Cardiac Markers

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Current Chest Pain Triage Methods
Result in Overadmissions and Missed MIs

US statistics

- 6 million visits to ER for chest pain
- Rule-outs (3.0 million)
- Suspicious (2.5 million)
- 5,000 deaths; 20% of ER malpractice $$

6,000 deaths; 20% of ER malpractice $$

Diagnostic ECG (0.5 million)

CCU/CPE/C Other

Other Dx (1 million)

Unstable Angina (1 Million)

AMI (0.5 million)

Unstable course (2.97 million)

Missed AMIs (30,000)

Treat or discharge

Other Dx (1 million)

30 billion in unnecessary costs
PATHOPHYSIOLOGY OF ACUTE CORONARY SYNDROME

Plaque Rupture
- markers of inflammation (C-reactive protein, amyloid protein A)

Intracoronary Thrombus
- coagulation factors and platelets

Ruptured Rind Flow
- perfusion or functional imaging (studies use radionuclide imaging 2-DE echocardiography)

Myocardial Ischemia
- early ischemic indicators (glycogen phosphorylase BB)

ECG: ST segment depression

Myocardial Necrosis
- classical biochemical markers (CK-MB, troponin, myoglobin)

ECG: ST segment elevation

History of Cardiac Markers

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1950</td>
<td>AST in AMI</td>
</tr>
<tr>
<td>1960</td>
<td>CK in AMI</td>
</tr>
<tr>
<td>1970</td>
<td>LDH in AMI</td>
</tr>
<tr>
<td>1980</td>
<td>Electrophoresis for CK and LD</td>
</tr>
<tr>
<td>1990</td>
<td>Monoclonal MB antibody</td>
</tr>
<tr>
<td>2000</td>
<td>cTnI in AMI</td>
</tr>
<tr>
<td>1950</td>
<td>RIA for myoglobin</td>
</tr>
<tr>
<td>1960</td>
<td>INH for CK-MB</td>
</tr>
<tr>
<td>1970</td>
<td>Optimized CK assay</td>
</tr>
<tr>
<td>1980</td>
<td>WHO criteria for AMI</td>
</tr>
<tr>
<td>1990</td>
<td>CK-MB mass assay</td>
</tr>
<tr>
<td>2000</td>
<td>cTnT in UA</td>
</tr>
<tr>
<td>1950</td>
<td>GUSTO trials</td>
</tr>
<tr>
<td>1960</td>
<td>cTnT &amp; cTnl for risk stratification</td>
</tr>
<tr>
<td>1970</td>
<td>POC testing</td>
</tr>
</tbody>
</table>

POC: Point-of-Care Testing
An Ideal Marker for Myocardial Injury Would Be

• Found in high concentrations in myocardium
• Released rapidly after the onset of pain
• Not be found in other tissues even in trace amounts or under pathological conditions
• Have a convenient diagnostic time window
• Reflect as much as possible the evaluation of myocardial damage

Serum Cardiac Markers of the Past

• Total CK Activity
• Aspartate Aminotransferase Activity
• Lactate Dehydrogenase Activity
• LD1/LD2 Ratio
CARDIAC MARKERS
OF THE PAST

Aspartate Aminotransferase (AST)
Creatine Kinase (CK)
Lactate Dehydrogenase (LDH)

Lactate dehydrogenase (LD)

- LD activity is measured by monitoring absorbance at $X = 340$ nm (NADH)
- Total LD activity has poor specificity
- LD has a molecular mass of 135 KDA and is a tetramer composed of heart and skeletal muscle subunits given rise to five isoenzymes
### Distribution of LD Isoenzymes in Human Tissue and Serum

<table>
<thead>
<tr>
<th>Tissue/Serum</th>
<th>LD1</th>
<th>LD2</th>
<th>LD3</th>
<th>LD4</th>
<th>LD5</th>
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</thead>
<tbody>
<tr>
<td>Serum</td>
<td>29</td>
<td>38</td>
<td>0</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Heart</td>
<td>52</td>
<td>29</td>
<td>16</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Kidney-cortex</td>
<td>38</td>
<td>32</td>
<td>17</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Erythrocyte</td>
<td>42</td>
<td>36</td>
<td>15</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Brain</td>
<td>25</td>
<td>25</td>
<td>34</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Lung</td>
<td>12</td>
<td>22</td>
<td>29</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>Spleen</td>
<td>6</td>
<td>11</td>
<td>35</td>
<td>28</td>
<td>20</td>
</tr>
<tr>
<td>Platelets</td>
<td>17</td>
<td>30</td>
<td>34</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>8</td>
<td>12</td>
<td>50</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>12</td>
<td>80</td>
</tr>
<tr>
<td>Skeletal Muscle</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>9</td>
<td>78</td>
</tr>
</tbody>
</table>

#### LD isoenzyme electrophoresis (normal)

LD-2 > LD-1 > LD-3 > LD-4 > LD-5
LD isoenzyme electrophoresis (abnormal)

LD-1 > LD-2

Cathode (-)  Anode (+)

LD1/LD2

POST AMI

Increase  8-12 HRS

LD1/LD2  ≥  1  48-72 HRS

Return to Normal  8-14 DAYS
Conditions Causing Flipped LD1/LD2 Without Acute Myocardial Infarction

- Hemolysis
- Megoblastic & Pernicious Anemia
- Renal Cortex Infarction
- Testicular Germ Cell Tumors
- Small Cell Lung Carcinoma
- Adenocarcinoma of the Ovary
- Acute Coronary Insufficiency (Unstable Angina)
- Exercise Induced Myocardial Ischemia
- Polymyositis
- Muscular Dystrophies
- Well Trained Athletes
- Rhabdomyolysis

Current Cardiac Markers

- CK-MB
- Myoglobin
- CKMB Isoforms
- Troponin I and T
Different Types of CK

CK: Dimer composed of 2 monomers: M (43,000 Da) and B (44,500 Da) ---- > CK BB or CK MB or CK MM

Role:
Creatine + ATP <-> ADP + Phosphocreatine + Energy
(muscular contraction)

CK BB = CK1 Increased in neurological diseases;
prostatectomy; digestive cancers

CK MB = CK2 Increased with AMI

CK MM = CK3 Increased in myopathy, hypothyroidy, polymyositis,
rhabdomyolysis, traumatism, intensive exercise, AMI

Distribution Of CK & CK Isoenzymes in Various Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Range of Total CK U/gm tissue</th>
<th>Range of CK Isoenzymes MM %</th>
<th>MB %</th>
<th>BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal Muscle</td>
<td>1080-3050</td>
<td>96-100</td>
<td>0-4</td>
<td>0</td>
</tr>
<tr>
<td>Heart Muscle</td>
<td>190-692</td>
<td>58-86</td>
<td>15-42</td>
<td>0-1</td>
</tr>
<tr>
<td>Brain</td>
<td>73-200</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Bladder</td>
<td>162</td>
<td>0-2</td>
<td>0-6</td>
<td>92-100</td>
</tr>
<tr>
<td>Placenta</td>
<td>250</td>
<td>19</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>Colon</td>
<td>200</td>
<td>0-5</td>
<td>0-4</td>
<td>95-100</td>
</tr>
<tr>
<td>Ileum</td>
<td>175</td>
<td>0.3</td>
<td>0-4</td>
<td>93-100</td>
</tr>
<tr>
<td>Stomach</td>
<td>170</td>
<td>0-5</td>
<td>4</td>
<td>96</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>140</td>
<td>96</td>
<td>4</td>
<td>22</td>
</tr>
<tr>
<td>Thyroid</td>
<td>32-34</td>
<td>7-11</td>
<td>0-6</td>
<td>90-96</td>
</tr>
<tr>
<td>Uterus</td>
<td>9-38</td>
<td>2-20</td>
<td>0-7</td>
<td>58-100</td>
</tr>
<tr>
<td>Kidney</td>
<td>10-50</td>
<td>0-13</td>
<td>0</td>
<td>87-100</td>
</tr>
<tr>
<td>Lung</td>
<td>13-24</td>
<td>0-39</td>
<td>56-93</td>
<td></td>
</tr>
</tbody>
</table>
CKMB

AFTER AMI

Increase  4-6 Hours
Peak      10-24 Hours
Return to Normal 48-72 Hours

Draw blood on admission, 4, 8, 16 and 24hr.
DIAGNOSTIC PERFORMANCE OF CKMB IN AMI

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity CKMB</th>
<th>Specificity CKMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission</td>
<td>31</td>
<td>94</td>
</tr>
<tr>
<td>2 Hr</td>
<td>68</td>
<td>92</td>
</tr>
<tr>
<td>4 Hr</td>
<td>80</td>
<td>91</td>
</tr>
<tr>
<td>6 Hr</td>
<td>96</td>
<td>94</td>
</tr>
</tbody>
</table>

Limitations of CKMB in AMI

Elevated CKMB Levels can be observed in:
- Skeletal Muscle Involvement
- Duchenne Muscular Dystrophy
- Polymyositis
- Alcohol Myopathy
- Thermal or Electrical Burn Patients
- Carcinomas
- Colon, Lung, Prostate, Endometrial
- Atypical CK Isoenzymes and CKBB
**CKMB IN AMI**

**Advantages:**
- Detects AMI 4-6 Hours After Chest Pain
- Methodology is Rapid and Automated
- Turnaround Time <60 Minutes

**Disadvantages:**
- Not Cardiac Specific

**CK isoforms**

- C-terminal lysine is removed from the M subunit--therefore, there are three isoforms of CK-3 (MM)
- $t_{1/2}$: CK-MB$_1$ > CK-MB$_2$
- Ratio of CK-MB$_2$ to CK-MB$_1$ exceeds 1.5 within six hours of the onset of symptoms
- Only method currently available is electrophoresis
Protocol for Early Detection of AMI

Draw Blood at 0, 1, 2, 3 hours

Measure CKMB Isoforms or Myoglobin

Re: Puleo & Roberts
New Engl. J. Med, 1 Sept 1994

• 1110 patients who came in Emergency Care Units for chest pain

• By using a ratio CKMB2/CKMBI \geq 1.5, as well as the CKMB2 value (0.5-1 UIL) in the 6 first hours after the onset of chest pain
## CKMB Isoforms and CKMB

<table>
<thead>
<tr>
<th>Time after onset, hr</th>
<th>CKMB Isoforms</th>
<th>CKMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>56</td>
<td>23</td>
</tr>
<tr>
<td>6</td>
<td>96</td>
<td>48</td>
</tr>
</tbody>
</table>
CK Isoforms in AMI

Advantages:
• Early detection of AMI with CKMB Isoforms

Disadvantages:
• Not Cardiac Specific
• Elevated in acute skeletal muscle trauma and in serum of marathon runners

Methodology Limitations
• Labor Intensive
• May not be able to detect small changes in CKMB Isoforms
• Requires careful interpretation of CK patterns
• Results not available in a timely fashion

Myoglobin

Definition:
• Oxygen-binding protein
• MW=17,800 kd
• Cytoplasmic
• Heart and Skeletal Muscle Tissue
Myoglobin

Present in Cardiac and Skeletal Muscle
Molecular Mass 17,800

<table>
<thead>
<tr>
<th>Post AMI</th>
<th>Myoglobin</th>
<th>CKMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase</td>
<td>Hrs</td>
<td>2-4</td>
</tr>
<tr>
<td>Peak</td>
<td>Hrs</td>
<td>5-9</td>
</tr>
<tr>
<td>Return to Normal</td>
<td>Hrs</td>
<td>24-36</td>
</tr>
</tbody>
</table>

Serum Myoglobin Levels in Various Conditions

Increased In:
- AMI
- Open heart surgery
- Exhaustive exercise
- Skeletal muscle damage
- Progressive Muscular Dystrophy
- Shock
- Renal Failure
- Following IM injection

Remains Normal:
- Chest pain without AMI
- CHF without AMI
- Cardiac catheterization
- Moderate exercise
Myoglobin in AMI

Advantages:
• Early Indicator of AMI
• Methodology - Automated
• Results Available in <60 Minutes

Disadvantages:
• Not Cardiac Specific
• Elevated in Trauma
• Skeletal Muscle Damage
• Exercise
• Impaired Renal Function

Temporal changes in CK-MB Isoforms, myoglobin and CK-MB
**Diagnostic Performance of Serum Cardiac Markers in AMI**

<table>
<thead>
<tr>
<th></th>
<th>CKMB</th>
<th>Isoforms</th>
<th>Myo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2 Hr</td>
<td>7</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>2-4 Hr</td>
<td>12</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>4-6 Hr</td>
<td>73</td>
<td>85</td>
<td>81</td>
</tr>
<tr>
<td>6-8 Hr</td>
<td>90</td>
<td>95</td>
<td>95</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CKMB</th>
<th>Isoforms</th>
<th>Myo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>93</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>Specificity</td>
<td>95</td>
<td>93</td>
<td>80</td>
</tr>
<tr>
<td>96</td>
<td>95</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>90</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>


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**Troponin Characteristics**

- **Troponin C (18 kd)**
- Calcium-binding subunit
- No cardiac specificity
- **Troponin I (26.5 kd)**
- Actomyosin-ATP-inhibiting subunit
- Cardiac-specific form
- **Troponin T (39 kd)**
- Anchors troponin complex to the Tropomyosin strand

The troponin complex consists of three different proteins (TnC, TnI, and TnT) that regulate the calcium-mediated contractile process of striated muscle.
Tissue specificity of Troponin subunits

- Troponin C is the same in all muscle tissue
- Troponins I and T have cardiac-specific forms, cTnI and cTnT
- Circulating concentrations of cTnI and cTnT are very low
- cTnI and cTnT remain elevated for several days
- Hence, Troponins would seem to have better specificity than CK-MB, and the long-term sensitivity of LD-1

<table>
<thead>
<tr>
<th></th>
<th>Post AMI</th>
<th>Troponin I</th>
<th>Troponin T</th>
<th>CKMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase Hrs</td>
<td>4-6</td>
<td>3-6</td>
<td>4-6</td>
<td></td>
</tr>
<tr>
<td>Peak Hrs</td>
<td>14-24</td>
<td>10-24</td>
<td>10-24</td>
<td></td>
</tr>
<tr>
<td>Return Days</td>
<td>5-7</td>
<td>6-10</td>
<td>2-3</td>
<td></td>
</tr>
</tbody>
</table>
## Specificity of cTnl, CK-MB Mass & Myoglobin In Noninfarct Patients with Chronic Renal Failure or Severe Polytrauma

<table>
<thead>
<tr>
<th>Pathology &amp; Markers</th>
<th>No. (%) of Positive Sera</th>
<th>Specificity %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Severe Polytrauma (24 Sera)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK-MB mass</td>
<td>14 (58)</td>
<td>42</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>21 (88)</td>
<td>12</td>
</tr>
<tr>
<td>cTnl</td>
<td>0 (0)</td>
<td>100</td>
</tr>
<tr>
<td><strong>Chronic Renal Failure (49 Sera)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK-MB mass</td>
<td>4 (8)</td>
<td>92</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>43 (88)</td>
<td>12</td>
</tr>
<tr>
<td>cTnl</td>
<td>0 (0)</td>
<td>100</td>
</tr>
</tbody>
</table>

## SERUM BIOCHEMICAL MARKERS OF 24 CHRONIC DIALYSIS PATIENTS WITHOUT ACUTE ISCHEMIC HEART DISEASE

Frequency Distribution of Cardiac Troponin I (cTnI) in Normals, Cardiac Non-Ischaemic (CNI), Congestive Heart Failure (CHF), Chest Pain of Non-Cardiac Origin (NC), and Surgical Trauma Patients (Surg)

![Frequency Distribution Chart]

### Diagnostic Performance of Troponin I and Troponin T for AMI

<table>
<thead>
<tr>
<th>Time</th>
<th>Troponin I</th>
<th>Troponin T</th>
<th>Troponin I</th>
<th>Troponin T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission</td>
<td>6</td>
<td>15</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>1 hr</td>
<td>25</td>
<td>38</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>2 hr</td>
<td>70</td>
<td>74</td>
<td>100</td>
<td>93</td>
</tr>
<tr>
<td>6 hr</td>
<td>96</td>
<td>97</td>
<td>99</td>
<td>93</td>
</tr>
<tr>
<td>12-24 hr</td>
<td>96</td>
<td>99</td>
<td>99</td>
<td>93</td>
</tr>
</tbody>
</table>
Troponin I, CKMB & Myoglobin

192 Patients With Chest Pain 59 Had An AMI
Clin Chem 45, 199-205, 1999

<table>
<thead>
<tr>
<th></th>
<th>Troponin I</th>
<th>CKMB</th>
<th>Myoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;6 hr</td>
<td>65</td>
<td>78</td>
<td>75</td>
</tr>
<tr>
<td>6-24 hrs</td>
<td>72-93</td>
<td>78-80</td>
<td>73-75</td>
</tr>
</tbody>
</table>

| Specificity %        |            |           |           |
| <6 hr                | 100        | 91        | 74        |
| 6-24 hrs             | 94-97      | 82-86     | 68-82     |

Release of Cardiac Markers in Myocardial Infarction

Days after onset of MI

LDH

Myoglobin

Reference Interval
Point-of-Care Testing For Cardiac Markers in the Emergency Department

- Solid Phase Chromatographic Immunoassay
- Quantitative and Qualitative Detection of CKMB, Myoglobin, Troponin I or T
- Whole Blood
- Assay Time-15-20 Minutes

Point-of-Care Troponin I & Troponin T
Hamm et.al., N Engl J Med 1997, 337,1648-83

773 Patients
47 AMI Patients

TnT positive-94%
TnI positive-100%
CK-MB positive- 91%

Unstable Angina Patients
TnT positive-22%
TnI positive-36%
CK-MB positive-5%
Use of Biochemical Markers for Detecting Myocardial Necrosis

• Myocardial Infarction redefined by joint European Society of Cardiology/American College of Cardiology (ESC/ACC) in 2000
• Recommendations
  – Tn – at least 1 value > cutoff in first 24 hrs
  – CK-MB – 2 successive samples > cutoff or 1 sample 2x cutoff
  – CK, AST, LDH – not recommended
• Testing Protocol
  – On admission, 6 – 9 hrs, and 12 – 24 hrs

JACC, 2000; 36:959-69