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Performance of LDL-C calculated with Martin's formula compared to the Friedewald equation in familial combined hyperlipidemia

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ABSTRACT

Background and aims: A novel method to estimate low density lipoprotein cholesterol (LDL-C) has been proposed by Martin et al. This may permit a more accurate estimation of cardiovascular risk, however, external validation is needed. Here, the performance of LDL-C using this new method (LDL-N) is compared with LDL-C estimated with Friedewald equation (LDL-F) in familial combined hyperlipidemia (FCHL), a common primary dyslipidemia in which apolipoprotein B containing particle composition is abnormal and interferes with LDL-C estimation.

Methods: A total of 410 FCHL subjects were included. LDL-C was estimated with both the Friedewald equation (LDL-F) and the novel formula (LDL-N). Apolipoprotein B levels and non- HDL-C were recorded. The correlation and concordance between LDL-F and LDL-N and both Apolipoprotein B and non-HDL-C levels were calculated. Analysis stratifying for triglyceride tertiles and FCHL lipid phenotypes was also carried out.

Results: The correlations between LDL-N and Apo B and non-HDL-C were $\rho = 0.777$ (95%CI 0.718–0.825) and $\rho = 0.735$ (95%CI 0.648–0.816), respectively. The corresponding correlations for LDL-F were $\rho = 0.551$(95%CI 0.454–0.637) and $\rho = 0.394$ (95%CI 0.253–0.537), respectively. In mixed dyslipidemia or isolated hypertriglyceridemia, these correlations were significantly better using LDL-N. With respect to concordance, LDL-N performed significantly better than LDL-F when considering apoB <90 mg/dL (κLDL-N = 0.495 *vs.* κLDL-F = 0.165) and non-HDL-C <130 (κLDL-N = 0.724 *vs.* κLDL-F = 0.253).

Conclusions: In FCHL, LDL-C estimation using Martin's formula showed greater correlation and concordance with non-HDL-C and Apo B compared with the Friedewald equation.

1. Introduction

Low density lipoprotein cholesterol (LDL-C) remains the principle goal of therapy in the management of dyslipidemia [1–4]. However, many people who achieve LDL-C goals still develop atherosclerotic disease due to residual risk [5]. In certain patients there is a mismatch between the concentration of LDL-C and the number of atherogenic particles, expressed as the number of lipoproteins containing apolipoprotein B. Low density lipoprotein (LDL) particles are heterogeneous with respect to the amount of cholesterol they carry [6]. One person may have large LDLs, rich in cholesterol, while a second person can have small LDLs, which contain only a small amount of cholesterol. Therefore, at the same concentration of LDL-C, the second person will have a greater number of atherogenic particles (LDLs), and consequently increased cardiovascular risk [6]. As a consequence of this discrepancy, several expert panels suggest the use of other parameters to improve the evaluation of cardiovascular risk and determine intensity of therapy. These include apolipoprotein B (ApoB) and non-high density cholesterol (non-HDL-C); both parameters are useful but not equivalent.

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LDL-C represents the mass of cholesterol within LDL particles, whereas the ApoB concentration represents the total number of circulating atherogenic particles [7]. The measurement of this parameter is standardized among laboratories and does not require fasting but it represents a significant additional cost to the patient. Non-HDL-C is calculated by subtracting the concentration of HDL-C from total cholesterol and represents the cholesterol contents of all the atherogenic lipoproteins. It is considered a good therapeutic goal because its value does not change regardless of lipid exchange between VLDL-C and LDL [8]. In summary, non-HDL-C represents the cholesterol content of atherogenic lipoproteins (VLDL, IDL, LDL and Lp(a)), whereas apolipoprotein B measures the total number of atherogenic particles. When the content of cholesterol in the LDL-C particles is normal, both parameters are consistent. This means that they are equal for reporting cardiovascular risk. However, when the cholesterol content in the LDL-C particles is higher or lower than normal, the two parameters are discordant and predict differing risks.

The superiority of ApoB and non-HDL cholesterol for the prediction of cardiovascular risk compared with LDL-C has been shown in several studies [9–14]. The assessment of ApoB and non-HDL cholesterol may be even more relevant in persons with atherogenic dyslipidemias characterized by triglyceride-rich lipoproteins, low levels of HDL-C and increased levels of small dense LDL-C particles, including type 2 diabetes, metabolic syndrome and certain primary dyslipidemias such as familial combined hyperlipidemia (FCHL). In these cases, the total number of LDL-C particles may be higher than the calculated LDL-C level. Thus, using the LDL-C goal alone may not be enough.

FCHL is the most common primary atherogenic dyslipidemia in Mexico, being present in approximately 14% of patients with premature coronary heart disease [15,16]. It is associated with other metabolic abnormalities including obesity, insulin resistance, diabetes and metabolic syndrome [17]. FCHL is characterized by hypercholesterolemia and/or hypertriglyceridemia and elevated apolipoprotein B levels, a fluctuating lipid profile and variable expression within the same kindred. LDL-C may not be the best treatment target in this population, given the frequent presence of hypertriglyceridemia, other lipid targets including non-HDL-C and ApoB levels are probably more relevant in FCHL.

Conventionally, LDL-C is calculated by the Friedewald equation, avoiding the need for an ultracentrifuge [18]. This equation estimates LDL-C as (total cholesterol) − (high-density lipoprotein cholesterol [HDL-C]) − (triglycerides/5) in mg/dL. The final term assumes a fixed ratio of triglyceride levels to very low-density lipoprotein cholesterol (TG:VLDL-C) of 5:1.This estimate is unreliable in patients with triglycerides $\geq$150 mg/dL due to this fixed triglyceride to VLDL-C ratio, and does not consider the variance of this ratio across different concentrations of triglycerides and non-HDL-C [18]. Martin et al. have developed a novel method for estimating LDL-C using an adjustable factor for the TG: VLDL-C ratio (using triglyceride and non-HDL-C concentrations), which offers a greater concordance with measurement of LDL-C by ultracentrifugation [19]. This novel method has not been validated in populations that are characterized by abnormal apolipoprotein B containing particle composition, such as in FCHL; this method might be particularly helpful in such population. The objective of this study is to evaluate the correlation and the concordance of LDL-C, as calculated with the Friedewald equation (LDL-F) and Martin's formula (LDL-N), with non-HDL-C and ApoB targets in patients with FCHL. The results will determine the usefulness of this new method of LDL-C estimation in patients with atherogenic dyslipidemia.

2. Materials and methods

2.1. Study population

Subjects with a previous diagnosis of familial combined hyperlipidemia (FCHL) attending the lipid Clinic at the Instituto Nacional de Ciencias Medicas y Nutricion, Salvador Zubirán (INCMNSZ) in Mexico City were included. All participants gave informed consent. The Human Research Ethics Committee of the INCMNSZ approved the study. All procedures were done in accordance with the Declaration of Helsinki.

2.2. Clinical evaluations

All participants completed a questionnaire which included demographic data, medical history, and lifestyle factors. Patients arrived with the results of a routine lipid profile taken a week before their clinic visit. Diagnostic criteria considered for FCHL were the presence of hypercholesterolemia (total cholesterol >200 mg/dL) or hypertriglyceridemia (triglycerides >150 mg/dL) along with the demonstration of hypercholesterolemia, hypertriglyceridemia and mixed hyperlipidemia in three different first degree relatives and apolipoprotein B level >90th percentile for the Mexican population (>108 mg/dL for men and >99 mg/dL for women). Exclusion criteria included history of an acute illness within the previous six weeks, pregnancy and the presence of any disease or medication known to significantly influence lipid parameters. A complete medical and family history, including use of medications was obtained from all subjects. Subjects were weighed on calibrated scales and height was determined with a floor scale stadiometer. Body mass index (BMI) was calculated as weight in kg divided by the squared product of height in meters.

2.3. Laboratory measurements

Blood samples were obtained after an 8–12 h fast. Plasma glucose concentration was measured by an automated glucose analyzer (Yellow Springs Instruments Co.), serum insulin concentration was measured by using a chemiluminescent immunoassay (Beckman Coulter Access 2). Lipid concentrations (cholesterol, triglycerides, and HDL cholesterol) and apo B measurements were performed using colorimetric assays (Unicel DxC 600 Synchron Clinical System Beckman Coulter). LDL-cholesterol was calculated with the Friedewald equation and the calculation proposed by Martin et al. [18].

2.4. Statistical analyses

Data are presented as mean ± SD or as median and interquartile range. Proportions and medians were compared between groups using the chi-square test and Mann Whitney-U tests. Variables with a parametric distribution were evaluated using Student's t-test. Spearman correlations were performed to evaluate the degree of linear association between LDL-C, LDL-N, apolipoprotein B and non-HDL cholesterol. Linear regression analyses were also performed using logarithmic transformation. Concordance between LDL-C, LDL-N, non-HDL cholesterol and apolipoprotein B targets was assessed using the kappa coefficient in the total population and in subpopulations. We also evaluated correlations and concordance across tertiles of triglyceride levels and according to the differing phenotypes of FCHL, namely isolated hypertriglyceridemia (IHTG), mixed dyslipidemia (MDLP) and isolated hypercholesterolemia (IHCT). Performance of the index was evaluated using areas under

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the receiving operating characteristic curve (Harrell's *c-statistic*) and 95% confidence intervals were estimated using bootstrap sampling drawing 2000 stratified random samples. To estimate differences between the AUC of the ROC curves, we performed non-parametric ROC tests using a stratified bootstrap sampling method using the *pROC* package from R version 3.4.3. Finally, we estimated thresholds for LDL-N and LDL-F using the Youden index in the *OptimalCutpoints* package in R. A two-tailed *p*-value <0.05 was considered significant as statistically significant. Statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS, version 21.0), R software (Version 3.4.4) and GraphPad Prism version 6.0.

3. Results

3.1. Study subjects

A total of 410 persons with a diagnosis of FCHL were included in the study. The mean age of participants was 49.5 ± 15.0 years, the mean BMI was 27.72 ± 4.28 kg/m^2 and 55.4% were women. Table 1 shows the laboratory characteristics of all study participants. Overall, 23.4% had a diagnosis of arterial hypertension and 25.1% had type 2 diabetes mellitus. Previous coronary heart disease was present in 2.7% of subjects and 2.2% had a history of stroke. In terms of lipid lowering treatment, 34.9% were on statins, 26.1% on fibrates and 4.4% on ezetimibe. Monotherapy was reported in 18.3%, combination therapy in 22.9% and dietary management alone in 58.8%. The number of patients achieving non-HDL-C and Apo B targets was recorded. One-hundred and fourteen (27.8%) patients had non-HDL-C <130 mg/dL, whilst only 18.8% had an ApoB level <90 mg/dL.

3.2. Differences across FCHL phenotypes

On comparing differences across the three FCHL phenotypes (namely isolated hypercholesterolemia, isolated hypertriglyceridemia and mixed dyslipidemia), there was no significant difference with respect to gender (p=0.128), family history of cardiovascular disease (p=0.614), hypertension (p=0.302), type 2 diabetes (T2D) (p=0.144), obesity (p=0.657) or previous myocardial infarction (p=0.275). We did not observe significant differences in biochemical parameters aside from the expected differences in lipid profiles.

Table 1
Biochemical characteristics of patients with diagnosed FCHL included in the study.

Parameter	Mean ± SD or median (IQR) N = 410
Age (years)	49.54 ± 15.01
Female sex (%)	227 (55.4%)
BMI (kg/m2)	27.72 ± 4.28
Systolic blood pressure (mmHg)	119.66 ± 14.97
Diastolic blood pressure (mmHg)	76.96 ± 9.00
Triglycerides (mg/dL)[a]	235.5 (160.8–381.0)
Total colesterol (mg/dL)[b]	198.70 ± 42.25
HDL-c (mg/dL)	42.32 ± 10.68
Non-HDL-c (mg/dL)	156.38 ± 42.58
LDL-F (mg/dL)	95.58 ± 34.06
LDL-N (mg/dL)	111.43 ± 28.62
Glucose (mg/dL)	104.96 ± 41.79
Insulin (mU/L)	12.80 (8.95–24.65)
Apolipoprotein B	111.26 ± 24.86

[a] Conversion factor for LDL-C, HDL-C and total cholesterol from mg/dL to mmol/L = 0.02585983966.
[b] Conversion factor for triglycerides from mg/dL to mmol/L = 0.01129050468.

3.3. Correlation of LDL-N and LDL-F with ApoB and non-HDL-C levels

There was a significant correlation between non-HDL-C and apolipoprotein B levels adjusted for age, sex, BMI, treatment modality and presence of T2D (ρ = 0.794, 95%CI 0.730–0.849). This correlation was higher for individuals with triglycerides <400 mg/dL (ρ = 0.861, 95%CI 0.818–0.898) and lower for subjects with triglycerides ≥400 mg/dL (ρ = 0.326, 95%CI 0.051–0.611). When this analysis was conducted according to FCHL lipid phenotype, we observed an improvement in correlation in isolated hypercholesterolemia (ρ = 0.838, 95%CI 0.776–0.903), followed by isolated hypertriglyceridemia (ρ = 0.787, 95%CI 0.640–0.877) and mixed dyslipidemia (ρ = 0.729, 95%CI 0.611–0.818).

There was a greater correlation between LDL-N and non-HDL-C (ρ = 0.735, 95%CI 0.648–0.816) compared with LDL-F (ρ = 0.394, 95%CI 0.253–0.537) (Fig. 1A). For individuals with triglyceride concentrations <400 mg/dL the adjusted correlation was still better for LDL-N (ρ = 0.959, 95%CI 0.946–0.968) compared to LDL-F (ρ = 0.870, 95%CI 0.833–0.900) (Fig. 1B). In the case of individuals with triglyceride concentrations ≥400 mg/dL the correlations for LDL-N (ρ = 0.061, 95%CI -0.145-0.280) and LDL-F (ρ = −0.116, 95%CI -0.335-0.138) were both much lower and lost statistical significance. When evaluating these correlations according to FCHL phenotype, we observed a good correlation in isolated hypercholesterolemia for both LDL-N (ρ = 0.990, 95%CI 983–0.994) and LDL-F (ρ = 0.977 95%CI 0.958–0.987). In isolated hypertriglyceridemia, the correlation was markedly better with LDL-N (ρ = 0.907, 95%CI 0.829–0.948) compared to LDL-F (ρ = 0.637, 95%CI 0.447–0.772). Finally, in mixed dyslipidemia, both correlations decreased significantly but the result was much better with LDL-N (ρ = 0.676, 95%CI 0.559–777) compared to LDL-F (ρ = 0.339, 95%CI 0.164–0.502).

With respect to apoB, there was a greater correlation with LDL-N (ρ = 0.777, 95%CI 0.718–0.825) compared to LDL-F (ρ = 0.551, 95%CI 0.454–0.637) (Fig. 1C). When comparing this correlation in individuals ≥400 mg/dL there was a better adjusted correlation for LDL-N compared to LDL-F (Fig. 1D). When the analysis was conducted according to lipid phenotype, LDL-N and LDL-F showed similar correlations with apoB in isolated hypercholesterolemia (ρ = 0.825, 95%CI 0.760–0.898 and ρ = 0.814, 95%CI 0.735–0.892, respectively). In isolated hypertriglyceridemia LDL-N showed a better correlation (ρ = 0.723, 95%CI 0.571–0.832) compared to LDL-F (ρ = 0.529, 95%CI 0.347–0.677). Finally, in mixed dyslipidemia, the correlation coefficient was also significantly better with LDL-N than with LDL-F (ρ = 0.769, 95%CI 0.694–0.831 vs. ρ = 0.562, 95%CI 0.454–0.664 respectively).

3.4. Concordance with respect to treatment goals comparing LDL-N vs. LDL-F

Given the importance of LDL-C treatment goals in patients with FCHL, we evaluated the concordance of the LDL-C targets in relation to apoB and non-HDL-C goals (Fig. 2A–D). When comparing an LDL-C goal <100 mg/dL with non-HDL-C <130 mg/dL, we observed a higher concordance for Martin's over Friedewald formula ($\kappa_{LDL\text{-}N} = 0.724$ *vs.* $\kappa_{LDL\text{-}F} = 0.253$); if the goal was set to a lower threshold (LDL-C <70 mg/dL, non-HDL-C <100 mg/dL), we observed a similar trend but with a reduced concordance ($\kappa_{LDL\text{-}N} = 0.674$ *vs.* $\kappa_{LDL\text{-}F} = 0.295$). When evaluating the goals based on ApoB, we observed a higher concordance for Martin's equation in both LDL-C <100 mg/dL and ApoB <90 mg/dL ($\kappa_{LDL\text{-}N}$=0.495 *vs.* $\kappa_{LDL\text{-}F}$=0.165) and with LDL-C <70 mg/dL and ApoB <80 mg/dL ($\kappa_{LDL\text{-}N} = 0.463$*vs.* $\kappa_{LDL\text{-}F} = 0.194$).

When we evaluated concordance based on triglyceride tertiles for the whole population, we observed a consistent decrease in

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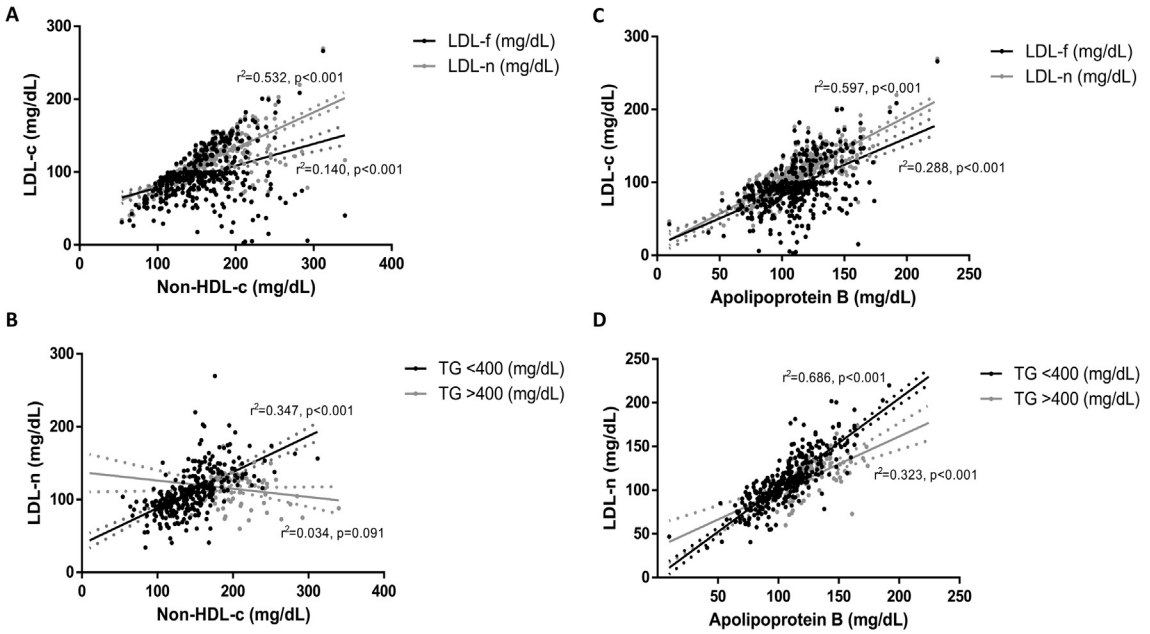

Fig. 1. Correlation between LDL-C estimated by the Friedewald equation and Martin's formula with ApoB and non-HDL-C in FCHL.
We observed a significant correlation between LDL-C estimated by the Friedewald formula (LDL-f) and Martin's formula (LDL-n) with non-HDL-C (A) and apolipoprotein B (C) that is higher for LDL-N and it remains higher even when further stratified by triglyceride levels (B and D).

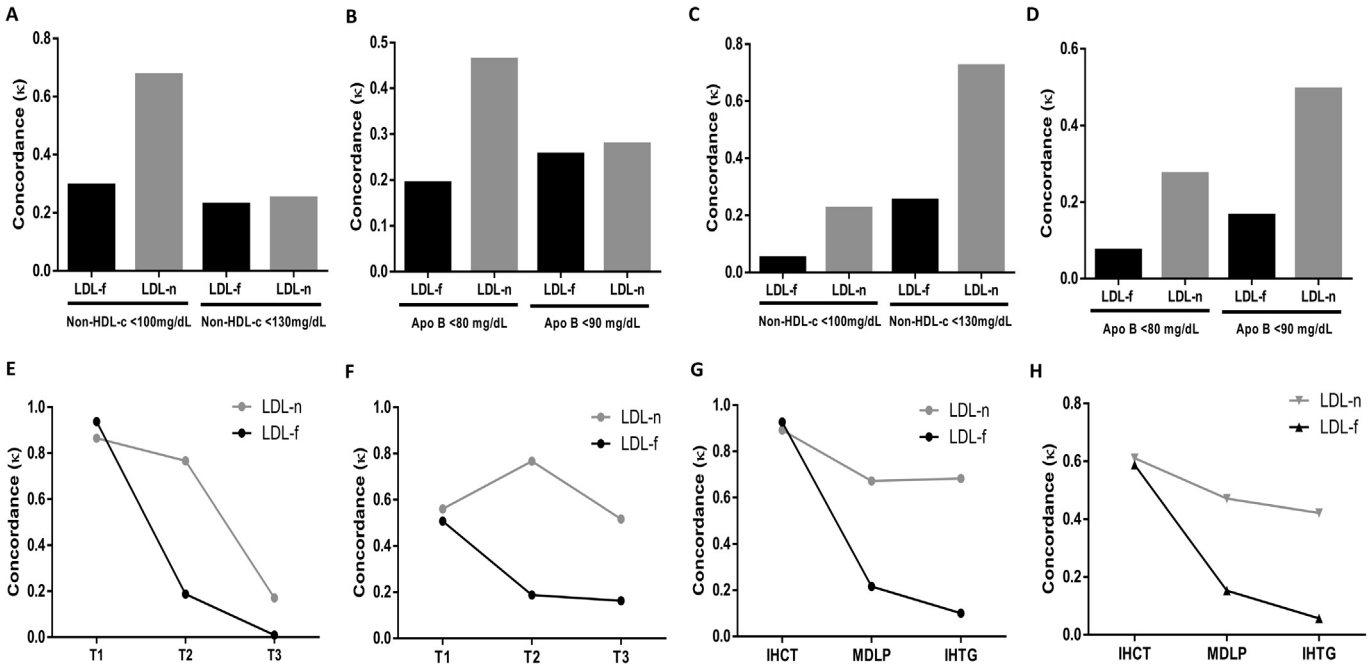

Fig. 2. Concordance (κ) between the Friedewald equation (LDL-F) and Martin's formula's (LDL-N) goals according to ApoB and non-HDL-C targets.
Concordance (κ) between LDL-f and LDL-n with targets of therapy for FCHL against LDL-c <70 (A and B) and against LDL-c <100 (C and D). The figure also shows concordance (κ) between LDL-f and LDL-n across terciles of triglyceride concentrations in FCHL against non-HDL-c <130 mg/dL (E) and apolipoprotein B < 90 mg/dL (F). Finally, we showed how both equations performed in different syndromes of FCHL including isolated hypercholesterolemia (IHCT), mixed dyslipidemia (MDLP) and isolated hypertriglyceridemia against non-HDL-c <130 mg/dL (G) and apolipoprotein B < 90 mg/dL (H).

concordance across tertiles (Fig. 2E–F) for both non-HDL-C and ApoB; however, concordance was maintained at higher levels for LDL-C estimated using Martin's formula (LDL-N) compared to the Friedewald equation (LDL-F). Finally, we evaluated concordance according to FCHL lipid phenotypes. We observed that concordance was nearly the same for patients with isolated hypercholesterolemia, but Martin's formula showed better concordance for patients with mixed dyslipidemia and isolated hypertriglyceridemia compared to the Friedewald equation for both non-HDL-C and ApoB targets (Fig. 2G–H) (see Fig. 3).

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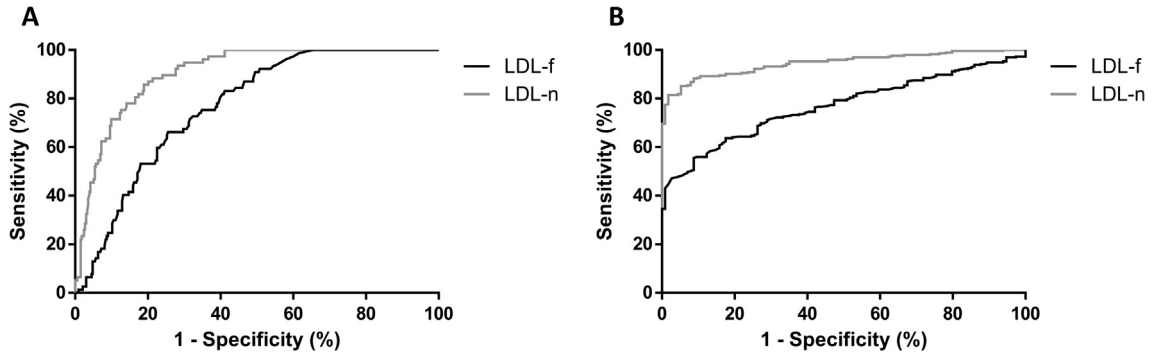

Fig. 3. Performance of LDL estimation comparing Martin's formula with the Friedewald equation to detect goals based on ApoB and non-HDL-C.
Here, we observe that the performance of LDL estimation is superior for Martin's formula (LDL-n) compared to the Friedewald equation (LDL-f) in FCHL using receiver operating characteristic (ROC) curves against apolipoprotein B < 90 mg/dL (A) and non-high density lipoprotein cholesterol <130 mg/dL (B).

3.5. Performance of LDL-N and LDL-F compared to Apo B and non-HDL-C

Finally, the area under the receiving operating characteristic curve (AUC of ROC) was estimated, to evaluate the performance of the LDL-C calculation using Martin's formula and the Friedewald equation. First, the accuracy of both estimations to detect non-HDL-C <130 mg/dL was evaluated; a significantly higher AUC for LDL-N (AUC 0.945 95%CI 0.925–0.966) compared to LDL-F (AUC 0.769 95%CI 0.727–0.813) was found ($p < 0.001$). A similar result was observed when comparing the AUC to detect ApoB <90 mg/dL; here, LDL-N also had a higher AUC (0.905 95%CI 0.874–0.934) compared to LDL-F (AUC 0.767 95%CI 0.716–0.814), reaching statistical significance ($p < 0.001$).

Subsequently, the Youden index was used to calculate the best LDL-N and LDL-F thresholds for the detection of target ApoB levels. A threshold of 99.2 mg/dL for LDL-N consistently detected ApoB levels <90 mg/dL (78.7% sensitivity, 88.3% specificity). In persons with TG < 400 mg/dL a similar threshold was identified, 99.2 mg/dL (~100 mg/dL) (79.2% sensitivity, 88.1% specificity). However, in individuals with TG > 400 mg/dL, a lower threshold of 81.8 (~80 mg/dL) was found to detect ApoB<90 mg/dL (92.8% sensitivity, 97.3% specificity). In the case of LDL-F, a threshold of 93.8 mg/dL detected ApoB levels <90 mg/dL; this had a lower sensitivity and specificity compared to the LDL-N threshold (58.8% sensitivity, 83.1% specificity). In persons with TG < 400 mg/dL, the corresponding LDL-F threshold was 93.6 mg/dL (68.0% sensitivity, 82.9% specificity). In contrast, in individuals with TG ≥ 400 mg/dL, the LDL-F threshold was significantly lower (15.2 mg/dL).

Finally, the best LDL-N and LDL-F thresholds for the detection of target non-HDL-C levels was estimated. A threshold of 101.2 mg/dL for LDL-N consistently detected non-HDL-C levels <130 mg/dL (85.2% sensitivity, 95.6% specificity). For LDL-F, a threshold of 97.2 mg/dL (55.8% sensitivity, 92.03% specificity) was identified. We were not able to carry out an analysis with triglyceride levels above and below 400 mg/dL, since no patient had a non HDL-C <130 mg/dL and TG ≥ 400 mg/dL. Instead we carried out this analysis using a threshold of 300 mg/dL. When considering a non-HDL-C target <130 mg/dL, in individuals with TG ≥ 300 mg/dL and <300 mg/dL, the thresholds for LDL-N were 85.2 mg/dL (94.0% sensitivity, 100.0% specificity) and 104.2 mg/dL (~100 mg/dL) (90.5% sensitivity, 99.1% specificity), respectively. The corresponding thresholds for LDL-F were 54.4 mg/dL (86.0% sensitivity, 100.0% specificity) and 97.6 mg/dL (81.6% sensitivity, 91.7% specificity) respectively.

4. Discussion

Familial combined hyperlipidemia is characterized by an overproduction of very low density lipoprotein particles and an innate variability in lipoprotein composition. Typically there is an atherogenic lipid profile, namely hypertriglyceridemia, hypoalphalipoproteinemia and the production of small dense LDL-C particles. The performance of the Friedewald equation in estimating LDL-C in these circumstances is not adequate (due to the moderate to severe hypertriglyceridemia). The possibility of an alternative formula which provides a superior estimation of LDL-C in this situation, is particularly appropriate in FCHL. In order to be considered an improvement, LDL-C estimation by Martin's formula would have to better reflect cardiovascular risk. An indirect measure of this is the degree of correlation and concordance with lipoprotein parameters known to be relevant in FCHL, namely non-HDL-C and apoB levels. Our results demonstrate that LDL-C estimated using Martin's formula (LDL-N) is an improvement over the traditional formula, showing a significantly greater correlation and concordance with both apoB and non-HDL-C targets in subjects with FCHL. Furthermore, in the setting of hypertriglyceridemia, even though the correlation and concordance with apoB and non-HDL-C becomes lower, LDL-N is still significantly better than the LDL-C estimated using the Friedewald equation (LDL-F). On analyzing FCHL lipid phenotypes, LDL-N and LDL-F perform similarly in the setting of isolated hypercholesterolemia. However, LDL-N is superior in the setting of mixed dyslipidemia and isolated hypertriglyceridemia in FCHL patients. The observation of decreased performance of both LDL-N and LDL-F in the setting of severe hypertriglyceridemia this is not entirely unexpected due to the presence of chylomicrons and highlights the problems associated with utilizing calculated LDL-C. With respect to FCHL phenotypes, the concordance with non-HDL-C and apoB was generally adequate, being only marginally lower in phenotypes with increased TG concentrations. In relation to sex, we observed differences in correlation and concordance for all evaluated parameters with consistent superiority for LDL-N over LDL-F; however, this could be attributable to significantly higher triglyceride values in male compared to female participants (Online Supplement).

In their original publication Martin et al. evaluated the concordance between LDL-N and directly measured LDL-C; they did not compare concordance with secondary measures of cardiovascular risk, specifically apoB and non-HDL-C. Martin et al. observed an improved concordance between LDL-N and ultracentrifugation

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measured LDL-C compared to LDL-F. In accordance with our findings, the authors stated that LDL-N performed best in the classification of LDL-C concentrations lower than 70 mg/dL, especially in patients with elevated triglyceride concentrations.

In FCHL, there exists a moderate correlation between non-HDL-C and apoB. This correlation improves with TG < 400 mg/dL but weakens in the presence of TG ≥ 400 mg/dL. In the presence of hypertriglyceridemia, ATP-III guidelines recommend the use of non-HDL-C as a secondary treatment goal once the LDL-C target is reached [20]. Up until now, it is unknown whether non-HDL-C and apoB are equivalent markers of cardiovascular risk. Sniderman et al. compared subjects with and without myocardial infarction when both parameters were discordant. When apoB > non-HDL-C, (when the apoB particles are poor in cholesterol), cardiovascular risk is increased. In contrast, when the non-HDL-C > apoB, (when apoB particles are rich in cholesterol), risk is lower than the reference concordant group. Therefore, these investigators concluded that when non-HDL-C and apoB are discordant, apoB was a more accurate marker of cardiovascular risk than non-HDL-C. This suggests that the atherogenic particle number is a more important determinant than the mass of cholesterol within LDL-C particles [21,22]. Therefore, when all three parameters are concordant, the clinical utility of these variables is similar. The moment they are discordant, cardiovascular risk can be under or overestimated if only LDL-C is considered [23–25].

Residual cardiovascular risk in FCHL may be indicated by discordance of LDL-C with apoB and non-HDL-C. In fact, both non-HDL-C and apoB predict overall cardiovascular risk better than LDL-C [21]. Otvos et al. reported that when there is discordance between apoB levels and LDL-C, only the number of particles is significantly associated with the incidence of cardiovascular events and the thickness of the carotid intima-media [6]. They concluded that when such a discrepancy exists, the risk attributable to LDL is best established by apoB levels. Patients with LDLs poor in cholesterol may have residual risk and, despite reaching LDL-C targets, they might continue to have high numbers of LDL particles. Discordance between LDL-C and non-HDL-C has also been reported. Masana et al. evaluated individuals, who having achieved LDL-C targets, continued to have uncontrolled non-HDL cholesterol levels [22]. They reported that 90% of patients with hypertriglyceridemia ≥400 mg/dL, showed LDL-C at target, but non-HDL-C was ≥130 mg/dL. Furthermore, 2 of every 5 patients with triglycerides ≥150 mg/dL and normal LDL-C levels had elevated levels of non-HDL-C. A recent study showed that approximately 20% of patients with Friedewald LDL-C <70 mg/dL have a LDL-C by Martin's formula of ≥70 mg/dL, and these individuals also have higher non-HDL-C and apoB concentrations [26]. Therefore, addressing accuracy of LDL-C estimation also addresses non-HDL-C and apoB discordance to an extent. Indeed, as shown by Sathiyakumar et al., when LDL-C is better estimated by Martin's formula and the LDL-C goal is achieved, then guideline non-HDL-C and apoB targets are also achieved in 98% or more of individuals and therefore are of modest additional utility in clinical management for individuals with elevated cardiovascular risk such as FCHL [27].

Our study has strengths and limitations. Firstly, this is a cross-sectional evaluation, which limits the possibility of establishing causality. Prospective studies with long-term follow-up to assess cardiovascular endpoints would aid in evaluating the relevance of discordant targets and evaluation of all three lipid parameters. Secondly, to evaluate the confounding effect of age, gender and lipid lowering treatment in correlations we adjusted for these variables; however, there exists the possibility of residual confounding. To the best of our knowledge, this is the first study validating LDL-N in FCHL, a high risk cardiovascular population in which the use of LDL-C estimation is problematic. In addition, it is noteworthy that validation in a cohort of FCHL patients is skewed towards subjects with a greater alteration in lipid profiles, as opposed to the general population. However, our results demonstrate that LDL-C estimation using Martin's formula is more useful than traditional methods in an atherogenic dyslipidemia with comorbid hypertriglyceridemia.

In conclusion, in FCHL, LDL-N offers improved correlation and concordance with apoB and non-HDL-C compared to LDL-F. LDL-N and LDL-F perform similarly when the lipid phenotype is restricted to isolated hypercholesterolemia. In FCHL, in the setting of elevated triglycerides, LDL-N outperforms LDL-F. An LDL-N threshold of 81.8 mg/dL could be used to identify patients at target without the need to measure ApoB levels in the setting of elevated TG.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

Research idea and study design: RM, ERR, CAAS, ICB; data acquisition: RM, ICB, ERR, ACGD; data analysis/interpretation: OYBC, RM, ERR; statistical analysis: OYBC, RM, AVV; manuscript drafting: OYBC, RM, CAAS, ICB, AVV; supervision or mentorship: CAAS, ICB, RM. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.atherosclerosis.2018.06.868.

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