordance superior (CSL) and inferior limit (CIL) described above.

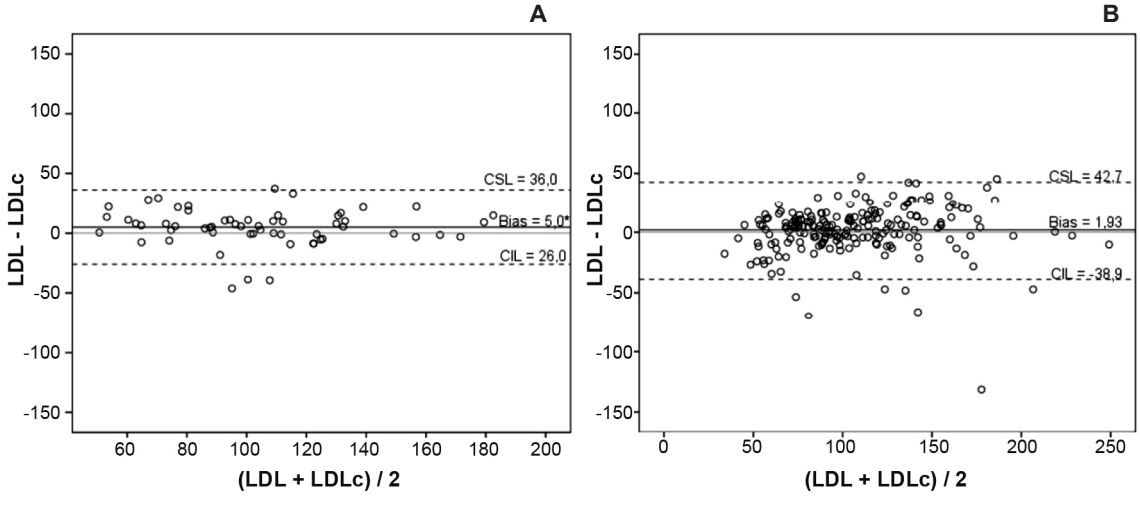

Figure 3
Bland and Altmann plots of average between measured and estimated LDL vs. difference between them in the 56 patients with uncontrolled diabetes (A) and 95 patients with controlled diabetes (B) (P = 0.005 and 0.248, respectively).
Bias and concordance superior (CSL) and inferior limit (CIL) described above.

Discussion

Studies over several decades have identified a direct relationship between LDL-c and the onset of new cases of CAD in previously healthy men and women.[9-11] The same relationship is observed for recurrent coronary events in patients with established CAD.[12-15] Dosing levels of LDL-c is extremely important, since the diagnosis and management of dyslipidemia is largely based on their concentrations. However, the gold standard method for its measurement (beta-quantification by ultra-centrifugation) requires expensive instrumentation not available in routine clinical laboratories, and it is laborious and time consuming.

Although there are several technical disadvantages of the FE - the need for fasting, relatively high imprecision secondary to accumulated variation of the other 3 measures, and recognized limitations in certain patients – the method is routinely used in clinical practice, and will only be replaced if other methods show clear advantages in performance, cost-effectiveness, or other financial benefits.

The FE is widely applied and performs remarkably well for most patients. However, differences between FE and directly measured LDL-c may arise in some conditions, such as higher TG levels. A recent study[16] has shown that FE tends to underestimate LDL-c most when accuracy is most crucial, especially if TG levels are above 150 mg/dl. Our study also showed that high TG levels makes FE underestimate LDL-c values. There is a significant proportion of patients with significant difference (> 20%) in test results, and this number exponentially increases with TG levels. Particularly in the presence of TG levels above 150 mg/dl, LDL-c underestimation is clinically relevant and may result in undertreatment.

A previous study[2] suggested that calculation of LDL-c by FE may be inaccurate for assessment of cardiovascular risk in patients with type II diabetes and may not be appropriate for management of lipoprotein abnormalities in those patients. In their study, the FE overestimated by > 10% the actual LDL-c concentration in 39% of patients with diabetes and underestimated the true value in 13% of patients, with only 48% accuracy. Hirany et al.[17] suggested that direct LDL-c assay could be more reliable, accurate, rapid and cost-effective method than the FE for LDL-c determination in individuals with diabetes.

On the other hand, Whiting et al.[18] found that FE was adequate for the measurement of LDL-c in diabetes of both type I and II, with a 68% FE accuracy for subjects with type II diabetes. In our study, FE was inaccurate among overall individuals with diabetes. However, when we divided patients with diabetes into good and poor disease control (HbA1C below and above 8 mg/dL, respectively), the first group behaved as if it did not have diabetes, with a very good correlation between FE and measured LDL-c. Median TG levels were similar in both groups.

Study limitations

We could not perform a multivariate analysis including TG values among patients with poor controlled diabetes, both due to the limited sample size among groups, and because TG levels are mathematically linked with variables in the study (FE). Although chances of losing statistical power is high (poor controlled diabetes usually is associated with higher TG levels), the message of our study is that patients with poor metabolic control should have their LDL-c measured instead of estimated.

Conclusion

Even though there is a good correlation between FE and directly measured LDL-c in general population, the first tends to underestimate LDL-c levels in individuals with high TG levels and poorly controlled diabetes. Considering this high-risk subset of patients, underestimation of LDL-c levels could result in undertreatment of dyslipidemia and predispose these patients to acute events. Patients with diabetes with a good disease control have good correlation between calculated and measured LDL-c, and performing FE in these patients is acceptable.

Most studies to date relating LDL-c levels and cardiovascular disease used FE to estimate LDL-c. Thus, future LDL-guided therapeutic studies may consider using direct LDL-c measurement to determine ideal targets.

Author contributions

Conception and design of the research: Vieira PL, Moriguchi EH, Polanczyk CA. Acquisition of data: Vieira PL, Smidt LFS, Jost MF, Furtado MV. Analysis and interpretation of the data: Vieira PL, Araújo GN, Telo GH, Smidt LFS, Jost MF, Furtado MV, Moriguchi EH, Polanczyk CA. Statistical analysis: Vieira PL, Araújo GN, Telo GH, Smidt LFS, Jost MF, Furtado MV. Obtaining financing: Vieira PL, Polanczyk CA. Writing of the manuscript: Vieira PL, Araújo GN,

Telo GH. Critical revision of the manuscript for intellectual content: Vieira PL, Araújo GN, Telo GH, Smidt LFS, Moriguchi EH, Polanczyk CA.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

References

1. 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.
2. Rubies-Prat J, Reverter JL, Sentí M, Pedro-Botet J, Salinas I, Lucas A, et al. Calculated low-density lipoprotein cholesterol should not be used for management of lipoprotein abnormalities in patients with diabetes mellitus. Diabetes Care. 1993;16(8):1081-6.
3. Johnson R, McNutt P, MacMahon S, Robson R. Use of the Friedewald formula to estimate LDL-cholesterol in patients with chronic renal failure on dialysis. Clin Chem. 1997;43(11):2183-4.
4. Howard BV. Lipoprotein metabolism in diabetes mellitus. J Lipid Res. 1987;28(6):613-28.
5. Reaven GM. Abnormal lipoprotein metabolism in non-insulin-dependent diabetes mellitus: pathogenesis and treatment. Am J Med. 1987;83(3A):31-40.
6. Stein R, Alboim C, Campos CES, Mello RGB, Rosito G, Polanczyk CA. Variability among cardiologists in the management of patients with ischemic heart disease. Arq Bras Cardiol. 2004;83(2):135-60.
7. Bland JM, Altman DG. Measuring agreement in method comparison studies. Stat Methods Med Res. 1999;8(2):135-66.
8. Bland M. Sample Q:3 size for a study of agreement between two methods of measurement. [Internet] (Accessed in 2013 Jan 15). Available from: http://www.users.york.ac.uk/~mb55/ meas/sizemeth.htm.
9. The Lipid Research Clinics Coronary Primary Prevention Trial results I. Reduction in incidence of coronary heart disease. JAMA. 1984;251(3):351-64.
10. Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA. 1986;256(20):2823-8.
11. Wilson PW, D'agostino RB, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation .1998.97(18):1837-47.
12. Pekkanen J, Linn S, Heiss G, Suchindran CM, Leon A, Rifkind BM, et al. Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without preexisting cardiovascular disease. N Engl J Med. 1990; 322(24):1700-7.
13. Rossouw JE, Lewis B, Rifkind BM. The value of lowering cholesterol after myocardial infarction. N Engl J Med. 1990;323(16):1112-9
14. Wong ND, Wilson PW, Kannel WB. Serum cholesterol as a prognostic factor after myocardial infarction: the Framingham Study. Ann Intern Med. 1991; 115(9):687-93.
15. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet. 2002;360(9326):7-22.
16. Martin SS, Blaha MJ, Elshazly MB, Brinton EA, Toth PP, McEvoy JW, et al. Friedewald-estimated versus directly measured low-density lipoprotein cholesterol and treatment implications. J Am Coll Cardiol. 2013;62(8):732-9.
17. Hirany S, Li D , Jialal I. A more valid measurement of low-density lipoprotein cholesterol in diabetic patients. Am J Med. 1997;102(1):48-53.
18. Whiting MJ, Shephard MD, Tallis GA. Measurement of plasma cholesterol LDL in patients with diabetes. Diabetes Care. 1997;20(1):12-4.