How to get the most from “liquid gold” samples

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Disclosures

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Biomarkers

• A major area of confluence between
  Basic
  Clinical, and
  Population health
  Research and Strategies

Enormous and often asynchronous growth in knowledge in these different domains
Why Determine a Biomarker(s)

• Can biomarker be measured: accurately and reproducibly, with assay that is accessible, and allows high throughput at reasonable cost?

• Does biomarker add new information: strong and consistent association with outcome(s), adding to usual methods of assessment

• Can aid clinical management: superior performance to existing diagnostic tests, associated risk is modifiable with specific therapy, or biomarker-guided therapy or monitoring enhances care

• It may enable identification of a new therapeutic target, eg from genetic epidemiology
# LIPID Biomarker Analyses

<table>
<thead>
<tr>
<th>Samples taken at</th>
<th>Used in</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>b. Definitive Studies</td>
</tr>
<tr>
<td>Year 1 Plasma</td>
<td>Definitive Studies</td>
</tr>
<tr>
<td>Year 2 Plasma</td>
<td>a. Pilot Studies</td>
</tr>
<tr>
<td>Year 4 Plasma</td>
<td>Pilot Studies</td>
</tr>
<tr>
<td>Year 5 Plasma</td>
<td>Pilot Studies</td>
</tr>
<tr>
<td>Year 6 Plasma, whole blood</td>
<td>a. Definitive Studies &amp; REST STORED</td>
</tr>
</tbody>
</table>
EVALUATION OF INCREMENTAL GAIN
(and the EVOLUTION of METHODOLOGY)

• Establish association: Hazard ratio

• Improve discrimination: Sensitivity, specificity, C-statistic (ability to distinguish between two individuals who will and who will not develop an event)

• Improve calibration: Goodness of fit. Compare deciles of observed and predicted risk

• Improve risk classification of low/intermediate/high risk groups: Net reclassification index (% moved to different group). Limitations now recognised.

• Mediation analyses

With aim of improved management decisions and outcomes
### Proportion of Treatment Effect Explained by On-Study Lipid Levels

<table>
<thead>
<tr>
<th>Lipid Parameters</th>
<th>Risk Reduction, † % (95% CI)</th>
<th>P</th>
<th>PTE, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD death and nonfatal MI (n=8202)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>25 (14–34)</td>
<td>&lt;0.001</td>
<td>NA</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>14 (−1–26)</td>
<td>0.069</td>
<td>48 (9–88)</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>13 (−2–26)</td>
<td>0.094</td>
<td>52 (10–94)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>22 (12–32)</td>
<td>&lt;0.001</td>
<td>11 (2–20)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>23 (12–32)</td>
<td>&lt;0.001</td>
<td>9 (1–17)</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>22 (12–32)</td>
<td>&lt;0.001</td>
<td>11 (3–19)</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>9 (−6–22)</td>
<td>0.233</td>
<td>67 (24–110)</td>
</tr>
<tr>
<td>Total cholesterol, HDL</td>
<td>9 (−7–22)</td>
<td>0.267</td>
<td>67 (27–106)</td>
</tr>
</tbody>
</table>

LIPID Biomarker Analyses: Important Collaborations

San Diego
Mainz
Hamburg
Sydney
Melbourne
Perth
Biomarkers in the MORGAM Cohorts

**Lipid related markers**
- Apolipoprotein A1
- Apolipoprotein B100
- Lipoprotein - associated phospholipase A2
  - activity
  - mass
- Paraoxonase-1

**Markers of vascular function and neurohumoral activity**
- (N-terminal pro) B-type natriuretic peptide
- C-terminal pro-vasopressin
- C-terminal pro-endothelin-1
- Mid-regional pro-adrenomedullin
- Mid-regional pro-atrial natriuretic peptide
- Tissue inhibitor of metalloproteinase-1

**Renal function markers**
- Creatinine
- Cystatin-C

**Metabolic markers**
- Adiponectin
- Leptin
- Insulin
- Ferritin
- Glucose

**Coagulation markers**
- D-Dimer

**Angiogenesis markers**
- Cardiac placental growth factor

**Necrosis markers**
- Creatine kinase-MB
- Troponin I

**Inflammatory markers**
- C-reactive protein
- Interleukin-18
- Interleukin-1 receptor antagonist
- Neopterin

**Markers of oxidative stress and antioxidants**
- Homocysteine
- Myeloperoxidase
- Vitamin B_{12}
- Active vitamin B_{12}
Some Important Considerations

Biological plausibility in itself is not enough

Carefully distinguish a risk factor and risk marker

Frequent correlations between markers

The need to consider and account for what are often many comparisons

Document analysis and statistical plan before embarking on analyses

Any ethical implications?
GBD Group: Benefits of CVD Intervention by Age

- Longer lifetime exposure
- Higher modifiable risk
- Healthy life-years
- Higher absolute risk
- Hospitalisation costs
- More comorbidities

CJL Murray et al, Lancet 2003;361:717-725
Trends in Age-Specific CHD Mortality Rates: USA
Genetic Epidemiology: Meta-analysis of log-linear effect of each unit long-term exposure to lower LDL-C on CHD risk

9 polymorphisms in 6 genes, selected for "exclusive" effect on LDL-C

Ference BA et al. JACC 2012; http://dx.doi.org/10.1016/j.jacc.2012.09.017
Comparative CHD Risk Reduction of Life-long* and Later LDL-C Lowering: Mendelian Randomisation and CTTC (RCT) Analyses

*CHD = CVD death, nfMI, cor. revasc. (where possible)

* 3-fold greater reduction in CHD risk/unit lower LDL-C

Ference BA et al. JACC 2012; http://dx.doi.org/10.1016/j.jacc.2012.09.017
The “....omics” Revolution
The Pragmatic Approach

“Kill them. For the Lord knows who are His.”

(When asked by a crusader how to distinguish the Cathars from the Catholics in Catalonia)

Arnaud Almaric, Abbott of Citeaux, ca 1209
Biomarker Application: Number Needed to Screen

\[ \text{NNS} = \frac{1}{[\text{Risk}^* \times \text{Rel. Risk for Marker} \times \text{RRR Treatment}] / P^{**}} \]

**where**

- \( ^* \) Risk predicted by base model
- \( ^{**} P \) = Proportion of people at risk level (R) who have the marker

CM Rembold BMJ 1998; 317: 307-12
Number Needed to Screen: Modelled for JUPITER

• NNS = \(\frac{1}{[0.10^{1} \times 1.5^{2} \times 0.44^{1}] } \times 0.14^{3}\)

= Approximately 90 subjects screened to prevent one major JUPITER CVD event over 10 years

\[1\] P. Ridker et al. NEJM 2008; 359: 2195-207
\[2\] J. Danesh et al. NEJM 2004; 350: 1387-97
A CONCEPTUAL FRAMEWORK

Risk assessment and management

Diagnosis

Personalised medicine

General population

At-risk individuals and groups

CVD patients

End-stage

New therapeutic targets

Societal Context

- Diabetes
- Chronic Kidney Disease
- Indigenous People

Acute Presentation
ACCS
Stroke

Modified from A Tonkin, Atherosclerosis and Heart Disease, 2003
A FRAMEWORK FOR STRATEGIES

- Prevent Modifiable Risk Variables
- Modifiable Risk
- Acute Care
- Prevent Recurrent Events

General population

At-risk individuals and groups

CVD patients

End-stage

Societal Context
- Diabetes
- Chronic Kidney Disease
- Indigenous People

Modified from A Tonkin, Atherosclerosis and Heart Disease, 2003
After Sargent et al. JCO 2005; 1387-97