

Advanced Lipoprotein Testing

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We are indebted to Dr Thomas Dayspring for his expert help in writing this *Heartbeat*, which will cover the “next level of lipid testing” necessary to assist us in better risk assessment and treatment of cholesterol. First we will cover the pathophysiology of dyslipidemia, which causes atherosclerosis. That will be followed by what routine lipid testing tells us and NCEP recommendations of why and when to treat. Then we’ll cover additional testing that will improve our ability to identify and treat risk, as well as when it should be used.

What are we treating?

Atherosclerotic plaque begins when lipoproteins invade the arterial wall (intima) and “dump” their sterol (both cholesterol and other non-cholesterol sterol) contents. If present in increased concentrations, all lipoproteins less than 70 nm in diameter have the ability to pass through the endothelium and invade the arterial wall. The “beta” lipoproteins (those that migrate with beta proteins in an electrophoretic field), have a single apolipoprotein B or “apoB” on their surface, and are the “atherogenic” lipoproteins, because apoB adheres to intimal proteoglycans. Once trapped, these particles are prone to oxidative and glycation forces—these modified beta-lipoproteins are ingested by macrophages which then become foam cells (the hallmark of atherosclerosis). Because of their small size, alpha-lipoproteins or “apoA1” (HDL), with surface apolipoprotein A1, can enter the intima but do not adhere to the proteoglycans, so their sterol content is “non-atherogenic. ApoB and apoA1 levels are lab measures which quantify the beta and alpha lipoproteins. In the large AFCAPS-TextCAPS trial¹, increased apoB and decreased apoA were the best predictors of baseline and on-treatment risk.

High CHD risk is directly related to too many beta-lipoproteins (ApoB) or too few alpha-lipoproteins (ApoA1).

The beta-lipoproteins consist of chylomicrons, chylomicron remnants, VLDL (very low density



lipoprotein), VLDL remnants, IDL (intermediate density lipoprotein), LDL (low density lipoprotein) and lipoprotein (a). All of the beta-lipoproteins can be atherogenic, but their respective concentrations vary. LDL particles compose 90% of an ApoB measurement. [Most of the above beta lipoproteins have very short half lives (hours), with the exception of the LDL, which lasts for 2 days or more.] *Presently we follow LDL-C levels as one of the surrogate markers for ApoB risk (cost and reliability of measurement considerations) and treat the LDL-C as the **primary goal** according to NCEP ATP III guidelines².*

The cholesterol inside of the beta-lipoproteins is called the non-HDL-C and is determined by subtracting HDL-C from the total cholesterol (TC) level. Thus, non-HDL-C is another estimation of beta-lipoprotein concentration or apoB.

LDL-C and non-HDL-C are NCEP surrogates for atherogenic beta-lipoproteins (ApoB).

If non-HDL-C is elevated, the patient probably has too many beta-lipoproteins. *Treatment of non-HDL-C is the **secondary goal** of the NCEP ATP III guidelines when triglycerides [TG] are > 200mg/dL. Non-HDL-C goal is 30mg/dL higher than the LDL-C goal based on coronary heart disease (CHD) risk assessment (see attached pages after references).*

All of the above lipoproteins come in heterogeneous mixtures of varying sizes (small to big diameters). All people have a predominant size (large or small) of LDL particles. Both LDL particle concentration (LDL-P) and size (often referred to as quality) are factors in determining the particle's atherogenicity. Too many LDL particles are a major CHD risk factor, but small particle excess is associated with a six-fold increase in risk, and large LDL excess with a doubling of risk. With respect to VLDL particles,

increased numbers of large are related to higher risk. Large VLDL, although too large (> 70 nm) to penetrate the endothelium, are associated with small, highly atherogenic LDL, lack of large HDL particles, hypercoagulation, and inflammatory markers. Smaller VLDL (or chylomicron) particles that have acquired cholesterol from HDL [via cholesterol ester transfer protein (CETP) transfer] are called *remnants*. TG-depleted, cholesterol-rich remnant lipoproteins are < 70 nm in diameter and, when present in increased numbers, can enter the arterial wall. NCEP uses increased VLDL-C (calculated as TG/5) as a surrogate of remnant lipoproteins. VLDL-C should be less than 30 mg/dL (TG/5).

Too many Large VLDLs, smaller remnant and LDLs (especially small) increase CHD risk.

As noted in AFCAPS and many other trials, persons who are at high risk for CHD are those that have elevated apoB or decreased apoA1. The goal of therapy in treating lipoprotein disorders is to prevent the beta-lipoproteins from invading the arterial wall. Many attempt to increase the alpha-lipoproteins concentration; however, functionality of HDL particles is also critical and cannot be determined by HDL concentration and size measurements.

So how do you determine if there are too many beta-lipoproteins and too few alpha-lipoproteins other than apoB and apoA1 (which tend not to be reimbursed)? Treatment errors are often made by looking solely at LDL-C, especially in patients with TG/HDL axis disorders—insulin resistance (IR) or metabolic syndrome (MetS) and type 2 diabetes (T2DM). Normal or slightly elevated LDL-C levels (rarely treated) are very frequently associated with elevated LDL particle concentrations (increased risk) if the LDL particles are small. Depending on their size, it takes 40-70% more small LDL particles to carry the same amount of cholesterol (LDL-C) as large particles. In both the Heart Protection Study (HPS)³ and PROVE-IT⁴ trials significant residual risk remained in some patients, with mean LDL-C levels of 70 and 62 respectively. If one has 62 mg of cholesterol in the LDL particles in a deciliter (dL) of plasma, it will take up to 70 % more very small LDL particles. In two patients with an LDL-C of 62mg/dL, the one with large LDL particles will likely have a normal (physiologic) LDL-P, and the one with very small particles will have an LDL-P that is 70% higher

(helping to explain the residual risk).

NCEP wants you to calculate the non-HDL-C (TC minus HDL-C) and use that value as the secondary goal of therapy in patients with elevated triglycerides. Non-HDL-C may be a better surrogate marker than LDL-C in abnormalities of the TG/HDL-C axis [dyslipidemia of IR]. (NCEP's non-HDL-C goal is < 160 mg/dL in primary prevention, < 130mg/dL in secondary prevention and < 100mg/dL in the very high-risk patient—30mg/dL higher than the LDL-C goal). No extra testing is required. Non-HDL-C certainly can help ascertain risk above that predicted by LDL-C. However, like all of these ratios, they pick up the patients in the middle of the proverbial bell shaped curve and miss those on the fringes. NCEP suggests that you calculate non-HDL-C in all patients with TG between 200 and 500 mg/dl because those patients are apt to be the patients with small LDL particles, increased LDL-P and an unremarkable LDL-C. The non-HDL-C values can alert the clinician that a person is at high risk despite the "normal" LDL-C level. A TG of 200 mg/dL or higher is associated with significant quantities of remnant lipoproteins that convey CV risk *substantially* above that predicted by LDL-C. One important caveat: *The non-HDL-C goal of therapy does not apply if the TG is > 500.*

More evaluation is needed in certain situations where TG is increased and LDL-C is near normal (abnormal TG/HDL axis). Elevated non-HDL-C is "the clinician alert" indicator that a person is high risk despite a "normal" LDL-C—it almost always means ↑ LDL-P and ↑ VLDL-P.

For more accurate risk assessments and to make sure our therapies maximally reduce risk, more advanced lipoprotein testing that **quantifies particle concentrations** may be necessary. As clinicians we have to know when cholesterol is going to invade the arterial wall, beginning the process of atherogenesis. Two things determine whether lipoproteins enter into the arterial wall:

- 1) The most important variable is the concentration of the apoB lipoprotein particles. If an apoB particle (VLDL, remnant, IDL or LDL) exists above a physiologic concentration, it will likely enter an artery, driven by the concentration gradient.

2) The size of the particles is also very important. For a lipoprotein to pass through the endothelial barrier, it must have a diameter < 70 nm. Smaller VLDLs, remnants, IDLs and LDLs all qualify. Since LDL particles are by far the most numerous of all the apoB particles (due to their 2 day half life) and also the smallest, they are the lipoproteins most likely to deliver sterols to the artery wall.

Of the 2 variables, only particle concentration has an independent statistically significant relationship to events and event reduction. Both LDL particle concentration (apoB) and size are listed by NCEP ATPIII as emerging risk factors. This is why advanced lipoprotein testing methods that do not provide particle concentration measurements are not much help in ascertaining risk or response to therapy. Knowing the size of the particles isn't enough if we don't know their concentrations.

We need to know both LDL size and LDL concentration (LDL-P) to estimate risk.

Advanced Testing

The only currently available tests that quantify lipoproteins are apolipoprotein measurements (apoB and apoA1) and nuclear magnetic resonance spectroscopy (NMR LipoProfile from LipoScience). This technology directly measures the lipoprotein particles responsible for CHD. It enhances clinical management of CHD risk by identifying patients whose risk is higher or lower than that assessed by our routine LDL-C and non-HDL-C testing. Treatment of at-risk patients can be improved by directing therapy to reduce overall numbers of LDL particles and small LDL particles, the primary causal agents of atherosclerosis.

By subjecting plasma (which needs no alteration by any reagents—no errors to be made) to magnetic waves, all lipoproteins can be sized and enumerated (concentrations can be determined). It is the only advanced lipoprotein test that does both (quantity and quality of all lipoprotein classes), and you get the all important particle concentrations without doing the costly ApoB test. You get both large and small VLDL (remnant) concentrations as well as HDL subparticle concentrations. No other technique offers you that. The NMR imaging-based method (commercially available and CLIA approved) has become the “Gold Standard” for the quantitation of

LDL particle size and LDL particle number (LDL-P) according to Dr Dan Rader in the chapter on lipids in Dr Eric Toprol's *Textbook of Cardiovascular Medicine*. NMR's determination of LDL-P gives a significantly better risk prediction than any other lipid variable that we can measure [not part of Atherotec's VAP (vertical auto-profile) which simply sizes particles].^{5 6 7 8}

LDL particle number (LDL-P) determined by NMR is considered by most experts to be the “Gold Standard” to determine *true* CHD risk.

LDL Particle Number (LDL-P): total number of cholesterol-carrying LDL particles (nmol particles/L). *LDL-P is linked more strongly to CHD than LDL cholesterol (LDL-C) and is an adjunct target of therapy.* For high-risk patients, the dual goal of LDL-P <1000 nmol/L and LDL-C <100 mg/dL is a reasonable alternative to the very-low optional LDL-C goal of <70mg/dL. For moderately high-risk patients, the suggested dual goal is LDL-P <1300 nmol/L and LDL-C <130 mg/dL.

LDL Size: the average diameter (nm) of the patient's LDL particles. Large LDL (Pattern A) is associated with lower CHD risk and small LDL (Pattern B) with higher CHD risk, the MetS, and increased risk of developing T2DM.

Large HDL: the concentration of the largest HDL subclass. Low levels are associated with higher CHD risk, MetS, and increased risk of developing T2DM.

Large VLDL: the concentration of the largest VLDL subclass. High levels of these large, triglyceride-rich particles are associated with higher CHD risk, MetS, and increased risk of developing T2DM.

NMR LipoProfile parameters most useful in assessing the risk of developing CHD and diabetes in patients without existing CHD are:

1. Elevated LDL-P (>50th percentile).
2. MetS, a high-risk condition recognized by a clustering of 2 or more of the following lipoprotein traits.
 - Small LDL
 - Reduced large HDL
 - Elevated large VLDL

LDL-P:

Goal for High-Risk Patient < 1000
Goal for moderately high-risk < 1300
Borderline high: 1300-1599

High risk: 1600-2000
Very high-risk: > 2000
(Suggested desirable goal < 700)

LDL Size:

Pattern A (large LDL)
23.0 – 20.6 → Lower risk
Pattern B (small LDL)
20.5 18.0 → Higher risk

Large HDL:

> 30 → Lower risk
30-11 → Intermediate risk
< 11 → Higher risk

Large VLDL:

< 7 → Lower risk
7-27 → Intermediate risk
> 27 → Higher risk

Recommendations:

Ascertain risk with the lipid profile: TC, TG, HDL-C and LDL-C (paying attention to TG, HDL-C).

- ↓ HDL-C/ ↑ TG & normal LDL-C → disorder of TG/ HDL axis (dyslipidemia of IR/ MetS).
- TC/HDL-C > 4 → higher risk.
- ↑ Non-HDL-C (TC minus HDL-C) almost always means ↑ LDL-P and ↑ VLDL-P.
- TG/HDL-C ratio > 3.8 Women, > 4 men → high chance of ↑ small LDL particles.

Treat to appropriate LDL-C and non-HDL-C goals per NCEP guidelines. If the patient is at moderate or high risk, then consider doing the NMR. Many will still have lipoprotein abnormalities.

Consider doing the NMR LipoProfile for LDL-P initially in:

- Those who have had a CHD event.
- Those calculated to be at high risk but unremarkable lipids or not meeting CHD risk equivalency.
- Those with strong family histories without obvious lipid abnormalities.

Patients expected to benefit from LDL-P testing are:

- Those with pre-existing heart disease with low baseline LDL-C levels.
- Those with T2DM/ MetS/IR or a family history of heart disease. Patients with abnormalities of the TG/HDL-C axis and/or high non-HDL-C (low HDL-C is enough) and other characteristics of the metabolic

syndrome are likely to have higher LDL-P levels than indicated by LDL-C testing. High TG may even be enough (>128mg/dL). In a just published editorial by Michael Criqui⁹: "There is a growing consensus about the importance of triglycerides, particularly in women, and we have shown in a national US sample¹⁰ that triglyceride level was the single most predictive component of MetS-NCEP for CVD in multivariate analysis".

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² Grundy SM et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation* July 13 2004; 110: 227-239.

³ Heart Protection Study (HPS) Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomized placebo-controlled trial. *Lancet*. 2002; 360: 7–22.

⁴ Cannon CP, Braunwald E, et al. Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 Investigators (PROVE-IT). Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med* April 8 2004; 350: 1495–1504.

⁵ Blake et al. Women's Health Study. *Circulation* 2002; 106: 1930-1937.

⁶ Kuller et al. Cardiovascular Health Study (CHS). *ATV B* 2002; 22: 1175-1180.

⁷ Rosenson et al. Pravastatin Limitation of Atherosclerosis in the Coronaries Trial (PLAC-I). *Am J Cardiol* 2002; 90: 89-94.

⁸ Chromwell C et al. Low-density-lipoprotein particle number and risk for cardiovascular disease. *Current Atherosclerosis Reports* 2004, 6:381–387

⁹ Criqui M. Obesity, risk factors and predicting cardiovascular risk. *Circulation* April 19 2005; 111:1869-1870.

¹⁰ Tankó LB et al. Enlarged waist combined with elevated triglycerides is a strong predictor of accelerated atherogenesis and related cardiovascular mortality in postmenopausal women. *Circulation* April 19 2005; 111: 1883–1890