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Comparison of the accuracy of the Friedewald, Martin, and Sampson formulas to estimate very low levels of low-density lipoprotein cholesterol

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Abstract

Introduction: The Martin (MF) and Sampson (SF) formulas have shown greater accuracy for low-density lipoprotein cholesterol (LDL-C) < 70 mg/dL compared to the Friedewald formula (FF); however, some disagreement is maintained. Non-high-density lipoprotein cholesterol (non-HDL-C) and apolipoprotein B (ApoB) are alternatives to assessing cardiovascular risk in patients with very low LDL-C. The objective was to evaluate the accuracy of FF, MF, and SF formulas to estimate LDL-C < 70 mg/dL vs. directly measured LDL-C (LDLd-C) and to compare non-HDL-C and Apo-B levels between the groups of patients with concordant vs. discordant LDL-C.

Material and methods: This was a prospective clinical study with measurements of lipid profile and LDLd-C in 214 patients with triglycerides < 400 mg/dL. For each formula, the estimated LDL-C was compared with the LDLd-C, and the correlation, the median difference, and the discordance rate were evaluated. Non-HDL-C and Apo-B levels were compared between the groups with concordant and discordant LDL-C.

Results: The estimated LDL-C was < 70 mg/dL in 130 (60.7%) patients by FF, 109 (50.9%) by MF, and 113 (52.8%) by SF. The strongest correlation was found between LDLd-C and Sampson estimated LDL-C (LDLs-C) ($R^2 = 0.778$), followed by Friedewald-estimated LDL-C (LDLf-C) ($R^2 = 0.680$) and Martin estimated LDL-C (LDLm-C) ($R^2 = 0.652$). Estimated LDL-C < 70 mg/dL was lower than LDLd-C, with the largest median absolute difference (25–75th) of –15 (–19 to –10) with FF. For estimated LDL-C < 70 mg/dL, the discordant rate was 43.8%, 38.1%, and 35.1%, reaching for 62.3%, 50.9%, and 50% when LDL-C < 55 mg/dL by FF, SF, and MF, respectively. Patients in the discordant group presented significantly higher levels of non-HDL-C and ApoB for all 3 formulas ($p < 0.001$).

Conclusion: FF was the most inaccurate formula to estimate very low LDL-C. Despite MF and SF showing better results, their frequency in underestimating LDL-C was still considerable. In patients with falsely low estimated LDL-C, apoB and non-HDL-C were significantly higher, reflecting its true high atherogenic burden. **(Endokrynol Pol 2023; 74 (2): 203–210)**

Key words: *LDL-C; Friedewald formula; Sampson formula; Martin formula; ApoB; non-HDL-C; estimated*

Introduction

The role of low-density lipoprotein cholesterol (LDL-C) and other atherogenic particles [apolipoprotein B (apo-B) containing lipoproteins] in the development of atherosclerotic cardiovascular disease (ASCVD) is well stablished [1, 2]. It is evident that a greater absolute reduction in LDL-C value and a shorter exposure time to high values are associated with a lower ASCVD risk [1].

For this reason, in 2021 the European Society of Cardiology (ESC) maintained the recommendation to use LDL-C levels to screen and diagnose ASCVD, as well as to guide therapeutic decision-making according to established cardiovascular risk [3]. For high-risk patients, an LDL-C target of < 70 mg/dL was proposed [3].

LDL-C can be measured directly by beta-quantification, the gold standard procedure based on ultracentrifugation and lipoprotein particle separation according to their densities [4]. However, despite being a more practical method and with a good approximation to the real value of LDL-C, this is a time-consuming, not totally accurate, and equally expensive assay to be widely available in clinical practice, especially when compared to the use of a formula [4, 5]. Therefore, LDL-C determination is generally estimated by measuring the remaining lipoproteins and triglycerides (TG).

The Friedewald formula is considered worldwide the gold standard for clinical estimation of LDL-C concentrations due to its great convenience, cost-effectiveness, and concordance with directly measured LDL-C (LDLd-C) in most patients [6].

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However, this equation loses accuracy in two increasingly common situations, underestimating the LDL-C values: when the TG level is > 400 mg/dL, because this formula considers a constant TG/very low-density lipoprotein cholesterol (VLDL-C) ratio of 5:1; and for very low LDL-C level, particularly < 100 mg/dL [6, 7]. Indeed, recent publications have shown that about 20% of patients with a Friedewald-estimated LDL-C (LDLf-C) < 70 mg/dL have LDLd-C values ≥ 70 mg/dL [5, 8, 9].

Two new formulas, the Martin/Hopkins and Sampson methods, recently developed in an attempt to overcome these barriers in the calculation of LDL-C, have shown more accurate results in individuals with low LDL-C (70 mg/dL) compared to the Friedewald formula [5, 8, 10].

Considering the importance of LDL-C in clinical decision-making for titration and/or introduction of aggressive LDL-C-lowering therapies to reach the low recommended values, the availability of reproducible and accurate laboratory assays for the estimation of low LDL-C becomes essential.

A reasonable alternative to assess the risk of ASCVD and determine a treatment target is the measurement of Apo-B and non-high-density lipoprotein cholesterol (non-HDL-C) plasma concentrations [3]. These two lipid parameters present the same strong relationship with the cardiovascular risk as LDL-C, because they encompass all atherogenic lipoproteins and therefore reflect the patient's atherogenic burden [2, 3].

Therefore, in this study we aimed to evaluate the accuracy of the Friedewald (FF), Martin (MF), and Sampson (SF) formulas to estimate low values of LDL-C (< 70 mg/dL) in comparison with LDLd-C, and to determine which equation has the highest concordance rate. The second goal was to compare the atherogenic burden, through the non-HDL-C and Apo-B levels, between the group of LDL-C concordant *vs*. LDL-C discordant patients.

Material and methods

In this prospective clinical study, we analysed 214 patients with lipid profile and LDLd-C measurements collected from 1 March 2021 to 9 April 2021 for different clinical reasons, in the clinical chemistry department of our centre. We included participants aged ≥ 18 years with an estimated LDL-C by FF, MF, and/or SF < 70 mg/dL, and we excluded patients with TG ≥ 400 mg/dL, according to specifications of the FF.

Total cholesterol, non-HDL-C, and TG were calculated by automated colorimetric enzymatic assay; LDLd-C and HDL-c by automated colorimetric enzymatic assay with polyanions; and Apo-B by an immunoturbidimetric method, all using the Roche Cobas Integra 400 Plus analyser [11, 12]. LDLf-C was estimated as total cholesterol (TC) minus HDL-C minus TG/5:

$$LDLf\text{-}C = TC - HDL\text{-}C - TG/5 \text{ [6]}$$

Martin estimated LDL-C (LDLm-C) was calculated using 1 of 180 different factors in the denominator for the TG/VLDL-C ratio, according to non-HDL-C and TG concentrations:

$$LDLm\text{-}C = LDL\text{-}C = TC - HDL\text{-}C - TG/specific\ factor \text{ [8]}$$

Finally, Sampson estimated LDL-C (LDLs-C) estimation was based on least squares, nonlinear regression analysis with TG, TC, and HDL-C values as independent variables:

$$LDLs\text{-}C = TC/0.948 - HDL\text{-}C/0.971 - (TG/8.56 + [TG \times Non\text{-}HDL\text{-}C]/2140 - TG^2/16100) - 9.44) \text{ [10]}$$

For each formula, the estimated LDL-C was compared with LDLd-C, and the correlation between the values, the median difference (estimated LDL-C minus LDLd-C), and the discordance rate defined as estimated LDL-C < 70 mg/dL and LDLd-C ≥ 70 mg/dL were evaluated. Then, a subanalysis to determine the proportion of concordance according to the estimated LDL-C (< 30 mg/dL, 30–54 mg/dL, and 55–70 mg/dL) was performed. We also verified the distribution of individuals across estimated LDL-C levels (< 30 mg/dL, 30 –54 mg/dL, and 55 to < 70 mg/dL) and 4 TG categories (< 100 mg/dL, 100–199 mg/dL, 200–299 mg/dL, and 300–399 mg/dL). Secondly, non-HDL-C and Apo-B levels were compared between the groups with concordant and discordant LDL-C values.

Statistical analysis was performed using the IBM Statistical Package for Social Sciences for Windows v.27 (IBM Corporation). Categorical variables were presented as number (n) and percentage (%). The normality of data distribution of numeric variables was evaluated through the Shapiro-Wilk test. Parametric continuous variables were described with mean ± standard deviation (SD), nonparametric variables were reported as median and interquartile (25–75th percentile) values, and the independent sample T-test and Wilcoxon-Mann–Whitney U test were employed to compare them, respectively. A scatter plot graph and Spearman's Rho were performed to calculate the correlation between LDL-C estimated by each formula and LDLd-C. The result was considered statistically significant for a p-value < 0.05.

Results

We included 214 patients, whose demographic and clinical characteristics are described in Table 1, with measurements of lipid profile including LDLd-C and with TG < 400 mg/dL. The estimated LDL-C was < 70 mg/dL in 130 (60.7%) patients by FF, 109 (50.9%) by MF, and 113 (52.8%) by SF (Tab. 2). Among patients with LDL-C < 70 mg/dL, the highest proportion had TG levels < 100 mg/dL in the 3 equations. However, in individuals with estimated LDL-C < 30 mg/dL, the vast majority presented TG values between 100 and 199 mg/dL (Tab. 2).

Comparing calculated LDL-C with directly measured LDLd-C, the strongest correlation was found between LDLd-C and LDLs-C ($R^2 = 0.778$), followed by LDLf-C ($R^2 = 0.680$) and LDLm-C ($R^2 = 0.652$) (Fig. 1).

Estimated LDL-C < 70 mg/dL was lower than LDLd-C, with a median absolute difference (25–75th) of –15 (–19 to –10) for LDLf-C, –10 (–14 to –5) for LDLm-C, and –11 (–14 to –8) for LDLs-C. No pattern of increasing median absolute difference with decreasing LDL-C values was identified in any equation (Tab. 3).

Table 1. *Demographic and clinical characteristics of patients*

Characteristics	All sample (n = 214)
Male (n, %)	123 (57.0)
Age (years)[a]	54.5 (24)
CV risk factors	
Hypertension (n, %)	113 (52.8)
Diabetes *mellitus* (n, %)	77 (35.9)
Dyslipidaemia (n, %)	111 (51.9)
Coronary disease (n, %)	42 (19.6)
Peripheral arterial disease (n, %)	22 (10.3)
Cerebrovascular disease (n, %)	12 (5.6)
Smoking (n, %)	10 (4.7)
CV risk	
Low risk (n, %)	70 (32.7)
Moderate risk (n, %)	23 (10.7)
High risk (n, %)	45 (21.0)
Very high risk (n, %)	76 (35.5)
Current therapy	
Statins (n, %)	114 (53.3)
Fibrate (n, %)	18 (8.4)
Ezetimibe (n, %)	23 (10.7)

[a]Data presented as median (interquartile range); CV — cardiovascular

Regarding the discordant rate for estimated LDL-C < 70 mg/dL, 43.8% of individuals with LDLf-C, 38.1% with LDLs-C, and 35.1% with LDLm-C had LDLd-C ≥ 70 mg/dL. The number of discordant values increased at lower levels of estimated LDL-C, reaching rates of 62.3%, 50.9%, and 50% for LDL-C < 55 mg/dL by FF, SF, and MF, respectively (Tab. 4).

Patients in the discordant group presented significantly higher levels of non-HDL-C for all 3 formulas (FF: 96.4 ± 21.1 mg/dL *vs.* 73.4 ± 18.6 mg/dL, p < 0.001; MF: 83.5 ± 8.0mg/dl *vs.* 71.6 ± 16.5 mg/dL, p < 0.001; SF: 86.67 ± 11.083 *vs.* 71.56 ± 16.57, p < 0.001) (Fig. 2). The same was verified with ApoB levels (FF: 79.8 ± 13.9 mg/dL *vs.* 64.3 ± 12.2 mg/dL, p < 0.001; MF: 72.9 ± 7.7mg/dL *vs.* 63.7 ± 12.1mg/dL, p < 0.001; SF: 73.86 ± 8.56 *vs.* 63.71 ± 12.12, p < 0.001) (Fig. 3).

Discussion

In our study, for estimated LDL-C <70 mg/dL, FF was the most inaccurate equation, with the highest mean absolute difference between estimated LDL-C and LDLd-C and with the highest discordance rate compared with SF and MF, reaching 62.3% of underestimated values for LDL-C < 55 mg/dL. In contrast, SF showed the strongest

Table 2. *Distribution of patients with very low estimated low-density lipoprotein cholesterol (LDL-C) levels across different LDL-C and triglyceride (TG) categories*

	Friedewald estimated LDL-C [mg/dL]			
	< 30	30 to 54	55 to < 70	Total
TG [mg/dL]				
< 100	1 (10.0)	22 (37.3)	25 (41.0)	48 (36.9)
100–199	4 (40.0)	17 (28.8)	21 (34.4)	42 (32.3)
200–299	1 (10.0)	14 (23.7)	5 (8.2)	20 (15.4)
300–399	4 (40.0)	6 (10.2)	10 (16.4)	20 (15.4)
Total	10 (7.7)	59 (45.4)	61 (46.9)	130
TG [mg/dL]	**Martin estimated LDL-C (mg/dL)**			
< 100	1 (20.0)	26 (63.4)	23 (36.5)	50 (45.9)
100–199	3 (60.0)	10 (24.4)	24 (38.1)	37 (33.9)
200–299	1 (20.0)	1 (2.4)	9 (14.3)	11 (10.1)
300–399	0	4 (9.8)	7 (11.1)	11 (10.1)
Total	5 (4.6)	41 (37.6)	63 (57.8)	109
TG [mg/dL]	**Sampson estimated LDL-C**			
< 100	1 (14.3)	25 (54.3)	22 (36.7)	48 (42.5)
100–199	3 (42.9)	10 (21.7)	27 (45.0)	40 (35.4)
200–299	1 (14.3)	6 (13.0)	9 (15.0)	16 (14.2)
300–399	2 (28.6)	5 (10.9)	2 (3.3)	9 (8.0)
Total	7 (6.2)	46 (40.7)	60 (53.1)	113

Data are presented as n (%)

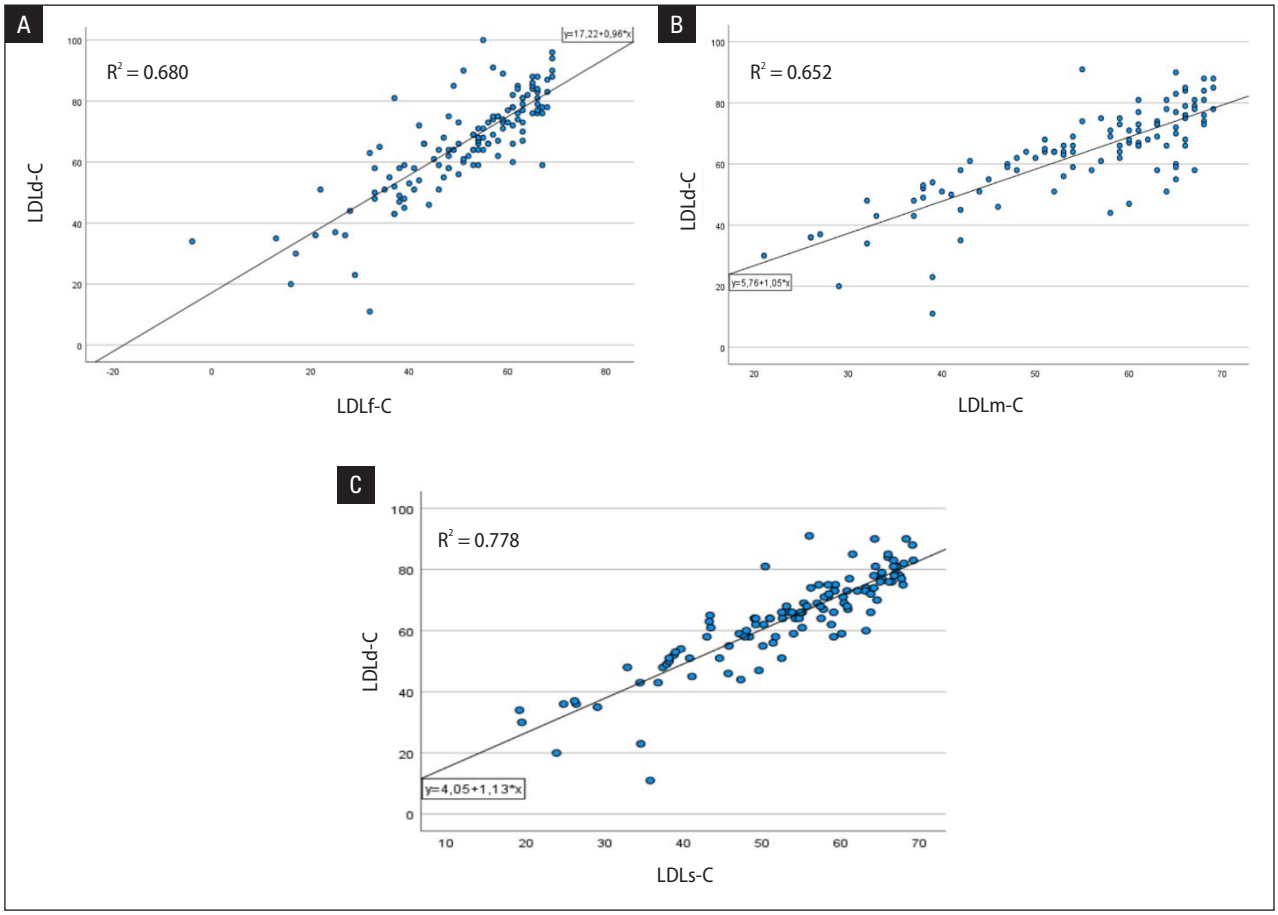

Figure 1. *Correlation between directly measured low-density lipoprotein cholesterol (LDLd-C) and Friedewald-estimated low-density lipoprotein cholesterol (LDLf-C) (***A***) Martin-estimated low-density lipoprotein cholesterol (LDLm-C) (***B***) and Sampson-estimated low-density lipoprotein cholesterol (LDLs-C) (***C***)*

correlation and the smallest mean absolute difference between calculated and directly measured LDL-C, but MF was the most accurate with fewer cases of underestimated LDL-C than SM and FF.

Elevated LDL-C concentration is considered a major risk factor for the development of cardiovascular disease, being consensual that "the lower the better" hypothesis allows for greater primary and secondary protection [3, 5, 13, 14]. Therefore, LDL-C remains internationally recognized as the main target for therapeutic decision-making, and according to 2021 ESC guidelines, lipid-lowering therapy should be introduced and/or titrated to maintain an LDL-C goal of <70 mg/dL and <50 mg/dL in high and very high cardiovascular risk patients, respectively [3]. These recommendations are based on a set of long-term scientific trials that considered LDL-C levels estimated by FF and included on-treatment patients with LDL-C ≥70 mg/dL [15–17].

In our sample, 21% and 35.5% of patients had high and very high cardiovascular risk, respectively. However, we verified that 43.8% of the sample with LDLf-C < 70 mg/dL actually had LDLd-C ≥ 70 mg/dL. Hence, these individuals are at risk of undertreatment by erroneously not starting and/or intensifying lower-lipid therapy or even by discontinuing drugs, and consequently they have lower cardiovascular protection. Likewise, clinical trials that have shown clinical benefit and safety in lowering LDL-C may have results based on falsely low estimated LDL-C values [14–17]. There is some controversy about the potential occurrence of adverse events with very low LDL-C levels. A post-hoc analysis of the Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial demonstrated a significantly higher incidence of hepatobiliary disorders, new-onset diabetes, and haematuria in rosuvastatin-treated patients with LDL-C ≤30 mg/dL *vs.* rosuvastatin-treated patients with LDL-C >30 mg/dL and placebo [18]. However, the LDL-C was estimated by FF, and, according our results, 80% of these individuals could have a discordant value.

It is already well established that the FF lost accuracy in estimating LDL-C in conditions of hypertriglyceridaemia and very low LDL-C levels, despite the fact that

Table 3. *Median absolute difference of estimated low-density lipoprotein cholesterol (LDL-C) by each formula and directly measured LDL-C (LDLd-C): overall and by LDL-C category assessment*

Estimated LDL-C [mg/dL]	Friedewald formula	Martin formula	Sampson formula
< 30	–14 (–23.8 to –7.8)	–10 (–10 to 0)	–10 (–11 to –6)
30 to 54	–14 (–19 to –10)	–11 (–14 to –6.5)	–11.5 (–13.3 to –6)
55 to < 70	–16 (–19 to –11)	–10 (–14 to –5)	–12 (–14 to –9)
Total	–15 (–19 to –10)	–10 (–14 to –5)	–11 (–14 to –8)

Data are presented as median (25–75th percentile)

Table 4. *Discordance rate between estimated low-density lipoprotein cholesterol (LDL-C) and directly measured LDL-C (LDLd-C) across estimated LDL-C categories*

	Directly measured LDL-C (mg/dL)					
	< 30	30 to 54	55 to < 70	≥ 70	Total	Discordance rate
LDLf-C [mg/dL]						
< 30	2 (20.0)	8 (80.0)	0	0	10	8 (80.0)
30 to < 55	1 (1.7)	15 (25.4)	36 (61.0)	7 (11.9)	59	43 (72.9)
55 to <70	0	0	11 (18.0)	50 (82.0)	61	50 (82.0)
< 55	3 (4.3)	23 (33.3)	36 (52.2)	7 (10.1)	69	43 (62.3)
Total (< 70)	3 (2.3%)	23 (17.7)	47 (36.2%)	57 (43.8%)	130	57 (43.8)
LDLm-C [mg/dL]						
< 30	1 (20.0)	4 (80.0)	0	0	5	4 (80.0)
30 to < 55	2 (4.9)	16(39.0)	23(56.1)	0	41	23 (56.1)
55 to <70	0	3 (4.8)	21(33.3)	39 (61.9)	63	39 (61.9)
< 55	3 (6.5)	20 (43.5)	23 (50.0)	0	46	23 (50)
Total (< 70)	3 (2.8)	23 (21.1)	44 (40.4)	39 (35.8)	109	39 (35.8)
LDLs-C [mg/dL]						
< 30	1 (14.3)	6 (85.7)	0	0	7	6 (85.7)
30 to < 55	2 (4.3)	17 (37.0)	26 (56.5)	1 (2.2)	46	27 (58.7)
55 to < 70	0	0	18 (30.0)	42 (70)	60	42 (70)
< 55	3 (5.7)	23 (43.4)	26 (49.0)	1 (1.9)	53	27 (50.9)
Total (<70)	3 (2.7)	23 (20.4)	44 (38.9)	43 (38.1)	113	43 (38.1)

Data are presented as n (%); LDLf-C — Friedewald-estimated low-density lipoprotein cholesterol; LDLm-C — Martin-estimated low-density lipoprotein cholesterol; LDLs-C — Sampson-estimated low-density lipoprotein cholesterol

the discordance rates in studies with larger samples are lower than ours, ranging from 15 to 35% [4, 5, 8–10]. For that reason, other equations have been developed in an attempt to overcome these limitations and increase the accuracy of the results. In 2013, the Martin/Hopkins formula was published and demonstrated that for TG lower than 400 mg/dL, overall concordance in guideline risk classification compared with LDLd-C was 91.7% for LDLm-C and 85.4% for LDLf-C [8]. Subsequently, Quispe *et al.* assessed only patients with LDL-C <70 mg/dL and concluded that, compared with FF, MF has higher correlation with LDLd-C, lower mean absolute difference, and lower discordant rate (6.3% *vs*. 22.9%) [5]. Still, the inaccuracy of the estimate with both formulas was greater the lower the LDL-C values, particularly FF [5]. In 2020, the Sampson equation was developed to improve the LDL-C results in patients with normolipidaemia and/or hypertriglyceridaemia, and it proved to be more accurate than MF and FF, with higher correlation, lower median absolute difference, and lower discordance rate [10]. However, when only patients with TG <400 mg/dL were considered, SF was similar to MF in underestimating LDL-C levels, maintaining an important superiority over FF [10].

Our publication is in agreement with the findings of these 2 large and very important studies (8, 10). In our sample of patients with TG < 400 mg/dL, FF was the most inaccurate in all parameters evaluated, and SF

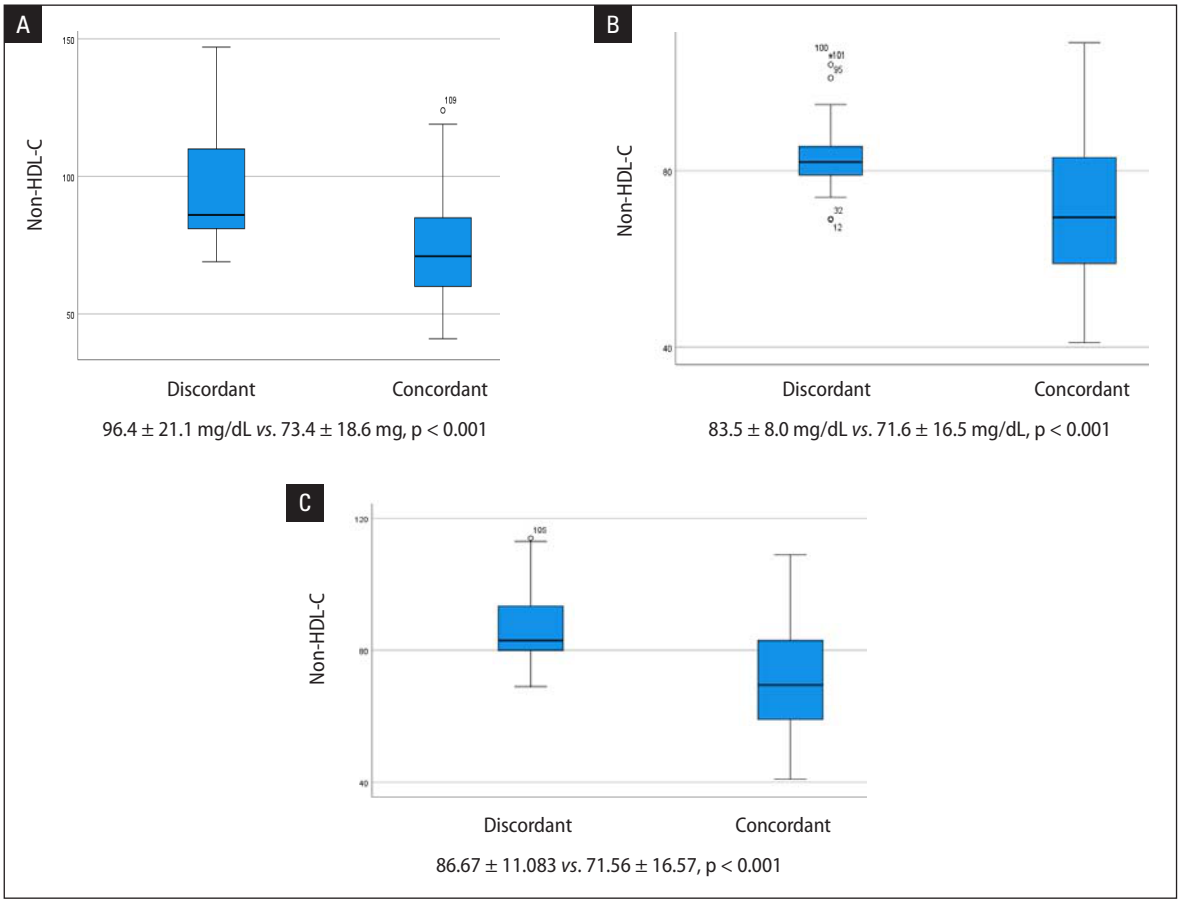

Figure 2. *Differences of non-high-density lipoprotein cholesterol (non-HDL-C) levels between patients with discordant vs. concordant low-density lipoprotein cholesterol (LDL-C) with Friedewald (***A***), Martin (***B***), and Sampson formulas (***C***)*

showed the highest correlation and the lowest absolute difference when compared to LDLd-C. However, MF proved to be the most accurate, with the lowest discordance rate, both for estimated LDL-C < 70 mg/dL and < 55 mg/dL. Our results are also in concordance with a recent publication that compared the 3 formulas in patients with very low LDL-C levels [19]. These authors found that MF, at LDL-C < 70 mg/dL and < 40 mg/dL, was more accurate and had less tendency to underestimate LDL-C, compared with FF and SF.

Despite the better results of MF and SF, their discordance rates of 35.8% and 38.1%, respectively, were still considerable. Patients at very high cardiovascular risk mainly have a small and dense LDL-C particle phenotype, which confers a greater atherosclerotic risk compared with the same mass of cholesterol with fewer and larger LDL particles [20]. However, equations currently available to estimate LDL-C, in addition to being limited to very low LDL-C levels, do not take into account the quantity or morphology of the LDL particles [6, 8, 10].

Furthermore, beyond LDL-C, there are many other atherogenic particles that induce arterial wall injury and increase the risk of atherosclerotic cardiovascular disease [3, 20]. All these particles contain one ApoB molecule, which makes their measurement an accurate marker of the atherogenic burden present in circulation [20, 21]. For that reason, several studies have been developed in an attempt to use apoB and non-HDL as alternative targets in the assessment of cardiovascular risk and in therapeutic decision-making [22, 23].

We showed that patients with discordant estimated and direct LDL-C presented significantly higher levels of non-HDL-C and ApoB for all 3 formulas. That suggests that individuals with underestimated LDL-C have higher atherogenic burden, in agreement with their direct LDL-C value >70 mg/dL. Currently, non-HDL-C levels are used as an input in the Systemic Coronary Risk Estimation 2 (SCORE2) and SCORE2-Older Persons (SCORE2-OP) risk algorithms, proposed by ESC [3]. Both ApoB and non-HDL levels are already considered reasonable alternatives to LDL-C in all patients, but especially in cases of hypertriglyceridaemia or very low LDL-C, with target values defined according to cardiovascular risk [3]. Even so, the use of non-HDL-C and ApoB in clinical practice for therapeutic adjust-

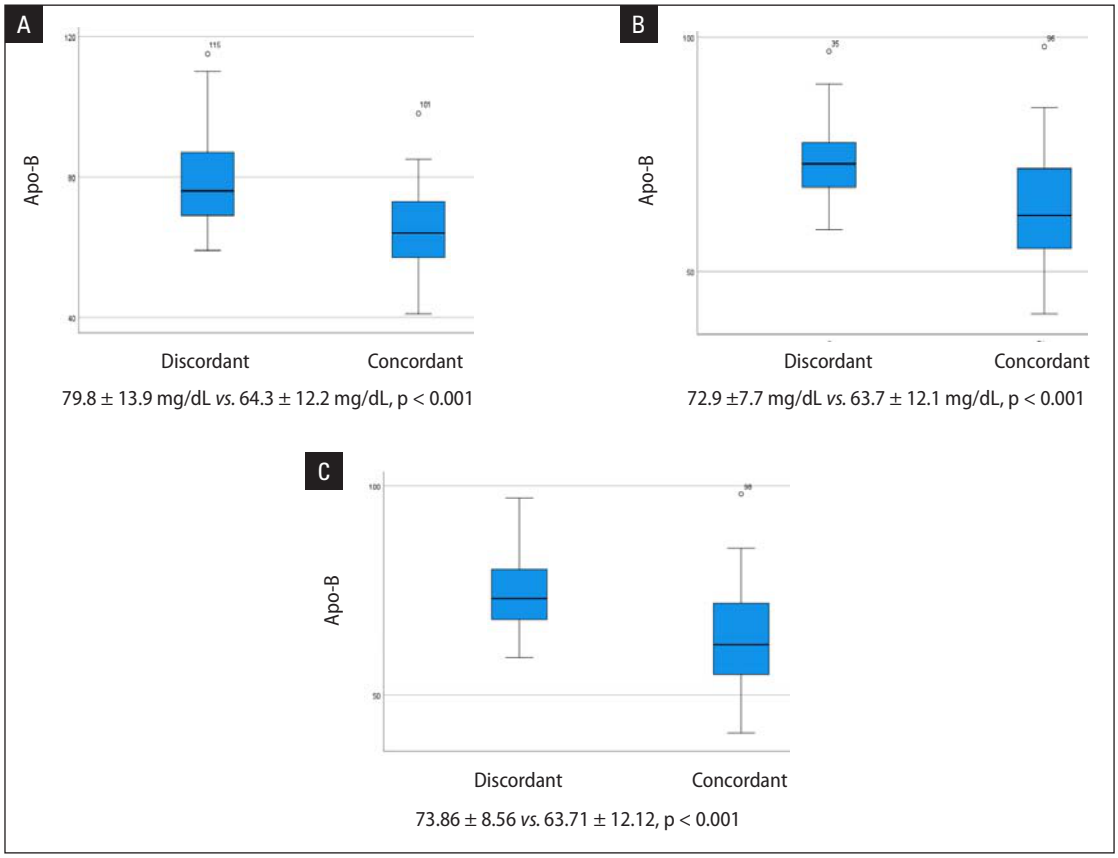

Figure 3. *Differences in apolipoprotein (Apo-B) levels between patients with discordant vs. concordant low-density lipoprotein cholesterol (LDL-C) with Friedewald (***A***), Martin (***B***), and Sampson formulas (***C***)*

ment requires some caution because they include all atherogenic particles, and lipid-lowering therapy acts only on the LDL-C receptor [20].

Our study has relevant limitations mainly due to its retrospective nature, its small sample and the lack of demographic, clinical, and therapeutic data. Moreover, the blood samples analysed in this study were collected for many different clinical purposes, either in outpatient or inpatient clinics, and information about their fasting status were not available. However, this is a real-life study, and this potential heterogeneity of fasting and non-fasting samples is representative of our real clinical practice. Another positive point of our work is its originality; the literature that compares these 3 formulas with LDLd-C and that evaluate the atherogenic burden in the group of patients with discordant LDL-C values is scarce.

In conclusion, FF was the most inaccurate formula to estimate LDL-C in patients with very low levels. Although MF and SF showed better results with higher correlation with LDLd-C and lower discordance rate, their frequency in underestimating LDL-C was still considerable, being higher for lower LDLd-C. For all 3 equations, in patients with falsely low estimated LDL-C compared to LDLd-C, the apoB and non-HDL-C were significantly higher, which reflects its true high atherogenic burden. Thus, the wide application of the FF in clinical practice should be reconsidered in individuals with a goal of LDL-C < 70 mg/dL, to prevent underestimation of cardiovascular risk. Its replacement by one of the new, more accurate formulas or by ApoB or non-HDL are alternatives. Prospective studies with larger samples are needed to validate these findings.

Authors' contributions

V.B.S. — study conception and design, drafting of manuscript, acquisition of data, analysis and interpretation of data; C.C. — acquisition of data, analysis and interpretation of data; J.C.O. — acquisition of data, analysis and interpretation of data; I.P. — acquisition of data, analysis and interpretation of data, critical revision

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Conflicts of interest

All authors have no conflicts of interest to declare with respect to the research and publication of this article.

Ethical disclosures

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References

1. Ference BA, Ginsberg HN, Graham I, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017; 38(32): 2459–2472, doi: 10.1093/eurheartj/ehx144, indexed in Pubmed: 28444290.
2. Pencina KM, Thanassoulis G, Wilkins JT, et al. Trajectories of Non-HDL Cholesterol Across Midlife: Implications for Cardiovascular Prevention. J Am Coll Cardiol. 2019; 74(1): 70–79, doi: 10.1016/j.jacc.2019.04.047, indexed in Pubmed: 31272554.
3. Visseren FLJ, Mach F, Smulders YM, et al. ESC Scientific Document Group, ESC Scientific Document Group, ESC Scientific Document Group, ESC National Cardiac Societies, ESC Scientific Document Group. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021; 42(34): 3227–3337, doi: 10.1093/eurheartj/ehab484, indexed in Pubmed: 34458905.
4. Maki KC, Grant JK, Orringer CE. LDL-C Estimation: The Perils of Living With Imperfection. J Am Coll Cardiol. 2022; 79(6): 542–544, doi: 10.1016/j.jacc.2021.12.005, indexed in Pubmed: 35144745.
5. Quispe R, Hendrani A, Elshazly MB, et al. Accuracy of low-density lipoprotein cholesterol estimation at very low levels. BMC Med. 2017; 15(1): 83, doi: 10.1186/s12916-017-0852-2, indexed in Pubmed: 28427464.
6. Tremblay AJ, Morrissette H, Gagné JM, et al. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972; 18(6): 499–502, indexed in Pubmed: 4337382.
7. Meeusen JW, Snozek CL, Baumann NA, et al. Reliability of Calculated Low-Density Lipoprotein Cholesterol. Am J Cardiol. 2015; 116(4): 538–540, doi: 10.1016/j.amjcard.2015.05.013, indexed in Pubmed: 26059865.
8. Martin SS, Blaha MJ, Elshazly MB, 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): 2061–2068, doi: 10.1001/jama.2013.280532, indexed in Pubmed: 24240933.
9. Whelton SP, Meeusen JW, Donato LJ, et al. Evaluating the atherogenic burden of individuals with a Friedewald-estimated low-density lipoprotein cholesterol <70 mg/dL compared with a novel low-density lipoprotein estimation method. J Clin Lipidol. 2017; 11(4): 1065–1072, doi: 10.1016/j.jacl.2017.05.005, indexed in Pubmed: 28826567.
10. Sampson M, Ling C, Sun Q, et al. A New Equation for Calculation of Low-Density Lipoprotein Cholesterol in Patients With Normolipidemia and/or Hypertriglyceridemia. JAMA Cardiol. 2020; 5(5): 540–548, doi: 10.1001/jamacardio.2020.0013, indexed in Pubmed: 32101259.
11. Allain CC, Chan CS, Richmond W, et al. Enzymatic Determination of Total Serum Cholesterol . Clin Chem. 1974; 20(4): 470–5.
12. Riepponen P, Marniemi J, Rautaoja T. Immunoturbidimetric determination of apolipoproteins A-1 and B in serum. Scand J Clin Lab Invest. 1987; 47(7): 739–744, doi: 10.3109/00365518709168939, indexed in Pubmed: 3685874.
13. Jarcho JA, Keaney JF. Proof That Lower Is Better--LDL Cholesterol and IMPROVE-IT. N Engl J Med. 2015; 372(25): 2448–2450, doi: 10.1056/NEJMe1507041, indexed in Pubmed: 26039520.
14. Baigent C, Blackwell L, Emberson J, et al. Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010; 376(9753): 1670–1681, doi: 10.1016/S0140-6736(10)61350-5, indexed in Pubmed: 21067804.
15. Deedwania P, Murphy SA, Scheen A, et al. Open-Label Study of Long-Term Evaluation against LDL Cholesterol (OSLER) Investigators, OSLER Investigators, LAPLACE-TIMI 57 Investigators. Efficacy, safety, and tolerability of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 in combination with a statin in patients with hypercholesterolaemia (LAPLACE-TIMI 57): a randomised, placebo-controlled, dose-ranging, phase 2 study. Lancet. 2012; 380(9858): 2007–2017, doi: 10.1016/S0140-6736(12)61770-X, indexed in Pubmed: 23141813.
16. Cannon CP, Blazing MA, Giugliano RP, et al. IMPROVE-IT Investigators. Ezetimibe Added to Statin Therapy after Acute Coronary Syndromes. N Engl J Med. 2015; 372(25): 2387–2397, doi: 10.1056/NEJMoa1410489, indexed in Pubmed: 26039521.
17. Raal FJ, Tuomilehto J, Sposito AC, et al. ODYSSEY CHOICE II Investigators, ODYSSEY LONG TERM Investigators. Monotherapy with the PCSK9 inhibitor alirocumab versus ezetimibe in patients with hypercholesterolemia: results of a 24 week, double-blind, randomized Phase 3 trial. Int J Cardiol. 2014; 176(1): 55–61, doi: 10.1016/j.ijcard.2014.06.049, indexed in Pubmed: 25037695.
18. Everett BM, Mora S, Glynn RJ, et al. Safety profile of subjects treated to very low low-density lipoprotein cholesterol levels (<30 mg/dl) with rosuvastatin 20 mg daily (from JUPITER). Am J Cardiol. 2014; 114(11): 1682–1689, doi: 10.1016/j.amjcard.2014.08.041, indexed in Pubmed: 25439449.
19. Sajja A, Park J, Sathiyakumar V, et al. Comparison of Methods to Estimate Low-Density Lipoprotein Cholesterol in Patients With High Triglyceride Levels. JAMA Netw Open. 2021; 4(10): e2128817, doi: 10.1001/jamanetworkopen.2021.28817, indexed in Pubmed: 34709388.
20. Sweeney Ty, Quispe R, Das T, et al. The Use of Blood Biomarkers in Precision Medicine for the Primary Prevention of Atherosclerotic Cardiovascular Disease: a Review. Expert Rev Precis Med Drug Dev. 2021; 6(4): 247–258, doi: 10.1080/23808993.2021.1930531, indexed in Pubmed: 34423130.
21. Shapiro MD, Fazio S. Apolipoprotein B-containing lipoproteins and atherosclerotic cardiovascular disease. F1000Res. 2017; 6: 134, doi: 10.12688/f1000research.9845.1, indexed in Pubmed: 28299190.
22. Mora S, Buring JE, Ridker PM. Discordance of low-density lipoprotein (LDL) cholesterol with alternative LDL-related measures and future coronary events. Circulation. 2014; 129(5): 553–561, doi: 10.1161/CIRCULATIONAHA.113.005873, indexed in Pubmed: 24345402.
23. Sniderman AD, Pencina M, Thanassoulis G. ApoB. Circ Res. 2019; 124(10): 1425–1427, doi: 10.1161/CIRCRESAHA.119.315019, indexed in Pubmed: 31070997.