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Original Article

Relationship of lipoprotein(a) molar concentrations and mass according to lipoprotein(a) thresholds and apolipoprotein(a) isoform size

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BACKGROUND: Lipoprotein(a) [Lp(a)] is reported as Lp(a) particle mass (mg/dL) or molar concentration of apolipoprotein(a) [apo(a)] (nmol/L), which is considered the gold standard. Values are often converted from one measurement to the other but the validity of this is unknown.

OBJECTIVES: To quantify the relationship between Lp(a) molar concentration and Lp(a) mass in the context of various Lp(a) level thresholds and apo(a) isoform size.

METHODS: In all samples, Lp(a) levels in molar concentration and apo(a) isoform size were determined at the Northwest Lipid Metabolism and Diabetes Research Laboratories (NLMDRL). Lp(a) mass levels were determined at the University of California, San Diego (UCSD) (1635 samples), by 5 commercially available assays: Denka 1 and Denka 2 (each 80 samples), 2 turbidimetric assays (2545 and 2673 samples, respectively), and an enzyme-linked immunosorbent assay (2605 samples). The ratios between Lp(a) molar concentration and mass (eg, nmol/L/mg/dL) were calculated and related to apo(a) isoform size.

RESULTS: The mean (SD) ratios for NLMDRL/UCSD, NLMDRL/Denka1, and NLMDRL/Denka2 were 2.42 (1.25), 1.64 (0.18), and 2.02 (0.22), respectively. The ratios for NLMDRL/UCSD, NLMDRL/Denka1, and NLMDRL/Denka2 increased by Lp(a) cutoffs, with ratios of 1.82, 1.52, and 1.87, respectively, for Lp(a) $<$ 75 nmol/L and 2.80, 1.89, and 2.24, respectively, for Lp(a) $>$ 125 nmol/L. For the commercial turbidimetric assays and enzyme-linked immunosorbent assay, the ratios ranged from $<$1 to $>$5.

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CONCLUSIONS: Lp(a) molar/mass ratios are threshold, method, and isoform dependent. A single factor between assays is not appropriate. These data support the transition of Lp(a) mass assays to molar concentration to improve diagnostic and clinical interpretation of Lp(a)-mediated risk.

Introduction

Lipoprotein(a) [Lp(a)] is a highly prevalent cardiovascular disease (CVD) risk factor that places an estimated 1.4 billion people at moderate-high risk.[1] Studies suggest that measuring Lp(a) in intermediate risk patients may reclassify 15% to 40% into either higher or lower risk categories.[2,3] Such information could influence treatment decisions and monitoring intensity of a large number of patients at risk for CVD. These and other findings have led to formal guidelines endorsing measurement of Lp(a) in intermediate and high-risk groups by the European Atherosclerosis Society/European Society of Cardiology[4] and the Canadian Cardiovascular Society.[5] Furthermore, the emergence of PCSK9 inhibitors that modestly lower Lp(a)[6,7] and antisense oligonucleotides targeting apolipoprotein(a) [apo(a)] that potently lower Lp(a),[8,9] as well as apheresis specifically for elevated Lp(a) levels,[10] has brought new therapeutic perspectives to the field, with the hope that reducing Lp(a)-mediated CVD events and calcific aortic valve stenosis may be feasible.[11,12]

The lack of global standardization of the different methods for measuring Lp(a) levels, which report results in different units, generates confusion in the care of patients with elevated Lp(a). Lp(a) is a complex lipoprotein that contains a single copy of apo(a) that varies greatly in molecular weight and carbohydrate content among individuals, as well as an low-density lipoprotein (LDL)-like particle that contains apolipoprotein B-100 (apoB-100) and variable amounts of cholesteryl esters, free cholesterol, triglycerides, and phospholipids. Lp(a) is generally reported in mass (as mg/dL) or apo(a) particle concentration (in nmol/L). Lp(a) mass assays reflect the mass of the entire Lp(a) particle including the mass of all of the components previously listed. These types of assays determine the content of apo(a) in Lp(a), but this value assumes that the lipid components are the same in all Lp(a) particles and that apo(a) makes up a fixed percentage of the content of the total Lp mass. In contrast, Lp(a) assays that measure apo(a) concentration circumvent the issue of the variable mass of apo(a) in Lp(a) particles and the values reflect the number of circulating Lp(a) particles. However, to convert the values from mg/dL to nmol/L or vice versa is not a straightforward process. This is very different from the standard conversion of cholesterol or other lipid values from mass units in mg/dL to molar concentration in mmol/L because the conversion factor is calculated for a single moiety with a defined molecular weight.

Driven by the need to help the clinicians in interpreting the Lp(a) results expressed in different units, prior recommendations suggested the use of a mean conversion factor of dividing the Lp(a) results expressed in nmol/L by 2.4 to convert the results in mg/dL, which was based on the approximate ratio of molar concentration/mass noted in prior studies.[13] This recommendation was based on expert opinion, but with the realization that this was only an approximate mean estimate and that a single factor is not appropriate.[14]

To formally assess the relationships of Lp(a) molar concentration and mass in different methods as function of isoform size, we performed the current analyses comparing results obtained by multiple Lp(a) mass methods to those obtained by a molar enzyme-linked immunosorbent assay (ELISA) method on the same samples.

Methods

Patient populations

Study 1

The first study consists of 169 subjects that were either screened and/or randomized into one of 3 clinical trials (two phase 1 trials and one phase 2 trial) aimed at assessing the effect of 2 antisense oligonucleotides, IONIS-APO(a)$_{Rx}$ and IONIS-APO(a)-L$_{Rx}$, directed against apo(a).[8,9] To be included in the current studies, subjects had to have samples tested with both Lp(a) mass and molar assays, as described below. Data from a total of 1635 blood samples were available for the study. Data from placebo and treatment groups were combined and included in the analyses as the analyses are indifferent to treatment assignment. Samples were drawn serially over time during each trial, with follow-up ranging from 106 to 190 days. The study designs and patient populations were recently described.[14]

Study 2

The second study was performed at the Northwest Lipid Metabolism and Diabetes Research Laboratories (NLMDRL) of the University of Washington and included 80 patients with measurement of Lp(a) by the NLMDRL method, as well as 2 commercially available assays using Denka reagents (termed Denka1 and Denka2), which utilized different calibrators. The Denka antibody is a polyclonal antibody and technically the assay is apo(a) isoform dependent. However, the values to the 5 independent assay calibrators are individually reassigned after comparison with the NLMDRL isoform independent assay to minimize the impact of apo(a) isoforms.[14]