Emerging Risk Factors for CVD: How to Assess Residual Risk

Ryan Bradley ND, MPH
Associate Director | Bastyr University Research Institute
Core Clinical Faculty | Bastyr University California
Director | Center for Diabetes & Cardiovascular Wellness
Overview

• Assessing CVD event risk
  • Traditional risk classification- Framingham & (now) Reynolds
  • Residual risk- How much is there?
    • INTERHEART

• Emerging risk factors- How do you evaluate them?
  • Criteria for evaluating emerging biomarkers

• Evaluation of select emerging risk factors
  • Lipoprotein measures: Apo B100: Apo A1, particle characteristics (i.e., number; density; size)
  • Methylation: Homocysteine, [MTHFR polymorphism]
  • Inflammation/ acute phase reactants: CRP, fibrinogen [SAA]
  • Atherosclerosis: Lp-PLA$_2$ [sICAM-1; IL-6]
  • Oxidative stress: oxLDL, GGT [f2-isoprostanes, etc.]
  • Endothelial function: Reactive Hyperemia Index (RHI)
  • Renovascular function: cystatin C
Traditional Cardiovascular Risk Assessment

- **Framingham Risk Score:**
  - Age
  - Gender
  - Total cholesterol
  - HDL
  - Smoking status
  - SBP

- **Reynolds Risk Score:**
  - Framingham under estimates risk in women
  - Reynolds re-classifies risk in 40%
  - Reynolds adds: hsCRP & A1c (if diabetes)
Impact of adding CRP in women

Figure 1. Risk factors for future cardiovascular events in apparently healthy women in the Women’s Health Study. Apo B = apolipoprotein B; CRP = C-reactive protein; HDL = high-density lipoprotein; IL-6 = interleukin-6; LDL = low-density lipoprotein; SAA = serum amyloid A; sICAM-1 = soluble intercellular adhesion molecule-1; TC = total cholesterol. (Reprinted with permission from *N Engl J Med*.)
Population Attributable Risk of First MI: INTERHEART

Risk Factors:
- Elevated ApoB:ApoA1
  - OR=3.25 (5th vs. 1st quin); PAR=49.2%
- Smoking
  - OR=2.87; PAR=35.7%
- HTN
  - OR=1.91; PAR=17.9%
- Abdominal obesity
  - OR=1.12 (top vs. lowest tertile); PAR=20.1%
- Diabetes
  - OR=2.37; PAR=9.9%
- Psychosocial factors
  - OR=2.67; PAR=32.5%

Protective Factors:
- Daily F&V Consumption
  - OR=0.70; PAR=13.7%
- Moderate alcohol consumption
  - OR=0.91; PAR=6.7%
- Regular Physical Activity
  - OR=0.86; PAR=12.2%

Total Population Attributable Risk (PAR): 90% in women & 94% in men

Yusuf et al., Lancet, 2004
Figure 2: Risk of acute myocardial infarction associated with exposure to multiple risk factors
Smk=smoking, DM=diabetes mellitus, HTN=hypertension, Obes=abdominal obesity, PS=psychosocial, RF=risk factors. Note the doubling scale on the y axis. The odds ratios are based on current vs never smoking, top vs lowest tertile for abdominal obesity, and top vs lowest quintile for ApoB/ApoA1. If these three are substituted by current and former smoking, top two tertiles for abdominal obesity and top four quintiles for ApoB/ApoA1, then the odds ratio for the combined risk factor is 129:20 (99% CI 90:24–184:99).
The Contribution of Lifestyle to All-Cause Mortality in CAD*

- Smoking Cessation:
  - RR = 0.64 (0.58, 0.71)

- Healthy Diet**: 
  - RR = 0.56 (0.42, 0.74)

- Moderate Alcohol:
  - RR = 0.80 (0.78, 0.83)

- Physical Activity:
  - RR = 0.76 (0.59, 0.98)

* CAD= Past MI, Angina, PCTA, CABG

** Healthy Diet=Low Sat’ d Fat, Regular Fish, Whole Grains, Nuts, F&V, reduced salt (<2400 mg/d)

Iestra et al., Circulation, 2005
Contribution of Lifestyle: INTERHEART

Figure 3: Reduced risk of acute myocardial infarction associated with various risk factors
Smk = smoking, Fr/vg = fruits and vegetables, Exer = exercise, Alc = alcohol. Note the doubling scale on the y-axis. Odds ratios are adjusted for all risk factors.

Yusuf et al., Lancet, 2004
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  • Endothelial function: Reactive Hyperemia Index (RHI)
  • Renovascular function: cystatin C
Criteria to Meet before Applying Emerging Biomarkers of CVD Risk

• Measurement provides independent risk prediction not captured by current standards/clinical norms.

• Treatment is available, validated and unique.

• Treatment improves clinical outcomes.

• Measurement and treatment are risk-balanced and cost effective.
Criteria Scoring Exercise

• Criteria:
  • Met: 2 points
  • Partially met: 1 point
  • Not met: 0 points

• Weighting based on your values:
  • E.g., Independent risk prediction & improves clinical outcomes >> unique treatment & risk-balanced and cost effective.
    • Met: 4 points
    • Partially met: 2 point
    • Not met: 0 points
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  • Lipoprotein properties
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  • Oxidative stress: oxLDL, GGT [f2-isoprostanes, etc.]
  • Endothelial function: Reactive Hyperemia Index (RHI)
  • Renovascular function: cystatin C
What properties of lipoproteins (LDL) can be measured?

- Calculated particle concentration (i.e., LDL-C)
- Apoprotein concentrations (ApoA, ApoB)
- Particle number (LDL-P)
- Physical size (nm or Å)
  - > or < 255 Å
- Density
- Cholesterol ester content
- Triglyceride (TG) content
- Oxidation state (oxLDL)
A Few Comments on LDL

• 1% reduction in LDL results in a 1-1.7% reduction in event RR\textsuperscript{1,2,3}

• Caveats:
  • LDL at birth: \sim 50 \text{ mg/dl}\textsuperscript{4}
  • Estimates of LDL requirement for peripheral cholesterol needs: \sim 25 \text{ mg/dl}\textsuperscript{5}

A Few Comments on HDL

• Plaque scavenger- “reverse cholesterol transport”
• ApoA corresponding lipoprotein
• 1% increase in HDL = 1% reduction in risk\textsuperscript{1,2}
• Caveats:
  • Difficult to increase if TG not normalized first
  • Evidence for drug-increased HDL leading to event protection is waning (especially when combined with statins)

ApoB

- Apoprotein on lipoprotein micelle, i.e. LDL
- ApoB100 vs. ApoB48
  - B100: hepatic synthesis- endogenously produced LDL & VLDL
  - B48: intestinal synthesis- packing of dietary cholesterol in chylomicrons
- Proxy for “non-HDL cholesterol”
- Strong correlation with LDL particle number, esp. with normal triglycerides
- B100 binds LDL receptor
  - B48 may not bind LDL receptor
ApoA

- Apoprotein on HDL lipoprotein micelle
- Strong correlation with HDL particle number
ApoB: ApoA

- Elevated ApoB:ApoA1: OR=3.25 (5th vs. 1st quin); PAR=49.2% (INTERHEART)

Yusuf et al., Lancet, 2004
Unique Treatment?

- Lowering LDL lowers apoB100
- Raising HDL increases apoA
- Lowering triglycerides lowers apoB100 via lowering VLDL
Criteria to Meet before Applying Emerging Biomarkers of CVD Risk: Apo B: Apo A

- Measurement provides unique independent risk assessment not captured by measurement per current standards/clinical norms

- Treatment is available, validated and unique

- Treatment improves hard clinical outcomes

- Measurement and treatment are risk-balanced and cost effective
LDL Particle Number (LDL-P)

• Criteria met:
  • Independent risk prediction?: Valid and better discriminates CVD risk than LDL-C\(^{1-4}\) major CVD case-control studies and prospective cohort studies
  • Treatment improves outcomes

• Criteria not met:
  • Unique treatment?: LDL-lowering treatment lowers LDL-P
  • Independent risk prediction?: Strong correlations with LDL and VLDL (therefore triglycerides), therefore testing less valuable for the cost due to high correlation with LDL and VLDL
  • Cost effective?

3. Rosenson Am J Cardiol. 2002
Criteria to Meet before Applying Emerging Biomarkers of CVD Risk: LDL-P

- Measurement provides unique/independent risk assessment not captured by measurement per current standards/clinical norms

- Treatment is available, validated and unique

- Treatment improves hard clinical outcomes

- Measurement and treatment are risk-balanced and cost effective
Does [LDL-P] Size Matter?
What causes differences in size?

1. **Diet**: sat’d fat & CHO increase triglycerides (TG) packaged in VLDLs

2. **Genes**: Cholesterol ester transport protein (CETP) transfers cholesterol esters to VLDL (removed from LDL)

3. CETP transfers TAGs to LDL (removed from VLDL)

4. Lipoprotein lipase acts on LDL and...

Voilá! small, dense LDLs

Fig. 1. Effect of increased plasma triglyceride (TG) on the possible mechanism of small-LDL formation. LPL, lipoprotein lipase.
How many patterns are there?

Table 1: Major Lipoprotein Class, Subclass, Density and Particle Size

<table>
<thead>
<tr>
<th>Name/Class</th>
<th>Acronym Subclass</th>
<th>Density Range (g/ml)</th>
<th>Particle Diameter (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(VLDL) Very Low Density Lipoprotein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>&lt;1.006</td>
<td>330-700</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1.006-1.010</td>
<td>300-330</td>
</tr>
<tr>
<td>(IDL) Intermediate Density Lipoprotein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td>1.008-1.022</td>
<td>285-300</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td>1.013-1.019</td>
<td>272-285</td>
</tr>
<tr>
<td>(LDL) Low Density Lipoprotein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td>1.019-1.023</td>
<td>272-285</td>
</tr>
<tr>
<td>IIa</td>
<td></td>
<td>1.023-1.028</td>
<td>265-272</td>
</tr>
<tr>
<td>IIb</td>
<td></td>
<td>1.028-1.034</td>
<td>256-265</td>
</tr>
<tr>
<td>IIIa</td>
<td></td>
<td>1.034-1.041</td>
<td>247-256</td>
</tr>
<tr>
<td>IIIb</td>
<td></td>
<td>1.041-1.044</td>
<td>242-247</td>
</tr>
<tr>
<td>IVa</td>
<td></td>
<td>1.044-1.051</td>
<td>233-242</td>
</tr>
<tr>
<td>IVb</td>
<td></td>
<td>1.051-1.063</td>
<td>220-233</td>
</tr>
<tr>
<td>(HDL) High Density Lipoprotein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td></td>
<td>1.063-1.100</td>
<td>98-130</td>
</tr>
<tr>
<td>2a</td>
<td></td>
<td>1.100-1.125</td>
<td>88-98</td>
</tr>
<tr>
<td>3a</td>
<td></td>
<td>1.125-1.147</td>
<td>82-88</td>
</tr>
<tr>
<td>3b</td>
<td></td>
<td>1.147-1.154</td>
<td>77-82</td>
</tr>
<tr>
<td>3c</td>
<td></td>
<td>1.154-1.203</td>
<td>72-77</td>
</tr>
</tbody>
</table>

Pattern A  Intermediate  Pattern B

© Berkeley Heart Labs
What predicts “Pattern B”?

• Cross-sectional study of 131 apparently health adults
• Associations with Pattern B?
  • +non-fasting TGs
  • -HDLs
• Prediction variables
  • Two-variable model:
    • HDL and total TG
  • Three-variable model:
    • HDL
    • Total cholesterol and
    • Total apoB

Table 5. Two- and Three-variable Regression Models that Predict Low Density Lipoprotein Density

<table>
<thead>
<tr>
<th>Model</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-variable model</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol and total triglyceride</td>
<td>0.35</td>
</tr>
<tr>
<td>HDL cholesterol and total triglyceride*</td>
<td>0.37</td>
</tr>
<tr>
<td>Three-variable model</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, HDL cholesterol, and LDL cholesterol</td>
<td>0.31</td>
</tr>
<tr>
<td>HDL cholesterol, LDL cholesterol, and LDL apo B</td>
<td>0.31</td>
</tr>
<tr>
<td>Total triglyceride, LDL cholesterol, and LDL apo B</td>
<td>0.35</td>
</tr>
<tr>
<td>Total cholesterol, total triglyceride, and LDL cholesterol</td>
<td>0.38</td>
</tr>
<tr>
<td>Total cholesterol, total triglyceride, and LDL apo B*</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Models in which variables, in addition to gender, age, smoking habits, and relative body weight, contribute significantly ($p<0.05$) to the prediction of LDL density.

*The best models for predicting LDL density.

HDL = high density lipoprotein, LDL = low density lipoprotein, apo = apolipoprotein.

Do small, dense LDLs really increase IHD Risk?

- Quebec Cardiovascular Study
  - 5-yr. cohort 4637 men
  - Nest case-control
- Evaluated sdLDL and IHD
  - Outcomes: new angina, coronary insufficiency, nonfatal MI, coronary death
  - Controls matched for age, BMI, smoking, alcohol use
- OR: 2.5 (95% CI: 1.2-5.2)
  - Did adjust for SBP, med use, FHx
  - Did not adjust for TG, HDL, or LDL particle number

Table 2: Prevalence of Traditional and Nontraditional Risk Factors Among Cases and Associated IHD Risk

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Prevalence in Cases, %</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated fasting insulin</td>
<td>81.9</td>
<td>6.5 (2.3-13.6)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Elevated apo B</td>
<td>66.8</td>
<td>2.7 (1.2-6.0)</td>
<td>.01</td>
</tr>
<tr>
<td>Fibrinol T3</td>
<td>76.3</td>
<td>3.5 (1.0-7.4)</td>
<td>.002</td>
</tr>
<tr>
<td>Reduced HDL-C</td>
<td>69.4</td>
<td>1.6 (0.2-0.3)</td>
<td>.04</td>
</tr>
</tbody>
</table>

“Small, dense LDL” - OR: 2.5 (95% CI: 1.2-5.2)
LDL Pattern & IHD: More in QCS

- Increased prediction when LDL size was added to model (BMI, systolic BP, diabetes, meds, age, HDL, LDL, log(TAG), log (Apo-a)& FHx)
  - as proportion of LDL<255Å
    - AUC: 76.8% vs 73.9%, p<0.001
  - as particle number of LDL <255Å
    - AUC: 77.4% vs. 73.9%, p<0.001

LDL pattern & carotid atherosclerosis: MESA

- MESA: Multiethnic Study of Atherosclerosis
  - 5538 participants
- Evaluated associations between carotid atherosclerosis (measured by IMT) and LDL pattern
- First to adjust for interclass correlations between small and large LDL particle number (LDL-P)
- Small and Large LDL-P were:
  - sdLDL and ldLDL negatively correlated with each other \( (r = -0.63) \)
  - sdLDL negatively correlated with HDL \( (r = -0.65, 0.67) \)
  - sdLDL positively correlated with TAGS \( (r = +0.57, -0.40) \)

Fig. 1. Mean IMT (y-axis) for increasing levels of large LDL particle concentration (LDL-p) are shown across increasing levels of small LDL-p. N is the number of individuals in each category.

Fig. 2. Mean IMT (y-axis) for increasing quintiles of large LDL particle concentration (LDL-p) adjusting for age (years) and sex (left panel) but not for small LDL-p showed no significant association between large LDL-p and IMT. After additionally adjusting for small LDL-p to account for the correlation between small and large LDL (right panel), there was a significant linear association between large LDL-p and IMT. P values for linear trend were obtained from linear regression models. Mean IMT values in the figure represent those for an average age man (61.4 years). To obtain values for a 61.4-year-old woman subtract 80 μm.

### sdLDL & ldLLDL: MESA

**Table 3**  
Association of LDL subclasses with IMT after adjusting for LDL subclass correlation

<table>
<thead>
<tr>
<th></th>
<th>Model 1(^a)</th>
<th>(P) value</th>
<th>Model 2(^b)</th>
<th>(P) value</th>
<th>Model 3(^c)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large LDL-p</td>
<td>36.6 (5.4)</td>
<td>&lt;0.001</td>
<td>23.8 (9.1)</td>
<td>0.009</td>
<td>30.3 (9.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Small LDL-p</td>
<td>52.2 (5.2)</td>
<td>&lt;0.001</td>
<td>38.7 (9.2)</td>
<td>&lt;0.001</td>
<td>34.8 (10.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-c</td>
<td>13.5 (7.7)</td>
<td>0.08</td>
<td>11.8 (7.8)</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-c</td>
<td></td>
<td></td>
<td>-17.3 (5.7)</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td></td>
<td>-1.6 (5.1)</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S.D. values for lipid and lipoprotein variables are shown in Table 2.

\(^a\) Model 1 included the two LDL subclasses, age (years), sex, race, hypertension, and smoking.

\(^b\) Model 2 included model 1 variables plus LDL-c.

\(^c\) Model 3 included model 1 variables plus LDL-c, HDL-c, and triglycerides.
LDL Pattern & CAD: EPI-Norfolk

- 25,663 participants for 6 years
  - Nested case-control eval. LDL size as predictor of CAD
  - Case definition: ICD-9 or CVD death
- 1003 cases and 1885 CAD-free controls

**Table 5** Odds Ratios for Future Coronary Artery Disease by Quartile of LDL Size, With and Without Adjustment for LDL Particle Number*

<table>
<thead>
<tr>
<th>LDL Size Quartile</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>p Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range (nm)</td>
<td>&lt;20.6</td>
<td>20.7–21.0</td>
<td>21.1–21.4</td>
<td>&gt;21.4</td>
<td></td>
</tr>
<tr>
<td>Unadjusted for LDL particle number</td>
<td>1.00</td>
<td>0.77 (0.62–0.97)</td>
<td>0.78 (0.60–0.95)</td>
<td>0.60 (0.47–0.76)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adjusted for LDL particle number</td>
<td>1.00</td>
<td>0.92 (0.72–1.16)</td>
<td>0.99 (0.77–1.28)</td>
<td>0.86 (0.65–1.15)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Odds ratios (95% confidence intervals) were calculated by conditional logistic regression adjusted for smoking and systolic blood pressure, with and without additional adjustment for low-density lipoprotein (LDL) particle number; †p for linear trend.

# Summary: Small, dense LDL & CVD Risk

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Outcome</th>
<th>Increased Unadjusted Risk?</th>
<th>Adjustment Variables</th>
<th>Attenuation of Risk?</th>
</tr>
</thead>
<tbody>
<tr>
<td>QCS-1998/2001</td>
<td>IHD</td>
<td>Yes</td>
<td>SBP, FHx, Med use (age, BMI, smoking, alcohol)</td>
<td>No</td>
</tr>
<tr>
<td>CHS-2002</td>
<td>IHD</td>
<td>Yes</td>
<td>SBP, FHx, Med use, diabetes, LDL, HDL, TAG, apo-A, BMI, age</td>
<td>No (increased AUROC for prediction)</td>
</tr>
<tr>
<td>ARIC-2004</td>
<td>Carotid IMT</td>
<td>Yes (women only)</td>
<td>Age, race, LDL particle number</td>
<td>Yes</td>
</tr>
<tr>
<td>MESA-2007</td>
<td>Carotid IMT</td>
<td>Yes (Caucasian only)</td>
<td>Age, smoking, BMI, TAG, HDL particle number</td>
<td>Yes</td>
</tr>
<tr>
<td>EPI-Norfolk-2007</td>
<td>CAD</td>
<td>Yes</td>
<td>Age, SBP, LDL particle number</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Unique Treatment?

• Available LDL treatment “improves” LDL pattern:
  • Statins$^1$
  • Fibrates$^2$
  • Niacin$^3$

Criteria to Meet before Applying Emerging Biomarkers of CVD Risk: LDL Patterns

• Measurement provides independent risk assessment not captured by measurement per current standards/clinical norms

• Treatment is available, validated and unique

• Treatment improves hard clinical outcomes

• Measurement and treatment are risk-balanced and cost effective
Lp(a)

- Lipoprotein a: ApoB covalently bound to glycylated Apo(a) through a disulfide bond
- Acts as an acute-phase reactant
Lp(a) & CVD Risk

- Levels > 30mg/dl increase risk approximately 3-fold in men (CHS)\(^1\); Higher risk in:
  - males,
  - young patients w/ familial hypercholesterolemia &
  - high risk patients (based on traditional risk factors)\(^1\)
- No evidence in females or older adults after adj. for other risk factors\(^1\)
- LDL reduction may offset risk\(^1\)
- Unique treatment?
  - Niacian lowers ~30%\(^1\);
  - L-Carnitine: ~13% reduction; 2g qd\(^2\); and
  - CoQ10: ~22% reduction; 120mg Qgel\(^3\)

Criteria to Meet before Applying Emerging Biomarkers of CVD Risk

- Measurement provides independent risk assessment not captured by measurement per current standards/clinical norms [*in select populations*]

- Treatment is available, validated and unique

- Treatment improves hard clinical outcomes

- Measurement and treatment are risk-balanced and cost effective
Homocysteine (Hcy)

• Metabolic byproduct of dietary methionine
• Vitamin B12, folic acid or vitamin B6 required for conversion of Hcy back into methionine (B12 & folic acid) or to cysteine (B6)
• Data in genetic “homocystinurics” suggests strong association with thromboembolic events
Homocysteine & IHD Risk

Hcy & Stroke Risk

Hcy & PAR

Population Attributable Risk

% of CHD Attributed

- Total Cholesterol
- LDL-C
- Homocysteine

JAMA. 1995. 274. 1049
RCT of 3749 with +1st MI; 40 months f/u; Primary endpoint: 2nd MI, stroke or death

Randomized to four arms:
- 0.8mg folic acid, 0.4 mg B12 & 40 mg B6
- 0.8mg folic acid and 0.4mg B12
- 40mg B6
- Placebo

Hcy lowered 27% in groups given folic acid + B12

No effect on primary outcome

Figure 1. Kaplan–Meier Estimates of the Probability of Reaching the Primary End Point during Follow-up. The primary end point was a composite of fatal and nonfatal myocardial infarction, fatal and nonfatal stroke, and sudden death attributed to coronary heart disease.
HOPE2

- RCT 5522 patients with vascular dz or diabetes; primary endpoint MI, stroke or CV-related death
- Randomized to 2.5 mg folic acid, 50 mg B6 and 1 mg B12 or placebo
- No difference in primary outcome
- Reduced risk of stroke in active tx arm: RR=0.75 (95% CI: 0.59-0.97)
- Active arm had increased hospitalization for angina: RR=1.24 (95% CI: 1.04-1.49)
Figure 2. Kaplan–Meier Estimates of the Proportion of Patients with the Composite Primary Outcome of Death from Cardiovascular Causes, Myocardial Infarction, or Stroke.

The relative risk of the composite primary outcome in the active-treatment group, as compared with the placebo group, was 0.95 (95 percent confidence interval, 0.84 to 1.07; P = 0.41 by the log-rank test).
Summary: Homocysteine

- PAR for Hcy is very low, if present
- Testing should be selective & treatment more so (>15)
- Unknowns:
  - Primary prevention data not available and may prove significant
  - Possible benefit for stroke prevention
  - Some design issues remain, i.e. B12 absorption in an elderly population
Criteria to Meet before Applying Emerging Biomarkers of CVD Risk: Homocysteine

- Measurement provides independent risk assessment not captured by measurement per current standards/clinical norms.

- Treatment is available, validated and unique.

- Treatment improves hard clinical outcomes (except possibly stroke).

- Measurement and treatment are risk-balanced and cost effective.
C-reactive protein & Fibrinogen

- Acute phase reactants indicative of inflammation
- Chronic elevations (lower levels) also impact CVD risk
- High co-linearity/correlation between CRP and fibrinogen (therefore no reason to ever measure both for residual risk assessment)
- Difficult to separate CRP reduction independent of LDL reduction
Figure 1. Additive value of high-sensitivity C-reactive protein after adjustment for traditional risk factors. Data are shown across all levels of low-density lipoprotein (LDL) cholesterol (right) and across all levels of calculated Framingham Risk (left). Adapted, with permission, from Ridker et al. (23).
Figure 4. C-reactive protein was an independent predictor of cardiovascular risk in 9 large prospective studies across diverse populations. ARIC = Atherosclerosis Risk in Communities; CHS = Cardiovascular Health Study; EPIC = European Prospective Investigation into Cancer and Nutrition; HPFS = Health Professionals Follow-up Study; MONICA = Monitoring Trends and Determinants in Cardiovascular Disease; NHS = Nurses’ Health Study; PHS = Physicians’ Health Study; WHS = Women’s Health Study.
Effect of Intensive Compared With Moderate Lipid-Lowering Therapy on Progression of Coronary Atherosclerosis
A Randomized Controlled Trial

Steven E. Nissen, MD
E. Murat Tuzcu, MD
Paul Schoenhagen, MD
B. Greg Brown, MD
Peter Ganz, MD
Bebek A. Vogel, MD
Tim Crowe, BS
Gail Howard, MS
Christopher J. Cooper, MD
Bruce Budoff, MD
Cindy L. Grines, MD
Anthony N. DeMaria, MD
for the REVERSAL Investigators

RCT (n=654)
• 40 mg/d pravastatin (PS) or
• 80 mg/d atorvastatin (AS)
• x 18 months

PS:
• 27% LDL reduction
• 5.2% reduction in hSCRP
• Progression of atheroma

AS:
• 47% LDL reduction
• 36.4% reduction in hsCRP
• Regression of atheroma in AS group

Nissen et al., JAMA. 2004
hsCRP & Regression of CAD

Figure 8. High-sensitivity C-reactive protein (hs-CRP) lowering with aggressive statin therapy slowed atherosclerotic progression and resulted in regression of atheroma volume as measured by intravascular ultrasonography in patients with coronary disease in the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial. ↓ = reduction greater than study median; ↑ = reduction less than study median; LDL-C = low-density lipoprotein cholesterol. (Based on data from N Engl J Med.26)
Justification for Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER)

Figure 1. Cumulative Incidence of Cardiovascular Events According to Study Group.

Panel A shows the cumulative incidence of the primary end point (nonfatal myocardial infarction, nonfatal stroke, arterial revascularization, hospitalization for unstable angina, or confirmed death from cardiovascular causes). The hazard ratio for rosuvastatin, as compared with placebo, was 0.56 (95% confidence interval [CI], 0.46 to 0.69; P<0.00001). Panel B shows the cumulative incidence of nonfatal myocardial infarction, nonfatal stroke, or death from cardiovascular causes, for which the hazard ratio in the rosuvastatin group was 0.53 (95% CI, 0.40 to 0.69; P<0.00001). Panel C shows the cumulative incidence of arterial revascularization or hospitalization for unstable angina, for which the hazard ratio in the rosuvastatin group was 0.53 (95% CI, 0.40 to 0.70; P<0.00001). Panel D shows the cumulative incidence of death from any cause, for which the hazard ratio in the rosuvastatin group was 0.80 (95% CI, 0.67 to 0.97; P=0.02). In each panel, the inset shows the same data on an enlarged y axis and on a condensed x axis.
Reclassification with CRP & Fibrinogen

• Evaluation of reclassification of CVD risk adding CRP and/or fibrinogen to determine incremental MI risk
  • 52 prospective studies analyzed including 246,669 participants
  • Results adjusted for statin indication based on traditional risk
  • Further adjustment for additional measures of acute inflammation via WBC count
• Reclassification index improved (statistically, but clinically?)
  • 1.52% for CRP
  • 0.83% for fibrinogen
• 30 events prevented over 10 years by measuring in 13,199 people
• Prevent 1 event/10 years/400-500 people screened

The Emerging Risk Factors Collaboration, NEJM, 2012
Unique treatment? hsCRP

- Some statins (e.g. rosvastatin)
- Niacin
- Fiber (30g/d) \(^1\)
- Pomegranate ?
- High AOX Capacity Diet?\(^2\)
- Smoking cessation

Criteria to Meet before Applying Emerging Biomarkers of CVD Risk: CRP/Fibrinogen

• Measurement provides independent risk assessment not captured by measurement per current standards/clinical norms

• Treatment is available, validated and unique

• Treatment improves hard clinical outcomes

• Measurement and treatment are risk-balanced and cost effective
Lipoprotein-associated Phospholipase A2 (Lp-PLA2)

- Secreted by macrophages, T cells and mast cells
- Resides in and is transported by lipoproteins (mostly LDL)
- Hydrolyzes oxidized phospholipids into two inflammatory mediators:
  - free oxidized lipid
  - lysophosphotidylcholine

- Conflicting data:
  - too little (due to genetic deficiency) increases risk
  - too much may also increase risk

- Highly correlated with LDL-C (adjustment for LDL-C in MONICA & WHI attenuated increased risk)
- Studies showing independence suggest limited usefulness (LDL<130; ARIC) and low excess risk (OR~1.2; WOSCOP)

Tsimikas et al. JACC. 2006
Figure 4. The role of lipoprotein-associated phospholipase A\textsubscript{2} (Lp-PLA\textsubscript{2}) in the breakdown of oxidized phospholipids. Reprinted, with permission, Macphee (44). LDL = low-density lipoprotein; oxLDL = oxidized low-density lipoprotein.

Tsimikas et al. JACC. 2006
Criteria to Meet before Applying Emerging Biomarkers of CVD Risk: Lp-PLA2

- Measurement provides unique/independent risk assessment not captured by measurement per current standards/clinical norms
- Treatment is available, validated and unique
- Treatment improves hard clinical outcomes
- Measurement and treatment are risk-balanced and cost effective
Oxidative Stress Biomarkers are Stressful

- F2-isoprostanes?
  - Limited measure of lipid peroxidation
  - Expensive to measure by GC-MS
  - Unreliable to measure by ELISA

- 8-hydroxydeoxyguanosine? (8-OHdG)
  - Limited measure of DNA oxidation
  - Overestimated by ELISA

- Protein carbonyls?
  - Limited measure of protein modification
  - Rapidly metabolized by kidneys

- Nitrotyrosine?
  - Limited measure of peroxynitrite activity
  - Expensive to measure by GC-MS
  - Unreliable to measure by ELISA

- RBC GSH?
  - Few population-based cohorts (ex. HIV+)
  - Cannot be measured from stored samples
  - Moderately expensive

- oxLDL?

- GGT?
oxLDL

- Formed from peroxidation of PUFA in the LDL
- Not a single entity
  - Research is heterogeneous due to differences in measurement methods
  - Debate continues re: whether it exists
- oxLDL (MDA-epitope) predictive of CAD in numerous studies
- Correlates with LDL + hsCRP
oxLDL + Traditional Risk Factors?

- Holvoet et al. conducted a case-control study in 178 CAD patients + 126 controls
  - 3.11 +/- 1.19 mg/dl (Cases) vs. 1.30 +/- 0.88 mg/dl (Controls); p<0.0001

- Calculated Global Risk Assessment Score based on Framingham factors and evaluated contribution of oxLDL to models
**oxLDL: Sens/Spec/PPV/NPV**

<table>
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<tr>
<th>Test performance</th>
<th>GRAS</th>
<th>+oxLDL</th>
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<td><strong>Sensitivity</strong></td>
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<td>Total</td>
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<tr>
<td>Women</td>
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GRAS= Global Risk Assessment Score

Pomegranate & oxLDL

• 90% reduction in oxLDL and 21% reduction in systolic BP
  • Dose: 2 oz. juice per day for 3 years\(^1\)

• 141% increase in antioxidant defenses (GSH), 56% reduction in lipid peroxides, reduced oxLDL uptake by macrophages by 39%
  • Dose: ~2oz. Juice per day x 3 months; Type 2 DM\(^2\)

1. Aviram et al. 2004
2. Rosenblat et al. 2006
Criteria to Meet before Applying Emerging Biomarkers of CVD Risk: oxLDL

- Measurement provides independent risk assessment not captured by measurement per current standards/clinical norms
- Treatment is available, validated and unique
- Treatment improves hard clinical outcomes
- Measurement and treatment are risk-balanced and cost effective

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Serum GGT activity: A valid biomarker of oxidative stress?

• No “gold standard” for systemic ox stress
• GGT activity increased in response to oxidative stress and demand for reduced GSH
• Other correlates with GGT:
  • + correlated with serum iron\(^1\) & heme iron intake, e.g. meat (pro-oxidant)\(^2\)
  • + correlated with F2-isoprostanes
  • - correlated with AOX and dietary patterns associated with protection
  • + correlated with HOMA-measured insulin resistance in DM, Pre-DM, and non-DM\(^3,4\)
• Predicts incident T2DM, HTN, CHF, CV events and total mortality

Multi-Ethnic Study of Atherosclerosis (MESA)

- N=6814, 44-84 yoa w/o known CVD but prevalent metabolic disease (DM2:11% and MetS:28%)
  - Caucasian: n=2,622
  - Chinese-American: n=803
  - Black: n=1,893
  - Hispanic/Latino-American: n=1,496

- GGT measurement: microplate assay, calibrated to 2 independent labs (r=0.99)
  - All within 95th percentile included in analyses
<table>
<thead>
<tr>
<th>Baseline Characteristic</th>
<th>GGT-Q1</th>
<th>GGT-Q2</th>
<th>GGT-Q3</th>
<th>GGT-Q4</th>
<th>GGT-Q5</th>
<th>P for trend</th>
</tr>
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<tr>
<td>(n=6,446)</td>
<td>&lt;24.5 U/l (N=1,289)</td>
<td>24.5-29.3 U/l (N=1,286)</td>
<td>29.3-35.1 U/l (N=1,292)</td>
<td>35.1-45.2 U/l (N=1,289)</td>
<td>45.2-97.7 U/l (N=1,290)</td>
<td>&lt;0.0001</td>
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<td>Mean (SD) or N (%)</td>
<td>Mean (SD) or N (%)</td>
<td>Mean (SD) or N (%)</td>
<td>Mean (SD) or N (%)</td>
<td>Mean (SD) or N (%)</td>
<td>Mean (SD) or N (%)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.4 (10.9)</td>
<td>63.5 (10.3)</td>
<td>62.8 (10.0)</td>
<td>62.1 (10.0)</td>
<td>61.0 (9.7)</td>
<td>&lt;0.0001</td>
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<td>Age &lt; 65 years</td>
<td>699 (54%)</td>
<td>647 (50%)</td>
<td>686 (53%)</td>
<td>726 (56%)</td>
<td>804 (62%)</td>
<td>Referent</td>
</tr>
<tr>
<td>Age ≥ 65 years</td>
<td>590 (46%)</td>
<td>639 (50%)</td>
<td>606 (47%)</td>
<td>563 (47%)</td>
<td>486 (38%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender</td>
<td>325 (25%) / 964 (75%)</td>
<td>523 (41%) / 763 (59%)</td>
<td>635 (49%) / 657 (51%)</td>
<td>724 (56%) / 565 (44%)</td>
<td>840 (65%) / 450 (35%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ethnicity</td>
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<tr>
<td>White</td>
<td>665 (52%)</td>
<td>543 (42%)</td>
<td>465 (36%)</td>
<td>435 (36%)</td>
<td>408 (32%)</td>
<td>Referent</td>
</tr>
<tr>
<td>Chinese</td>
<td>194 (15%)</td>
<td>173 (13%)</td>
<td>164 (13%)</td>
<td>123 (13%)</td>
<td>119 (9%)</td>
<td>0.66</td>
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<tr>
<td>Black</td>
<td>226 (18%)</td>
<td>308 (24%)</td>
<td>389 (30%)</td>
<td>430 (33%)</td>
<td>395 (31%)</td>
<td>&lt;0.0001</td>
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<td>Hispanic</td>
<td>204 (16%)</td>
<td>262 (20%)</td>
<td>274 (20%)</td>
<td>301 (23%)</td>
<td>368 (29%)</td>
<td>&lt;0.0001</td>
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<td>Smoking Status</td>
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<tr>
<td>Never</td>
<td>746 (58%)</td>
<td>663 (52%)</td>
<td>695 (54%)</td>
<td>615 (48%)</td>
<td>533 (41%)</td>
<td>Referent</td>
</tr>
<tr>
<td>Past</td>
<td>432 (34%)</td>
<td>483 (38%)</td>
<td>435 (34%)</td>
<td>498 (39%)</td>
<td>525 (41%)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Current</td>
<td>105 (8%)</td>
<td>135 (11%)</td>
<td>159 (12%)</td>
<td>172 (13%)</td>
<td>228 (18%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pack Years</td>
<td>21.1 (23.2)</td>
<td>21.6 (21.6)</td>
<td>24.8 (27.1)</td>
<td>23.4 (25.5)</td>
<td>24.5 (33.7)</td>
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<td>Current Alcohol</td>
<td>699 (71%)</td>
<td>669 (68%)</td>
<td>672 (66%)</td>
<td>719 (66%)</td>
<td>782 (71%)</td>
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<td>Drinks/Week</td>
<td>2.4 (3.7)</td>
<td>3.5 (5.1)</td>
<td>3.5 (5.1)</td>
<td>4.4 (6.8)</td>
<td>5.9 (8.0)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Total Intentional Exercise (MET-min/Wk)</td>
<td>1499 (2131)</td>
<td>1529 (2286)</td>
<td>1548 (2196)</td>
<td>1558 (2580)</td>
<td>1609 (2841)</td>
<td>0.75</td>
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<tr>
<td>Glucose (mg/dl)</td>
<td>85.0 (80-91)</td>
<td>88.0 (82-95)</td>
<td>91.0 (84-100.5)</td>
<td>92.0 (85-103)</td>
<td>93.0 (86-106)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>3.8 (2.7-5.5)</td>
<td>4.8 (3.3-7.2)</td>
<td>5.6 (3.8-8.6)</td>
<td>6.2 (4.1-9.4)</td>
<td>7.1 (4.4-11.1)</td>
<td>&lt;0.0001</td>
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<tr>
<td>HbA1C (%)</td>
<td>5.4 (5.1-5.6)</td>
<td>5.5 (5.2-5.8)</td>
<td>5.5 (5.2-5.9)</td>
<td>5.6 (5.3-6.0)</td>
<td>5.6 (5.3-6.1)</td>
<td>&lt;0.0001</td>
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<tr>
<td>HOMA-IR</td>
<td>0.8 (0.6-1.2)</td>
<td>1.0 (0.7-1.6)</td>
<td>1.3 (0.8-2.1)</td>
<td>1.4 (0.9-2.3)</td>
<td>1.6 (1.0-2.7)</td>
<td>&lt;0.0001</td>
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<td>Waist circumference (cm)</td>
<td>91.9 (14.3)</td>
<td>96.2 (14.3)</td>
<td>99.0 (14.0)</td>
<td>101.1 (13.8)</td>
<td>101.4 (13.4)</td>
<td>&lt;0.0001</td>
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<td>Diabetes Medications</td>
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<td>None</td>
<td>1,218 (94%)</td>
<td>1,184 (92%)</td>
<td>1,159 (89%)</td>
<td>1,150 (89%)</td>
<td>1,124 (87%)</td>
<td>Referent</td>
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<tr>
<td>Insulin</td>
<td>11 (1%)</td>
<td>19 (2%)</td>
<td>20 (2%)</td>
<td>21 (2%)</td>
<td>33 (3%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Oral</td>
<td>60 (5%)</td>
<td>83 (6%)</td>
<td>113 (9%)</td>
<td>118 (9%)</td>
<td>133 (10%)</td>
<td>&lt;0.0001</td>
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<td>Total cholesterol (mg/dl)</td>
<td>192.0 (32.6)</td>
<td>192.8 (35.7)</td>
<td>193.5 (35.3)</td>
<td>195.3 (34.8)</td>
<td>196.8 (37.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>114.1 (29.4)</td>
<td>116.6 (31.1)</td>
<td>118.3 (31.0)</td>
<td>119.6 (31.9)</td>
<td>118.7 (32.5)</td>
<td>&lt;0.0001</td>
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<td>HDL-cholesterol (mg/dl)</td>
<td>56.7 (15.3)</td>
<td>52.4 (15.5)</td>
<td>49.4 (14.1)</td>
<td>48.4 (13.3)</td>
<td>48.0 (13.8)</td>
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<td>Triglycerides (mg/dl)</td>
<td>94.0 (68-127)</td>
<td>104.0 (74-146)</td>
<td>111.0 (78-158)</td>
<td>119.0 (83-174)</td>
<td>132.0 (89-191)</td>
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<td>Lipid Medications</td>
<td>171 (13%)</td>
<td>210 (16%)</td>
<td>216 (17%)</td>
<td>234 (18%)</td>
<td>218 (17%)</td>
<td>0.03</td>
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<td>SBP (mm Hg)</td>
<td>121.9 (21.6)</td>
<td>125.7 (22.0)</td>
<td>127.7 (21.7)</td>
<td>128.1 (21.0)</td>
<td>129.2 (20.5)</td>
<td>&lt;0.0001</td>
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<td>DBP (mm Hg)</td>
<td>68.3 (10.0)</td>
<td>70.4 (9.9)</td>
<td>72.4 (10.1)</td>
<td>74.4 (10.1)</td>
<td>73.9 (10.2)</td>
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<td>Hypertension (≥140/90 or treatment)</td>
<td>466 (36%)</td>
<td>531 (41%)</td>
<td>591 (46%)</td>
<td>642 (50%)</td>
<td>650 (50%)</td>
<td>&lt;0.0001</td>
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</table>
Figure 1a. Odds Ratio* of the Metabolic Syndrome by GGT Quintile in Ethnic Subgroups

*Adjusted for site, age, gender, waist circumference, alcohol, smoking and exercise plus ethnicity in entire cohort

Figure 1b. Odds Ratio* of Type 2 Diabetes by GGT Quintile in Ethnic Subgroups

*Adjusted for site, age, gender, waist circumference alcohol, smoking and exercise plus ethnicity in entire cohort

Figure 1c. Odds Ratio* of Metabolic Syndrome by GGT Quintile in Subgroups Defined by Age < or ≥ 65

*Adjusted for site, age, gender, waist circumference, alcohol, smoking and exercise plus ethnicity in entire cohort

Figure 1d. Odds Ratio* of Type 2 Diabetes by GGT Quintile in Subgroups Defined by Age < or ≥ 65

*Adjusted for site, age, gender, waist circumference, alcohol, smoking and exercise plus ethnicity in entire cohort
GGT: Inflammation, Oxidation & Endothelial Dysfunction

Figure 1a. Trends in C-reactive Protein (CRP) by GGT Quintile

- Q1 = <24.5 U/L
- Q2 = 24.5-29.3 U/L
- Q3 = 29.3-35.1 U/L
- Q4 = 35.1-42.2 U/L
- Q5 > 45.2 U/L

GGT Quintile

p<0.0001 for all linear trends; adjusted for age, sex, site and ethnicity (in the entire cohort)

Figure 1b. Trends in Soluble Interleukin-1 (sICAM-1) by GGT Quintile

- Q1 = <24.5 U/L
- Q2 = 24.5-29.3 U/L
- Q3 = 29.3-35.1 U/L
- Q4 = 35.1-42.2 U/L
- Q5 > 45.2 U/L

GGT Quintile

p<0.0001 for all linear trends; adjusted for age, sex, site and ethnicity (in the entire cohort)

Figure 1c. Trends in Interleukin-6 (IL-6) by GGT Quintile

- Q1 = <24.5 U/L
- Q2 = 24.5-29.3 U/L
- Q3 = 29.3-35.1 U/L
- Q4 = 35.1-42.2 U/L
- Q5 > 45.2 U/L

GGT Quintile

p=0.0001 for trend in the entire cohort and White and Hispanic subgroups; p=0.001 for Black and p=0.14 for Chinese; adjusted for age, sex, site and ethnicity (in the entire cohort)

Figure 1d. Trends in Oxidized LDL (oxLDL) by GGT Quintile

- Q1 = <24.5 U/L
- Q2 = 24.5-29.3 U/L
- Q3 = 29.3-35.1 U/L
- Q4 = 35.1-42.2 U/L
- Q5 > 45.2 U/L

GGT Quintile

p=0.0001 for trend in entire cohort and White subgroup; p=0.10 for Chinese; p=0.14 for Black; and p=0.11 for Hispanic; adjusted for age, sex, site and ethnicity (in the entire cohort)
Criteria to Meet before Applying Emerging Biomarkers of CVD Risk

• Measurement provides **independent risk assessment** not captured by measurement per current standards/clinical norms

• Treatment is available, **validated and unique**

• Treatment **improves hard clinical outcomes**

• Measurement and treatment are **risk-balanced and cost effective**
Endothelial Dysfunction

• Early, continuous disease process of vascular inflammation and reduced NO

• Effects accumulate and result in:
  • ischemia,
  • plaque formation, and
  • modified metabolic signaling
EndoPAT Procedure

3 Phases:
- Pre-occlusive,
- Occlusive, and
- Post-occlusive/re-perfusion

Final result = Reactive Hyperemia Index (RHI)
- post-occlusive diameter : pre-occlusive diameter
EndoPAT, RHI and Risk

Outcomes Response Curve
EndoPAT vs. Framingham Risk Score

Cardiac hospitalization (%)

- High risk & endothelial dysfunction
- High risk & normal endothelial function
- Low risk & endothelial dysfunction
- Low risk & normal endothelial function

Years from EndoPAT study

Figure 5
Sensitivity: RHI vs. cIMT

Fig. 1. Comparison of peripheral circulation markers between normal subjects (n=13) and patients with CAD (n=40). The patients had impaired forearm hyperemia and greater carotid IMT compared with control subjects. There was no difference in ABI between the groups. **p<0.01, ***p<0.001 compared with normal subjects.

Fig. 2. The sensitivity of subclinical markers in the younger (n=16) and older patients (n=24). Both forearm hyperemia and albuminuria were sensitive markers irrespective of age, whereas carotid ultrasound was less sensitive in the younger age group (p<0.01). The sensitivity of ABI was very low in these patients.
RHI adds to Reynolds Risk Score to Identify Ischemic Heart Disease in Women

Figure 5: ROC Curves to Identify NOCAD
Receiver-operating characteristic (ROC) curves for Reynolds Risk Score (RRS) and RHI-PAT index to identify patients with NOCAD among women without obstructive CAD. Abbreviations as in Figures 1 and 4.

Figure 4: ROC Curves to Identify Obstructive CAD and IHD
Receiver-operating characteristic (ROC) curves for the Reynolds Risk Score (RRS) and IHD-PAT index to identify patients with obstructive CAD and patients with IHD in stable women complaining of chest pain. AUC = area under the curve; other abbreviations as in Figure 1.

Figure 1. Plaque composition in patients with normal (*black bars*) or abnormal (*gray bars*) reactive hyperemia index in all vessel segments pooled (*n* = 594).
RHI & Plaque Composition

Figure 2. Intravascular ultrasound of normal and abnormal reactive hyperemia indexes and intravascular ultrasound virtual histology for fibrous (green), fibrofatty (greenish-yellow), necrotic core (red), and dense calcium (white) tissues. The patient with abnormal reactive hyperemia index has more necrotic core and dense calcium tissue and less fibrous and fibrofatty tissue compared to the patient with normal reactive hyperemia index. (For interpretation of the references to color in this figure legend, please refer to the web version of the article).

Criteria to Meet before Applying Emerging Biomarkers of CVD Risk: RHI

- Measurement provides independent risk assessment not captured by measurement per current standards/clinical norms

- Treatment is available, validated and unique

- Treatment improves hard clinical outcomes

- Measurement and treatment are risk-balanced and cost effective
Cystatin C

- Biomarker for glomerular filtration rate (GFR)
- Unlike creatinine-estimated GFR, Cystatin C is independent of age, sex & lean muscle mass
  - Cysteine protease inhibitor produced & excreted by all human cells
  - Freely filtered by glomerulus & metabolized in prox. tubule
- May be elevated in hypothyroidism & depressed in hyperthyroidism
Figure 1. Mortality from All Causes According to Quintile of Measures of Renal Function.

For cystatin C, creatinine, and estimated glomerular filtration rate (GFR), the fifth quintile was subdivided into three roughly equal groups, labeled 5a, 5b, and 5c.
Cystatin C & CVD-related Mortality

- Highest quintile (after adj. for traditional risk factors):
  - CV-related Death: HR=2.27 (95% CI: 1.73-2.97)
  - MI: HR=1.48 (95% CI: 1.08-2.02)
  - Stroke: HR=1.47 (95% CI: 1.09-1.96)

Shlipak et al. NEJM. 2005
Cystatin C & Death in those w/o CKD?

Cystatin C +/- CKD: CV-events & death

Figure 3. Cardiovascular events and deaths per 1000 years.

- No chronic kidney disease and low cystatin C level
- No chronic kidney disease and high cystatin C level
- Chronic kidney disease

Shlipak et al. NEJM. 2005
Criteria to Meet before Applying Emerging Biomarkers of CVD Risk: Cystatin C

- Measurement provides unique/independent risk assessment not captured by measurement per current standards/clinical norms

- Treatment is available, validated and unique

- Treatment improves hard clinical outcomes

- Measurement and treatment are risk-balanced and cost effective

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## Summary of Emerging Risk Factors for CVD

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0=red, 1=orange, 2=green; scored double for “unique risk assessment” and “improved outcomes” *limited populations or outcomes, thus ½ possible points awarded

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Conclusions: Assessing CVD Risk

• **Start here:**
  - Traditional risk factors of CVD are important, deserve detection and treatment for reduction
    - LDL, HDL, and BP still account for 67.1% of PAR for MI

• **The go here:**
  - Total INTERHEART factors (smoking, stress, lifestyle, etc.) account for 90-94%
  - Until lifestyle factors in place, and treatment goals met, why do anything else?

• **Then consider:**
  - First tier:
    - CRP
    - Endothelial function assessment (RHI)
    - Lipoprotein particle number (although consider waiting until LDL:HDL optimized)
  - Second tier:
    - oxLDL
    - GGT (although co-linear with CRP and oxLDL)
    - Cystatin C
  - Select ordering:
    - Hcy for stroke risk assessment
    - Lp(a) in select high-risk populations

• Emerging risk factors should be incorporated cautiously until validated by prospective data on outcomes, co-lateral risk, and costs.
HEART DISEASE RISK

THE PAIN MAY BE DUE TO YOUR YIN AND YANG
BEING OUT OF CELESTIAL ALIGNMENT. BUT HUMOUR
ME AND LET'S SEE IF YOUR BROKEN LEG IS PART
OF THE PROBLEM.

Diet, Stress, LDL, HDL, Triglycerides & Blood Pressure