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Comparing Measures of HDL: On the Right Path with the Wrong Map

Jeffrey W. Meeusen*

HDL cholesterol is often referred to as the "good" cholesterol. This is due to HDL involvement in transport of cholesterol from vascular plaques and peripheral tissues back to the liver during reverse cholesterol transport. Additional protective aspects attributed to HDL include antioxidant, antiinflammatory, vasodilatory, and antithrombotic activities.

This variety of HDL physiological functions is made possible by structural and compositional heterogeneity of circulating HDL particles. Direct measures of HDL activity are available but remain limited to research applications at present. Reverse cholesterol transport studies have demonstrated that the size and composition of HDL particles can influence the mechanism and capacity for cholesterol efflux and other potential protective activities *(1)*. Thus, measurement of HDL size and composition may provide a surrogate for protective HDL activity.

In this issue of *Clinical Chemistry*, investigators from the Boston Heart Diagnostics group report a comparison of their HDL mapping method with 4 different clinical methods for HDL subfractionation *(2)*. Comparing these methods is not a trivial task. The authors used a clever approach by normalizing the relative amount of large, medium, and small HDL to the total HDL reported by each method. Passing–Bablok and Bland–Altman plots for each pairing of methods were then used to compare normalized large, medium, or small HDL among 98 subjects. In this way, correlation and bias between the various methods were quantifiable.

Unfortunately, the approach of normalization cannot adequately address some confounders. First, the methods of separation are not the same. Some methods separate subfractions according to HDL size while others separate them according to HDL density. Second, and perhaps more critical, the definitions of large, medium, and small HDL are not consistent, even among methods that separate according to the same parameter (Fig. 1) *(3)*.

Finally, each method measures a different component of HDL [i.e., apolipoprotein A1 (apo A1), cholesterol content, or particle number]. The authors assume that changing the relative amount of one HDL component should require an equal change in another component. There is no evidence to support this assumption and some evidence actually contradicts this view *(4)*.

For example, consider the relationship between apo A1 and HDL particle concentration. Apo A1 is the primary apolipoprotein of HDL. Unlike apo B, which always has 1 copy per LDL particle, the stoichiometry for apo A1 is variable. Studies have shown that each HDL contains between 1 and 5 copies of apo A1. There are some steric constraints that dictate that in order for the number of apo A1 copies per each HDL particle to increase, HDL size must also increase. However, the obverse is not necessarily true. An increase in HDL size does not require an increase in the number of apo A1 molecules per HDL *(5)*.

Cholesterol is another example of differing quantitative behavior for different measures. Hydrophobic cholesteryl esters are carried in the lipid core of HDL. Free cholesterol is dispersed along the lipoprotein surface (Fig. 1). The current understanding of reverse cholesterol transport is that cholesterol uptake drives the growth of small lipid-poor preß-1 HDL to nascent α-HDL and then to large lipid-rich HDL *(4)*. The simple math of surface area and volume suggests that minimal increases in concentration of larger HDL particles should allow for exponential increases in HDL cholesterol.

According to the reported 2D electrophoresis data, particle size increases 44% between small HDL (modal diameter 7.62 nm) and large HDL (modal diameter 10.97 nm). The relative increase in HDL surface area and volume are 108% (181 nm^2 to 378 nm^2) and 201% (230 nm^3 to 691 nm^3), respectively. Thus, a substantial increase in cholesterol capacity can be accommodated by a relatively small increase in concentration of large vs small particles. Unfortunately, the ultracentrifuge method does not distinguish medium from small HDL so the comparison data are not available.

Considering these limitations, it is unsurprising that none of the Passing–Bablok comparisons reported by Asztalos et al. comes close to the "optimal regression line." The authors single out NMR and a pre-ß1-HDL method from Sekisui Diagnostics as particularly incongruent. In both cases, these conclusions were based on a

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.
* Address correspondence to the author at: 200 First St. SW, Rochester, MN 55905. Email meeusen.jeffrey@mayo.edu.
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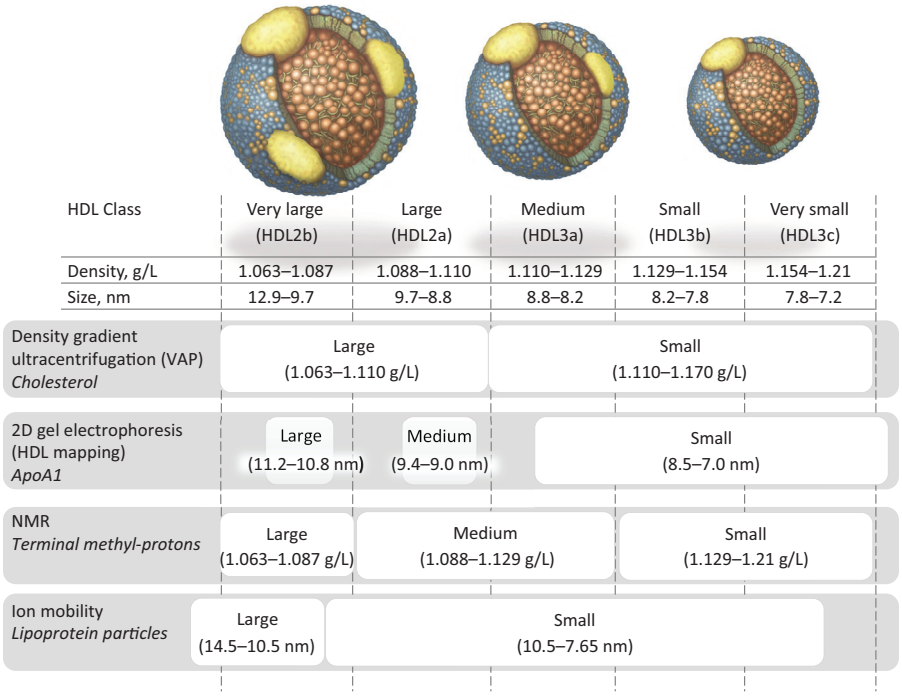

Fig. 1. HDL methods separate subfractions by different means, classify sizes with different limits, and measure different components of HDL.

Each HDL particle can have between 1 and 5 copies of apolipoprotein A1. Cholesterol esters are stored in the core while free cholesterol is dispersed on the HDL surface.

slightly weaker correlation with their own 2D electrophoresis method.

Comparison of HDL subfraction methods is certainly of interest. However, by normalizing the data and focusing on reported distributions of large, medium, and small HDL, the more informative data are lost. Thus, the primary conclusion of the study is that if you measure different parameters by different methods in subfractions created by different means and defined by different limits, you get different answers.

Recent reports suggest that the functional activity of HDL stems from the protein and lipid composition of HDL *(4)*, and that the functionality of a given subfraction can be altered without a change in size *(6)*. Comparison of apo A1, cholesterol, and particle number within a given HDL subfraction could potentially provide insight on prognostic phenotypes. To this end, there are pearls to be gleaned from the original (nonnormalized) data reported.

Both ion mobility (6.8 μmol/L) and NMR (6.2 μmol/L) measured a similar concentration of large HDL particles. Converting the concentration of large HDL apo A1 (2D electrophoresis) from milligram per deciliter to micromole per liter (assuming 28 kDa/apo A1) would suggest that there are between 1.7 and 1.8 copies of apo A1 per large HDL. The average concentration of small HDL particles was between 18.6 μmol/L (ion mobility) and 19.7 μmol/L (NMR). Combining these data with 2D electrophoresis suggests approximately 0.72 copies of apo A1 per small HDL.

Considering the 3% to 9% imprecision for particle number, and the 14% to 24% imprecision for small HDL by the 2D electrophoresis method, these ratios corroborate the expected stoichiometry for large and small HDL. While the normalization of data prevents a more informed analysis, there is a reported distribution range of 5%–50% large HDL by apo A1 across all patients. In those same patients, there is a distribution of large HDL particles of 1%–55%. Thus, it would seem that there is an interindividual variability in the number of apo A1s per large HDL particle. Additional granularity of data in a cohort with well-characterized outcomes could provide clinically meaningful phenotypes.

The goal of HDL subfraction testing is to better identify patients with dysfunctional HDL and therefore increased risk of coronary artery disease. Current data regarding protective effects of specific HDL subfractions are conflicting. Increases in small HDL (α3 and pre-ß1

HDL) measured by 2D electrophoresis were positively associated with coronary heart disease *(7)*. However, a vertical auto profile (VAP) study found that small HDL cholesterol was inversely associated with increased incidence of myocardial infarction and mortality *(8)*. Furthering the confusion are data that suggest that medium and large but not small HDL particle concentration correlates with cholesterol efflux capacity *(9)*.

In the absence of a gold standard method or even comparable methods, future comparisons of HDL subfractions focused on associations with HDL function and patient outcomes would be more beneficial. Attempts to identify HDL function by measuring subfractional distribution of isolated components are akin to relying strictly on anatomy to define the significance of coronary disease. In extreme cases this works, but when there is ambiguity, physiology is essential.

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