Assessing cardiovascular risk through use of inflammation testing

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Response to Injury Hypothesis

Lipids

Inflammation

50% of individuals who experience a heart attack or stroke have 'normal' lipid levels

Blood Pressure
Blood Glucose
Oxidation
Smoking
Age/Gender

Inflammation
The benefits of a multi-marker approach

- Individuals with impaired fasting glucose have a higher incidence of unrecognized myocardial infarctions.¹
- Silent heart attacks account for over 45% of all heart attacks.²
- A multi-marker strategy that combines biomarkers across various pathobiological axes provides incremental prognostic information for prediction of cardiovascular death.³


The standard of care utilizes a risk factor approach to identify risk

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Odds Ratio (adj. for all other risk factors 99% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoker</td>
<td>2.9</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.4</td>
</tr>
<tr>
<td>High BP</td>
<td>1.9</td>
</tr>
<tr>
<td>Abdominal Obesity</td>
<td>1.4</td>
</tr>
<tr>
<td>Lack of Exercise</td>
<td>1.3</td>
</tr>
<tr>
<td>High Cholesterol</td>
<td>3.3</td>
</tr>
</tbody>
</table>

The standard of care utilizes a risk factor approach to identify risk.

Inflammation testing can identify:
1. Those with 'hidden risk'
2. Those at greatest risk
3. Those who are 'worried well'
F₂-Isoprostanes (F₂-IsoPs)  Risk of Disease

Prostaglandin-like compounds formed from free radical mediated oxidation of arachidonic acid and excreted in the urine.

The 'gold standard' for measuring oxidative stress in the body.

F₂-Isoprostanes (F₂-IsoPs)

• Lifestyle markers (inversely related to conditioning)
  Exercise daily & eat healthy
  Sedentary lifestyle, eat poorly & smoke

Smoking increases F₂-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

Oxidized LDL (OxLDL)  Risk of Disease

Measures protein damage due to oxidative modification of the ApoB subunit on LDL cholesterol

LDL Oxidation is the initiating event for foam cell formation

High OxLDL levels precede development of metabolic syndrome and insulin insensitivity

OxLDL (OxLDL)

Poor Diet
Lack of Exercise
Lifestyle Risks

Metabolic syndrome
Early Onset Disease

Diabetes
Cardiovascular Disease
Chronic Disease

OxLDL is beyond F2-IsoPs which marks lifestyle risk and identifies altered LDL biology increasing risk for chronic disease

Inflammation testing

Low Risk
Risk of Disease

Moderate
Risk
Presence of Disease

High Risk
Disease Activity
ADMA/SDMA

Presence of Disease

Asymmetric dimethylarginine (ADMA)
Symmetric dimethylarginine (SDMA)

A measurement of endothelial dysfunction due to NO deficiency

Nitric oxide maintains endothelial health and is cardioprotective

- Reduced LDL oxidation
- Reduced artery wall thickening
- Reduced free radical formation
- Reduced inflammation in artery wall
- Improved dilation of blood vessels
- Reduced blood clotting

Nitric oxide maintains endothelial health and is cardioprotective

Measuring endothelial health – Past and present

Microalbumin
- Examines endothelial health in the kidneys due to reduced vascular integrity
- Structural damage
- Low Microalbumin = CV risk
- High Microalbumin = Renal risk

ADMA/SDMA
- Examines systemic endothelial health associated with reduced NO production
- Chemical damage
- High ADMA = CV risk
- High SDMA = Renal risk

Use as a surrogate for endothelial health in other vascular beds
Regulation of NO production by ADMA and SDMA

- ADMA directly blocks eNOS to inhibit NO production
- SDMA indirectly blocks NO production by inhibiting the availability of free L-Arginine.

ADMA and SDMA are excreted/degraded by distinct mechanisms, and therefore manifest differently

- ADMA is cleared gradually by degradation while SDMA is cleared rapidly through the urine
- Therefore, ADMA identifies endothelial dysfunction and CV risk whereas SDMA identifies renal insufficiency and subsequent renal failure

Individuals with elevated ADMA are 1.5x more likely to experience all-cause mortality

Framingham Offspring Study

- ‘Asymptomatic’ individuals (n = 3,320)
- Follow-up: ~11 yrs.

Table A: Associations of ADMA and SDMA with Mortality

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile 1</td>
<td>0.7 (0.1-4.8)</td>
<td>1.0 (0.0-7.6)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>1.2 (0.0-7.6)</td>
<td>1.0 (0.0-7.6)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>1.8 (0.0-15.0)</td>
<td>1.0 (0.0-7.6)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>3.5 (0.0-33.0)</td>
<td>1.0 (0.0-7.6)</td>
</tr>
</tbody>
</table>

Death rates decreased across increasing quartiles of Arginine and the Arg/ADMA ratio

AtheroGene Study
- Individuals w/ known CAD (n=1,874)
  - Microalbumin: Elevated ADMA
- Individuals who went on to have an event (n=114; or 6%)
  - Follow-up: ~3 yrs.

Individuals with known CAD and elevated ADMA are 2.5x more likely to experience a CV-related event

<table>
<thead>
<tr>
<th>Hazard Ratio According to Trofolds of Baseline ADMA</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00 (per 1.00 fold)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>


LURIC Study
- Individuals w/ & w/o CAD (n=3,238)
  - Follow-up: 6 yrs.

Individuals with unstable CAD plus elevated ADMA are 2.5x more likely to experience CV-related mortality

<table>
<thead>
<tr>
<th>Hazard Ratio for death from cardiovascular causes according to ADMA</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
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<td>1.00 (per 1.00 fold)</td>
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Measuring endothelial health – Past and present

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  - Low Microalbumin = CV risk
  - High Microalbumin = Renal risk
  - Use as a surrogate for endothelial health in other vascular beds

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  - Chemical damage
  - High ADMA = CV risk
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Microalbumin Presence of Disease

The quantification of small amounts of albumin in the urine that can identify microvascular integrity and endothelial dysfunction.

May suggest the presence of systemic endothelial dysfunction – an early indicator of heart disease.

Individuals with microalbumin levels above the gender-specific median had a nearly 3-fold increased risk of CVD

The Framingham Heart Study
- 1,568 nonhypertensive, nondiabetic offspring participants free of CVD
- Mean age 55 yrs. (58% women)
- ~6 yrs. follow-up
- Median: Men: ≥3.9 μg/mg
  Women: ≥7.5 μg/mg
- CHL reports the aforementioned gender-specific cut-offs


Elevated levels of microalbumin are a robust independent continuous risk factor for CV events and death

The HOPE Study
- 5,545 (w/o DM + history of CVD)
- ~3,498 (w/ DM + at lease 1 risk factor)

High-sensitivity C-reactive Protein (hsCRP)

A highly sensitive quantification of C-reactive protein - an acute phase protein - that may be associated with the presence of heart disease.

hsCRP Presence of Disease

To date, increased hsCRP levels have not been associated with vulnerable plaque suggesting that it is a marker of atheroma burden more so than plaque activity.

hsCRP

• An acute-phase protein released into the blood by the liver during inflammation
• Compared to standard CRP testing, hsCRP testing can more accurately detect lower concentrations of CRP making it more useful in predicting a healthy person’s risk for cardiovascular disease
• hsCRP is a well documented clinical marker of general and cardiac-related inflammation
Interpreting hsCRP results

<table>
<thead>
<tr>
<th>hsCRP mg/dL</th>
<th>Relative Risk</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1.0</td>
<td>Low Risk</td>
<td>Reflect low CV risk</td>
</tr>
<tr>
<td>1.0-3.0</td>
<td>Moderate Risk</td>
<td>Doubles risk for coronary events (compared with levels &lt;1 mg/L)</td>
</tr>
<tr>
<td>3.0-10.0</td>
<td>High Risk</td>
<td>Elevated vascular risk, 4-6x ↑ risk of diabetes</td>
</tr>
<tr>
<td>&gt;10.0</td>
<td>High Risk</td>
<td>Can reflect acute infection</td>
</tr>
</tbody>
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Acute Infection - hsCRP > 10.0 mg/dL
- Viral infections (10-40 mg/L)
- Active inflammation and bacterial infections (40-200 mg/L)
- Severe bacterial infections/bums (>200 mg/L)

How to Interpret hsCRP results

- Elevated levels should be confirmed at least 1 month later
- To date, increased hsCRP levels have not been associated with vulnerable plaque suggesting that it is a marker of atheroma burden more so than plaque activity.

Baseline hsCRP levels in ‘apparently healthy’ men can predict the risk of first MI or ischemic stroke

Physicians’ Health Study
- 1,086 men (>8 yrs)
- hsCRP measured at baseline

**hsCRP is a stronger predictor of cardiovascular events in women than LDL-C**

**Women's Health Study**
- 28,345 women (8 yrs.; 15,745 were not on HRT)
- hsCRP and LDL-C measured at baseline

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**Inflammation testing**

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**The two sides of vulnerable plaque**

**Outside the vessel wall**
MPO can be used to tell us if circulating white blood cells are being activated in response to fissures, erosions or warming plaque increasing the risk of vulnerable plaque rupture.

**Inside the vessel wall**
Lp-PLA₂ Activity can be used to tell us if there is active inflammation within the vessel wall that could contribute to vulnerable plaque formation.
Lp-PLA₂ Activity

- A vascular-specific inflammatory enzyme implicated in the formation of atherosclerotic plaque
- Bound primarily to small, dense LDL - highly atherogenic
  - In arterial wall, Lp-PLA₂ releases chemicals that generate an immune response to damaged LDL

Lp-PLA₂ is associated with risk of CHD and all vascular mortality

- Less distinct associations with ischemic stroke and the aggregate of non-vascular mortality were documented.

The two sides of vulnerable plaque

Outside the vessel wall
MPO can be used to tell us if circulating white blood cells are being activated in response to fissures, erosions or warning plaque increasing the risk of vulnerable plaque rupture

Inside the vessel wall
Lp-PLA₂ can be used to tell us if there is active inflammation within the vessel wall that could contribute to vulnerable plaque formation
Myeloperoxidase (MPO) Disease Activity

- Enzyme produced and stored within polymorphonuclear (PMNs) leukocytes and monocytes
- Generates anti-microbial oxidants that:
  - Invade PMN leukocytes and monocytes to kill bacteria and other pathogens
  - Damage surrounding vasculature
  - Are enriched within human atherosclerotic plaque

Myeloperoxidase (MPO)

- An enzyme synthesized and stored within polymorphonuclear (PMNs) leukocytes and monocytes
- MPO generates potent anti-microbial oxidants
  - That invade PMN leukocytes and monocytes to kill bacteria and other pathogens
  - That can also damage surrounding vasculature
  - That are enriched within human atherosclerotic plaque

Myeloperoxidase (MPO)

- MPO contributes to endothelial dysfunction
  - Diminishes nitric oxide bioavailability
- MPO contributes to cholesterol accumulation
  - Modifies LDL = atherogenic
  - Modifies HDL = dysfunctional
- Specific marker of arterial vulnerable plaque/erosions/fissures
  - Contributes to plaque instability by activating protease cascades that ultimately degrade the collagen cap
  - Increasing levels signify increasing risk for cardiac events

Time release of various inflammatory biomarkers

EPIC/Norfolk Study: MPO levels predict the risk of CAD in subjects otherwise associated with low risk

MPO levels are independently associated with incident CHD in a healthy population

The MONIKA/KORA Augsburg Study
- Population-based case-cohort study
- Middle-aged, healthy men and women
- 333 subjects with incident CHD (non-cases: 1727)
- Mean follow-up: ~10 years

MPO levels are independently associated with incident CHD in subjects otherwise associated with low risk.
MPO levels increase with the severity of CAD

Case-controlled Study
- 847 patients with angiographically proven CAD

MPO and CRP have combined utility in predicting CV mortality risk in patients with evidence of CAD

- Patients with either a high MPO or high CRP elevated had a 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

Elevated MPO levels can predict the incidence of death or MI in individuals with ACS

The CAPTURE Trial
- 1,090 patients with ACS
- Follow-up 6 months


The two sides of vulnerable plaque

Clinical Interpretation

Low Lp-PLA₂ and High MPO
Less active artery wall, but an unstable collagen cap

High Lp-PLA₂ and Low MPO
Active artery wall, but a stable collagen cap

High Lp-PLA₂ and High MPO
Active artery wall, and an unstable collagen cap

Anatomical and biological assessment of CV risk

- **Anatomy (structure)**
  Important, but may be hard to follow

- **Biology (pathophysiology)**
  Important, and can be measured routinely

*Together, both provide additive insight into a patient's risk for heart disease*


Elevated MPO levels predict a significantly higher incidence of cardiovascular events in patients with PAD

- MPOx >183.7 pM had higher hsCRP levels versus MPOx ≤183.7 pM

Measurement of MPO, in addition to ABI, improved the ability to identify PAD patients at risk for MI and stroke.


Inflammation testing

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Moderate Risk
High Risk
Risk of Disease
Presence of Disease
Disease Activity