Biochemical Cardiac Tests
Before, During & After the Infarct

Heart Disease
- Lifetime risk of Coronary Heart Disease
  49% men
  32% women
- Leading cause of death in both groups
- Most risk factors are modifiable
Atherosclerotic plaque rupture
Established Biochemical Markers

- Lipids
  - LDL cholesterol, HDL cholesterol
  - Apoproteins – Apo (B), Apo (A), LP (a), Apo (E)
- Diabetes mellitus
- Metabolic syndrome

Non-Biochemical Markers

- Age
- Male gender
- Family history
- Cigarette smoking
- Hypertrophy
- Hypertension
- Psychosocial factors
  - Physical inactivity
  - Diet
  - Obesity
  - Left ventricular
  - Chronic kidney disease
Lipid Risk Factors

- Total cholesterol / LDL cholesterol / Apo B
  - LDL-C is most important atherogenic particle
  - Many studies with statins and other drugs have demonstrated decreases in CHD in secondary prevention trials

Apo B

- Only apoprotein on LDL but also found on other atherogenic particles, VLDL and IDL
- Most (but not all) studies have shown Apo (B) to be better as a marker of CHD than LDL-cholesterol
HDL Cholesterol (Apo A-1)

- HDL-C consistently found to be a negative risk factor
- Apo (A-1) questionable additional benefit over HDL-C and other risk factors

IDL (Apo E)

- Atherogenic particle with cholesterol and triglycerides
- Normally rapidly cleared
- Levels found in Type III dyslipidemia (Apo E receptor defects)
- Cholesterol and triglycerides

How do you treat?

- According to Adult Treatment Panel III (ATP III)
  - Complete lipid profile on all adults
  - Calculate 10 year risk of CHD based on:
    - Age
    - Sex
    - Total cholesterol, HDL cholesterol
    - Blood pressure
    - Smoking
How do you treat … (continued)

- Treatment goals (ie., LDL-C) based on global 10 year risk

<table>
<thead>
<tr>
<th>LDL-C target</th>
<th>Known CAD or equivalent 10 yr risk &gt; 20% (2.0 mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 2 risk factors 10 yr risk &lt; 20% (3.4 mmol/L)</td>
<td></td>
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<tr>
<td>0-1 risk factor 4.1 mmol/L</td>
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</table>

Special risk assessment for:
- Known CAD
- Diabetes mellitus
- Family history premature CAD
- Metabolic syndrome

Highly Sensitive C-Reactive Protein (hs-CRP)

- Inflammatory biomarker with physiologic role in the immune system
- Independent of other risk factors
- Appears to have a role in progression of atherosclerosis
hs-CRP … (continued)

- Role – to lower treatment goals (i.e., LDL-C) in individuals with moderate CHD risk
- Role in primary prevention currently controversial

- CRP lowered by:
  - Statins, fibrates
  - Weight loss
  - Increased physical activity
  - Smoking cessation

Homocysteine

- Amino acid, with role in folate/vitamin B metabolism
- Elevated levels associated with increased risk of vascular disease of all types
- Several postulated mechanisms to explain increased risk

Homocysteine … (continued)

- Screening not generally recommended
  - Folate now added to all grain products
  - No study has demonstrated that lowering homocysteine levels leads to improved clinical outcomes
Homocysteine … (continued)
- Role – patients with premature atherosclerosis or family history of premature CHD
- Treatment with folate and B12 supplementation

LP (a)
- Specialized form of LDL linked to a protein that resembles plasminogen
- Net effect is that
  - Thrombolysis is impaired by plasminogen analogue
  - Atherogenesis enhanced by LDL

LP (a) … (continued)
- Important when:
  - Markedly increased cholesterol
  - Familial combined hyperlipidemia
  - Strong family history of premature CHD
- Can be treated with Niacin
Other Markers

- Fibrinogen
- Plasminogen Activator-I
- Protein C
- Factor V Leiden
- Antithrombin III
- Tissue Plasminogen Activator
- Von Willibrand Factor

CARDIAC MARKERS:
- Creatine kinase
- Myoglobin
- Cardiac Troponin

Characteristics of an Ideal Marker for Myocardial Injury

- Be specific for myocardial injury
- Be sensitive to small injuries
- Be rapidly released following injury
- Be long enough lasting in the blood to permit delayed diagnosis
Characteristics cont’d

- Produce blood levels that are proportionate to infarct size
- Permit risk assessment
- Be technically easy to measure

Estimated Clinical Sensitivity & Specificity of Cardiac Markers for AMI

<table>
<thead>
<tr>
<th>Marker</th>
<th>2-4 h</th>
<th>8-24 h</th>
<th>24-72 h</th>
<th>&gt;72 h</th>
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</thead>
<tbody>
<tr>
<td>Myoglobin</td>
<td>70</td>
<td>95</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>CK-MB</td>
<td>95</td>
<td>90</td>
<td>95</td>
<td>98</td>
</tr>
<tr>
<td>Troponin</td>
<td>99</td>
<td>75</td>
<td>95</td>
<td>98</td>
</tr>
</tbody>
</table>
Causes of Increased CK

- **Myocardial diseases**
  AMI, heart trauma, myocarditis, after heart surgery
- **Skeletal muscle diseases and injury**
  Muscular dystrophy, rhabdomyolysis, polymiositis, IM injections, seizures, trauma (e.g., MVA)
- **Miscellaneous**
  Hypothyroidism (poor clearance), malignant hyperthermia, prolonged hypothermia, cerebral injury

Creatine Kinase

- Catalyzes conversion of creatine phosphate:
  creatine phosphate + ADP → creatine + ATP
- Molecular weight 84 kDa.

Myoglobin

- Oxygen-bearing heme protein found in the skeletal muscles and heart.
- Molecular weight 17 kDa. No tissue isoenzymes.
- Early marker of myocardial infarction (~3 h before CK-MB).
- Cleared by renal filtration.
- Non-specific AMI marker. Increased in patients with skeletal muscle disease and chronic renal failure (retention)
Case Study

- 47 year old male
- Insulin dependent diabetic
- Seen at doctor’s office with chest pain for past several hours

Laboratory

- Hematology - normal
- Lipids - normal
- FBS - 9.3 mmol/L (3.6 - 6.0)
- HbA1c - 0.095 g/gHb (0.048 - 0.062)

Laboratory

- CK - 191 U/L (< 165)
- Troponin - 0.2 ug/L (< 0.06)
Summary

- High risk male
- Equivocal CK
- Ischemic ECG
- Positive Troponin

Course in Hospital

- Mildly elevated troponins for one week
- Significant elevation to 1.2 ug/L day 9
- Coronary angiogram “significant triple vessel disease”
Treatment Included

- Low molecular weight heparin
- ASA
- Morphine
- Nitroglycerine

Summary

- Troponin result consistent with findings on angiography
- Predicted infarct 9 days post-admission
Troponin Complex

- Troponin C, I and T
- Controls force of muscle contraction
- Not an enzyme

Specificity

- Cardiac forms of Troponins I and T do not exist in other tissues
- Cardiac Troponin I has a 31 amino acid sequence that skeletal troponin does not have

Specificity cont’d

- The “B” isoenzyme of CK is the fetal form and stressed muscle (eg., hypoxic diseased) may revert to this form
- 1-2% of skeletal CK is of the “MB” isoenzyme type
Specificity cont’d

- Troponin is useful for cardiac diagnosis in cases of trauma or muscle breakdown
- CKMB is not

Sensitivity

- Troponin concentration in muscle tissue is high (~ 13 times higher than CKMB)
- Healthy people have no detectable Troponin (there is always some CKMB detectable)

Sensitivity cont’d

- We now detect a significant number of patients who are:
  - CKMB negative
  - Troponin positive
Sensitivity - One Study
Normal CK/CKMB - Increased Troponin?
- 91 patients
  - normal CK/CKMB
  - ECG ischemia
  - chest pain at rest

22 patients
Troponin I increased
  - 2 deaths (9.1%)  - 4 MIs (18.2%)

69 patients
No increase
  - 0 deaths (0%)  - 4 MIs (5.8%)

Troponin
- Time to detection: 2 - 6 hours
- Persistence in blood: 5 - 10 days

Emergency Treatment of Chest Pain
WHO Criteria for AMI

- History is typical if severe and prolonged chest pain is present.
- Unequivocal ECG changes, abnormal Q or QS waves, evolving injury lasting >1 d.
- Unequivocal serial enzyme changes, or initial rise and subsequent fall of levels. Change must be properly related to the particular enzyme and the delay between onset and sampling.


ESC/ACC Redefinition of AMI

1. Typical rise and gradual fall (troponin) or more rapid rise and fall (CK-MB) of biochemical markers of myocardial necrosis with at least 1 of the following:
   a) ischemic symptoms;
   b) development of pathologic Q waves;
   c) ECG changes indicative of ischemia (ST segment elevation or depression;
   d) coronary artery intervention
2. Pathologic findings of AMI

Beckman Access cTnI CV Profile
AMI Diagnosis

- Maximum concentrations of cardiac troponin exceeding the 99th percentile (with acceptable precision) for a reference control group on at least 1 occasion during the first 24 h after a clinical event (Class I).

Risk Assessment

- In patients with a clinical syndrome consistent with ACS, a maximal concentration of cardiac troponin >99th percentile (with acceptable precision) should be considered indicative of increased risk of death or recurrent ischemic events (Class I).

- In patients with a high clinical probability of ACS, a maximal concentration of cardiac troponin >99th percentile (without stringent requirements for precision) may be recognized as indicative of increased risk of death or recurrent ischemic events. (Class IIb).
Other Uses of Troponin

- Determination of size of infarct
- Determination of success of refusion
- Two negative Troponins 6 hours apart are good (but not absolute) evidence of no recent acute coronary syndrome

Pitfalls

- Testing too early
- Assay interferents
- Standardization
Reporting Comments

- <0.06
  - Negative for myocardial injury
- 0.06 – 0.49
  - Low level positive suggestive of myocardial injury, possibly evolving MI. Suggest repeat in 6 – 8 hours as clinically indicated.
- >0.49
  - Positive to myocardial injury most likely MI

Patient Summary

- 70 year old male
- Atypical chest pain
Troponin < 0.06

Patient Summary

- 65 year old male
- Chest pain
Troponin I = 6.6

Patient Summary

- 68 year old male
- Known heart disease
- Chest pain X one week
- Troponin I - < 0.06

Patient Summary

- 58 year old male
- Chest pain 2 days ago
Key Points About Troponin

- Troponin is a protein which is:
  - Present in high concentration in muscle
  - Regulates the force of muscular contractions
  - Is composed of 3 sub units I, T and C
- The Troponin I and Troponin T found in heart muscle is significantly different from Troponins found in non-cardiac muscle

Key Points About Troponin cont’d

- There is no Troponin present in the blood unless there is heart damage
- Troponins are both more sensitive and more specific than CKMB in terms of its diagnostic ability with respect to myocardial damage
Key Points About Troponin cont’d

- Troponin are first detectable 2 – 6 hours after heart damage has occurred, and last 5 – 10 days
- Negative results can occur if testing is done too early
- The decision to use clot dissolving medication is usually based on factors other than Troponin level

B-Type Natriuretic Peptide for Congestive Heart Failure

Congestive heart failure (CHF)

A condition where the heart is unable to supply the body with enough oxygen-rich blood to accommodate the body’s needs during exercise and at rest. As a result of decreased heart function, body fluids may build up in the lungs and limbs.
**Etiology of CHF**

- **Ischemia**: 69%
- **Hypertension**: 13%
- **Idiopathic**: 11%
- **Other**: 7%

(CHF (N=6,063) - SOLVD Registry)

**Clinical symptoms of CHF**

- Shortness of breath
- Orthopnea (difficulty breathing when lying down)
- Peripheral edema (swelling of ankles, legs, and arms)
- Pulmonary edema
- General fatigue and weakness

**The CHF epidemic in the United States**

- 400,000 - 700,000 new cases each year
- 10% prevalence in those over 65 years
- 1/2 are asymptomatic
- 11 million office visits
- 3.5 million hospitalizations
- 250,000 deaths with cost of $30+ billion
- Increasing prevalence of CHF in the US population
- Only 1/3 of the population has controlled hypertension
- No decline in the incidence of MI in males and females
Natriuretic peptides
- The natriuretic peptides are a family of 3 related forms.
- They are increased in CHF due to enhanced atrial & ventricular synthesis.
- ANP: 28-aa peptide found in the atrium of the heart
- BNP: 32-aa peptide found in the brain and ventricles of the heart
- CNP: 22 aa peptide found in the brain and CNS
- Urodilatin: 32 aa peptide, is the renal form of ANP

Structure of the natriuretic peptides

Natriuretic peptides and vasopressin: counterbalance

Hypertension

BNP, ANP
- Vasodilation
- Natriuresis/diuresis

Renin, aldosterone
- Vasoconstriction
- Salt/water retention
- Increase heart rate/contractility

Hypotension
Release of BNP from cardiac myocytes

- preproBNP (134 aa)
- proBNP (108 aa)
- signal peptide (26 aa)
- myocyte
- secretion
- NT-proBNP (1-76)
- BNP (77-108)

BNP/NT-proBNP for clinical studies
- Correlation with other CHF Criteria
  - NYHA Classification
  - Echocardiography
- Diagnosis of CHF in ED patients
- Prognostic potential of BNP
- Screening
- Monitoring CHF therapy

New York Heart Association (NYHA) Classification System

- Class I: Asymptomatic
- Class II: Cardiac symptoms on light activity
- Class III: Cardiac symptoms at rest
- Class IV: Inability to carry out any activity

NYHA = New York Heart Association; ED = Emergency Department; SOB = shortness of breath
The "Breathing Not Proper" study

- 1586 patients presenting to the ED with shortness of breath
- Data recorded: history, physical exam, lab tests
- Initial assessment by ED physicians
- BNP measured
- Followup assessment: 2 cardiologists with access to all tests (echos), hospital course, response to treatment, etc.
Heart failure: diagnosis

- Plasma BNP or NT-proBNP testing should be performed to confirm the diagnosis of HF in patients with suspected HF but with presenting signs and symptoms that are ambiguous or with confounding disease states (such as COPD) (Class IIa).

Heart failure: replacement of other tests?

- In diagnosing patients with heart failure, plasma BNP or NT-proBNP testing should not be used to replace conventional assessment of the degree of left ventricular structural or functional abnormalities (e.g., echo, invasive hemodynamic assessment) (Class IIa).
Heart failure: Drug monitoring

- Although there is evidence from pilot studies to suggest that BNP or NT-proBNP can be useful to guide therapy in patients with mild-to-moderate HF, at this time, routine testing is not indicated for therapeutic decisions (Class IIb).

Biomarkers of Cardiac Ischemia

What is ischemia?

- Oxygen supply diminishes with disease progression.
- Oxygen demand changes daily and during life.
- Ischemia occurs when $O_2$ demand exceeds supply.
### Biochemical Events after AMI

<table>
<thead>
<tr>
<th>Coronary artery occlusion</th>
<th>Myocardial ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anoxia</td>
<td>ATP pump failure</td>
</tr>
<tr>
<td>Lack of collateral blood flow</td>
<td>Leakage of ions, e.g., potassium</td>
</tr>
<tr>
<td>Reversible damage</td>
<td>Accumulation of metabolites</td>
</tr>
<tr>
<td>Irreversible damage</td>
<td>Leakage of metabolites, e.g., lactate</td>
</tr>
<tr>
<td>Cell death &amp; tissue necrosis</td>
<td>Membrane damage</td>
</tr>
<tr>
<td></td>
<td>Leakage of enzymes, e.g., LDH</td>
</tr>
</tbody>
</table>

### Ideal ischemic marker characteristics

- Cardiac specific
- Rises soon after plaque rupture
- Elevated over a sustained period of time
- Easy to measure, fast assay TAT
- Diagnostic utility verified by clinical studies

### Next generation cardiac marker

1. Cardiac marker needed to rule out acute coronary syndromes on blood collected at presentation.
2. Markers needed that have high sensitivity for ischemia (unstable angina and silent ischemia).
3. Triage of patients using ischemic markers will likely reduce unnecessary admissions and wrongful discharges.
Ischemia Modified Albumin (IMA)
- Normal albumin can bind metals at its N-terminus
- During ischemia, free radicals alter the binding site, decreasing binding ability
- Assays using cobalt as the metal can detect changes

IMA ... (continued)
- Positive test – ischemia
- Negative test (together with negative troponin and negative ECG) has a 99% negative predictive value for a coronary event

IMA ... (continued)
- Rapidly cleared
- Not specific for cardiac ischemia
- Other causes of positive results
Other Ischemic Markers

- Unbound free fatty acids (uFFA)
- BNP
- Choline
- Pregnancy associated plasma protein A
- Modified form of LDL