Cardiac Biomarkers: When to Test? – Physician Perspective

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Introduction
A biomarker is a substance used as an indicator of a biologic state. It is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic response to a therapeutic intervention. In a patient having a triad of chest pain, ECG changes, and elevation of cardiac biomarkers while chest pain is highly variable and subjective, ECG and the biomarker abnormality lend objectivity to help define the diagnosis of acute coronary syndrome (ACS) or acute myocardial infarction (AMI).

What are they?
Cardiac biomarkers are substances that get released into the blood when the heart is damaged. Cardiac biomarkers can be detected in blood by a specialised immunoassay. They are used to help diagnosis, evaluate, and monitor patients with suspected acute coronary syndrome (ACS).

Cardiac biomarkers are not necessary for the diagnosis of patients who present with ischaemic chest pain and diagnostic ECG with ST segment elevation. But cardiac biomarkers are very useful in patients of non-diagnostic ECG. The patients with ischaemic chest pain and diagnostic ECG with ST segment elevation may be candidates for thrombolytic therapy or primary angioplasty. Treatment should not be delayed to wait for cardiac marker results, especially since the sensitivity is low in the first 6 hours after symptom onset.

The most common cardiac biomarkers used in the evaluation of acute coronary syndrome are troponin T and I, CK-MB, myoglobins. Out of these troponin T and I are the markers of choice for detecting the heart damage. However, other cardiac biomarkers are less specific for heart and may be elevated in other conditions like severe muscle injury, liver disease, and kidney disease. Many other potential cardiac biomarkers are being researched but their clinical utility has yet to be established.

Cardiac biomarker tests must be available to the doctor 24 hours a day, 7 days a week with rapid turn-around time of one hour. Some of these tests may be performed in the emergency room or at the patient’s bedside. Furthermore, the introduction of newer cardiac troponin assays with increased sensitivity and lower cut-off levels have rendered traditional early markers such as myoglobin and CK-MB isoforms unnecessary for early diagnosis of myocardial infarction.

Usefulness of cardiac biomarkers
1. Diagnosis of acute myocardial infarction/acute coronary syndrome by detecting myocardial damage.
2. Prognostic value and risk stratification of:
   - Patients with acute coronary syndrome.
   - Patients undergoing pre- and post-reperfusion/coronary interventions.
   - Patients with congestive heart failure, renal disease, etc.

Types of cardiac biomarkers
1. Cardiac troponin I and T
2. CK-MB
3. Myoglobin
4. BNP
5. hs-CRP
6. Myeloperoxidase
7. Ischaemia modified albumin.

Cardiac troponins
The cardiac troponins regulate the interactions of actin and myosin and are more cardiac specific than CK-MB.
cardiac troponin complex consist 3 subunits: TnT, TnI and troponin C (Tn C). Monoclonal antibody based immunoassays have been developed to detect cardiac-specific TnT and cardiac specific TnI, because the amino-acid sequences of skeletal and cardiac isoforms of both TnT and TnI have sufficient dissimilarity. But troponin C has amino acid sequences similar to those of skeletal and cardiac isoform, so that no immunoassays have been developed for chemical purposes. Therefore, the term "cardiac troponin" refers to either TnT or TnI or to both. Cardiac troponins are used mainly to aid in the diagnosis of chest pain patients with non-diagnostic ECG; they are also used as prognostic indicators of a MI and to identify patients having an increased risk from cardiac events resulting in death.

**Laboratory range definitions**

(a) Cut-off is at 99th percentage of normal reference population.

(b) Cardiac troponin levels are undetectable in normal subjects, this 99th percentile corresponds to < 0.06.

(c) Heparin in sample can result in lowered value.

Troponin levels begin to rise 3 - 4 hours after the onset of acute coronary syndrome, and roughly 80% of patients with ACS will have a positive value at 3 hours. These levels remain elevated for 7 to 10 days. There is a direct relationship between the degree of elevation of troponin value and long-term outcome after episode of acute coronary syndrome.

**Troponin T level**

Level less than < 0.07 mg/ml: Negative.

Level 0.07 to 0.5 mg/ml: consistent with possible cardiac damage and possible increased clinical risk.

Level > 0.5 mg/ml: consistent with cardiac damage and increased clinical risk.

Cardiac troponins are sensitive, specific, and provide prognostic information for patients with ACS. Therefore, troponins are currently the cardiac markers of choice for patients with ACS. They also have greater sensitivity for smaller degree of myocardial damage than detectable by CK-MB assays. In patients with clinical history suggestive of ACS, even slight elevations of TnI and TnT can identify patients with increased risk of complications who could potentially benefit from aggressive management strategies like Gp II b/IIIa administration and/or coronary interventions. Apart from ACS, troponin levels are also increased in same causes such as:

1. Pulmonary embolus
2. Myocarditis
3. Cardiac contusion
4. Congestive cardiac failure
5. Cardioversion or radiofrequency ablation
6. Septic shock
7. Chemotherapy (adriamycin, 5-fluorouracil)
8. Renal failure
9. Hypothyroidism

**Creatine kinase (CK)**

Creatine kinase is an enzyme responsible for transferring a phosphate group from ATP to creatine. It is composed of M and B subunits that form CK-MM, CK-MB and CK-BB isoenzymes. Total CK is not cardiac specific but CK-MB is a sensitive as well as a specific marker for myocardial infarction. CK-MB begin to rise 4 - 6 hour after myocardial infarction, peak at 24 hours and return to normal within 48 - 72 hours. CK-MB estimation is useful not only for diagnosis of MI but also for the diagnosis of reinfarction.

**Relative index**

The formula for calculation of the relative index ratio is: CK-MB/total CK x 100.

Relative index is helpful in differentiating false-positive elevation of CK-MB arising from skeletal muscle. A ratio less than 3 is consistent with skeletal muscle source. Ratio greater than 5 is indicative of cardiac source, and a ratio between 3 and 5 represents a gray zone. The diagnosis of myocardial infarction must not be based on an elevated relative index alone. The relative index may be elevated in clinical settings when either the total CK or the CK-MB is within normal limits. The relative index is only clinically useful when both the total CK and the CK-MB levels are increased. Relative index improves specificity of CK-MB elevation for myocardial infarction.
CK-MB isoforms
The CK-MB isoenzymes exists as two isoforms: CK-MB1 and CK-MB2. CK-MB2 is the tissue form and initially is released from myocardium after myocardial infarction. It is converted peripherally in serum to the CK-MB1 isoforms. The ratio of CK-MB2/CK-MB1 is calculated. Normally the tissue CK-MB1 isoform predominates, so the normal ratio is < 1. A result is positive if CK-MB2 is elevated and ratio is more than 1.7. CK-MB2 is detected in serum within 2 - 4 hours after onset of symptoms and peaks at 6 - 9 hours, so it is used as an early marker for myocardial infarction. It has a sensitivity of 92% at 6 hours after symptom onset compared with 66% for CK-MB13.

Causes other than ACS of false-positive CK-MB
1. Significant skeletal injury
2. Myocarditis
3. Blunt chest trauma
4. Cardiac catheterisation
5. Shock
6. Cardiac surgery
7. Post-cardio-pulmonary resuscitation

Cardiac markers in CRF
(a) Troponin
A very high prevalence (30 - 70%) of TnT positive results have been reported in asymptomatic patients with CRF who are on dialysis. TnI is also elevated in CRF but less frequently (5%). Hence TnI has more specificity for diagnosis of acute myocardial infarction than TnT in the setting of CRF.

(b) CK-MB
About 30 - 50% of renal failure patients exhibit an elevation in the MB fraction (> 5%) without evidence of myocardial ischaemia. Unlike troponin, CK-MB however is dialysable, and levels are decreased after dialysis.

Myoglobin
Myoglobin is an oxygen storing protein found in skeletal and cardiac muscle. It is released into circulation as early as one hour after myocardial injury. Myoglobin typically rises 2 - 4 hours after onset of myocardial injury, peaks at 8 - 12 hours and returns to normal within 24 hours. Thus, myoglobin is the earliest marker but it lacks cardiac specificity.

Myoglobin should not be used alone as a method for diagnosing myocardial infarction but it should be supplemented with more cardiac-specific markers such as troponin I or troponin T15,14. Serial sampling every 1 - 2 hours can increase the sensitivity and specificity.

Table I summarises the currently used cardiac markers.

Emerging cardiac markers
1. hs-C-reactive protein (hs-CRP)
CRP is a non-specific marker of inflammation, and is considered to be directly involved in coronary plaque atherogenesis. CRP is a useful prognostic indicator in patients with ACS. Elevated CRP levels are an independent predictor of cardiac death, AMI, and CHF. Measurement of hs-CRP should be done in the fasting or non-fasting state in metabolically stable patients free of infection or acute illness. The cut-offs for hs-CRP using standardised assays categorises patients as follows:

Low-risk: < 1.0 mg/l
Average risk: 1.0 - 3.0 mg/l
High-risk: > 3.0 mg/l
Very high-risk: > 10 mg/l

Levels of hs-CRP greater than 3 mg/l also predict recurrent coronary events, thrombotic complications after angioplasty, poor outcome in the setting of unstable angina, and vascular complications after bypass surgery (CABG). Additionally, hs-CRP has prognostic usefulness in cases of acute ischaemia, even without troponin level elevation, suggesting that an enhanced inflammatory response at the time of hospital admission can determine subsequent plaque rupture. These findings explain why individuals with elevated hs-CRP levels are more likely to benefit from aggressive interventions compared to those with low hs-CRP levels15. hs-CRP levels correlate only modestly with underlying atherosclerotic disease, but indicate an increased propensity for plaque disruption and/or thrombosis.
Table I: Currently used cardiac biomarkers.

<table>
<thead>
<tr>
<th>Marker</th>
<th>What it is</th>
<th>Tissue source</th>
<th>Reason for increase</th>
<th>Time to increase</th>
<th>Time back to normal</th>
<th>When/how used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac troponin</td>
<td>Regulatory protein complex, two cardiac specific isoforms: T and I</td>
<td>Heart</td>
<td>Injury to heart</td>
<td>3 to 4 hours</td>
<td>Remains elevated for 7 to 10 days</td>
<td>To diagnose myocardial infarction, assess degree of damage</td>
</tr>
<tr>
<td>CK-MB</td>
<td>Myocardium related isoenzymes of CK</td>
<td>Heart primarily, but also skeletal muscle</td>
<td>Injury to heart and skeletal muscle cells</td>
<td>4 to 6 hours after heart, muscle injury; peak at 24 hour</td>
<td>Returns to normal within 48 - 72 hour</td>
<td>Less specific than troponin, may be ordered when troponin is not available; also used to diagnose re-infarction</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>Oxygen storing protein</td>
<td>Heart and skeletal muscle</td>
<td>Injury to heart and skeletal muscle</td>
<td>2 - 4 hours after injury peak in 8 - 12 hrs</td>
<td>Returns to normal within 24 hours</td>
<td>Sometimes, in addition to troponin to provide early diagnosis</td>
</tr>
</tbody>
</table>

2. **B type natriuretic peptide (BNP)**

BNP is secreted by right and left ventricular myocytes and released in response to stretch, volume overload, and elevated filling pressures. Serum levels of BNP are elevated in patients with asymptomatic LV dysfunction as well as symptomatic HF. The presence of acute heart failure (HP) in patients with ACS is a well known predictor of adverse cardiac events. Therefore it is not surprising that an elevated BNP level is a marker of CHF and is also a predictor of adverse cardiac events in patients with ACS. In addition, the severity of ischaemia is directly proportional to elevation in BNP. A serum BNP of < 100 pg/ml has a good negative predictive value and typically excludes HF as primary diagnosis in dyspnoeic patients. BNP levels correlate with the severity of HF and predict survival.

3. **Myeloperoxidase (MPD)**

Myeloperoxidase is a leukocyte enzyme that generates reactant oxidant species and has been linked to prothrombotic oxidised lipid production, plaque instability, lipid-laden soft plaque creation and vasoconstriction from nitrous oxide depletion. Elevated MPD levels independently predicted the increased risk of major adverse cardiac events including MI, reinfarction, need for revascularisation, or death at 30 days and 6 months, even among those with negative cardiac troponin I and T. Myeloperoxidase may be a useful early marker in the emergency department (ED) based on its ability to detect plaque vulnerability that precedes acute coronary syndrome.

4. **Ischaemic modified albumin (IMA)**

IMA is a novel marker of ischaemia produced when circulating serum albumin contacts ischaemic heart disease. IMA can be measured by albumin cobalt binding assay that is based on ischaemic modified albumin inability to bind to cobalt. This assay has received the FDA approval for use with troponin and ECG. It is not widely available but may become useful some day for identifying patients at higher risk of ACS. In a recent metaanalysis, this test has high sensitivity and negative predictive value of IMA for detecting ACS especially in combination with troponin and ECG measurements, but specificity is very poor.

However, IMA level is also increased in patients with liver cirrhosis. Certain infections, and advanced cancer within reduces the specificity of this assay further.

Which is the best cardiac biomarker?

The best marker depends on the time taken to give positive results from onset of symptoms. The earliest markers are myoglobin and CK-MB isoforms. CK-MB and troponins are ideal markers in the intermediate period of 6 to 24 hours. It is important that the clinicians realise that troponins are not early markers. Only 35% of patients with NSTEMI (non-ST elevation myocardial infarction) have positive troponins at baseline evaluation.

Time schedule for cardiac biomarker testing

The sample time at 3 - 4 hours is useful in the chest pain observation unit where rapid triage and early diagnosis are essential. In other patients admitted for ACS, biomarkers drawn at the 3 to 4 hours time interval are not as important as the 6 to 9 hours sample. The recent ACC/AHA guidelines for the treatment of patients with unstable angina and NSTEMI recommend a baseline
sample upon ED arrival and a repeat sample 6 - 9 hours after presentation. Serial sampling that become positive in the 12 to 24 hours time window are unlikely unless the patient has ongoing symptoms of ischaemia after admission. AMI can essentially be ruled-out in patients with negative serial marker results through the 6 - 9 hours period after presentation. This later recommendation from the ACC/AHA guidelines represents a significant change in the standard of care for ruling-out AMI.1,22

The following table outlines the recommended sampling frequency after ED admission for various cardiac markers.

**Table II: Cardiac marker sampling frequency.**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Baseline</th>
<th>3-4 hr</th>
<th>6-9 hr</th>
<th>12-24 hr</th>
<th>&gt; 24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB isoform, myoglobin</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK-MB, TnI, TnT</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>(only if very high-risk)</td>
<td></td>
</tr>
<tr>
<td>Late presenters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

**Conclusion**

Cardiac biomarkers are released into the blood stream after myocardial injury or during pressure or volume overload and myocardial dysfunction. They play an important role not only in the diagnosis of patients of acute myocardial infarction/acute coronary syndrome but also for risk stratification and prognostication of such patients. However, abnormal values should be interpreted carefully in the proper clinical context.

**References**