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Discordance analysis of Apolipoprotein B and non-high density lipoprotein cholesterol as markers of cardiovascular risk in the INTERHEART study

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ABSTRACT

Objectives: Whether non-HDL-C and apoB are equivalent markers of cardiovascular risk remains controversial. Only when apoB particles *in toto* contain either more or less cholesterol than normal – that is, when their composition is discordant – could apoB and non-HDL-C predict risk differently. Accordingly, this study tests within the INTERHEART data base whether apoB or non-HDL-C are equivalent markers of risk when the two markers are discordant.

Methods: The INTERHEART study is a standardized case-control study of acute myocardial infarction with blood samples in 9345 cases and 12,120 controls from 52 countries. To produce comparability, the concentrations of non-HDL-C and apoB are expressed as percentiles (P) within the study population. Concordance is defined as the phenotype when P Non-HDL-C = P apoB (that is, apoB particles contain a normal mass of cholesterol). Discordance is defined either as the phenotype when P Non-HDL-C > P apoB (cholesterol-enriched apoB particles) or P Non-HDL-C < P apoB (cholesterol-depleted apoB particles). The OR of cases to controls was determined for both discordant groups and compared to the ratio of cases to controls in the concordant group, which was the reference group. An OR > 1 means that risk is greater in the discordant than in the reference phenotype whereas an OR < 1 means the cases are less common in the discordant phenotype than in the reference group.

Results: When discordance was defined as percentiles within 5%, a definition that produced equal numbers of discordant and concordant individuals, the OR for P Non-HDL-C > apoB (cholesterol-enriched apoB particles) was 0.72 (0.67–0.77 95% CI) indicating risk was less than the reference concordant group whereas the OR for P Non-HDL-C < apoB (cholesterol-depleted apoB particles) was 1.58 (1.38–1.58 95% CI) indicating risk was significantly greater than the reference concordant group. The same findings were reproduced using all definitions of discordance from 1% to 10%. Moreover, the pattern of findings was consistent amongst the ethnic groups that made up the overall study population.

Conclusion: Discordance analysis demonstrates that apoB is a more accurate marker of cardiovascular risk than non-HDL-C.

1. Introduction

Apolipoprotein B (apoB) and non-high density lipoprotein cholesterol (non-HDL-C) have been claimed to be more accurate measures of the risk of cardiovascular events than low-density lipoprotein cholesterol (LDL-C), the conventional index to estimate the concentration of the atherogenic lipoproteins in plasma. Indeed, ATPIII recommended non-HDL-C as an alternate target of therapy to LDL-C in hypertriglyceridemic patients [1] and the most recent Canadian and European guidelines recommend using either apoB or non-HDL-C as alternate targets to LDL-C [2,3].

However, two contradictory meta-analyses of the predictive power of the three markers in prospective observational studies have appeared. The Emerging Risk Factor Collaboration (ERFC), a patient-level evaluation of subjects, has published two analyses of their results [4,5] and there were no significant differences in accuracy in the prediction of cardiovascular risk amongst LDL-C, non-HDL-C and apoB. Indeed, none of these three markers was superior to total cholesterol. The second meta-analysis [6], which was based on published reports, demonstrated that non-HDL-C was superior to LDL-C as a predictor of vascular risk and that apoB was

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superior to non-HDL-C. In only 3 of the studies included in the ERFC analysis were the data on apoB and non-HDL-C published and accessible for our meta-analysis [6] and therefore only 3 of the studies were in both reports. There was, therefore, virtually no overlap in the materials included in the two meta-analyses. Nevertheless, the difference in results is striking and should be resolved. Accordingly, this study applies a different method – discordance analysis – to try to clarify the issue.

The absolute risk of LDL is determined by the absolute level of LDL. Accordingly, the risk predicted by any biomarker of LDL relates to the concentration of the biomarker in plasma. ApoB particles differ substantially in the mass of cholesterol they contain per particle. When the mass of cholesterol in LDL particles is normal, non-HDL-C and apoB are defined as concordant and the two markers must equally informative of cardiovascular risk because their concentrations in plasma, relative to the population, are the same [7,8]. However, when the mass of cholesterol in LDL particles is either greater than normal or less than normal, non-HDL-C and apoB are discordant [9] and only then could they predict risk differently.

The conventional approach to test the potential superiority of one biomarker over another compares their predictive powers in all subjects, which in this case would include those who are concordant as well as those who are discordant. But the test of two tests is when they disagree, not when they agree [10]. In this instance, by including the concordant subjects, the ability to determine which marker might be superior in the subjects in whom they are discordant is diluted. Accordingly, we examined the relationship of apoB and non-HDL-C to risk in the INTERHEART study [11], a large case-control study of the determinants of the risk of myocardial infarction with participants from all the major regions of the world, to determine which provided more accurate risk prediction when the cholesterol and apoB measurements were discordant.

2. Methods

Details of the design and methods of the INTERHEART study have been published previously [11]. INTERHEART consisted of 15,152 cases with a first acute myocardial infarction and 14,820 age and sex-matched controls without known cardiovascular disease. Subjects were recruited from 262 centers in 52 countries. Non-fasting blood samples were obtained from 9345 cases and 12,120 controls. Concentrations of total cholesterol, HDL cholesterol, apo A1 and apoB were measured with the Roche Hitachi 917 analyzer and concentrations of non-HDL cholesterol were calculated as total cholesterol minus HDL cholesterol. Apolipoprotein concentrations were measured using the Tina-quant apoB and apo A1 kits (version 2, with the IFCC SP3-07 reference standard and IFCC SP1-01 reference preparations), which are WHO standardized methods for measurement of apoB and apo A1. Cholesterol concentrations were measured with an enzymatic colorimetric method (CHOD-PAP) with cholesterol esterase, cholesterol oxidase, and 4 amino-antipyrine. Concentrations of HDL cholesterol were measured with a homogeneous enzymatic colorimetric assay (HDL-C plus, 2nd generation) that uses cholesterol esterase and cholesterol oxidase coupled with polyethylene glycol to the amino groups.

3. Statistical methods

Age, risk factors and laboratory results of apoB and non-HDL-C were summarized based on mean and standard deviation, with other demographics summarized by reporting number (n) and percent. Proportions and means were also compared using Chi-square and t test respectively. Concordance categories were derived based on matched percentiles. For example, when a 5 percentile difference was of interest, the difference in concentration expressed as percentile of the population between apoB and non-HDL was computed for each individual. If the difference in percentile was less or equal to 5, the individual was classified as concordant and if the difference was greater than 5, the individual was classified as discordant. In the group in which P Non-HDL-C $<$ P apoB, the apoB particles will contain less cholesterol than the reference concordant group. Accordingly, the number of apoB particles will be underestimated by non-HDL-C, the apoB percentile will be higher than the non-HDL-C percentile, and the risk predicted by apoB will be higher than the risk predicted by non-HDL-C. Similarly, when cholesterol mass per particle is increased – the discordant group with cholesterol-enriched apoB particles – the P Non-HDL-C will be greater than the P ApoB and the risk estimated by non-HDL-C will be greater than risk estimated by the number of apoB particles.

Discordance analysis tests whether non-HDL-C or apoB correctly identifies risk in the two discordant groups. Within each group, there will be a range of particle numbers and concentrations for non-HDL-C and apoB for the individuals within that group and their individual risks will differ. However, it is the risk for the group that is calculated and compared to the other groups. Identical procedures were used for other cut-offs from 1% to 10%.

Pearson's coefficient of correlation was used to identify the degree of linear association between apoB and non-HDL-C. Odds ratios were computed using a logistic regression model with appropriate adjustment. In each group, the ratio of cases/controls was computed with the ratio in the concordant group as the reference. The Odds ratio for each discordant group was calculated using the concordant group as the reference. A ratio >1 indicates the discordant phenotype is associated with increased risk compared to the reference concordant group whereas a ratio <1 indicates the discordant phenotype is associated with decreased risk compared to the reference concordant group. The overall Odds ratios were adjusted for age, sex and ethnicity whereas the ethnic specific Odds ratios were adjusted for age and sex only. All analyses were performed using SAS version 9.2 in UNIX and Figures were prepared using S-PLUS 8.1 for Windows.

4. Results

The INTERHEART study population ($n = 20{,}852$) had a mean age ($\pm$SD) of 57.3 ($\pm$12.2) years and 24% were women. The clinical and biochemical characteristics of the three groups are detailed in Table 1. The great majority of subjects in all groups was male but there were significantly more were males in the cholesterol-depleted apoB particle group compared to the cholesterol-enhanced apoB particle group. Diabetes, hypertension, smoking, percent with a WHR in the top tertile, depression – all were most frequent in the cholesterol-depleted apoB particle group.

With regard to plasma lipid and apolipoprotein levels, the concentration of non-HDL-C was greatest in the P Non-HDL-C $>$ P apoB group and least in the P Non-HDL-C $<$ P apoB group, whereas the reverse was the case for the levels of apoB. Accordingly, plasma cholesterol, non-HDL-C, LDL-C were highest in the former, the cholesterol-enriched discordant apoB group whereas, by contrast, apoB was highest and apoA-I lowest in the latter, the cholesterol-depleted discordant apoB group. Of interest, triglyceride levels parallel the non-HDL-C levels not the apoB levels. A high triglyceride, therefore, is a better surrogate for a high non-HDL-C than for a high apoB. As anticipated, the LDL-C/apoB ratio is highest in the P Non-HDL-C $>$ P apoB group, and least in the P Non-HDL-C $<$ P apoB group. The results of the non-HDL-C/apoB ratio are of particular interest. This value is highest in P Non-HDL-C $>$ P apoB cholesterol-enriched apoB particle group and least in the P non-HDL-C $<$ P apoB cholesterol-depleted apoB particle group.