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Lipoprotein(a)-cholesterol levels estimated by vertical auto profile correlate poorly with Lp(a) mass in hyperlipidemic subjects: Implications for clinical practice interpretation of Lp(a)-mediated risk

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BACKGROUND: Lipoprotein(a) [Lp(a)] is generally measured as total mass of the entire particle or as apolipoprotein(a) particle number.

OBJECTIVE: The cholesterol content of Lp(a) [Lp(a)-C)] can be estimated by the vertical auto profile (VAP) method. We assessed whether this is an accurate surrogate measurement of Lp(a) mass.

METHODS: VAP-Lp(a)-C and VAP-high density lipoprotein cholesterol (HDL-C) estimated by the VAP technique, Lp(a) mass, oxidized phospholipids on apolipoprotein B-100 (OxPL-apoB) that primarily reflect OxPL on Lp(a), and HDL-C measured by enzymatic methods were measured in 552 hypercholesterolemic patients at baseline and 24 weeks after therapy with niacin monotherapy (N = 118), ezetimibe/simvastatin monotherapy (n = 155), or ezetimibe/simvastatin (10/20 mg) + niacin (to 2 g) (N = 279) in a randomized, double-blind trial.

RESULTS: VAP-Lp(a)-C correlated only modestly with Lp(a) mass at baseline ($r = 0.56$, $P < .001$) and 24 weeks ($r = 0.56$, $P < .001$), explaining only 31% of the association. VAP-Lp(a)-C correlated with HDL-C at baseline ($r = 0.34$, $P < .001$) and 24 weeks ($r = 0.30$, $P < .001$) and with VAP-HDL-C at baseline ($r = 39$, $P < .001$) and 24 weeks ($r = 0.33$, $P < .001$). In contrast, Lp(a) mass did not correlate with HDL-C at baseline ($r = 0.06$, $P = .12$) and 24 weeks ($r = -0.01$ $P = .91$). Lp(a) mass correlated strongly with oxidized phospholipids on apolipoprotein B-100 at baseline ($r = 0.81$, $P < .001$) and 24 weeks ($r = 0.79$, $P < .001$). VAP-Lp(a)-C levels increased linearly with HDL-C and VAP-HDL-C quartiles ($P < .001$ for both) but Lp(a) mass did not. Quantitating the percent of cholesterol present on Lp(a) by dividing VAP-Lp(a)-C by Lp(a) mass revealed that 25% of patients had a percentage >100, which is not possible.

CONCLUSIONS: VAP-Lp(a)-C is a poor estimate for Lp(a) mass and likely reflects the content of HDL-C in the overlapping density spectrum of Lp(a) and HDL. These data suggest that patients with prior VAP-Lp(a)-C measurements may have misclassification of Lp(a)-related risk.

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Introduction

Lp(a) consists of apolipoprotein(a) [apo(a)] covalently bound to apoB on low-density lipoprotein (LDL) and is the major lipoprotein carrier of oxidized phospholipids (OxPL).[1,2] Lp(a) measurements as either total particle mass at levels >30 mg/dL[3] or particle number >75 nmol/L[4] predict incident myocardial infarction, coronary artery disease,[5] peripheral artery disease,[6] stroke, calcific aortic valve disease,[7] and faster progression of preexisting aortic stenosis.[8]

Lp(a), by virtue of containing apolipoprotein B-100 (apoB-100) also contains free and esterified cholesterol in a similar proportion to LDL (~50% of mass). However, because apolipoprotein(a) is a very large glycoprotein, and often larger than apoB-100 (which is approximately 550 kDa), the relative proportion of cholesterol on Lp(a) is lower and estimated to be approximately 30% to 45% of total Lp(a) mass.[9–12] The cholesterol content of Lp(a) contributes to the laboratory measurement of "LDL-C," as all clinical assays, including direct LDL assays, cannot differentiate LDL-C vs Lp(a)-C.[13] Therefore, as Lp(a) mass increases, so does the relative contribution of Lp(a)-C to the laboratory measurement of "LDL-C." For example, when Lp(a) mass is 100 mg/dL and LDL-C is 100 mg/dL, the contribution of Lp(a)-C to "LDL-C" is 30% to 45% of Lp(a) mass, or ~33 to 45 mg/dL, and estimated true LDL-C is 55 to 66 mg/dL.

Lp(a)-C, determined by the vertical auto profile (VAP) method (VAP-Lp(a)-C) has been a widely used clinical method for estimating Lp(a)-related risk.[12] However, whether it is a precise, accurate, and reliable surrogate for Lp(a) mass or in predicting cardiovascular disease is not known. Appropriate Lp(a) measurements are needed for population cutoffs, clinical risk prediction, and classification of patients into proper risk categories.[14]

VAP-Lp(a)-C is determined by measuring the cholesterol content of the Lp(a) fraction of plasma separated by ultracentrifugation.[15] The VAP technology uses nonequilibrium density ultracentrifugation to resolve plasma lipoproteins in a single tube and spin, followed by continuous analysis of cholesterol concentrations, and hence separates by flotation rate, which is a function of both lipoprotein density and size. The different classes of lipoproteins are continuously but sequentially flowed out of the spin tube and mixed with reagents for enzymatic colorimetric cholesterol determination. The raw data are represented as absorbance over time, and software algorithms are used to decompose the absorbance profile into discrete lipoprotein-associated cholesterol levels. VAP-Lp(a)-C has been compared with more traditional forms of Lp(a) measurements infrequently, and anecdotal experiences from lipidology clinics have raised concerns regarding its accuracy. Here, we examine how VAP-Lp(a)-C compares with Lp(a) mass in a hyperlipidemic population undergoing lipid-lowering therapy.

Material and methods

Patients and study design

Plasma samples were obtained from the previously completed study by Guyton et al.[16] This was a randomized, double-blind study in which patients of ages 18 to 79 years with LDL-C levels 130 to 190 mg/dL and triglyceride levels <500 mg/dL were initially randomized to 3 arms: extended release niacin titrated up to 2 g (N), ezetimibe 10 mg/simvastatin 20 mg (E/S), or triple combination therapy of E/S/N after an initial 4-week washout period. The samples included in this study reflect trial completers (75%) that included 552 patients with complete baseline and 24-week on-treatment plasma samples for VAP-Lp(a)-C, VAP-high density lipoprotein cholesterol (HDL-C), HDL-C, Lp(a) mass, and oxidized phospholipids on apolipoprotein B-100 (OxPL-apoB), and full characteristics of these study subjects were described by Yeang et al.[17]

Measurement of Lp(a) mass

Lp(a) mass quantification was performed by the University of California San Diego (UCSD) assay with a double-antibody enzyme-linked immunosorbent assay as previously described.[18] Plasma from each sample was diluted 1:400 and added to microtiter wells coated with the monoclonal antibody MB47 (5 μg/mL). Biotinylated LPA4 (1 μg/mL), a monoclonal antibody, was added to determine the amount bound detected by a chemiluminescent technique. The coefficient of variation of the assay is 6.0% to 7.4%. This assay has been validated previously and the methodology and standardization were recently described in detail.[19]

A second, commercially available, Food and Drug Administration-approved Lp(a) mass assay (Polymedco, Inc, Cortlandt Manor, NY) was also used in a subset of 75 patients on niacin monotherapy, 88 patients on E/S monotherapy, and 175 patients on E/S/N (approximately 62% of total group) with complete data on HDL-C by VAP, Lp(a)-C by VAP, and Lp(a) mass.

Measurement of OxPL-apoB levels

OxPL-apoB levels were measured in a chemiluminescent immunoassay using the murine monoclonal antibody E06 that recognizes the phosphocholine (PC) group on oxidized but not on native phospholipids (Taleb et al[20] and references therein). A 1:50 dilution of plasma was added to microtiter wells coated with the apoB100-specific monoclonal antibody MB47, and biotinylated E06 was then used to determine the content of OxPL-apoB. These values were recorded as relative light units and then converted to nanomolar (nM) PC-OxPL using a standard curve of nM PC equivalents, as recently described.[6,21] Because each well contains equal numbers of apoB100 particles, the OxPL-apoB value reflects the content of OxPL per apoB100-containing lipoprotein.