Advanced Lipid Testing: Why, When, and How?

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Duality of Interest

Dr. Brinton has received:

• **Research** funding: Amarin, Aurora Foundation, NIH

• Honoraria as **consultant/advisor**: Alexion, Amarin, Amgen, Araelez, Kowa, Merck, PTS Diagnostics, Regeneron, Sanofi-Aventis

• Honoraria as **speaker**: Alexion, Amarin, Amgen, AstraZeneca, Genzyme, Janssen, Kowa, Merck, Regeneron, Sanofi-Aventis, Takeda
Advanced Lipid Testing: Talk Outline

• History of cholesterol hypothesis and lipid measurements
• Need to go beyond LDL-C (non-HDL-C vs advanced lipid testing)
• Different types of advanced lipid testing
• Referral to a lipidologist (vs become one)?
• Subclinical atherosclerosis imaging (CAC vs other?)
Cholesterol and Lipoprotein Measurement History

- 1908 Ignatowsky: intake of meat & dairy linked to CHD in humans
- 1913 Anitschkow: high-fat diets cause arteriosclerosis in rabbits
- 1952 Abell: simplified measurement of serum TC
- 1966 Gofman: Analytic Ultracentrifugation (ANUC)
- 1972 Friedewald: LDL-C estimation by apoB particle precipitation
- 1972 Lindgren: density gradient ultracentrifugation (DGUC)
- 1973 Mackenzie Preparative isoelectric focusing (IEF)
- 1980 Chung & Segrest: Vertical (UC) Automated Profile (VAP)
- 1981 Blanche & Krauss: Gradient Gel Electrophoresis (GGE)
- 1984 Cheung: Apoprotein immunoaffinity chromatography
- 1992 Bodwell: Reflotron method for HDL-C
- 2000 Otvos: nuclear magnetic resonance spectroscopy (NMR)
- 2008 Caulfield & Krauss: Ion Mobility (IM)
ASCVD Risk Prediction: Patients vs Populations

“A majority of middle-aged patients who experienced a first myocardial infarction (MI) had a traditional risk factor profile which would not have qualified them for preventive medical therapy.” ¹

“Although current risk estimates work very effectively in populations, variation of estimated risk leads to misclassification of true risk in individual patients.” ²

“Even risk algorithms based on established risk factors are limited in predictive power for individuals. More effective prediction tools are needed.” ³

As little as 25% of premature CHD is attributable to elevated LDL-C\textsuperscript{2,3,4}

Lipid Treatment Reduced ASCVD Risk by ~30%, Leaving ~70% Residual Risk

Many Patients With Reduced LDL-C Still Have ASCVD Events

Cholesterol lowering worked (~7%)

Cholesterol lowering did NOT work (~24%)

Lipoprotein - Composition

- **Cholesteryl Ester** (cholesterol bound to 1 fatty acid)
- **Triglycerides** (glycerol bound to 3 fatty acids)
- **Free Cholesterol** (cholesterol not bound to fatty acid)
- **Apolipoproteins**
  - Proteins attached to the outer lipid layer of the particle by means of a lipophilic domain
- **Phospholipid**
  - Polar head group bound to 2 fatty acids
- **Protein**
- **Hydrophilic core**
  - Inner core of water insoluble lipids, mainly cholesteryl esters, triglycerides, and fat soluble vitamins
Heart disease patients often have elevated Remnants, LDL (increased small dense LDL), and Lp(a) and decreased large HDL particles.
Major (and minor) Players in the “Advanced Lipid Profile” Arena

- Direct LDL-C (several labs)
- LDL size (several labs)
- Apo B and Apo A-I (most labs)
- Lp(a) (most labs)
- Beta-quantification (few labs)
- LDL-P & HDL-P (by NMR, mainly LabCorp)
- HDL subfractions (Boston Heart + others)
- Sterol balance (mainly Boston Heart)
- Inflammatory markers: hsCRP, LpPLA2, MPO, other? (several labs)
- PCSK9 levels (few labs)
Friedewald LDL-C is Inaccurate if TG > 100 mg/dL!

(Comparison: Direct LDL-C)

Serum Triglyceride Level (mg/dL)

% Error in LDL-C

<100 mg/dL 100-149 mg/dL 150-199 mg/dL 200-399 mg/dL

Data from 1.2 million patients. Direct LDL-C by DGUC= density gradient ultracentrifugation.
LDL-C vs small, dense LDL as Factors in Atherogenesis

Fewer and Larger LDL Particles

100 mg/dL

More and Smaller LDL Particles

100 mg/dL

↑ Atherogenesis

- ↓ Hepatic clearance
- ↑ Entry into subendothelial (SE) space (= artery wall)
- ↑ Retention in SE space
- ↑ Oxidation
- ↑ Macrophage uptake
- ↑ Atherogenicity per particle

LDL-C does predict ASCVD in familial hypercholesterolemia (FH) and other nL-TG states
Small, Dense LDL-C Strongly Predicts ASCVD Even When LDL-C does Not!

Hoogeveen RC. ATVB. 2014;34;1069-1077
Ion Mobility Separates Lipoproteins by Size and Directly Measures Particle Concentrations

Apo B and Apo A-I Best Predict CVD Risk

INTERHEART Data from 52 Countries. McQueen, MJ. Lancet 2008;372:224-33.
Apo B: Better Than LDL-C & Non-HDL-C

1. Even in HTG (except type III) >90% of apo B is on LDL [1,2]

2. Apo B beats LDL-C for CHD risk:
   - Prospective epidemiologic trials [3-12]
   - Residual CHD risk w/ statin or fibrate Rx [13-17].

3. Apo B > Non-HDL-C for CHD risk:
   - Prospective epidemiologic trials [8,11,18]
   - Residual CHD risk w/ statin or fibrate Rx [13-15].

**Lipoprotein(a) Structure**

- Produced by liver but assembly unclear
- Similar to LDL in lipid content and composition, and accepts oxidized lipids from LDL
- Apo(a) linkage to Apo B-100 via disulfide bond
- **Apo(a):**
  - Highly polymorphic glycoprotein
  - Variable number of kringle IV₂ repeats (3-50), Lp(a) size inverse to levels
  - Homologous with plasminogen and high Lp(a) interferes with clot lysis
  - Pro-oxidant
  - Cleared poorly by LDL-R
  - No ↓ levels: Statins, CAI, BAS, etc.
  - NA, ERT, PCSK9-I, mipomersen & CETP-I

*After: Koschinsky ML and Marcovina SM. Curr Opin Lipid 2004; 15:167-174*
Type-III Dysbetalipoproteinemia: Best Dx by Beta-Quantification

- Confers very high ASCVD risk, and generally responds well to Rx
- Men & PM women > others (E2 protects)
- More common than low prevalence of E2/2 + palmar xanthoma indicates
- Dx: CE-enrichment of VLDL
- How to measure VLDL-C?
  - Friedewald (=TG/5) LOUSY!
  - Direct LDL-C—fair
  - IM, NMR, DGUC—fair to good?
  - Beta-quant (UC)—gold standard
NMR Lipoprotein Analysis

Each subclass NMR signal comes from the aggregate number of terminal methyl groups on the lipids in the particle shell and core.

The number of methyl groups in a particle of given size is unaffected by lipid compositional variation.

Otvos JD. Handbook of Lipoprotein Testing. AACC Press 2000
Each lipoprotein subclass broadcasts a unique NMR "sound"

Simultaneous "ringing" of the plasma lipoproteins produces a recorded signal

Recorded plasma signal

Sizes of contributing subclass signals (derived by computer analysis) give the subclass concentrations
Relations of LDL Particles and LDL Cholesterol to Levels of HDL Cholesterol and Triglycerides

Framingham Offspring Study

Cromwell WC and Otvos JD. *Curr Athero Reports* 2004;6:381-387
LDL-P Often High with Low LDL-C (MESA)
(<100 mg/dL; <30th percentile; n=1631)

LDL-P Low
n=1115
(68%)

LDL-P Not Low
n=516
(32%)

LDL-C and LDL-P in MESA (n=5,598)

Discordance Between LDL-C and LDL-P in MESA

“Discordance” was defined as a difference of ≥12 percentile, to make 50% of subjects discordant.
LDL-P and LDL-C Discordance in MESA
Relations with Incident CVD Events (n=319)

LDL-C underestimates LDL-attributable risk
LDL-C overestimates LDL-attributable risk

MetSyn

<table>
<thead>
<tr>
<th>MetSyn</th>
<th>LDL Size</th>
<th>LDL-C</th>
<th>LDL-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-P &gt; LDL-C</td>
<td>54%</td>
<td>20.3</td>
<td>104</td>
</tr>
<tr>
<td>Concordant</td>
<td>33%</td>
<td>20.7</td>
<td>117</td>
</tr>
<tr>
<td>LDL-P &lt; LDL-C</td>
<td>16%</td>
<td>21.1</td>
<td>130</td>
</tr>
</tbody>
</table>

LDL-P Differs from Apo B (explains only ~1/2 of variability)

HDL Testing

- Great inverse predictor in general population, but
- The more we learn the less we know
- Apo A-I may be useful, but when?
- HDL subfractions may be useful, but when?
- HDL function may be useful, but unclear when and not yet available
Why does Statin Response Vary?

- Answer: Less inhibition of cholesterol synthesis, and/or more upregulation of cholesterol absorption.
Cholesterol Balance vs LDL Removal

All major LDL-lowering treatments → ↑LDL-receptor activity
Most do this by → ↓hepatic cholesterol, but PCSK9-I work by
→ ↓LDL-R destruction by PCSK9

↓Dietary cholesterol & saturated fat
→ ↓hepatic cholesterol → ↑LDL-R

Statins → ↓chol
synthesis → ↓hepatic cholesterol → ↑LDL-R
BUT tend to → ↑chol absorption
(also → ↑PCSK9
→ ↑LDL-R destruction)

• Ezetimibe
→ ↓intestinal chol absorp → ↓hepatic chol → ↑LDL-R
• Bile-acid-sequest
→ ↓intestinal bile re-absorp → ↑bile synth
→ ↓hepatic chol
→ ↑LDL-R
BUT tend to → ↑chol synthesis

Stable vs Vulnerable Plaque

**Stable Plaque**
- Less Lp-PLA₂ accumulation (dark color)
- Thick fibrous cap with high collagen content
- Minimal necrotic lipid pool
- Less inflammation

**Vulnerable Plaque**
- High Lp-PLA₂ accumulation (dark color)
- Thin fibrous cap with low collagen content
- Large necrotic lipid pool
- More inflammation

ARIC- Additive Risk for Incident CHD and Stroke by Lp-PLA₂ When Added to hs-CRP

Heart Attack¹

<table>
<thead>
<tr>
<th>hsCRP top tertile</th>
<th>hsCRP bottom tertile</th>
<th>Lp-PLA₂ top tertile</th>
<th>Lp-PLA₂ bottom tertile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>1.0</td>
<td>1.2</td>
<td>1.4</td>
</tr>
</tbody>
</table>

(n>12,000, 6-8 yr f/u, 203 coronary events, LDL < 130 mg/dL)

p=0.001, 95% CI 1.7-10.3

Stroke²

<table>
<thead>
<tr>
<th>hs-CRP top tertile</th>
<th>hs-CRP bottom tertile</th>
<th>Lp-PLA₂ top tert.</th>
<th>Lp-PLA₂ bott. tert.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>11.4</td>
<td>5.5</td>
</tr>
</tbody>
</table>

(n>12,000, 6-8 yr f/u, 194 ischemic strokes)

p<0.001, 95% CI 3.1-41.4

Lp-PLA₂, in combination with hs-CRP, demonstrates increased clinical utility

Adjusted for demographics, current smoking status, blood pressure, diabetes and HDL- ARIC Study data

¹ Ballantyne et al, Circulation. 2004
² Ballantyne et al, Arch Intern Med. 2005
PCSK9

- Produced by liver in response to SREBP and cholesterol levels
- Destroys LDL-receptor
- Destroys other receptors (scavenger, etc.)
- Found in the brain (? Function there)
- Levels *increase* with statins
- Levels decrease greatly (to zero) with PCSK9-mAb
- Testing NOT yet shown to be useful
## Recent Buyouts of Advanced Lipid Profile Companies

<table>
<thead>
<tr>
<th>Company</th>
<th>Purchased By</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berkeley Heart Labs (Ion Mobility)</td>
<td>Quest (Celera)</td>
<td>2011 (2007)</td>
</tr>
<tr>
<td>Lipo Science (NMR)</td>
<td>LabCorp</td>
<td>Nov. 2014</td>
</tr>
<tr>
<td>Boston Heart Labs (2D HDL map, sterol balance, etc.)</td>
<td>Eurofins</td>
<td>Dec. 2014</td>
</tr>
<tr>
<td>Health Diagnostic Labs (IR index, etc.)</td>
<td>True Health</td>
<td>Sept. 2015</td>
</tr>
<tr>
<td>Atherotech (VAP)</td>
<td>Bankrupt, closed, no buyer—reverted to UAB</td>
<td>Feb. 2016</td>
</tr>
<tr>
<td>Cleveland Heart Labs (MPO, etc.)</td>
<td>Original owner</td>
<td>NA</td>
</tr>
</tbody>
</table>
## Parameters for ASCVD Risk Prediction

<table>
<thead>
<tr>
<th>sdLDL-C</th>
<th>Cholesterol in sdLDL particles (LDL $d$ 1.044-1.063 g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoB</td>
<td>Serum/plasma apoB (# apoB/atherogenic particles)</td>
</tr>
<tr>
<td>NonHDL-C</td>
<td>Cholesterol in all apoB-containing/atherogenic particles</td>
</tr>
<tr>
<td>LDL-P</td>
<td>Number of LDL particles determined by NMR</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Cholesterol in all LDL particles ($d$ 1.019-1.063 g/mL)</td>
</tr>
</tbody>
</table>

**sdLDL ≈ apoB > nonHDL-C > LDL-P > LDL-C**

1. Tsai MY et al. Arterioscler Thromb Vasc Biol 2014; 34:196-201,  
Coronary Artery Calcium – Used to Assess Risk, CIMT Used to Follow Patients

“The Best Predictor of Disease is the Presence of Disease”

NO CALCIFICATION
Score = 0

MODERATE CALCIFICATION
Score = 89

SIGNIFICANT CALCIFICATION
Score = 1894
Coronary Artery Calcium Improves Risk Classification in Younger Populations

Andre R.M. Paixao, MD, Colby R. Ayers, MS, Abdallah El Sabbagh, MD, Monika Sanghavi, MD, Jarett D. Berry, MD, MS, Anand Rohatgi, MD, MSCS, Dharam J. Kumbhani, MD, SM, Darren K. McGuire, MD, Sandeep R. Das, MD, MPH, James A. de Lemos, MD, Amit Khera, MD, MSc

![Graph showing adjusted incidence of CHD events over years with a p-value of <0.001.](image_url)
All-cause Mortality in Different Age Groups Stratified by Increasing CAC

Mortality/1000 person years

Age

<45 45-54 55-64 65-74 ≥75

CAC=0 □  CAC 1-100 □  CAC 101-400 □  CAC>400 □

Tota-Maharaj, Blaha, et al.  
*European Heart Journal.* 2014.
**Advanced Lipid Testing: Summary**

- Basic lipid panel LDL-C is ok for most uses, *but*
- Need to look at Non-HDL-C, especially in HTG or if non-fasting
- Apo B useful if discordant with Non-HDL-C but not clear when to test
- Apo(a) useful for
  - Bad family history
  - Bad personal history
  - All 20 prevention?
- Other testing in selected cases (consult/be a lipidologist!)
- CAC useful if
  - Over ~40/50 (men/women) but primary prevention *only*
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