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High-density lipoprotein subclass measurements improve mortality risk prediction, discrimination and reclassification in a cardiac catheterization cohort

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ABSTRACT

Background and Aims: Recent failures of HDL cholesterol (HDL-C)-raising therapies to prevent cardiovascular disease (CVD) events have tempered the interest in the role of HDL-C in clinical risk assessment. Emerging data suggest that the atheroprotective properties of HDL depend on specific HDL particle characteristics not reflected by HDL-C. The purpose of this study was to determine the association of HDL particle concentration (HDL-P) and HDL subclasses with mortality in a high-risk cardiovascular population and to examine the clinical utility of these parameters in mortality risk discrimination and reclassification models.

Methods: Using nuclear magnetic resonance spectroscopy, we measured HDL-P and HDL subclasses in 3972 individuals enrolled in the CATHGEN coronary catheterization biorepository; tested for association with all-cause mortality in robust clinical models; and examined the utility of HDL subclasses in incremental mortality risk discrimination and reclassification.

Results: Over an average follow-up of eight years, 29.6% of the individuals died. In a multivariable model adjusted for ten CVD risk factors, HDL-P [HR, 0.71 (0.67–0.76), p = 1.3e-24] had a stronger inverse association with mortality than did HDL-C [HR 0.93 (0.87–0.99), p = 0.02]. Larger HDL size conferred greater risk and the sum of medium- and small-size HDL particles (MS-HDL-P) conferred less risk. Furthermore, the strong inverse relation of HDL-P levels with mortality was accounted for entirely by MS-HDL-P; HDL-C was not associated with mortality after adjustment for MS-HDL-P. Addition of MS-HDL-P to the GRACE Risk Score significantly improved risk discrimination and risk reclassification.

Conclusion: HDL-P and smaller HDL subclasses were independent markers of residual mortality risk and incremental to HDL-C in a high-risk CVD population. These measures should be considered in risk stratification and future development of HDL-targeted therapies in high-risk populations.

1. Introduction

The biological role of HDL in cardiovascular disease (CVD) remains unclear. Whereas epidemiological studies consistently demonstrate an inverse relation of HDL cholesterol (HDL-C) with CVD, pharmacological interventions that raise HDL-C fail to result in improved cardiovascular outcomes [1–4]. Moreover, a recent large Mendelian randomization study failed to identify any relation between genetic variants of high HDL-C and improved CVD risk [5]. These findings have raised serious doubts about the biological relation between HDL-C and CVD; they clearly demonstrate that the health benefits of HDL metabolism extend beyond HDL-C alone.

New data suggest that the atheroprotective properties of HDL – such as its antioxidant effects, removal of cellular cholesterol and production of nitric oxide – depend on specific HDL particle characteristics that are not well represented by HDL-C (a measure of HDL dominated by the contribution of larger, more cholesterol-rich HDL subclasses) [6]. HDL particle concentration (HDL-P), an

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alternate measure of HDL that attributes equal weight to all HDL subclasses, may better represent the biological relation between HDL and clinical risk. For instance, in individuals not on lipid-lowering medications in the Multi-Ethnic Study of Atherosclerosis (MESA), even after adjustment for HDL-C and LDL particles (LDL-P), HDL-P is inversely associated with carotid intimal thickness and incident CVD [7]. Similarly, in the Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) study, HDL-P is inversely associated with incident CVD in both placebo- and rosuvastatin-treated individuals. This association persists after adjustment for HDL-C, suggesting that HDL-P may be a marker of residual CVD risk in individuals on statin therapy [8]. Notably, in both of these studies, HDL-C is no longer associated with CVD after adjusting for HDL-P. There are similar findings in the Heart Protection Study (HPS) and VA-HIT study in patients with established coronary disease [9,10]. This apparently discordant relation between HDL-C and HDL-P is further illustrated in MESA and in a recent Chinese study that demonstrate a positive association between the HDL-C/HDL-P ratio and risk of progression of carotid atherosclerosis. This suggests that cholesterol-overloaded HDL particles may have impaired atheroprotective properties and therefore that HDL subclasses differing in size or density may have differential associations with clinical outcomes [7,11].

The data are conflicting regarding which HDL subclasses are associated with decreased risk of clinical outcomes. Earlier studies indicate that larger HDL subclasses confer more cardioprotection; however, more recent studies suggest that smaller HDL subclasses are associated with improved cardiovascular risk [9,12–15]. While HDL-P and HDL subclasses have been considered in relation to intermediate CVD phenotypes and CVD outcomes, the few studies that have examined all-cause mortality as an endpoint suggest that HDL subclasses have different relations with CVD than with mortality [16].

The CATHGEN biorepository at Duke University collected blood samples from 9344 individuals presenting to the catheterization laboratory from 2001 to 2010 for concern of ischemic heart disease. These individuals were followed after enrollment for clinical events. The overall 5-year mortality rate was 21% [17]. As such, this high-risk population was ideal for further investigating the relation of HDL-P and HDL subclasses with all-cause mortality.

We tested the hypothesis that HDL-P and HDL subclasses would be associated with all-cause mortality, independent of HDL-C levels; we also hypothesized that in clinical risk prediction models HDL-P and HDL subclasses would improve mortality risk discrimination and reclassification.

2. Methods

2.1. Study population

The CATHeterization GENetics (CATHGEN) biorepository at Duke University has collected blood samples from sequential consenting individuals undergoing coronary catheterization for suspicion of ischemic heart disease from 2001 to 2010. Details of the biorepository have been previously described [17,18]. Clinical information was obtained from the Duke Databank for Cardiovascular Disease. Available data include symptom histories; clinical characteristics and medical history; angiographic data; and in most subjects, fasting chemistry data within 1 year preceding cardiac catheterization. Individuals enrolled in the biorepository had routine yearly follow-up after enrollment catheterization. Follow-up included mortality (verified via National Death Index search and supplemented by Social Security Death Index search), myocardial infarction (MI), stroke, rehospitalization, coronary revascularization procedures, smoking, and medication use. Coronary artery disease (CAD) was defined as $\geq$1 epicardial vessel with $\geq$75% stenosis on enrollment catheterization in individuals with no history of CAD or coronary artery bypass grafting (CABG). Incident events were defined as all-cause death at any time during the follow-up period.

2.2. Laboratory methods

Lipoprotein particle concentrations and sizes were measured in 3972 CATHGEN individuals by NMR spectroscopy at LipoScience, Inc (Raleigh, NC) using the LipoProfile-3 algorithm [19,20]. The 3 measured HDL subclasses had the following estimated particle diameter ranges: large HDL-P, 9.4–14 nm; medium HDL-P, 8.2–9.4 nm; small HDL-P, 7.3–8.2 nm. In some analyses, the medium and small HDL subclasses (HDL particles with diameters <9.4 nm) were combined and named MS-HDL-P. Mean HDL sizes are mass-weighted averages [14]. Standard lipids including triglycerides were measured with an Olympus AU680 chemistry analyzer using Beckman Coulter reagents. LDL-C was measured using a direct homogeneous assay.

2.3. Statistics

Continuous variables are presented as mean ± SD and dichotomous variables as percentages. Follow-up time is presented as median time with interquartile range. Lipoprotein particle levels were Z-transformed to obtain hazard ratios in terms of each population standard deviation change in particle value.

Differences in baseline characteristics between those who did and did not experience all-cause death were determined using Student's t-test. The associations of baseline HDL parameters with time to all-cause death were quantified using Cox proportional hazard models, adjusted for age, race, sex, diabetes, hypertension, LDL-C, smoking status, BMI, CAD and EF. Proportional hazard assumptions were tested by introducing time-dependent covariates into the model and testing for the interaction of time and particle measures. Pearson correlation coefficients were used to determine the correlations between HDL parameters.

Likelihood ratio tests, yielding χ^2 statistics, were used to assess the improvement in risk discrimination resulting from the addition of HDL parameters to multivariable clinical models and from the addition of MS-HDL-P to the GRACE Risk Score in individuals with complete data available on all of the variables used in the GRACE Risk Score (N = 3209). The GRACE Risk Score is a registry-based clinical risk prediction tool originally developed to estimate the cumulative six-month risk of death or MI in individuals presenting with acute coronary syndrome [21]. An updated GRACE Risk Score was developed to estimate all-cause mortality or the combined outcome of all-cause mortality or MI at 1 and 3 years [22]. This revised score improves risk discrimination to a greater degree for all-cause mortality than it does for the combined outcome: it was thus suitable for our current study. Variables include: age, heart rate, systolic blood pressure, creatinine, cardiac arrest at admission, ST segment deviation on electrocardiogram, abnormal cardiac enzymes and signs/symptoms of CHF. To assess the usefulness of MS-HDL-P in risk discrimination, we determined the χ^2 statistic from the likelihood ratio test of models containing the GRACE Risk Score variables with and without the addition of MS-HDL-P. Using the risk categories of <5%, 5% to <10%, 10% to <20% and $\geq$20%, the net reclassification index (NRI) and integrated discrimination improvement (IDI) were calculated for individuals who experienced all-cause death and for those who did not during the follow-up period.

Statistical analyses were performed using SAS Version 9.4 (Cary,