LIPID CASE 273 What else is new? LDL-P LDL-C Discordance

This case will get into the issue of how two lab parameters can have high correlation (r value) but be grossly discordant in individual patients. Correlation without concordance can lead a clinician astray. I received an e-mail from a person (~ 30 year old Caucasian male) who presumed he was very healthy until he had advanced cardiovascular testing done at Health Diagnostic Labs in Richmond VA (for whom I am an advisor). This normotensive man noted he uses Nexium 40mg as needed, Krill oil 1000 mg daily and Centrum Cardio daily (multivitamin with added phytosterols), Pycnogenol 50mg daily (a pine tree bark extract that supposedly acts as an antioxidant, a natural anti-inflammatory, selectively binds to collagen and elastin, and finally, it aids in the production of endothelial nitric oxide. He notes that he goes to the gym regularly but do mostly weight training with VERY little cardio. He has no serious family history but my mother is hypoglycemic. He was very happy when he saw the following lipid profile (all in mg/dL):

TC = 175 LDL-C = 94 HDL-C = 43 Trig = 112 VLDL-C = 22.4 Non-HDL-C 132
TC/HDL-C = 4.0

Then of course some things of concern started to appear:

Apolipoprotein B = 87 mg/dL [the 32nd percentile Framingham Offspring (FOS) Cut point]
Apolipoprotein A-1 138 mg/dL (optimal > 132 High risk < 114)
Apo B:Apo A-1 Ratio 0.63 (desirable < 0.60 High risk > 0.80)

NMR LipoProfile
LDL-P = 1420 nmol/L (50th percentile FOS cut point; the 65th percentile MESA cut point)
Small LDL-P = 656 nmol/L
Total HDL-P = 33.5 umol/L
LDL-P/HDL-P = 42
LDL size 20.8 nm Pattern A is 20.6 or higher
No large VLDLs
Large HDL-P < 0.7 (very, very low)
HDL size very small
LP-IR score elevated > 50

Additional Lipid markers:

sdLDL = 22 mg/dL (more accurately called sdLDL-C)
% sdLDL 24 (the % of total cholesterol in small LDL particles per dL)
HDL2 = 11 mg/dL (more accurately correctly called HDL2-C)
Lp(a) Mass 15 mg/dL
Lp(a)-C not performed when Lp(a) mass is normal

Inflammation & Myocardial markers:

- Myeloperoxidase 280 pmol/L
- Lp-PLA2 172 ng/ml
- hs-CRP 0.39 mg/L
- Fibrinogen 226 mg/dL
- NT-proBNP 29 pg/dL
- High sensitivity Cardiac Troponin-I = 0.5

Genetic studies:

- Apolipoprotein genotype E 3/3
- CYP2C19 *1/*1
- Factor V Leiden Arg/Gln
- Prothrombin Mutation G/G
- MTHFR Genotype C/C

Insulin resistance markers:

- Insulin 3
- FFA 0.37
- Glucose 83
- Adiponectin 1.4 (low)

Other tests:

- Vitamin D 43 ng/ml
- Homocysteine 9 umol/L
- Creatinine 0.9 mg/dL
- Omega-3 Index= 12.1%

The e-mail concluded with the following statement: "My concern is my LDL-P (1420), Apo-B (87), % sdLDL (22), HDL-P (33.5), HDL2 (11) and Factor V (Arg/Gln) and Adiponectin (1.4). I What do I need to do in order to improve my numbers? I certainly do not want to be heading towards a pre-diabetic
stage."

DAYSPRING DISCUSSION:

As always let's look at this man from an NCEP ATP-III point of view. That is easy: he has zero cardiovascular risk factors and a wonderful lipid profile. Therefore, he does not qualify for Framingham risk scoring. So pat him on the back, tell him to keep up the lifestyle and advise him that he is immortal if he wears seat belts. But he had to ruin everything and go and get more advanced testing which suggests he is not exactly the total picture of cardiovascular health suggested by the lipid concentration panel. There is little reason at this time to think that anything ATP IV will recommend (when it is released at an AHA Plenary Session in November) would be of benefit in this case. I am assuming ATP-IV will not be recommending particle measurements in seemingly low

risk folks, but quoting Bill O'Reilly "I could be wrong." (wishful thinking). But now that we have these measurements we surely cannot ignore them. The 2008 ADA/ACCF consensus advises lipoprotein quantification using measured apoB (via protein immunoassay not by calculation) or LDL-P in patients with cardiometabolic risk. However does this man have cardiometabolic risk? The low adiponectin, very low large HDL-P, elevated LP-IR score and low HDL2-C would support it, but the lack of large VLDL-P, borderline small LDL-P and Pattern A LDL phenotype would not. If we used the 2009 AACC (American Association of Clinical Chemistry: Lipoproteins and Vascular Disease Division) guidelines, regardless of insulin resistance presence, they advocate management using apoB or LDL-P. However their low risk patient goal of therapy for apoB is 100 (the 55th percentile FOS cut point) and for LDL-P is < 1400 nmol/L. Using that criteria, this patient is at apoB goal and minimally above LDL-P goal. However if we look at the more contemporary MESA (MultiEthnic Study of Atherosclerosis) cut points and LDL-P of 1400 is the 65th percentile (too darn high).

Let's look a bit more closely at other markers:

Total LDL-P 1420 slight discordance with apoB
Small LDL-P 656 (mild elevation with < 400 considered ideal)
LDL-P/HDL-P ratio = 42

LDL size 20.8 Pattern A is 20.6 or higher
No large VLDLs

When I see discordance between LDL-C and LDL-P I know there are only three explanations:

1) Small LDLs (takes 40-70% more small than large LDLs to carry a given LDL-C)
2) TG-rich, cholesterol-poor LDLs of any size: it takes many more cholesterol-depleted LDLs than cholesterol-rich LDLs to carry a given LDL-C.
3) Statin induced depletion of LDL particles: this is why statins are so much better at lowering LDL-C than LDL-P.

In the case at hand only reason number 2 makes sense. he does not have small LDL and he is not on a statin which excludes reasons 1 and 3. An LDL-TG value would be very revealing. Believe it or not an LDL-TG is a better risk predictor than LDL-C. Circulation. 2004;110:3068-3074

In both the Women's health Study and the VA-HIT trial (Circulation. 2006;113:1556-1563) LDL-P/HDL-P ratio was the best predictor of risk and significantly out predicted the apoB/apoA-I ratio or TC/HDL-C ratio. A level of 42 would equate to those in the second of four quartiles of risk (with quartile 4 being the highest risk). For those of you who would like to incorporate this ratio into your practice here are the values:

Quartile I (lowest risk) 13.9 - < 40.5
Quartile II 40.5 to 51.25

Quartile III 51.3 - 61.2
Quartile IV (highest risk) 61.3 - 127

Total HDL-P 33.5 This places him in the MESA 60th percentile (a tad low)
ApoA-I is at lower limits of normal (138 mg/dL)
Large HDL-P < 0.7 almost (non-existent)
HDL2-C = 11 (bit low)
Very small HDL size

Be careful comparing apoA-I to HDL-P. Although both are used as a measure of HDL particle number or concentration, they do not measure the same thing. Although they correlate well, discordance can be a problem. Keep in mind that HDL particles contain from 1 to 4 molecules of apolipoprotein A-I per HDL particle (unlike apoB where there is one molecule per VLDL, IDL, LDL, chylomicron). So since there is not a fixed apoA-I per HDL particle ratio; thus apoA-I may not be an exact way to count HDL particles. Since the larger HDLs carry more apoA-I particles, people with predominantly large HDLs may have a higher apoA-I than HDL-P. But also know that NMR spectroscopic analysis cannot detect prebeta HDLs (as they have so few lipids and NMR is based on detecting lipid methyl groups). However since prebeta HDLs make up at most 5% of total HDL-P they do not contribute a lot to total HDL-P. In this case there is a minor discordance between the apoA-I of 138 mg/dL and the total HDL-P of 33.5 umol/L.

So why is his direct HDL-C measurement normal at 43 mg/dL but his total HDL-P a bit low. Usually people with low HDL-P findings have low HDL-C. Why is the HDL-C not lower? How much faith should we have in the reported HDL-C? If one just looks at the available US assays please take note what was just published by an expert panel chaired by Robert Rosenson in Clinical Chemistry 57:3;392–410 (2011) where it is stated:

"In the past 10 years, most laboratories have switched to direct (homogenous) assays that do not involve physical separation of HDL from other lipoproteins. There are currently 7 different direct HDL cholesterol assays, which use several different approaches for either shielding or selectively consuming cholesterol on non-HDL lipoprotein fractions (Table 1). Direct HDL cholesterol assays are fully automated and precise and require less labor. Therefore, they have largely replaced older assays. It remains uncertain whether direct HDL cholesterol assays have clinical utility comparable to that of chemical-based precipitation methods (23–25). 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 "

This patient's concentration of large HDL-P is very low, his HDL size is very small but his HDL2 is minimally low at 11. Desirable is 12 or greater. Should not the HDL2 be a lot lower? As you all know HDL2 and HDL3 are classifications from ultracentrifuge or electrophoretic separation of HDL species where 2 are the larger particles: there are two HDL2 subspecies a larger 2a and smaller 2b. I want all to be very clear on what exactly an HDL2 measurement is. Labs that report this parameter are a bit deceiving. They want

clinicians to think it is a quantitative measurement of large HDL-P even though it is not. The NMR separation reports both total and large HDL particle numbers (concentration: i.e. # of particle per liter of plasma). HDL2 is not an HDL2-P test but rather HDL2-C or in other words it is a measurement of the cholesterol trafficked within the large HDL species that exist in a deciliter (dL) of plasma. Like everything else when you compare lipid measurements (how much lipids are in a dL of particles) with lipoprotein measurements (how many lipoproteins are in a liter of plasma) there can be discordance. You might have just a few very large HDLs which would keep HDL2-C high, but in reality have a reduced large HDL-P. This can be due to variant subtleties of hepatic lipase, endothelial lipase or secretory lipase (enzymes that hydrolyze HDL core TG or surface phospholipids). As is virtually always the case, risk follows particle measurements better than lipid measurements. Also recognize that an HDL can be large, not because it is packing lots of cholesterol but it might be packing excess triglycerides. Typically large TG-rich HDLs are subject to rapid lipolysis (hydrolysis of its core TG) by the lipases mentioned above and become small, dense HDLs (HDL3 species). But due to lipase variants (isoforms) that may not be the case in all humans. It would be very interesting to have an HDL-TG level in this patient. Not to be any more confusing but the advanced HDL technique used by Boston Heart Lab calls the smaller HDL3 particles alpha-HDL 4 (the smallest) and alpha HDL 3 and larger more mature HDL2 as alpha-HDL 2 and alpha HDL1 (the largest). The vertical auto profile reports HDL particle cholesterol content, not any HDL-P.

Bottom line: HDL-C or subparticle HDL-C depend on HDL particle number, exact HDL size but also HDL core composition (how much TG and how much cholesterol is present - keeping in mind HDL under normal circumstances should carry very little TG). Much better to bet on apoA-I and HDL-P measurements and avoid HDL-C or HDL subparticle cholesterol levels.

Might as well also talk about why the lack of large HDL-P is such an important independent predictor of risk **IN DRUG NAIVE PATIENTS** (few seem to know that caveat). Of course such independence depends on what you adjust it against. The most common reason at risk patients lack large HDL species is that they are insulin resistant and the HDL becomes TG-enriched (acquiring TG via cholesteryl ester transfer protein or CETP in exchange for core HDL cholesteryl ester or CE). The subsequent TG-rich, CE-poor HDL upon the action of hepatic and endothelial lipase becomes very small and dense, is subject to break up and renal excretion of the released apoA-I. Therefore drug naive patients who lack large HDL species will also usually have a low total HDL-P but virtually always have a high LDL-P or apoB (the source of the acquired TG). Such patients will have elevated LDL-P/HDL-P (apoB/apoA-I) ratios. Most of the risk is really due to the increase in LDL-P. That is why the ADA/ACCF guideline state that the proper first line treatment of IR patients with low HDL-C is a statin (our best LDL-P, apoB lowering medication). Moral of the story: the next time you see a reduced HDL2-C or reduced large HDL-P be sure to look at LDL-P (and /or apoB) and you will clearly understand that the major reason low large HDL parameters are related to risk, is that they exist in a milieu of IR and very high LDL-P (the world's # 1 CV risk factor).

Why do I say that HDL2-C and large HDL-P are only risk factors in drug naive patients. Because different drugs modulate HDL particles in very different ways. Please check out: http://www.lipidcenter.com/pdf/Drugs_and_HDL_Subfractions.pdf Fibrates by upregulating hepatic scavenger receptors which delipidate the CE from large mature HDLs and convert them to smaller cholesterol-depleted HDL species (HDL3). Paradoxically, fibrates reduce HDL size but very significantly increase small and total HDL-P. There is usually some increase in HDL-C but not what you would expect in face of the significant increase in total HDL-P. If you are unaware of that fact, in your fibrate treated patients you might see very low levels of large HDL-P or HDL2-C and think the fibrate was making things worse - whereas if you looked at total HDL-P you would see the fibrates making things better by increasing it. Conversely on a niacin you would see increased levels of large HDL-P, HDL-C, HDL2-C and total HDL-P, but the increase in total HDL-P would be no better and often a tad less than that seen in fibrates. So again, paradoxically niacin is superior to fibrates in increasing large HDL-P, Total HDL-C, HDL2-C but is not superior in increasing total HDL-P. Right now we know there is Level I evidence outcome benefit when fibrates are given to IR patients with TG > 200 and reduced HDL-C (Helsinki, VA HIT). At this point in time we do not know that about niacin and CV outcomes and will not until the AIM-HIGH study is completed (the first Level I trial niacin has ever been in). I think we would all be shocked if statin/niacin did not reduce events in AIM-HIGH (a study of patients with TG-HDL axis disorder patients). I case anyone thinks I have a bias with respect to fibrate or niacin use, please note that every day I take 2000 mg of extended release niacin (Niaspan) as well as 135 mg of fenofibric acid (Trilipix). I have my butt covered! Note: I do not believe there are any efficacy differences between the prodrug fenofibrate or its active form fenofibric acid.

The LP-IR scores towards IR solely because of the lack of large HDL. Other parameters that would worsen the score would be the presence of large VLDL-P and increased small LDL-P and reduced LDL size. Why are they not present in this case? The very low adiponectin would suggest significant insulin resistance. I have a few thoughts. Did everyone notice the incredibly high Omega 3 index of 12%. That is the highest I have ever seen. Eskimos tend to be in the 9% range. Desirable is > 8%. The patient is taking Krill oil. In this formulation omega-3 fatty acids are delivered as components of phospholipids not as omega-3 ethyl esters (as with Lovaza). Either formulation will over time raise the Omega 3 index. Since it is FDA monitored, Lovaza is very pure without any potential toxic additives and there is no FDA guarantee of that with food additive Omega-3s. Please note that use of Lovaza at 1000 mg to raise the omega-3 index would be an off-label use of the product. Is it possible that the patient may not be showing large VLDL-P or making small LDLs because the very high level of Omega-3 (achieved with only 1000 mg per day over time) has drastically lowered the TG and thus increased LDL size and reduced large VLDL-P? That would be pure speculation on my part as typically there is a threshold effect and 4000 mg of Omega -3 are required to see a TG and lipoprotein benefit. But maybe this guy is simply an over absorber of omega-3 and he has very high levels despite the lower dose. Lots of people, take 4000 mg and do not achieve an Omega 3 Index of 12%. I'll bet anyone with an Omega-3 index of 12% will have TG benefits related to the omega-3s. I do a lot of Omega-3 index testing

(www.omegaquant.com) and have never ever seen a level of 12%. Please note I talk about this index a lot (based on the science) and I have no financial association of any type with Omegaquant.

Lastly I do not support the use of meds enriched with phytosterols such as Centrum Cardio unless you know if the patient can or cannot absorb noncholesterol sterols. Anyone taking such a phytosterol enriched product who is an over absorber of sterols will be raising their sitosterol, and campesterol levels and these are major predictors of risk (Nutrition, Metabolism & Cardiovascular Diseases 2006;16, 13e21 and J. Lipid Res. 2009;50: 1927–1935). If you feel you have to use sterols (instead of nonabsorbable stanols to help lower LDL-C and LDL-P you should monitor noncholesterol sterols (Boston Heart Lab www.bostonheartlab - cholesterol balance test).

If I look at all of the other numbers provided by Health Diagnostic Labs, the inflammatory markers are fine. He is a heterozygote for Factor V Leiden. *Wikepedia states it is the most common hereditary (autosomal dominant) hypercoagulability disorder amongst Eurasians. The risk of developing an abnormal blood clot averages about 1 in 1,000 per year in the general population, the presence of one copy of the factor V Leiden mutation increases that risk to 1 in 125 to 1 in 250. Having two copies of the mutation may raise the risk as high as 1 in 12*. He must not smoke and take VTE precautions and advise future physicians and surgeons of this and should alert first degree relatives to be tested. It is not an atherosclerotic risk factor.

So we have to make a clinical decision: Ignore the discordance and continue current lifestyle (? minus the plant sterols), or start medication. I prefer not to guess about the absorption status. I'd measure markers of sterol absorption: if OK he can safely use the sterols. I am not comfortable with the recommendations of AACC calling for an apoB of 100 mg/dL and an LDL-P of 1400 nmol/L. Those recommendations are based on cut point data from Framingham Offspring collected in the late 1980s. I do not think they have much relevance in 2011. I'd prefer the more contemporary MESA cut points. Thus since this patient has an excellent lifestyle, I'd start a statin to further reduce the LDL-P to ~ 1000 nmol/L or less.