Inflammatory Biomarkers to Define Cardiovascular Risk

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Inflammatory Biomarkers to Define Cardiovascular Risk
Biomarker risk strategy

Long-Term Risk → Mid-Term Risk → Near-Term Risk

- Life long
- Decade(s)
- Years

- Classic Lipid Panel
- Advanced Lipid Testing
- Inflammatory Markers
Cleveland HeartLab mission statement

• **Mission**: Through increasing our understanding of the **inflammatory** related molecular pathways involved in disease we define novel biomarkers that enable physicians

  ✓ Better define patients at risk
  ✓ Identify the source and cause of the risk
  ✓ Provide tools to monitor therapeutic response

Minimize cardiovascular events & improve outcomes
Mission: Through increasing our understanding of the inflammatory related molecular pathways involved in disease we define novel biomarkers that enable physicians

- Better define patients at risk
- Identify the source and cause of the risk
- Provide tools to monitor therapeutic response

Minimize cardiovascular events & improve outcomes
Why monitor inflammation?

Hazard Ratios for Incident Cardiovascular Events in the JUPITER Trial According to Achieved Concentrations of LDL Cholesterol and hsCRP After Initiation of Rosuvastatin Therapy

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>7832</td>
<td>1.11</td>
</tr>
<tr>
<td>LDL≥70mg/dL, hsCRP≥2 mg/L</td>
<td>1384</td>
<td>1.11</td>
</tr>
<tr>
<td>LDL&lt;70mg/dL, hsCRP≥2 mg/L</td>
<td>2921</td>
<td>0.62</td>
</tr>
<tr>
<td>LDL≥70mg/dL, hsCRP&lt;2 mg/L</td>
<td>726</td>
<td>0.54</td>
</tr>
<tr>
<td>LDL&lt;70mg/dL, hsCRP&lt;2 mg/L</td>
<td>2685</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Treatment benefits occur when you reduce both LDL and hsCRP\(^2\)

Why monitor inflammation?

Atherosclerosis is a chronic inflammatory disease\(^1\)

Markers of inflammation help refine cardiovascular risk estimation

FRANKLIN H. EPSTEIN, M.D., Editor

ATHEROSCLEROSIS — AN INFLAMMATORY DISEASE

RUSSELL ROSS, PH.D.

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Why monitor inflammation?

Studies continue to show the value of assessing the *degree* of inflammation in a patient.
Why monitor inflammation?

A patient’s degree of inflammation can change quickly and should be monitored routinely

ATPIII – Potential of inflammatory biomarkers to more properly assess hidden CVD risk and should be an adjunct to traditional risk factor assessment
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  ✓ Provide tools to monitor therapeutic response

  Minimize cardiovascular events & improve outcomes
Cleveland HeartLab – Inflammatory panel

Spectrum of Risk

- Oxidation
- Plaque deposition
- Plaque growth/vulnerable plaque
- Plaque rupture
- Acute coronary syndrome

- F2-Isoprostanes (F2-Isops)
- hsCRP (high-sensitivity C-reactive protein)
- Microalbumin
- MPO (myeloperoxidase)
- Lp-PLA2
- Troponin T
- CK-MB

- Inflammation
- Myeloperoxidase
- Thickening
- Plaque
- Calcium build-up
- Vulnerable plaque
- Stiffening wall
- Rupture
- Blood clot
F$_2$-Isoprostanes (F$_2$-IsoPs)

- **Low Risk**
  - Lifestyle
  - Endothelial dysfunction

- **Moderate Risk**
  - Oxidation
  - Plaque deposition
  - Plaque growth/vulnerable plaque

- **High Risk**
  - Plaque rupture
  - Vessel vulnerable plaque
  - WBC activation
  - Acute coronary syndrome

- **Markers**
  - F$_2$-IsoPs
  - Microalbumin
  - hscRP
  - MPO
  - Lp-PLA$_2$
  - Troponin T
  - CK-MB

- Processes
  - Inflammation
  - Myeloperoxidase
  - Thickening
  - Plaque
  - Calcium build-up
  - Vulnerable plaque
  - Stiffening wall
  - Rupture
  - Blood clot
What are $F_2$-IsoPs?

- Prostaglandin-like compounds formed from free-radical initiated peroxidation of arachidonic acid (essential free fatty acids)
What are $F_2$-IsoPs?

- Lifestyle markers (inversely related to conditioning)

- Exercise daily & eat healthy
- Sedentary lifestyle, eat poorly & smoke

Low $F_2$-IsoPs  
High $F_2$-IsoPs
Clinical implications of $F_2$-IsoPs testing

Cigarette smoking and predisposition to disease

- Smoking increases $F_2$-IsoPs production levels which can be reduced with cessation

Case-controlled study

- 10 smokers
  - > 1.5 packs/day
- 10 non-smokers
  - Matched for age and sex
- Plasma samples

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High-sensitivity C-reactive protein (hsCRP)
Clinical implications of hsCRP testing

Cardiovascular events

• Baseline hsCRP levels in *apparently healthy* men can predict the risk of first myocardial infarction or ischemic stroke\(^1\)

Physicians’ Health Study

• 1,086 men (>8 yrs)
• hsCRP measured at baseline

Clinical implications of hsCRP testing

Cardiovascular events (cont’d)

• hsCRP is a stronger predictor of cardiovascular events in women than LDL-C and adds prognostic information to Framingham risk scores\(^1\)

Women’s Health Study

• 28,345 women (8 yrs.; 15,745 were not on HRT)
• hsCRP and LDL-C measured at baseline

New FDA indication for CRESTOR (Rosuvastatin)

• February 8, 2010:

**CRESTOR will now be indicated for the primary prevention of cardiovascular disease in individuals who have no clinically evident heart disease but are at an increased risk of heart disease due to the combined effect of the following risk factors:**

- Age ($\geq 50$ years in men; $\geq 60$ years in women)
- An elevated high-sensitivity C-reactive protein level ($\geq 2$ mg/L)
- Presence of at least one additional cardiovascular risk factor
  - High blood pressure
  - Low HDL-C
  - Smoking
  - Family history of premature heart disease
Urinary Microalbumin

**Low Risk**
- Lifestyle
  - Endothelial dysfunction

**Moderate Risk**
- Oxidation
- Plaque deposition
- Plaque growth/vulnerable plaque

**High Risk**
- Vessel vulnerable plaque
- WBC activation
- Plaque rupture
- Acute coronary syndrome
- Troponin T
- CK-MB

**Markers**
- F_{2}-IsoPs
- hsCRP
- Microalbumin
- MPO
- Lp-PLA_{2}

**Processes**
- Inflammation
- Myeloperoxidase
- Thickening
- Plaque build-up
- Vulnerable plaque
- Stiffening wall
- Rupture
- Blood clot
Clinical implications of urinary microalbumin testing

Cardiovascular events and death

- Elevated levels of microalbuminuria are a robust independent continuous risk factor for cardiovascular events and death\(^1\)

The HOPE study

- 5,545 (w/o DM; history of CVD)
- 3,498 (w/ DM + at least 1 risk factor)

Clinical implications of urinary microalbumin testing

Cardiovascular events and death

- Urinary microalbumin $\geq$ sex specific median experienced a nearly 3-fold increased risk of CVD compared to those below median\(^1\)

The FHS

- 1,568 nonhypertensive, nondiabetic Framingham offspring participants free of CVD
- Mean age 55 yrs. (58% women)
- ~6 yrs. follow-up
- Median:  
  Men: $\geq 3.9 \, \mu g/mg$
  Women: $\geq 7.5 \, \mu g/mg$
- Reference range for CHL: $< 30 \, mg/g$. The more sensitive values are annotated w/in our report.

Lipoprotein-Associated Phospholipase-A$_2$ (Lp-PLA$_2$; The PLAC® Test)

**Low Risk**
- Lifestyle

**Moderate Risk**
- Endothelial dysfunction

**High Risk**
- Vessel vulnerable plaque
- WBC activation

Processes leading to Acute coronary syndrome:
- Oxidation
- Plaque deposition
- Plaque growth/vulnerable plaque
- Plaque rupture
- Troponin T CK-MB

**Markers**
- F$_2$-IsoPs
- hsCRP
- Microalbumin
- MPO
- Lp-PLA$_2$
Lipoprotein-Associated Phospholipase-A₂ (Lp-PLA₂; The PLAC® Test)

What does Lp-PLA₂ measure?
- The amount of plaque within the artery wall due to accumulation of oxidized LDL
Clinical implications of The PLAC® Test

- Elevated Lp-PLA₂ levels are independently associated with high stroke risk in individuals who have low LDL-C levels.

The ARIC study
- 960 middle-aged men and women
- Follow-up ~6-8 yrs.

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Myeloperoxidase (MPO)

- **Low Risk**
  - Lifestyle
  - Endothelial dysfunction

- **Moderate Risk**
  - Oxidation
  - Plaque deposition
  - Plaque growth/vulnerable plaque

- **High Risk**
  - Vessel vulnerable plaque
  - WBC activation
  - Plaque rupture
  - Acute coronary syndrome

- Key markers:
  - F$_2$-IsoPs
  - Microalbumin
  - hsCRP
  - MPO
  - Lp-PLA$_2$
  - Troponin T
  - CK-MB

- Processes:
  - Inflammation
  - Myeloperoxidase
  - Thickening
  - Plaque
  - Calcium build-up
  - Vulnerable plaque
  - Stiffening wall
  - Rupture
  - Blood clot
Introduction to Myeloperoxidase (MPO)

- An enzyme synthesized and stored within polymorphonuclear leukocytes (PMNs) and monocytes

- MPO generates potent anti-microbial oxidants
  - Used by invading PMNs and monocytes to kill bacteria and other pathogens
  - However, oxidation products can damage surrounding vasculature, and are enriched within human atherosclerotic plaque

- MPO is a specific marker of plaque vulnerability
Myeloperoxidase (MPO)

What does MPO measure?

- The amount of leukocyte activity in response to arterial inflammation or erosion
- Increased levels of MPO indicate increased risk for plaque rupture
Time release of various inflammatory biomarkers
Clinical implications of MPO testing

- A single, initial measurement of MPO independently predicts the early risk of MI and MACE independent of myocardial necrosis\(^1\)

<table>
<thead>
<tr>
<th>Myeloperoxidase Quartile</th>
<th>(\leq 119.4) pM</th>
<th>(119.4-197.9) pM</th>
<th>(198.0-393.9) pM</th>
<th>(\geq 394.0) pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major adverse cardiac event</td>
<td>odds ratio (95 percent confidence interval)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 30 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>1.0</td>
<td>1.7 (1.02–2.8)(\dagger)</td>
<td>3.2 (2.0–5.4)(\ddagger)</td>
<td>4.7 (2.8–7.7)(\ddagger)(\dagger)</td>
</tr>
<tr>
<td>Patients persistently negative for troponin T</td>
<td>1.0</td>
<td>2.2 (1.1–4.6)(\dagger)</td>
<td>4.2 (2.1–8.4)(\ddagger)</td>
<td>4.1 (2.0–8.4)(\ddagger)(\dagger)</td>
</tr>
<tr>
<td>At 6 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>1.0</td>
<td>1.6 (1.0–2.7)</td>
<td>3.6 (2.2–5.8)(\ddagger)</td>
<td>4.7 (2.9–7.7)(\ddagger)(\dagger)</td>
</tr>
<tr>
<td>Patients persistently negative for troponin T</td>
<td>1.0</td>
<td>1.9 (1.0–3.8)</td>
<td>4.4 (2.3–8.4)(\ddagger)</td>
<td>3.9 (2.0–7.7)(\ddagger)(\dagger)</td>
</tr>
</tbody>
</table>

Time release of various inflammatory biomarkers

Example only

MI Patient

Myeloperoxidase
Myoglobin
CK-MB
C-reactive Protein
Troponin I
B-type Natriuretic Peptide

Apparent Healthy
Stable Angina
0 - 3
3 - 6
6 - 12
12 - 24
24 - 48
[hours after pain onset]
Clinical implications of MPO testing

- MPO levels increase with the severity of CAD, and more accurately predicts ACS than hsCRP\(^1\)

Clinical implications of MPO testing

- Elevated MPO levels predict cardiovascular mortality at 13 yrs in patients with angiographic evidence of CAD\(^1\)

\[\text{Log-rank test: } p=0.007\]

First tertile (lowest)

Second tertile

Third tertile (highest)

HR: 2.38 (95% CI: 1.47-2.98) for top vs bottom MPO tertile

\(^1\)Modified from Heslop CL et al. Myeloperoxidase and C-reactive protein have combined utility for long-term prediction of cardiovascular mortality after coronary angiography. *J Am Coll Cardiol*. 2010; 55:1102-1109.
Clinical implications of MPO testing

- MPO and CRP have combined utility in predicting cardiovascular mortality risk in patients with angiographic evidence of CAD\(^1\)

![Graph showing cumulative survival over time with different MPO and CRP levels.](image)

- Patients with either a high MPO or high CRP elevated had 5.3-fold higher mortality risk.
- Patients with high levels of both MPO and CRP had a 4.3-fold risk vs. patients with only one elevated marker.

Log-rank test: p<0.001 for trend

\(^1\)Modified from Heslop CL et al. Myeloperoxidase and C-reactive protein have combined utility for long-term prediction of cardiovascular mortality after coronary angiography. J Am Coll Cardiol. 2010; 55:1102-1109.
Time release of various inflammatory biomarkers

Example only

Apparent Healthy

Stable Angina

0 - 3

3 - 6

6 - 12

12 - 24

12 - 24

24 - 48

[hours after pain onset]
Clinical implications of MPO testing

- In apparently healthy individuals, mean MPO levels were greater according to increasing CAC categories, and the risk for CVD increased by quartiles of MPO\textsuperscript{1}

\textsuperscript{1}Wong ND et al. Myeloperoxidase, subclinical atherosclerosis, and cardiovascular disease events. \textit{J Am Coll Cardiol Img.} 2009; 2: 1093-1099.
Clinical implications of MPO testing

- In apparently healthy individuals, moderate and significant CAC (≥100) and MPO levels (≥257 pm) demonstrated increased risk for CVD

MPO levels ≥ 257 pm remained an independent predictor of CVD events even after adjusting for various risk factors (HR: 1.9, p=0.04)

\[ \text{Log-rank test for trend } p<0.001 \]

\[ \begin{array}{c|c|c|c}
MPO<257 \text{ pm} & MPO≥257 \text{ pm} \\
--- & --- \\
3.0\% & 3.9\% \\
3.2\% & 14.0\% \\
7.1\% & \\
\end{array} \]

\[ \text{CAC 0-9} \quad \text{CAC 10-99} \quad \text{CAC≥100} \]

\textsuperscript{1}Wong ND et al. Myeloperoxidase, subclinical atherosclerosis, and cardiovascular disease events. \textit{J Am Coll Cardiol Img.} 2009; 2: 1093-1099.
Clinical implications of MPO testing

- Elevated MPO levels predict a significantly higher incidence of cardiovascular events in patients with PAD\(^1\)

\[ \text{MPOx} > 183.7 \text{ pM had higher hsCRP levels versus MPOx} \leq 183.7 \text{ pM} \]

Clinical implications of MPO testing

- Measurement of MPO, in addition to ABI, improved the ability to identify PAD patients at risk for MI and stroke\(^1\)

Lp-PLA$_2$ and MPO identify unique patients
Elevated MPO and ATPIII

Note: The example above is from an academic institution which uses MPO in algorithms based upon peer reviewed publications and discussions of existing literature. They incorporate many of the proprietary markers as part of patient care, and use the results to impact not only the ATPIII goals for LDL lowering, but also global preventative, cardiovascular risk management, including therapeutic lifestyle goals of nutrition and exercise, blood pressure goals, platelet prophylaxis therapy triggers, and stroke prevention efforts.
Cleveland HeartLab – Inflammatory Panel

Spectrum of Risk

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- Plaque growth/vulnerable plaque
- Plaque rupture
- Acute coronary syndrome

Markers:
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Minimize cardiovascular events & improve outcomes
Monitoring Tools

• Nitrotyrosine - anti-inflammatory effect of statin therapy
• DysHDL – portion of HDL that is oxidized and not part of plaque regression
• CoQ10 – mitochondrial energetics in primary prevention and heart failure
• Oxidized Fatty Acids – diagnosis and monitor NASH
CoQ10 and Statin Therapy

• There are recurrent concerns about the effects of HMG-CoA reductase therapy and CoQ10 levels

• Decreases in CoQ10 is thought to be related to statin myopathy
Statin Therapy Inhibits CoQ10 Synthesis
CoQ10 and Statin Therapy

- **CoQ10 Group (n=24)**
  - Before: CoQ10 (100mg/day)
  - 4 week: CoQ10 (100mg/day)
  - 8 week: Atorvastatin (10mg/day)
  - 12 week: Atorvastatin (10mg/day)
  - 4 week after withdrawal

- **Placebo Group (n=25)**
  - Before: Placebo
  - 4 week: Placebo
  - 8 week: Placebo
  - 12 week: Placebo
  - 4 week after withdrawal
CoQ10 Decreases with Statin Therapy

<table>
<thead>
<tr>
<th>Time</th>
<th>Total CoQ10 (µmol/L)</th>
<th>CoQ10 $p$</th>
<th>Placebo $p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>1.113 ± 0.444</td>
<td></td>
<td>1.180 ± 0.282</td>
</tr>
<tr>
<td>4 week</td>
<td>2.402 ± 0.825**</td>
<td>0.731 ± 0.201***</td>
<td></td>
</tr>
<tr>
<td>8 week</td>
<td>2.402 ± 0.738***</td>
<td>0.711 ± 0.256***</td>
<td></td>
</tr>
<tr>
<td>12 week</td>
<td>2.531 ± 0.874***</td>
<td>0.691 ± 0.234***</td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>0.866 ± 0.430*</td>
<td>0.762 ± 0.325***</td>
<td></td>
</tr>
</tbody>
</table>

Atherosclerosis. 2007 195:e182-9
Role of CoQ10 in ETC
CoQ10 Supplementation and Statin Myopathy

Pain Severity Score

Coenzyme Q10

Vitamin E

Am J. Cardiol. 2007 99:1409-12
CoQ10 Supplementation and Statin Myopathy

<table>
<thead>
<tr>
<th>Tolerated Dose</th>
<th>CoQ10 + Simvastatin</th>
<th>Simvastatin</th>
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</thead>
<tbody>
<tr>
<td>40</td>
<td>16 (73%)</td>
<td>13 (59%)</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>2 (9%)</td>
</tr>
<tr>
<td>0</td>
<td>6 (27%)</td>
<td>4 (18%)</td>
</tr>
</tbody>
</table>

Am J. Cardiol. 2007 100:1400-03
## CoQ10 Supplementation and Statin Myopathy

<table>
<thead>
<tr>
<th></th>
<th>CoQ10 + Simvastatin</th>
<th>Simvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in Myalgia Score</td>
<td>6.0 (2.1-8.8)</td>
<td>2.3 (0-12.8)</td>
</tr>
<tr>
<td>Change in CoQ10 (umol/L)</td>
<td>1.7 (0.3 to 2.2)</td>
<td>-0.5 (-0.6 to -0.3)</td>
</tr>
</tbody>
</table>
ApoA1 and Infarct Size
ApoA1 Null Have Low Cardiac CoQ10

![Graph showing CoQ10 levels in different conditions.](image)

* p<0.001, n=3
CoQ10 Supplementation Normalizes Infarct Size in ApoA1-/-
ApoA1 and Infarct Size

• Mouse data suggest that ApoA1 levels regulate CoQ10 levels
• IP injection of CoQ10 (bypassing the gut) leads to normalization of myocardial CoQ10
• These data are consistent with the concept that ApoA1 regulates CoQ10 absorption from the GI tract
Clinical data ?
Changes in ApoA1 Based on Statin

Atherosclerosis 2011 217:158-64
Changes in CoQ10 Levels Based on Changes in ApoA1

suggest ApoA1 levels predict CoQ10 levels with r value of ~0.56

Atherosclerosis 2011 217:158-64
Emerging HDL Panel From Cleveland HeartLab

- HDL
- DysHDL
- ApoA1
- CoQ10
Utility of HDL Panel

• HDL
  – Low HDL classic risk factor
  – *Target for therapy*

• DysHDL
  – Define if normal/high HDL is protective in given patient
  – Insight into benefits associated with modulation of HDL
  – *Monitor therapeutic benefit*
Utility of HDL Panel

• ApoA1
  – Secondary measure of HDL
  – May define CoQ10 absorption
  – *Monitor therapeutic response*

• CoQ10
  – Statin myopathy / CHF *treatment target*
  – Can define statin or approach
  – Further indication for need to raise HDL/ApoA1
  – *Therapeutic target*
Approach

• New patient with hypercholesterolemia
  – Low HDL
    • Quantify CoQ10 levels/ApoA1
    • If low CoQ10 pre-treatment consider
      – CoQ10 supplementation
      – Consider HDL raising therapies

• Patient titrated on statin
  – Should we target HDL?
    • ApoA1 and CoQ10 low
      – CoQ10 supplementation
      – Target HDL and follow CoQ10 levels
Approach

• “Doc, I have read a lot about CoQ10”

“Well you are on a statin which can lower CoQ10 levels, that said you are tolerating your statin well, but let’s look into it because if it is low you may be at increased risk for a bigger heart attach if/when the time comes …..”
CHL is an advanced cardiovascular disease management company

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