LIPID CASE 272 New HDL nomenclature

Some exciting stuff on High Density Lipoproteins has happened: advanced students and those trying to really develop an understanding of these enigmatic lipid transportation vehicles have always been challenged by the fact that there are numerous laboratory technologies on assaying HDL. Even worse, each of these technologies uses different terminologies which can obviously impede understanding and coherent discussion of HDL. So Robert Rosenson got together a world class panel of HDL experts and they sat down and wrote a terrific paper on HDL nuances and proposed a new nomenclature. This is must reading for all Lipidologists. I applaud Bob Rosenson and his team for tackling this issue. Please get a hold of the manuscript entitled HDL Measures, Particle Heterogeneity, Proposed Nomenclature, and Relation to Atherosclerotic Cardiovascular Events. Robert S. Rosenson, H. Bryan Brewer, Jr., M. John Chapman, Sergio Fazio, M. Mahmood Hussain, Anatol Kontush, Ronald M. Krauss, James D. Otvos, Alan T. Remaley, and Ernst J. Schaefer. Clinical Chemistry 57:3 392–410 (2011).

Right now those interested in evaluating HDLs using available testing one can order a lipid profile, or apolipoprotein A-I, or HDL subparticle cholesterol testing (VAP - density gradient ultracentrifugation), or HDL electrophoretic separation by size (Berkeley: separation by HDL size via segmented gradient gel electrophoresis) or 2 dimensional gradient gel electrophoresis with apoA-I immunoblotting (Boston Heart Lab), or ion mobility transfer (Quest) or finally nuclear magnetic resonance (NMR) separation by particle size and number (LipoScience). Here is the existing nomenclature (the paper reviews this in detail)

1) Lipid profile HDL-cholesterol (how much cholesterol is within all of the HDLs in a dL of plasma). The paper will tell you how inaccurate this technology is.
2) ApoA-I (any major lab) Each HDL has 1-4 molecules of apoA-I so this is a less than perfect measure of HDL particle counts
3) VAP: Total HDL-C, HDL2 and HDL3. In reality what you get are not HDL2-P and HDL3-P but HDL2-C and HDL3-C (how much cholesterol is trafficked within HDL2 and HDL3 subparticles. VAP provides no particle quantification measure. For specific detailed comment on this technology with respect to HDLs please download the data supplement word file in addition to the article pdf at Clinical Chemistry web site
4) Berkeley: measures HDL size as a distribution of five subclasses (HDL 2b, 2a, 3a, 3b, and 3c). Provides no particle quantification.
5) Boston Heart Lab: apoA-I, prebeta HDL, and all four alpha HDL species (alpha 1-4)
6) LipoScience: Total HDL-P, Large HDL-P, HDL size. They no longer report medium HDL-P and small HDL-P and no longer use H1 through H5 on the report form where 1 was the smallest and 5 the largest HDL particle.
7) Quest Ion Mobility Transfer: HDL2b

Have any of you wondered why past nomenclature used HDL2 (larger) and 3 (smaller). Has anyone ever heard about HDL1? Go read the full article. Live and learn.

What you need to know is that high density lipoproteins are not only the smallest of all lipoproteins but by far the most numerous. ApoB particle number or concentrations are measured in nanomoles and HDL species in micromoles. That is a big difference. HDLs are like all lipoproteins, lipid-filled particles with a core of mostly cholesteryl ester (CE) with a tiny amount of triglycerides (TG) with a one molecule thick surface of phospholipids (PL) and free cholesterol (more appropriately called unesterified cholesterol, abbreviated as FC). The lipids are enwrapped with numerous apolipoproteins of which the most important are apolipoprotein A-I and A-II. However there can be up to 40-50 other surface proteins on various HDL particles. HDL-C is simply the amount of cholesterol trafficked within all of the HDLs that exist in a dL of plasma. Despite the fact that HDL-C correlates with CHD risk, it is not a specific goal of therapy in NCEP. The new HDL paper states the following which should shock most of you who rely on HDL-C values:

*"In a recent study of 175 individuals with a wide variety of lipid disorders, **NONE** of the 7 current direct assays met the minimum total error goal of less than 12% established by the National Cholesterol Education Program. Furthermore, inaccurate HDL cholesterol results from direct assays were found to significantly compromise the accurate classification of CVD risk based on estimated LDL cholesterol."*

HDLs start life as free apolipoprotein A-I that is secreted into plasma by hepatocytes or jejunal enterocytes or trafficked into plasma as a surface component of chylomicrons. ApoA-I by attaching to membrane cholesterol efflux transporters (ATP binding cassette transport AI or ABCA1) rapidly acquire phospholipids (phospholipidation) and a few molecules of free (unesterified cholesterol) and become pre-beta HDL species (formerly called nascent or discoidal). By the way the tissues that express the most ABCA1 are the liver and jejunum which explains why the vast majority of HDL-cholesterol is of hepatic and jejunal origin, not peripheral cells and certainly not the arterial wall. Any cholesterol acquired from arterial plaque cellular transporters (ABCA1, ABCG1, SR-B1) is trivial and does not affect serum HDL-C (even though that is a cardioprotective function of HDL species). The process where HDLs delipidate foam cells is called macrophage reverse cholesterol transport.

The cholesterol now in the prebeta HDL is then esterified using the enzyme lecithin cholesterol acyl transferase or LCAT which simply transfers a fatty acid from the sn2 position (sn stands for stereospecific number) of a surface phospholipid molecule to the #3 position (the -OH) group on the cholesterol molecule. The now very hydrophobic CE molecules tries to get away from aqueous plasma and moves deep into the tiny HDL core which initiates the process of changing the particle shape from discoidal to spherical. Of course changing a flat surface into a sphere also allows many more lipid molecules to enter, as a spherical particle which has a volume of 4/3pi radius cubed can traffic many more molecules (CE and TG) than a disk. Once this transformation occurs and more lipidation with FC and CE further fills the prebeta HDL it enlarges and is then called an alpha 4 HDL. This is a larger discoidal HDL but as it fills up it can attach to a different cellular membrane cholesterol efflux transporter called ABCG1 and accept more FC, most of which will rapidly get esterified. The alpha 4 HDL continues to evolve or

remodel into a fully spherical, larger (mature) alpha 3 HDL. This lipidation process continues (note: the HDL can also acquires additional cholesterol from free diffusion from cells). HDLs can also give up CE in exchange for TG from other HDLs or apoB lipoproteins via the cholesterol ester transfer protein (CETP) route. Replacing CE with TG changes the core of the HDL and causes further enlargement as a TG molecule is much larger (takes up more room) than CE. Ultimately alpha HDL 2 and alpha HDL 1 (the largest of the HDL species) are created. Large HDLs are susceptible to delipidation at cells that express scavenger receptors B1 or SR-B1 (hepatocytes, enterocytes, steroidogenic tissue, adipocytes, placenta) or subject to hepatic endocytosis via the holoparticle receptor (beta chain apoA-I synthase) or LDL receptors (which use the apoE on HDL as a ligand). If you are wondering where does the HDL acquire all of the phospholipids needed for its surface as it expands: they arrive on phospholipid transfer protein (PLTP) which acquires the PL from the large TG-rich apoB particles (chylomicrons and VLDLs) as they undergo lipolysis (hydrolysis of their core TG). As those particles lose their TG they collapse freeing up their vast surface collection of PL (which attach to PLTP and in effect become building blocks for small HDL species. Tiny HDLs (prebeta or alpha 4 HDL) can also attach to each other and fuse creating larger HDL species. PHEW Got all that?

In essence HDL particles are in a constant state of dynamic remodeling, lipidating and delipidating meaning acquiring and giving up their core lipids (God only knows how many times over their 6 day half life they fill up and empty). The amount of core CE and surface FC in or on HDLs rapidly changes. You now should understand why a serum HDL-C tells you absolutely nothing about this complex remodeling process nor does it have any correlation with the outdated-reverse cholesterol transport concept, i.e. that HDLs simply traffic peripherally acquired cholesterol back to the liver:

HDLs lipidate at primarily at the liver and intestine but also can lipidate at peripheral cells and arterial plaque macrophages (if they exist). The HDLs traffic their cholesterol to steroidogenic tissues (adrenal cortex and gonads) and adipocytes, hang around for several days (as a circulating source of cholesterol for the adrenal cortex) and/or they deliver cholesterol to the liver or intestine where delipidation or endocytosis can occur (direct RCT). The HDLs can also, using CETP, send CE to apoB particles in exchange for TG. The apoB particles (the vast majority of which are LDLs) carry it back to the liver (in a process called indirect RCT) or tragically into the arteries if there are too many apoB particles.

In the new consensus paper, the authors "propose a new HDL nomenclature based on density and size of the particles (see Table 2 in the manuscript). In addition, they compare these terms with other designations
available in the literature. In this nomenclature the HDL particles are termed:

VERY LARGE, LARGE, MEDIUM, SMALL and VERY SMALL

Wow - not terribly scientific sounding but it sure cannot get simpler than that

Using this new paper I will discuss the following case. A clinician contacted me and stated: "I have a patient who is really puzzling me. She's now 59 years old, and three years ago had a big anterior wall MI and had PTCA of her left anterior descending (LAD) artery. She also had fairly significant calcific, nonobstructive plaque in her other vessels. She has a very positive family history of CAD in multiple siblings and both her parents. She is an avid exerciser, very thin, and nonsmoker (and those things were all in place for years before her MI). Her lipids before her MI showed TC anywhere from 210 to 262, but she had HDL-C values ranging from 74 to 126 mg/dL. Her TG have always been under 100 mg/dL. About a year before her MI, her TSH was less than .03, and free T4 elevated at 3.25. An endocrinologist felt she had Grave's, but she never followed up on treatment. Her TSH and free T4 were similar at the time of her MI, and then were not checked again until recently. Initially she was treated with Vytorin"

"She's been off Vytorin since June of 2009 because of myalgia and was not restarted on another statin. Her TC went 215 to 251 when she went off the Vytorin. Over the past 6 months the TC jumped almost another 100 points. She was just sent to me because her TC went from 251 to 341 in six months with no change in diet or meds. When I saw her, I got an NMR and a TSH. TSH is normal, but after talking with our endocrinologist, I'm going to check a free T4 and a thyroid hormone stimulating immunoglobulin. Her NMR-LipoProfile (still off a statin) was as follows:"

TC 374 TG 136 HDL-C 104 LDL-C 243.
Total LDL-p = 1553. LDL size = 22.1 (very large).
Total HDL-P was 44.7 (very high) Large HDL-P = 20.5 (very high)
All the lipoprotein Markers associated with Insulin Resistance were good

DAYSPRING DISCUSSION: Before we chat about her HDL parameters lets approach her as we should all persons. What is her risk? Stupid question. Her MI makes her either high or very high risk. Are there additional risk factors on top of the MI that would push her into the very high risk range? They would be poorly controlled HTN, smoking, extreme elevation of a lipid value, metabolic syndrome, or T2DM. well I guess the LDL-C of 243 would count as an extreme lipid value (it is the 99th percentile population cut point for LDL-C). So let's call her very high risk. Why the drastically and steadily rising cholesterol? The lady has FH and the cholesterol values tend to worsen significantly as time goes on. Is she an over absorber or over synthesizer of cholesterol? both? Neither, meaning she cannot clear her LDL particles (defective LDL receptors, defective apoB or PCSK9 gain of function mutation)? One would have to order markers of synthesis (desmosterol and lathosterol) or absorption (sitosterol or campesterol) to be sure about synthesis/absorption.

Let's not forget LDL-C is actually IDL-C plus LDL-C plus Lp(a)-C. So I'd measure Lp(a) mass and Lp(a)-C in anyone with such a horrific family history.

In case you are surprised the high HDL-C did not protect here do not be. The very high HDL-C is obviously not a negative risk factor in this case. Using the new HDL nomenclature this woman has a high total HDL-P due to increased concentration of both small and very large HDL particles. Bill Cromwell shoed years ago that HDL-C values > 45 mg/dL are due to high HDL-P, but levels > 45 are mostly to the increasing size of the HDL. Could her very large HDLs be dysfunctional or proatherogenic? Maybe, maybe not. Using data from EPIC-Norfolk and IDEAL Trial we now have a way of understanding how some folks with HDL-C > 90 suffer from CV events. In those studies the hyperalphalipoproteinemia patients with events had low apoA-I (HDL-P): the very high HDL-C was explained by the very large HDL size. Those without events had normal sized HDL particles and thus had very high apoA-I (HDL-P) see J Am Coll Cardiol 2008;51:634–42. In the case at hand the total HDL-P is very high, thus it is not likely that dysfunctional HDLs are at play.

Clearly she qualifies for aggressive lipid-modulating drug therapy, but she could not tolerate the Vytorin. Would not you think with such a horrible LDL-C that her LDL-P should be a lot higher than it is. Yes if she had normal sized and composed LDLs. But her LDLs are gigantic at 22.1 nm. It always takes less large particles rather than small of any lipoprotein to traffic a given lipid concentration. If this person had smaller LDLs her LDL-P would be dramatically higher as it takes many more small compared to large LDLs to carry a given LDL-C. Her cells are full of cholesterol and thus it is not a surprise her HDLs are aggressively lipidating (accepting cholesterol from those cells) causing her HDLs to be large and causing total HDL-P (and almost certainly her apoA-I) as well as her large HDL-P to be very high. Cells full of cholesterol upregulate a lot of ABCA1 which transfer the intracellular cholesterol into prebeta and alpha 4 HDLs and also upregulate ABCG1 which effluxes cholesterol into alpha 3 and 2 HDLs. Always keep in mind that over absorption of cholesterol by the jejunum (due to over expression of the Niemann Pick C1 Like 1 protein or NPC1L1) will also cause upregulation of jejunal ABCA1 which facilitates enterocyte lipidation of HDLs. Thus high LDL-C and HDL-C often means there is over absorption of cholesterol (thus increasing the likelihood that ezetimibe or Zetia would be very efficacious when added to a statin).

So how to treat: First lets rule out hypovitaminosis D - no one yet knows if this is a predictor or cause of statin-myopathy, but it is unhealthy and if low I'd normalize it. She appears euthyroid to me. Obviously she needs a lot of apoB (LDL-P) lowering. Try a different statin monotherapy first (remember ezetimibe as well as statin is associated with myopathy). I'd try the hydrophilic rosuvastatin (Crestor) starting at low dose and trying to titrate upwards over time because of its hydrophilicity but pitavastatin (Livalo) is becoming the go to statin for many lipidologists when statin myopathy is present. Certainly do not give up on statins until you try Livalo. Statins can also be used intermittently (off label) to achieve better tolerance. Many still try ubiquinone (Co Q10) but it is expensive has no proven efficacy and I have stopped trying it in my patients. If she could tolerate a different statin, ultimately Zetia would have to be re-added it is unlikely LDL-P goal can be achieved with statin monotherapy. Ultimately she is going to need some combo of statin (even very intermittently), ezetimibe and the bile acid

sequestrant colesevelam (Welchol). I'd throw in a plant stanol (remember never a sterol), namely Benecol.

The clinician in this case stated: "I'm starting a statin, even though she is hesitant because of the severe myalgia she had with of Vytorin. I'm explained to her that some of that muscle aching might have been related to the thyroid abnormality. " I'll buy that. If she was previously hypothyroid, she would have been very prone to myalgia. Thus rechallenging with a statin makes sense now that she is euthyroid.