Cardiac Biomarkers

Felicia Ikolo, Ph.D.

Department of Biochemistry, SGU
Email: fikolo@sgu.edu   Tel. ext #: 3425

Office hours: 2.00 p.m. – 3.30 p.m. Mon., Wed. & Fri., or by appointment at other times
Objectives

- Discuss the effects of necrosis and inflammation on serum proteins and enzyme levels
- Describe isozymes in general
- Differentiate the isozymes of creatine kinase (CK) and lactate dehydrogenase (LDH) based on tissue location and subunit composition
- Discuss the utility of serum biomarkers following myocardial infarction
  - use of serum cardiac troponins I and T as markers
  - use of CK/CKMB ratio for evaluation of myocardial infarction
  - Time frame of serum cardiac biomarkers (myoglobin, cardiac troponin, CK-MB).
- cTnI and cTnT are the new cardiac biomarkers
- Present information about the medical use of cardiac biomarkers in MI and non-MI situations.
Cardiac Biomarkers

- **Biomarkers** are measurable and quantifiable biological parameters which serve as indices for health and physiology assessments.
  

- **Cardiac biomarkers** are mostly **enzymes** and **proteins** which are assayed to assess the health and physiological state of the heart.
  
  - Cardiac biomarkers indicate the amount of damage to the heart.
  
  - The level of a specific enzyme or protein in the serum often correlates with the extent of tissue damage.
Ideal Cardiac Biomarker

• Early appearance, but measurable later on.

• Accurate, specific, precise detection

• Readily available, fast results

• Cost-effective
Serum versus Plasma

- **Plasma** is the fluid, noncellular part of blood.
  - Plasma is the liquid, cell-free part of blood, that has been treated with anti-coagulants.

- **Serum** is the part of blood which is similar in composition to plasma but exclude blood clotting factors.
  - Serum is obtained by centrifugation of whole blood after it has been allowed to clot.
  - Laboratory assays of enzyme activity most often use serum.

[http://labtestsonline.org/lab/photo/blood1/start/3](http://labtestsonline.org/lab/photo/blood1/start/3)
Enzymes as Biological Catalysts

• Most reactions in cells are catalyzed by enzymes

• Enzymes are reused during the course of a reaction.

• Enzymes are physiologically important because they speed up the rates of reactions that would otherwise be too slow to support life.

• Increases in the levels of enzymes with a wide tissue distribution gives a less specific indication of the site of cellular injury and limits their diagnostic value.
Isoenzymes

• Most isoenzymes (also called isozymes) are enzymes which catalyze the same reaction.

• They do not necessarily have the same physical properties because of genetically determined differences in their amino acid sequence.
  
  – Hence, isoenzymes may contain different numbers of charged amino acids and may, therefore, be separated from each other by electrophoresis.

• Different organs often contain characteristic proportions of different isoenzymes.

• The pattern of isoenzymes found in the serum may serve as a means of identifying the site of tissue damage.
Which would be the preferred sample for estimating enzymes of the cardiovascular system?

A. Whole blood
B. Blood clot
C. Serum
D. Plasma
E. Urine

Correct answer: C. Serum
Cardiovascular disease (CVD)

- **CVD**: collective term used to describe any disorder of the heart and circulatory system. The most common cardiovascular diseases are:
  - coronary artery disease and
  - stroke.

- **Coronary artery disease**: due to a build up of fatty deposits and cholesterol on the inner walls of the artery. This build up can eventually lead to stenosis or narrowing of the arteries and eventually blockage.
  - If the blockage remains and is not cleared within 12 hours, this will result in cell death (necrosis) in the effected area.
  - When this process occurs in the heart it is called a **heart attack** or **myocardial infarction (MI)**.
Clinical Symptoms of MI

• Radiating chest pain or discomfort (may be different in women).

• Shortness of breath

• Other symptoms:
  – breaking out in a cold sweat,
  – nausea, or light-headedness,
  – upper body discomfort in one or both arms, the neck, jaw, or stomach.

• In patients with diabetes, it is not unusual for myocardial ischaemia (deficient supply of blood) and MI to be silent. That is, to occur without any symptoms due to associated nerve damage.
Key Tests for the Diagnosis of MI

- Electrocardiogram (ECG or EKG)
- Blood test—screens for cardiac biomarkers
- Nuclear scan
- Coronary angiography (or arteriography)
Serum Enzymes & Proteins as Biomarkers in the Cardiovascular system

• The measurement of the serum levels of numerous enzymes and proteins has been shown to be of diagnostic significance.

• The presence of these proteins and/or enzymes in the serum indicates that tissue or cellular damage has occurred resulting in the release of intracellular components into the blood.
Measurement of Cardiac Biomarkers

• Measurement of cardiac biomarkers help to diagnose, stratify risk, monitor and manage patients.

• Serial testing of one or more cardiac biomarkers is necessary to ensure that:
  – a rise in blood levels is not missed and
  – to estimate the severity of a heart attack.

Appearance of creatine kinase (CK) and cardiac troponin in plasma after a myocardial infarction.
Cardiac Biomarkers of the past

- Lactate dehydrogenase (LD or LDH).
- Aspartate aminotransferase (AST)
- Myoglobin
- Creatine kinase
Lactate dehydrogenase (LD or LDH)

• An enzyme used in the past, along with aminotransferases, to diagnose acute MI

• LDH is non-specific for cardiac tissue
  – 5 major LD isoenzymes, LD1–LD5
  – The pancreas, kidneys, stomach tissue and red blood cells (RBCs) also contain LDH-1. Platelets contain LDH2, LDH3 & LDH4
  – Cardiac tissue contains LDH-1 and LDH-2.

• In acute MI, LDH levels rise at about 10 hrs, peak at 24-48 hrs, and remain elevated for up to 8 days
Aspartate aminotransferase (AST)

• Also called serum glutamate oxaloacetate transaminase (SGOT)

• AST is an enzyme found in high concentration in the heart and the liver.

• AST catalyzes the reversible transfer of an α-amino group between aspartate and glutamate
  – an important enzyme in amino acid metabolism.

• AST is non-specific and no longer used in the assessment of myocardial damage
Myoglobin

• A heme-protein present in heart and skeletal muscles

• Functions:
  – as a reservoir for oxygen, and
  – as an oxygen carrier which increases the rate of transport of oxygen within the muscle cell.

• Released from damaged tissues

• Increases occur more rapidly than creatine kinase.

• Presently not utilized on its own for assessing MI because of:
  – Its short plasma half-life (rapid metabolism) and
  – Its lack of specificity for cardiac tissue.

• It is used together with troponins and/or CK-MB
Creatine Kinase

- Creatine kinase (CK) is an enzyme, formerly known as creatine phosphokinase (CPK)
- An intracellular enzyme present in greatest amounts in skeletal muscle, myocardium, and brain; smaller amounts occur in other visceral tissues
- Catalyses a reversible reaction:

![Creatine Kinase Reaction](http://en.wikipedia.org/wiki/File:Creatine_kinase_rxn.png)
Subunits and Isoenzymes of Creatine Kinase

• CK is made up of two different subunits:
  – B
  – M

• These two CK subunits make different isoenzymes:
  – CK-MM (mainly in muscle)
  – CK-BB (brain) and
  – CK-MB (high percentage in heart).
Approximate values of CK Isoenzymes

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<th>Location</th>
<th>CK-MM %</th>
<th>CK-MB %</th>
<th>CK-BB %</th>
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- Elevations in total CK may be from a variety of non-cardiac conditions.
- An elevation in total CK is not specific for MI.
New Cardiac Biomarkers

• Heart-type fatty acid binding protein (H-FABP)

• CK-MB

• Troponin
**Heart-type fatty acid binding protein (H-FABP)**

- H-FABP helps in the intracellular uptake of long chain fatty acids in the myocardium.
- Due to its small size (15kDa) and location (cytoplasm of cardiac myocytes), it is released rapidly into the blood following myocardial damage.
- Combining H-FABP and cTnT provides a significant improvement in the diagnosis of patients presenting with MI within 4 and 12 hours.
- H-FABP is still being evaluated for its utility and not generally evaluated in MI.
**CK-MB**

- Highly specific for cardiac tissue
- Starts to rise 4-6 hours after onset of MI and shows a sharp peak.
- Peaks at about 12 hrs
- Levels return to baseline at 24-48 hrs
- If level normalizes and then rises again, CK-MB levels can be used to indicate re-infarction

Appearance of creatine kinase (CK) and cardiac troponin in plasma after a myocardial infarction.
Elevated CK-MB levels in non-MI cases

• Elevated CK-MB values may be seen in
  – Significant skeletal muscle injury
  – Cardiac injury other than MI
    • Blunt chest trauma like sports injury
    • Some surgical procedures
    • Cocaine abuse
CK:CK-MB ratio

• Proposed to improve specificity for use in diagnosis of MI

• The higher the ratio of CK-MB to CK, the more likely MI has occurred.

• Ratios > 2.5-3 have been proposed for MI
Troponin

- A complex of three regulatory proteins

- Troponin has three subunits:
  - **TnC** which binds calcium ions to produce a conformational change in TnI
  - **TnT** binds to tropomyosin and interlocks them to form a troponin-tropomyosin complex
  - **Tnl** binds to actin in thin myofilaments, thereby holding the troponin-tropomyosin complex in place.

- Attached to tropomyosin which sits in the groove between actin filaments in striated muscle

- **Integral in non-smooth muscle contraction in the skeletal and in the cardiac muscle.**
Cardiac Specific Serum Troponin

• Troponin C is not measured because it does not have an isoform that is specific for the heart
  – it could come from the heart or the skeletal muscle

• Cardiac specific cTnI and cTnT are measured as heart injury markers.

• Serum levels of total CK, CK-MB, and cTnI or cTnT can be used to diagnose or exclude MI in patients with current chest pain, especially when the ECG is inconclusive.
Elevations of cTnl and cTnT

- cTnl levels begin to rise 2-3 hours after onset of MI

- Elevations of cTnl and cTnT may persist for up to 10 days after MI
  - can be measured several days after the MI, when CK-MB is back to baseline.

- The percentage of CK-MB of total CK is used as indicator for the severity

- >3% CK-MB of total CK and increase of cTnl or cTnT may indicate MI.
Which of the following would be the ideal cardiac biomarker 4 days after the onset of clinical symptoms?

A. CK-MB
B. Total CK
C. H-FABP
D. cTnl
E. Myoglobin

D. cTnl
Release Kinetics of some cardiac biomarkers

Interpretation of normal serum cardiac troponin levels

• Cardiac troponin levels are normally low in serum, because they are intracellular and only released from dead myocytes.
  – Troponin I : <10 µg/L
  – Troponin T : 0–0.1 µg/L

(http://www.nlm.nih.gov/medlineplus/ency/article/007452.htm)

• Normal troponin levels 12 hours after chest pain has started mean a heart attack is unlikely.

• Angina is a type of chest discomfort caused by poor blood flow through the blood vessels of the heart muscle with normal cardiac troponin levels
Elevated troponin in non-MI cases

- Serum troponin levels may be elevated in situations other than MI.
  - In non-MI cases, elevated troponin levels is without clinical evidence of ischaemia
  - MI shows troponin peak

- Possible causes of elevated troponin values may include:
  - myocarditis (inflammation of the heart muscle),
  - an arrhythmia (abnormal heart rhythm) or
  - pulmonary embolism (blood clot lodged in the lung).
  - high blood pressure (hypertension),
Laboratory Techniques for detecting MI

• Electrophoresis for CK isoenzymes

• Radioimmunoassays for troponin and CK-MB

• ELISA for CK isoenzymes, troponin & H-FABP

• Lateral flow chromatography immunoassay: measures Myoglobin, CK-MB and cTnI in one sample
Definition of MI

• Evidence of a significant increase in troponin (or CK-MB) concentration with time.

• Evidence of ischaemia: a sudden reduction of heart muscle blood supply with clinical symptoms of MI:
  – Chest pain or discomfort.
  – Shortness of breath
  – Other symptoms—breaking out in a cold sweat, nausea, or light-headedness, upper body discomfort in one or both arms, the neck, jaw, or stomach

• New ECG changes or new imaging changes.

• The Joint European Society of Cardiology/American College of Cardiology Committee
  
  http://eurheartj.oxfordjournals.org/content/21/18/1502.long
A 48 year old male with a history of hypertension and high serum cholesterol presents to the emergency department with chest pains for 60 minutes. He describes a substernal (below the sternum) chest pressure “like an elephant on my chest” associated with shortness of breath and diaphoresis. His ECG result is consistent with myocardial infarction. Which of the following laboratory results would be expected?

A. Elevated myoglobin, elevated troponin I, and elevated CK-MB

B. Normal myoglobin, elevated troponin I, and normal CK-MB

C. Elevated myoglobin, normal troponin I, and normal CK-MB

D. Normal myoglobin, normal troponin I, and elevated CK-MB

E. Normal myoglobin, normal troponin I, and normal CK-MB

C. Elevated myoglobin, normal troponin I, and normal CK-MB
Follow up to question

- During myocardial infarction, certain cardiac biomarkers are released into the bloodstream early and others late.

- Myoglobin is non-specific enzyme which only takes 30 minutes to elevate in the serum after the onset of myocardial infarction.

- cTnI, cTnT and CK-MB are elevated 3-4 hours after onset, but can be measured earlier.

- cTnI and cTnT will stay elevated for 7-10 days and CK-MB for only 3-4 days.

References

  *Enzymes: pages 53 – 68
  *Structure and function of myoglobin: page 26

  *Biomarkers of Myocardial Necrosis – Past, Present and Future: Pages 5 – 20

  *Acute Myocardial Infarction: pages 342 – 349
References continued.


References continued.

