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Discordance between apolipoprotein B, calculated low density lipoprotein-cholesterol and non-high density lipoprotein cholesterol measurements in plasma

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Abstract

Introduction: Routine estimation of the majority of the atherogenic risk attributable to lipoproteins is done by the measurement of cholesterol content of the low density lipoprotein (LDL) as LDL-cholesterol (LDL-C). Non-high density lipoprotein cholesterol (n-HDL-C) and Apolipoprotein B (Apo B) measurements have also been used as indices of risk as they account for other atherogenic molecules beyond LDL. We evaluate for discordance between these indices.

Methodology: Fasting plasma total cholesterol, triglycerides, high density lipoprotein cholesterol, Apo B and glucose were measured on healthy non-diabetic participants. Low density lipoprotein- Cholesterol, non-HDL-C and BMI were calculated. Low density lipoprotein- Cholesterol LDL-C, apolipoprotein B and non-HDL cholesterol were grouped into percentiles. Individuals were discordant if either their LDL-C or non-HDL values belonged to a percentile category different from their percentile category for Apo B.

Results: A discordant result (apolipoprotein B /LDL-C or apolipoprotein B /non-HDL-C) was seen in 55 (22%) of the 252 participants. Discordance was more frequent between apolipoprotein B and non-HDL cholesterol, occurring in 50 (20%) persons than between apolipoprotein B and LDL-C, 21 (8.4%). Discordance was associated a body mass index (BMI) $\geq$ 25kg/m^2 ($p = 0.039$) and $\geq$ 30kg/m^2 ($p = 0.008$) and the median BMI of persons who were discordant was also higher than those who were not 26.2 kg/m^2 vs. 25.0 kg/m^2, $p = 0.018$, respectively.

Conclusion: Discordance between Apo B and the calculated LDL-C and non-HDL is common among overweight and obese persons. It may provide a useful insight into the presence of atherogenic small dense LDL particles among these persons.

Keywords: *Discordance, Apolipoprotein B, LDL-Cholesterol, non-HDL-Cholesterol*

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Résumé

Contexte: L'estimation routinière de la majorité du risque athérogène attribuable aux lipoprotéines est réalisée par la mesure de la teneur en cholestérol de la lipoprotéine de basse densité (LBD) sous forme de LBD-cholestérol (LBD-C). Les mesures du cholestérol des lipoprotéines de non-haute densité (n-HDL-C) et d'apolipoprotéine B (Apo B) ont également été utilisées comme indices de risque car elles représentent d'autres molécules athérogènes au-delà des LBD. Nous évaluons pour la discordance entre ces indices.

Méthodologie: Le cholestérol total plasmatique à jeun, les triglycérides, le cholestérol des lipoprotéines de haute densité, l'Apo B et le glucose ont été mesurés sur des sujets non diabétiques sains. Les lipoprotéines de basse densité, le cholestérol non HDL et l'IMC ont été calculés. Les lipoprotéines de basse densité, le cholestérol LBD-C, l'apolipoprotéine B et le cholestérol non-HDL ont été regroupés en percentiles. Les individus étaient discordants si leurs valeurs LBD-C ou non-HDL appartenaient à une catégorie de percentile différente de leur catégorie percentile pour Apo B.

Résultats: Un résultat discordant (apolipoprotéine B / LBD-C ou apolipoprotéine B / non-HDL-C) a été observé chez 55 (22%) des 252 participants. La discordance était plus fréquente entre l'apolipoprotéine B et le cholestérol non HDL, survenant chez 50 (20%) personnes qu'entre apolipoprotéine B et LBD-C, 21 (8,4%). La discordance était associée à un indice de masse corporelle (IMC) $\geq$ 25 kg/m^2 ($p = 0,039$) et $\geq$ 30 kg/m^2 ($p = 0,008$) et l'IMC médian des personnes discordantes était également plus élevé que celui de ceux qui n'étaient pas 26,2 kg/m^2 contre 25,0 kg/m^2, $p = 0,018$, respectivement.

Conclusion: La discordance entre l'Apo B et le LBD-C calculé et non-HDL est fréquente chez les personnes en surpoids et obèses. Il peut fournir un aperçu utile de la présence de petites particules de LBD dense athérogènes parmi ces personnes.

Mots clés: *Discordance, Apolipoprotéine B, LBD-cholestérol, non-HDL-cholestérol*

Introduction

The Framingham Heart Study provided strong evidence that there are risk factors for the development of atherosclerotic cardiovascular disease (ASCVD) [1]. Amongst these risk factors, disorders of lipid and lipoprotein metabolism are especially critical in the pathogenesis of atherosclerotic disease. The fatty streak, which is thought to be the initial lesion in atherosclerosis, is an accumulation of lipid-containing foam cells in the endothelium of the arterial wall [2]. The progression of this streak to form an atheroma is also a function of the inability of the cholesterol reverse transport mechanisms to remove lipids from the developing lesion at a rate that exceeds that at which they enter the arterial wall [3]. In view of this central role of lipids in the pathogenesis of CVD, it has been recommended that lipid screening be done for all adults after 20 years of age. This should involve the fasting measurement of total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol [4].

LDL – Cholesterol is the primary target of cholesterol lowering therapy [4]. This is because it is a surrogate marker of the lipoprotein LDL, which is considered the most atherogenic of all the lipid carrying lipoproteins [5]. LDL is however a heterogeneous group of molecules consisting of distinct subclasses which vary in size, density and chemical composition [6]. Two distinct phenotypes have been described. Majority of healthy persons have phenotype A which is characterised by large buoyant LDL (lbLDL) particles. Phenotype B, which is seen in a small subset of healthy people have small dense LDL (sdLDL) particles [7]. The sdLDL particles are thought to be more atherogenic compared to the lbLDL particles.

The lbLDL particles contain more cholesterol than the sdLDL particles implying that at a given level of LDL-Cholesterol, persons with a predominance of sdLDL have more of the atherogenic LDL particles and are at a higher risk of CVD, than individuals with more of the lbLDL [8]. This underlines a weakness in using LDL-C measurements as estimates of CVD risk. In addition to the aforementioned reason, this residual risk may also be further explained by the fact that LDL itself is not the only atherogenic lipoprotein. Other pro-atherogenic lipoproteins include chylomicron remnants, VLDL remnants, IDL and Lipoprotein (a). The contribution of these other lipoproteins is neither accounted for nor adequately estimated by LDL-C measurements.

To improve the risk prediction of the traditional lipid profile and capture the contribution of the non-LDL pro-atherogenic lipoproteins, the calculation of non-HDL-C has been used [9]. Non-HDL-C is calculated as total cholesterol minus HDL-C and reflects the cholesterol content of all the atherogenic lipoprotein particles. Several studies have highlighted the increased capability of non-HDL-C over LDL-C in predicting increased risk of CVD[10-12]. These have resulted in its inclusion in the newer recommendations of the National Lipid Association as a co-primary target along with LDL-C. However, similar to LDL-C, the accuracy of this calculated index is influenced by the heterogeneity of VLDL and LDL particles. When these particles are either cholesterol enriched or depleted, its ability to act as a surrogate of the sum of all atherogenic lipoproteins is affected.

Unlike the indirect measurement of all the atherogenic lipoproteins provided by non-HDL-C, Apolipoprotein B provides a direct assessment of these macromolecules. This is because it is an integral part of all atherogenic lipoprotein particles with each carrying a single apolipoprotein B particle on their surface. This fact underlies the clinical utility of apolipoprotein B as a marker of cardiovascular risk. Several studies have shown the superiority of apolipoprotein B over both LDL-C and non-HDL-C in predicting likelihood of cardiovascular events. Sniderman *et al*, performed a meta-analysis of published epidemiological studies with estimates of the relative risks of non-HDL-C and apolipoprotein B of fatal or nonfatal ischemic cardiovascular events. They concluded that over a 10-year period, an apolipoprotein B strategy would prevent 500 000 more events than a non-HDL-C strategy. This suggests that cardiovascular risk is more closely related to the number of atherogenic particles than to the total mass of cholesterol within them [13].

The above evidence suggests that despite the strong correlation that is frequently observed between LDL-C, non-HDL-C and apolipoprotein B, they are not of equivalent clinical value. This would further mean that there are circumstances where there is significant disagreement between values obtained by these 3 parameters in an individual. This is defined as discordance. Assessments of the degree of discordance, including descriptions of prevalence and associations, in a population should inform risk assessment for CVD. The present study aims to define the level of discordance that exists between LDL-C, and non-HDL-C with apolipoprotein B. This

may give an estimate in the degree of over or under-estimation of CVD risk that may be present in the use of these parameters among an apparently healthy Nigerian population.

Materials and methods

Study population

This was a cross-sectional study. Participants were recruited from the staff of the University College Hospital, Ibadan. They were apparently healthy and aged between 30 and 65 years. Persons with diabetes, on hypolipidemic agents or oral contraceptives were excluded. After consent for participation was obtained, a structured questionnaire was used to obtain information on demographic and social and clinical characteristics.

Laboratory measurements

Venous blood was obtained into EDTA bottles for fasting TC, TG, HDL–C and Apoprotein B 100 measurements. LDL – C was calculated using the Friedewald formula (LDL-C = TC minus [HDL-C plus TG/5]) while non-HDL – C was calculated as TC – HDL–C. Fluoride oxalate specimens were also collected for fasting glucose studies.

All analyses were carried out on the Landwind C100 plus automated analyzer (Landwind Medicals, Schenzen, China). Total Cholesterol, LDL Cholesterol, HDL Cholesterol and Triglycerides were measured by enzymatic methods while apolipoprotein B was measured by immunoturbidimetry.

Statistical analysis

Statistical Analysis was performed using Statistical Package for Social Sciences (SPSS) version 21. Statistical significance was set at $p< 0.05$.

Ethical approval was obtained from the University of Ibadan/University College Hospital, Ibadan Ethics Committee.

Results

Two hundred and fifty two (252) apparently healthy adults were recruited for the study. They included 89 males (35.3%) and 163 females (64.7%) with mean (SD) ages of 42.0 (8.5) years and 47.3 (10.3) years respectively. The difference in the ages of the 2 genders was not statistically significant. The mean age (SD) for all the participants was 45.4 (10.0) years. Twenty six persons (10.4%) were hypertensive and 52.7% were either overweight or obese.

Table 1 shows the distribution of the lipid and lipoprotein indices in the study population. The range of values for LDL-C, non-HDL-C and apolipoprotein B were 1.24 – 5.9mmol/L, 1.37 – 6.44 mmol/L and 0.62 – 2.57 µmol/L respectively. Values greater than the 75th percentile were observed in 191 (76.4%), 188 (75.2%) and 188 (75.2%) of the values for LDL-cholesterol, apolipoprotein B and non-HDL cholesterol respectively. Table 2 shows the Spearman's correlation of LDL-C, non-HDL-C and apolipoprotein B with clinical and biochemical parameters. The correlation studies show that these 3 parameters had significant associations with age, BMI, Total Cholesterol and Triglycerides. Non-HDL-C was the only parameter significantly associated with systolic blood pressure, diastolic blood pressure and fasting plasma glucose while LDL-C and apolipoprotein B were significantly associated HDL-C.

A discordant result (apolipoprotein B /LDL-C or apolipoprotein B /nonHDL-C) was seen in 55 (22%) of the participants. Table 3 shows the pattern

Table 1: Distribution of Lipid and Lipoprotein metrics

	LDL-C (mmol/L)	non-HDL-C(mmol/L) (mmol/L)	Apo B (µmol/L)
Mean,(SD)	3.34 (0.82)	3.71 (0.92)	1.88 (0.5)
Range	1.24 – 5.9	1.37 – 6.44	0.62 – 3.55
Median	3.23	3.59	1.79
Interquartile Range	2.74 – 3.83	3.05 – 4.28	1.52 – 2.17

Definition of discordance

Discordance was defined as used in previous reports.[14, 15] Values for LDL-C, apolipoprotein B and non-HDL cholesterol were grouped into 2nd, 20th 50th and 80th percentile. Individuals were considered discordant for if either their LDL-C or non-HDL values belonged to a percentile category which was higher or lower than that for the Apo B.

Table 2: Correlation of Lipid and Lipoprotein metrics with Clinical and Biochemical Parameters

	LDL-C	non-HDL-C	Apo B
	rho p value	rho p value	rho p value
Age, years	0.196, 0.002	0.215, 0.001	0.194, 0.002
BMI, kg/m²	0.193, 0.002	0.221, 0.000	0.183, 0.004
Systolic BP	0.079, 0.211	0.125, 0.048	0.088, 0.168
Diastolic BP	0.102. 0.109	0.143, 0.023	0.107, 0.091
Total Cholesterol	0.954, 0.000	0.972, 0.000	0.949, 0.000
Triglycerides	0.228, 0.000	0.368, 0.000	0.230, 0.000
HDL-C	0.133, 0.035	0.104,0.102	0.129, 0.041
LDL-C	-- --	0.972, 0.000	0.994, 0.000
non-HDL-C	0.972, 0.000	-- --	0.967, 0.000
Apolipoprotein B	0.994, 0.000	0.967, 0.000	-- --
FPG	0.111, 0.080	0.139, 0.027	0.107, 0.092

of discordance between values of apolipoprotein B and those of LDL-Cholesterol while Table 4 shows that of apolipoprotein B and non-HDL cholesterol. Discordance was more frequent between persons with discordance between Apolipoprotein B and non-HDL cholesterol, 27 (54%) were due to non-HDL-C values occurring in a lower percentile. In both comparisons, discordance was more frequently

Table 3: Discordance of Apo B and LDL-C percentiles

Apolipoprotein B percentiles	≤ 2nd	> 2nd - ≤ 20th	> 20th - ≤ 50th	> 50th - ≤ 80th	>80th	Total
< 2nd	5 (83.3)	1(16.7)	0 (0.0)	0 (0.0)	0 (0.0)	6 (100.0)
> 2nd - < 20th	0 (0.0)	41 (97.6)	1 (2.4)	0 (0.0)	0 (0.0)	42 (100.0)
> 20th - < 50th	0 (0.0)	4 (5.3)	69 (90.8)	3 (3.9)	(0.0)	76 (100.0)
> 50th - < 80th	0 (0.0)	0 (0.0)	3 (4.0)	68 (90.7)	4 (5.3)	75 (100.0)
>80th	0(0.0)	0(0.0)	0 (0.0)	5 (9.8)	46 (90.2)	51 (100)

Values are n (%)

apolipoprotein B and non-HDL cholesterol, occurring in 50 (20%) persons. The number of persons with discordance between apolipoprotein B and LDL-C observed for values within the 20th and 80thpercentiles, occurring in 9.2% and of values obtained in this range. This corresponds to a

Table 4: Discordance of Apo B and nonHDL-C percentiles

Apolipoprotein B percentiles	≤ 2nd	> 2nd - ≤ 20th>	20th - ≤ 50th	> 50th - ≤ 80th	>80th	Total
< 2nd	5 (83.3)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	6 (100.0)
> 2nd - < 20th	0 (0.0)	35 (83.3)	7 (16.7)	0 (0.0)	0 (0.0)	42 (100.0)
> 20th - < 50th	0 (0.0)	10 (13.2)	69 (90.8)	3 (3.9)	(0.0)	76 (100.0)
> 50th - < 80th	0 (0.0)	0 (0.0)	7 (9.3)	58 (77.3)	10 (13.3)	75 (100.0)
>80th	0(0.0)	0 (0.0)	0 (0.0)	10 (19.6)	41 (80.4)	51 (100)

Values are n (%)

was 21 (8.4%). 16 (6.4%) persons had a discordant percentile classification for both Apolipoprotein B and LDL-C as well as Apolipoprotein B and nonHDL-C. Of the persons with discordance between Apolipoprotein B and LDL-C, 13 (61.9%) had LDL-C values occurring in a lower percentile and for concentration of 2.66 and 3.93 mmol/L for LDL-C and 2.97 and 4.40 mmol/L for non-HDL-C.

There was a strong association between Apolipoprotein B /LDL-C discordance and Apolipoprotein B /non-HDL-C discordance (p <0.0001). Discordance (either ApoB/LDL-C or

ApoB/non-HDL-C) was associated a body mass index (BMI) $\geq$ 25kg/m^2 ($p = 0.039$) and $\geq$ 30kg/m^2 ($p = 0.008$). The median BMI of persons who were discordant was also significantly higher than persons who were not discordant, 26.2 kg/m^2 vs. 25.0 kg/m^2, $p = 0.018$, respectively.

Discussion

Appropriate estimation of an individuals' CVD risk allows for the appropriate interventions, whether lifestyle modification or pharmacologic therapy as indicated by risk category. A discordance between Apolipoprotein B and the more routinely used LDL-C and nonHDL-C indicates inappropriate risk estimation (under- or overestimation) and inappropriate interventions. About one out of every five (22%) of our study participants had a discordant result with either an LDL-C and/or nonHDL-C value that occupied a different percentile category with the correspondingly measured Apolipoprotein B value. In more than 50% of these persons with discordant results, Apolipoprotein B results were in a higher percentile when compared to either LDL-C or nonHDL-C. This suggests that risk estimation using either of the latter 2 parameters in these persons will result in an underestimation of risk and inappropriate intervention. This has consequences for long-term cardiovascular health of these persons. This is supported by the longitudinal CARDIA (Coronary Artery Risk Development in Young Adults) study which followed up persons aged between 18 and 30 years for 25 years[16]. It reported that persons with Apolipoprotein B values greater than median and with LDL-C or nonHDL-C values lower than median (discordant) had a higher likelihood of having year 25 evidence of coronary artery calcium than in those persons in whom all the parameters were below the median (concordant). While this may provide evidence of the better predictive value of ApolipoproteinB, it also implies that the management of CVD risk in these persons would have been suboptimal if dependent on just LDL-C or nonHDL-C alone.

Our data also suggests that individuals are likely to have discordant results if they were overweight and yet more likely if they were obese. This may guide the selection of persons who in addition to the routine and traditional lipid studies should have apolipoprotein B measurements performed for optimal risk assessment. That a raised BMI may serve as a clinical predictor of discordance was also suggested by the results of Mora et al among participants in the Women's Health Study [14]. They noted that individuals who had Apolipoprotein B values greater than the median value and LDL-C lower than the median value of their study population had a higher BMI compared with individuals who had both Apolipoprotein B and LDL-C concordantly below the median. These findings are consistent with changes in the structure/composition of LDL that is observed in obesity, particularly an increase in the number of small dense LDL particles [17]. Ohmura *et al* [18] demonstrated that, relative to their ApolipoproteinB content, small dense LDL particles had significantly lower free cholesterol and cholesterol ester when compared to large buoyant LDL. This would provide a pathophysiological explanation to our observation. Thus the presence of ApoB/LDL discordance may guide the management of dyslipidaemia by helping to identify individuals who despite having desirable LDL-C cholesterol values may have increased concentrations of the atherogenic small dense LDL particles. These persons may then be offered appropriate lipid lowering interventions which they may not have received if LDL-C alone was the main guide for therapy.

There are methodological reasons for a using a surrogate marker to detect the presence of small dense LDL particles. The conventional approach has relied on either analytical ultracentrifugation (UC) or gradient gel electrophoresis (GGE). Also previously used in LDL class separation are tube gel electrophoresis, nuclear magnetic resonance, high performance liquid chromatography with gel filtration columns, ion mobility analysis, dynamic light scattering and direct homogenous assays [19]. The low cost options (UC, GGE) may require up to 72 hours of separation time and 10 mls of plasma while the ones with shorter duration of analysis are typically high costing. In addition, there is significant heterogeneity in the identified LDL subclasses as to make comparison across methods difficult due to current poor standardization across the different methods [19]. This in contrast to Cholesterol and Apolipoprotein B methods that have had international reference preparations/methods available for over 2 decades [20, 21]. This suggests that discordance as an index for assessing for the presence of small dense LDL may provide a reproducible index.

In conclusion, discordance is common among apparently healthy adults, especially those who are overweight and obese. It may provide an insight in to the presence of the atherogenic small dense LDL particles in circulation.

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