Biomarkers of myocardial infarction

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Cardiovascular system, 2014
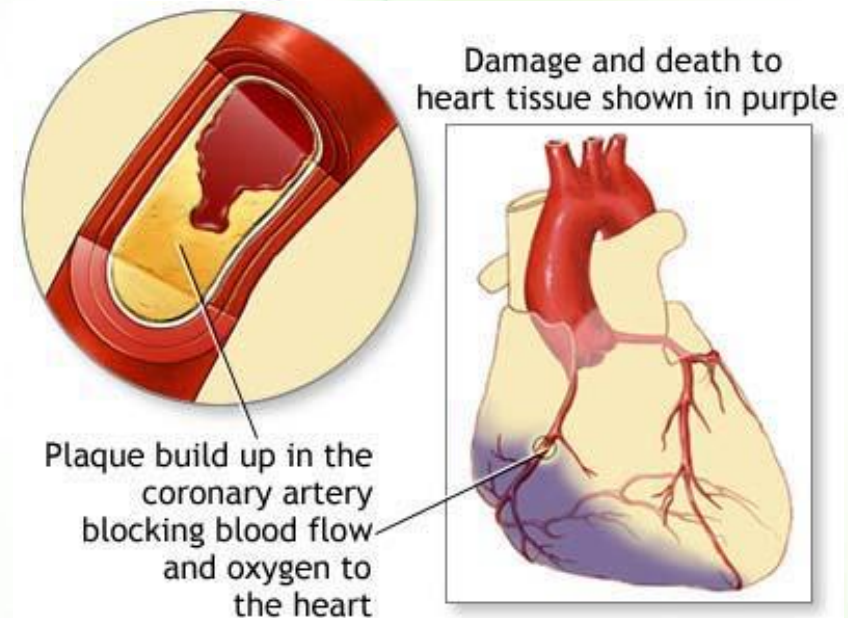
Resources:
- **This lecture**
- **Hand-outs**

Acute Myocardial Infarction

- A rapid development of myocardial necrosis caused by prolonged ischemia (a critical imbalance between the oxygen supply and demand of the myocardium) resulting in an irreversible myocardial injury.

  - After the onset of myocardial ischemia, histological cell death takes as little as 20 min.

  - Complete necrosis of myocardial cells requires at least 2–4 hours or longer.

  - A healed infarction usually takes at least 5–6 weeks.

Damage and death to heart tissue shown in purple

Plaque build up in the coronary artery blocking blood flow and oxygen to the heart

@ ADAM, Inc.

Biochemical Changes

ischemia to myocardial muscles (with low O_2 supply)

↓

anaerobic glycolysis

↓

increased accumulation of Lactate

↓

decrease in pH

↓

activate lysosomal enzymesa

↓

disintegration of myocardial proteins

↓

cell death & necrosis

clinical manifestations
(chest pain)

BIOCHEMICAL MARKERS
release of intracellular contents
to blood

ECG
changes

What is a molecular biomarker?

A molecular alteration that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention

Criteria for ideal markers for MI

- Specific: no false positive **(present in the myocardium absent in nonmyocardial tissues)**
- Sensitive: no false negative **(produced at high concentrations that can be measurable)**
- Prognostic: relation between plasma level & extent of damage
- Persists longer: can diagnose delayed admission
- Reproducible
- Simple, inexpensive
- Quick
- Acceptable (by patient and clinician)
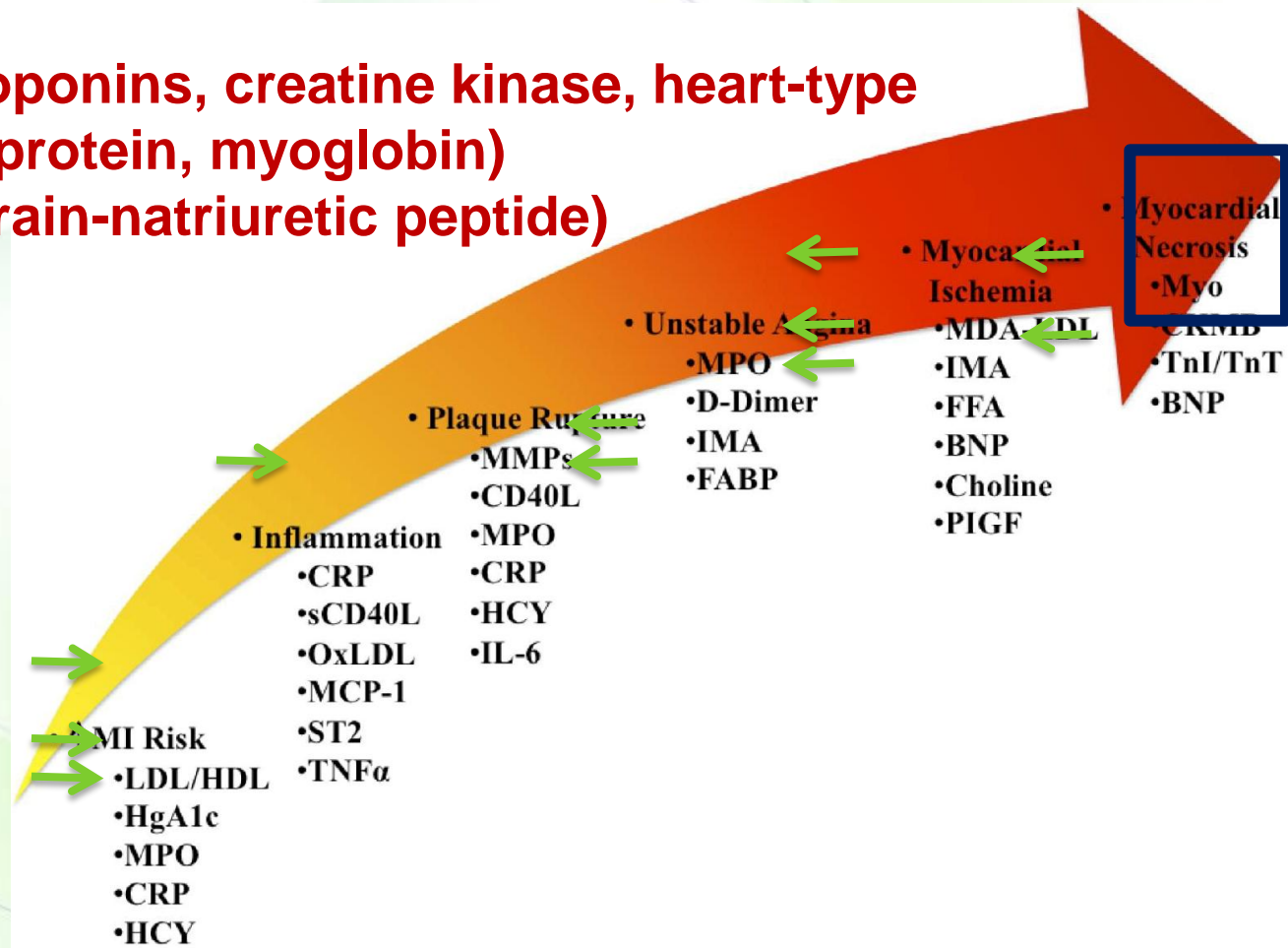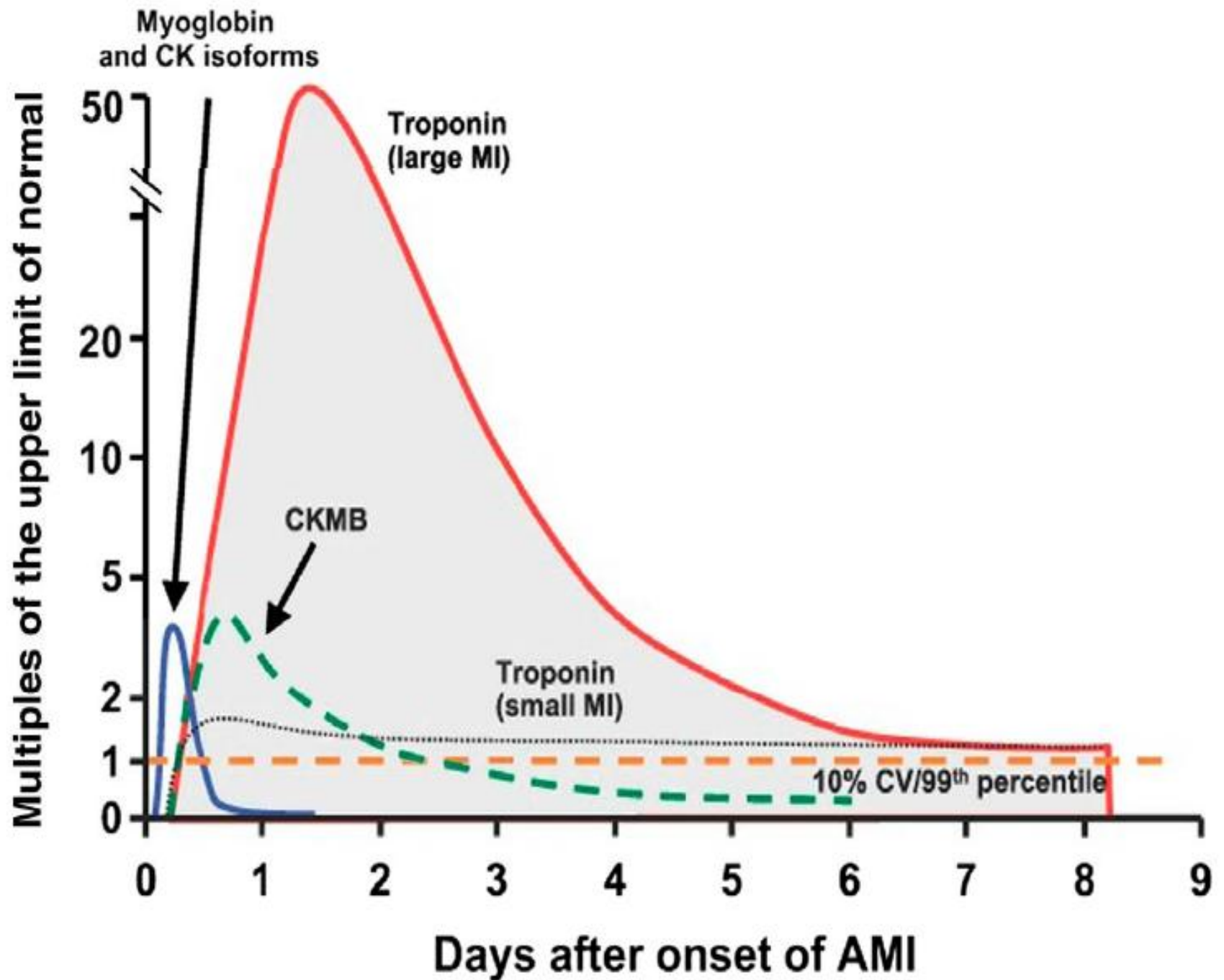
What are biomarkers good for?

- Diagnostic (yes or no; infarct vs. reinfarct)
- Differentiating (AMI, skeletal muscle damage, other cardiac conditions, renal disease, etc.)
- Risk-stratifying (low- vs. high-risk)
- Prognostic (degree of severity; infarct size)

MI biomarkers

- **Inflammation (C-reactive proteins)**
- **Oxidative stress (myeloperoxidase)**
- **Extracellular matrix remodeling (proteases)**
- **Neurohormones**
- **Myocyte injury (troponins, creatine kinase, heart-type fatty acid binding protein, myoglobin)**
- **Myocyte stress (Brain-natriuretic peptide)**
- **New biomarkers**

• Myocardial Necrosis
  •Myo
  •CKMB
  •TnI/TnT
  •BNP

• Myocardial Ischemia
  •MDA-LDL
  •IMA
  •FFA
  •BNP
  •Choline
  •PIGF

• Unstable Angina
  •MPO
  •D-Dimer
  •IMA
  •FABP

• Plaque Rupture
  •MMPs
  •CD40L
  •MPO
  •CRP
  •HCY
  •IL-6

• Inflammation
  •CRP
  •sCD40L
  •OxLDL
  •MCP-1
  •ST2
  •TNFα
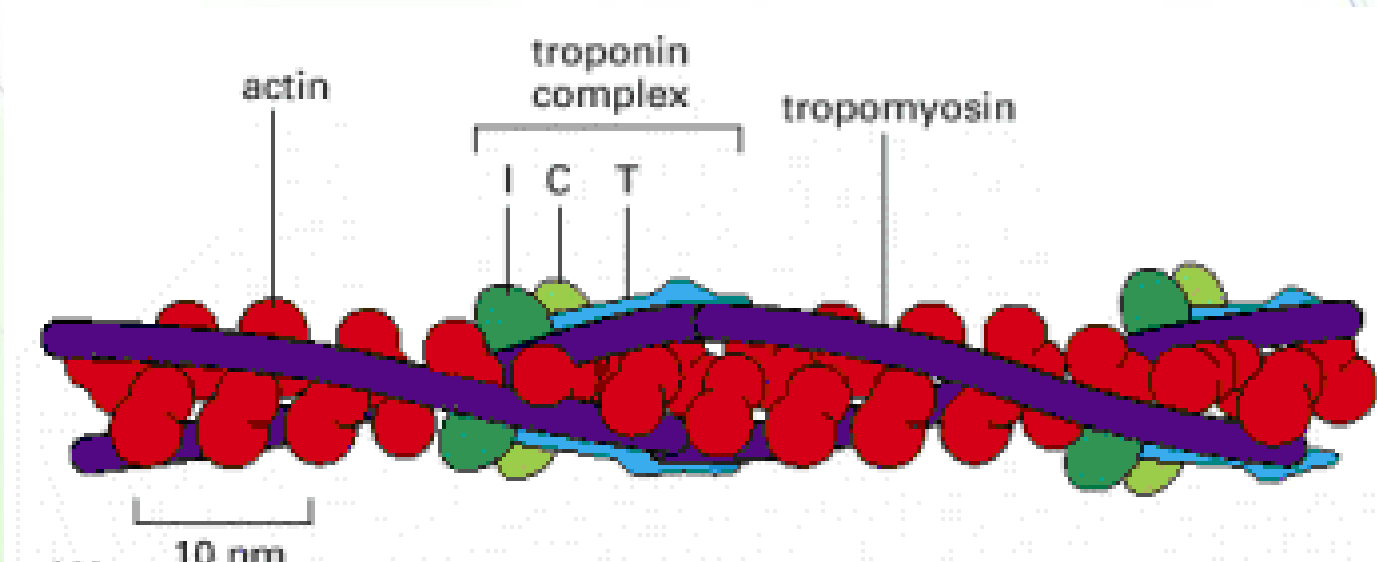
• MI Risk
  •LDL/HDL
  •HgA1c
  •MPO
  •CRP
  •HCY

TROPONINS

THE GOLD STANDARD

Structure

- It is associated with tropomyosin, which forms a continuous chain along each actin thin filament.
- It is a complex of the three subunits:
  - TN-T: tropomyosin binding subunit
  - TN-I: myosin ATPase inhibiting subunit
 - TN-C: calcium binding subunit

Troponin isoforms

- Normal people have virtually nil levels of troponin in serum.
    - Positive results: MI or **chronic** disease.
- Troponin I and T are highly specific for myocardial injury.
    - Levels in a healthy person are negligible.
    - cTNI indicates only cardiac troponin.
    - cTNT may cross-react with troponin found in other muscles:
        - non-cardiac injury such as skeletal myopathies and with renal failure.

Troponins in MI

- Increase within 4-6 hours after onset of MI
- Stays elevated: 5–10 days (cTnI) or 5–14 days (cTnT)

If the earlier results are negative and clinical suspicion remains high, serial sampling at 3–6 h later, and after 12 h is recommended.
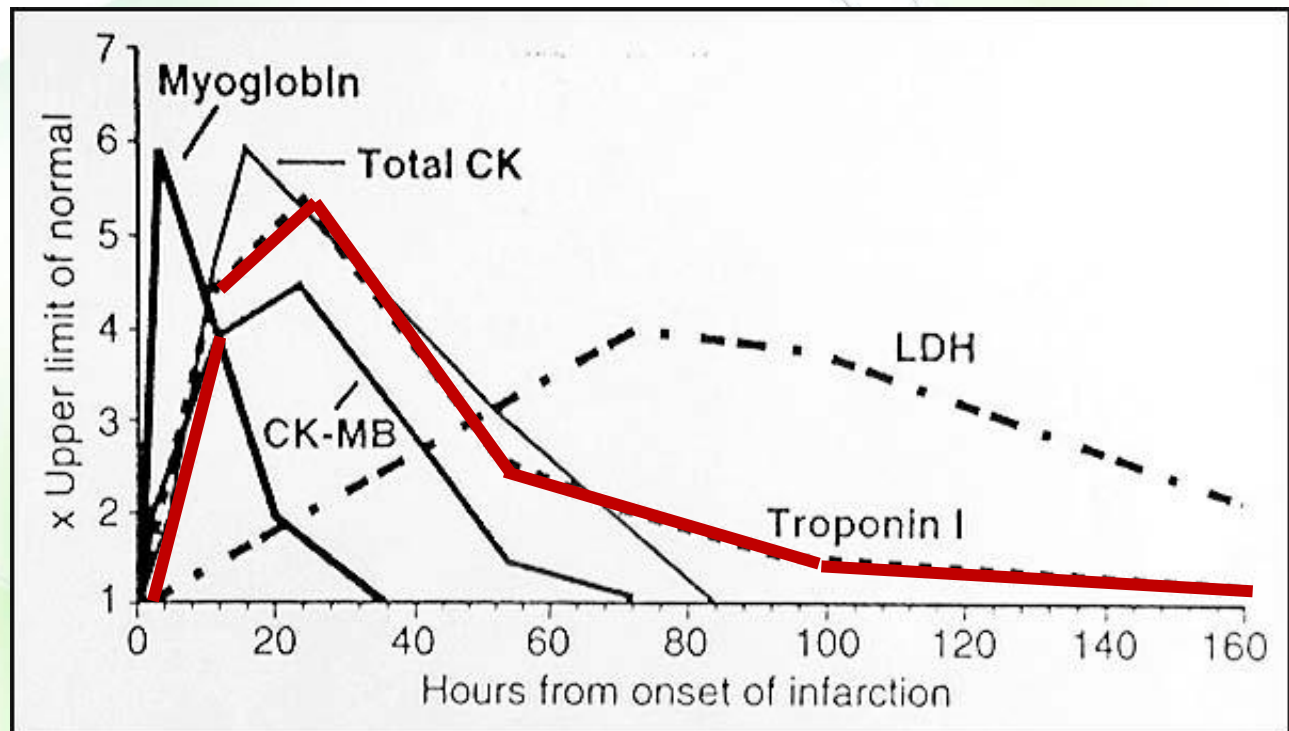
Why is release of troponin prolonged?

- Most is bound to the contractile apparatus of the cardiomyocyte.

- 3% of cTnI and 6% of cTnT exist free in the cytoplasm.

- The initial elevation of cTnI or cTnT is thought to be a function of the free cytosolic form.

- The prolonged elevation is caused by degradation of the contractile pool.

Advantages

- High sensitivity.
- Fewer false-positive.
- Prognostic of death from acute coronary syndrome.
- Troponin can remain elevated up to 10 to 14 days after an event, helping to diagnose patients who have delayed seeking treatment.
 - Nonetheless, re-elevations are easily seen, allowing reinfarction to be diagnosed unambiguously.

- **It lacks sensitivity in the early hours of AMI.**
- **Pulmonary embolism, congestive heart failure, and myocarditis can all lead to cardiac troponin elevation.**
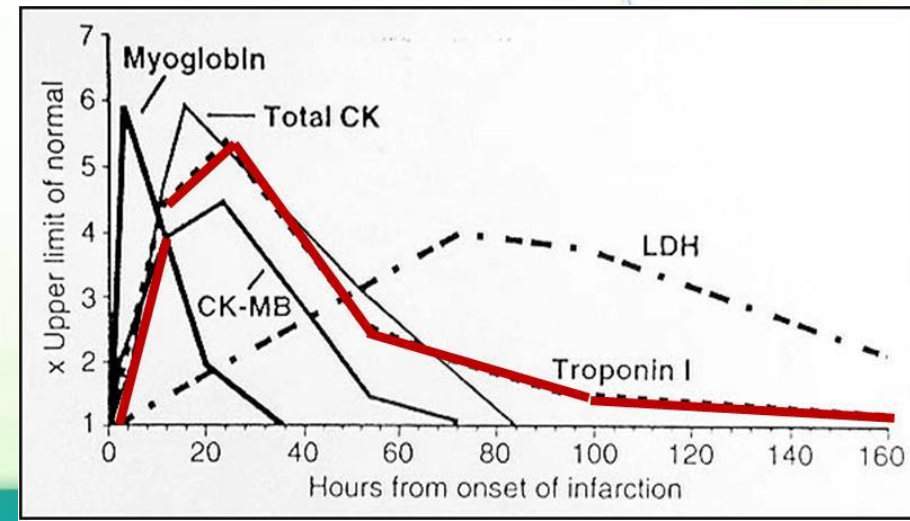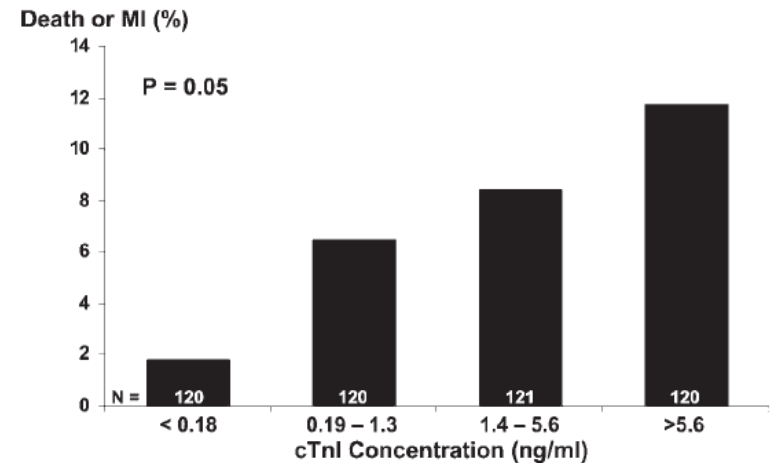
Acute vs. chronic conditions

- It is important to distinguish acute causes of cTn elevation from chronic elevations that tend not to change acutely.

- This is done by demonstrating a rising and/or falling pattern (in acute cases) rather than continuously high elevations in chronic cases.
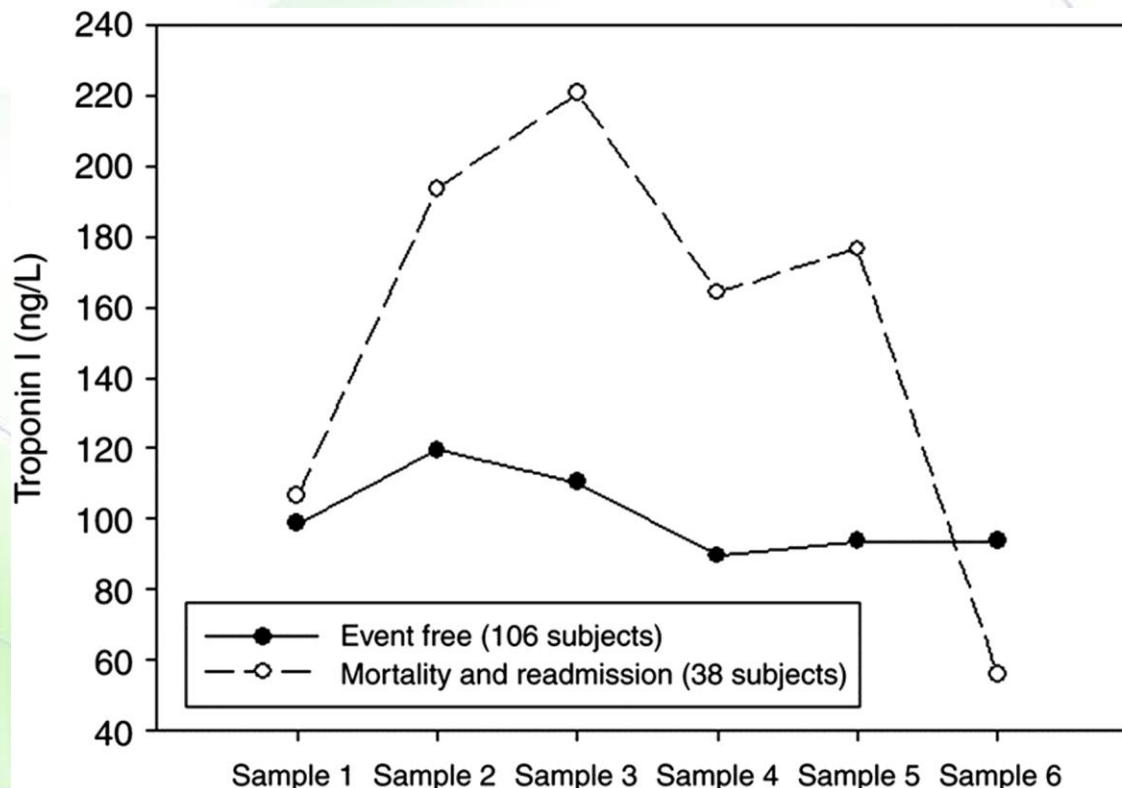
then use that of creatine kinase (measured by mass assay).

Sources of energy

Phosphagen system

8-10 seconds (100 m)

Sprinter

Glycogen-lactic acid system

1.3–1.6 minutes (400 m)

Swimmer

Aerobic respiration
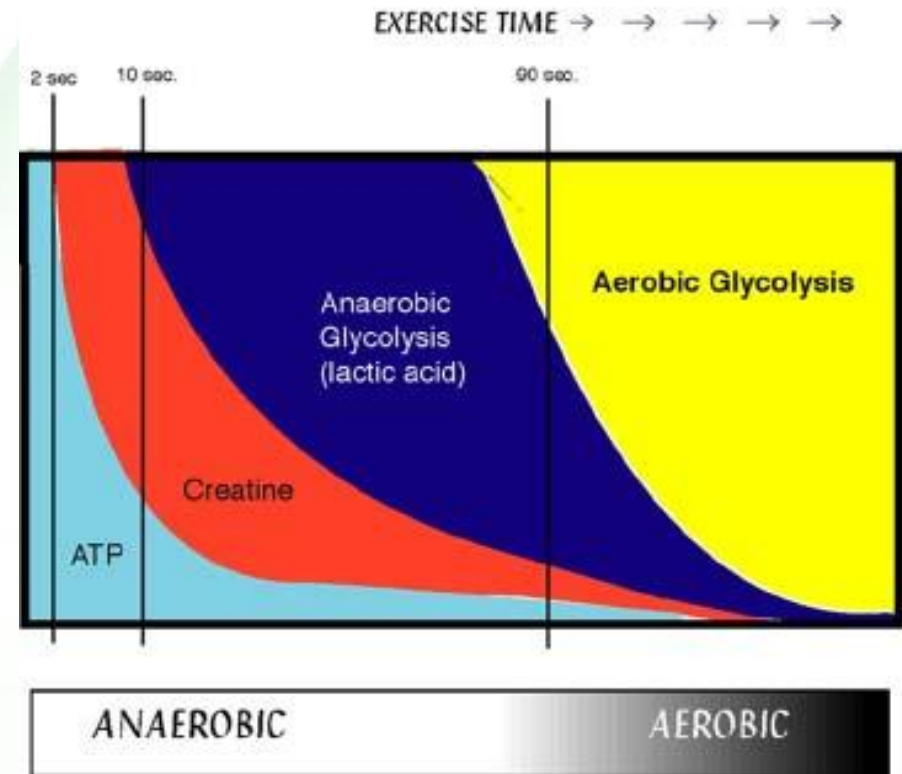
Marathon runner — Unlimited time (15 Km)

©2000 How Stuff Works

PCr

COO⁻
CH₂
N
H₃C NH—P—O⁻
H₂N⁺

ADP ATP

creatine kinase

creatine

COO⁻
CH₂
N
H₃C NH₂
H₂N⁺

2.6% of PCr reserves per day

Pᵢ + H₂O

creatinine

1.1% of creatine reserves per day

H₂O
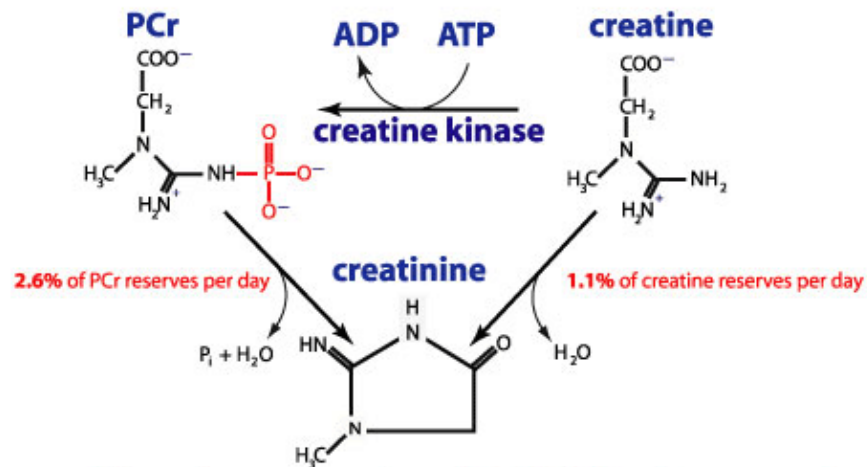
Dominant Energy Pathways for Exercise of Differing Durations

EXERCISE TIME → → → → →

2 sec   10 sec.   90 sec.

Anaerobic Glycolysis (lactic acid)

Aerobic Glycolysis

Creatine

ATP

ANAEROBIC   *AEROBIC*

CK isozymes

Serum	Skeletal Muscle	Cardiac Muscle	Brain
0 trace BB <6% MB >94% MM	0 trace BB 1% MB 99% MM	0% BB **20% MB** **80% MM**	97% BB 3% MB 0%MM

CK-MB

- Increase: 3 and 12 h after the onset of MI
- Peaks at 24 hr
- Reverts to normal values within 48–72 h
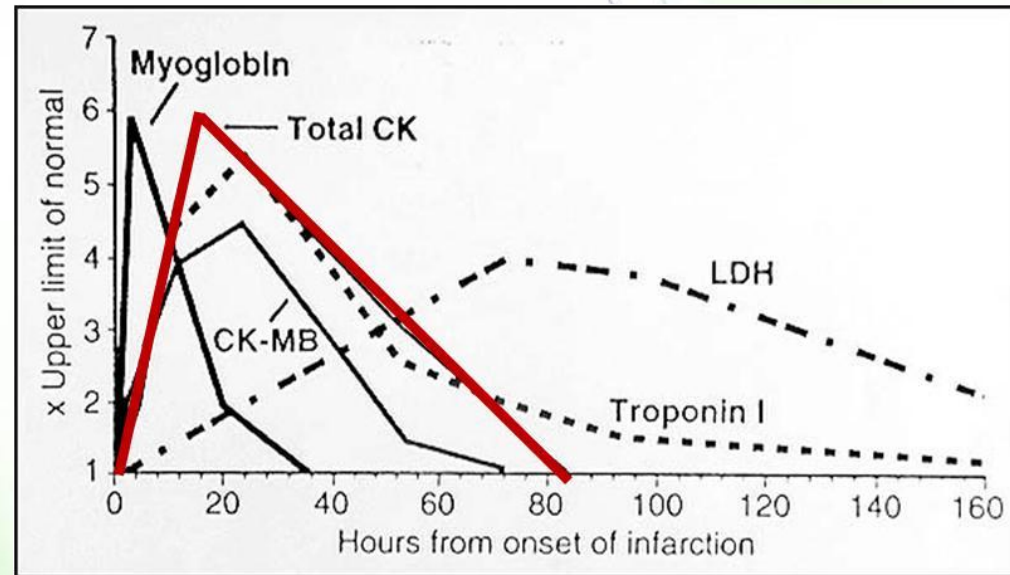
It is useful for diagnosing reinfarction.
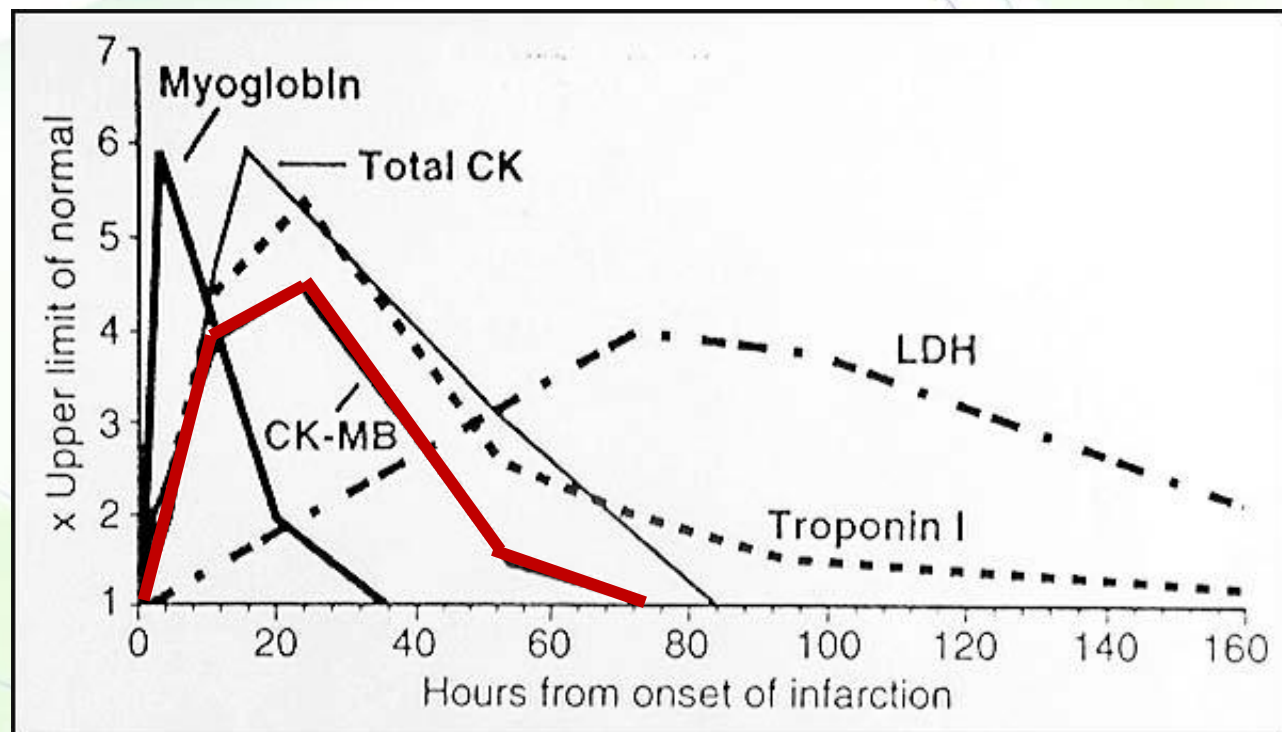
Cardiac relative index
(improves specificity, but may limit sensitivity)
RI = (CK-MB mass / Total CK) x 100

- **Ratio <3 = skeletal muscle source**
- **Ratio > 5 = cardiac source**
- **Ratio of 3-5: definitive diagnosis can be established with serial determinations to detect a rise**

Total CK can be elevated

- False positive (for MI) CK elevation can be seen in:
  - Significant skeletal muscle injury
  - Significant CNS damage (Stroke/Trauma)
  - Occasionally from GI, renal, urologic disease
  - Others: i.m. injection, hypothermia, exercise, intoxication and drug abuse
- Dose-related side effect in statin treatment
  - Statin-related increases in CK mainly affect MM isozyme

CK-MB isoforms and MI

- Two isoforms called 1 (plasma) and 2 (cellular)
- 2 to 1 ratio of > 1.5 can useful for early MI detection
  - It requires a skilled technician.
  - False positive results with congestive heart failure and other conditions can occur.

Types of infarction

- "Incident MI": the individual's first MI.
- "Reinfarction": MI occuring within the first 28 days after an incident event.
- "Recurrent MI": MI occurring after 28 days following an incident MI.

Diagnosis of reinfarction

- In patients in whom reinfarction is suspected from clinical signs or symptoms following the initial MI, an immediate measurement of cTn is recommended.

- A second sample should be obtained 3–6 h later.

  - If the cTn concentration is high, but does not change or is not decreasing, s second sample is needed and diagnosis of reinfarction requires a 20% or greater increase of the cTn value.

MYOGLOBIN (Mb)

- it is an early marker that can be detected 1–2 hours after symptom onset, and remains elevated for up to 24 hours.

- It is sensitive in the absence of concomitant skeletal muscle trauma or renal failure.

- Specimens collected serially every 1-2 hours during the first 2-10 hours after infarction.

- Levels that double within 1-2 hours are highly suggestive of AMI.
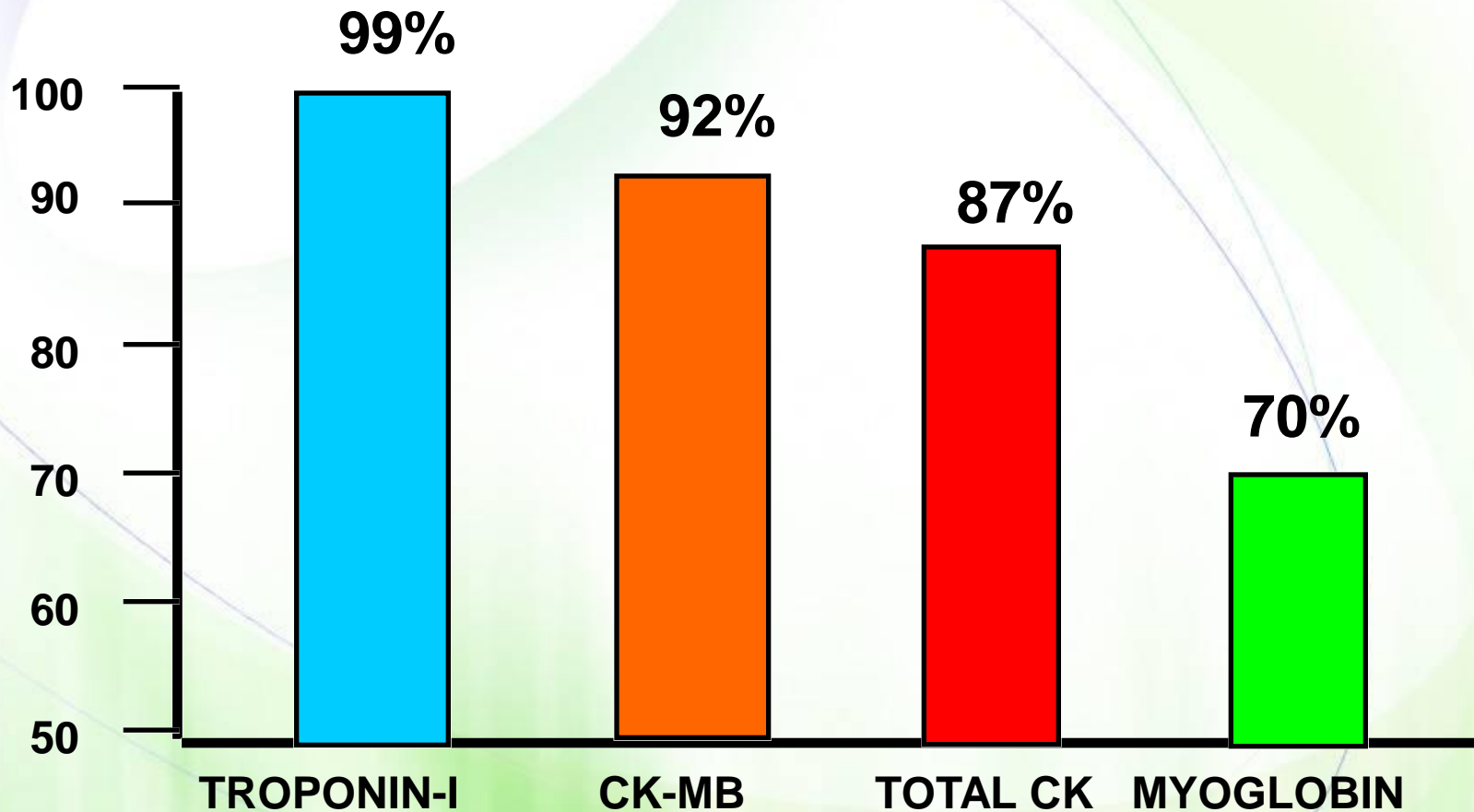- Suited to excluding AMI at the earliest phase.
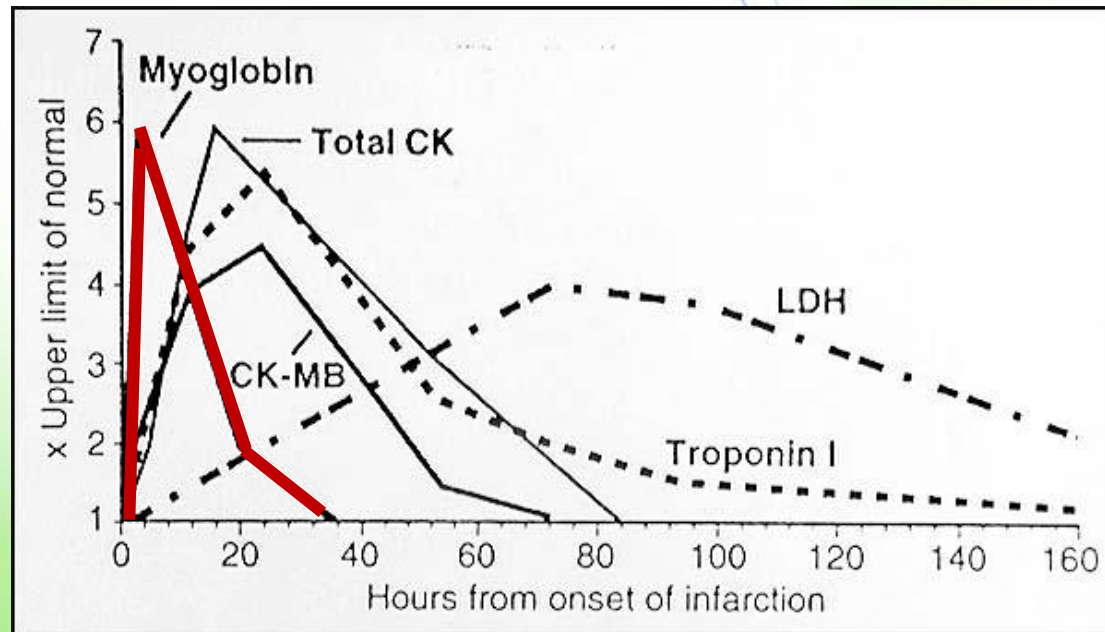
But...

- low-specificity for MI
  - in patients with renal failure or skeletal muscle trauma
- Rises and falls rapidly in the setting of MI
- The level may normalize in patients that present >24 hours after symptom onset
  - indicated for the diagnosis of re-infarction
- Therefore,
  - potentially useful for ruling out but not for confirming the diagnosis of AMI
  - Is used in combination with CK-MB or cTn

SPECIFICITY OF CARDIAC MARKERS

Summary

MARKER	TISSUE SOURCE	PHYSIOLOGIC FUNCTION	"DIAGNOSTIC WINDOW"	CLINICAL UTILITY
Cardiac Troponin I	Cardiac muscle	Muscle contraction regulatory protein; bound to tropomyosin and actin	Rise 4-8 hr Peak: 14- 18 hr Normal: 5-9 days	Highly specific for myocardial injury Useful for patients with atypical symptoms or those who delay seeking medical attention Potential to diagnose AMI in patients who also have concomitant skeletal muscle trauma/disease Potential usage to risk stratify angina pectoris

| MARKER	TISSUE SOURCE	PHYSIOLOGIC FUNCTION	"DIAGNOSTIC WINDOW"	CLINICAL UTILITY
Cardiac Troponin T (cTnT)	Cardiac muscle; regenerating skeletal muscle	Same as above	Rise: 4-8 hr Peak: 14-18 hr Normal: >14 days	As above for cTnI

MARKER	TISSUE SOURCE	PHYSIOLOGIC FUNCTION	"DIAGNOSTIC WINDOW"	CLINICAL UTILITY
Creatine Kinase (CK) Total Activity	Skeletal muscle Cardiac muscle Skeletal muscle	Rephosphorylation of ADP, forming ATP in muscle contraction	Rise: 6-8 hr Peak: 24-36 hr Normal: 3-4 days	Limited diagnostic (increased in various diseases) CK isoenzyme analysis is more useful for diagnosis
CK-MB Isoenzyme, Mass (amount, not activity)	Cardiac muscle Skeletal muscle to a lesser extent	Same as above	Rise: 4-6 hr Peak: 12-24 hr Normal: >48 hr	Mass assay of CK-MB isoenzyme, the current "gold standard" for early diagnosis of AMI
CK-MB Isoforms and Isoforms ratio	Same as above	Same as above	Rise: 2-6 hr Peak: 6-12 hr Normal: 24-36 hr	Early marker of AMI

Old biomarkers

Aspartate aminotransferase
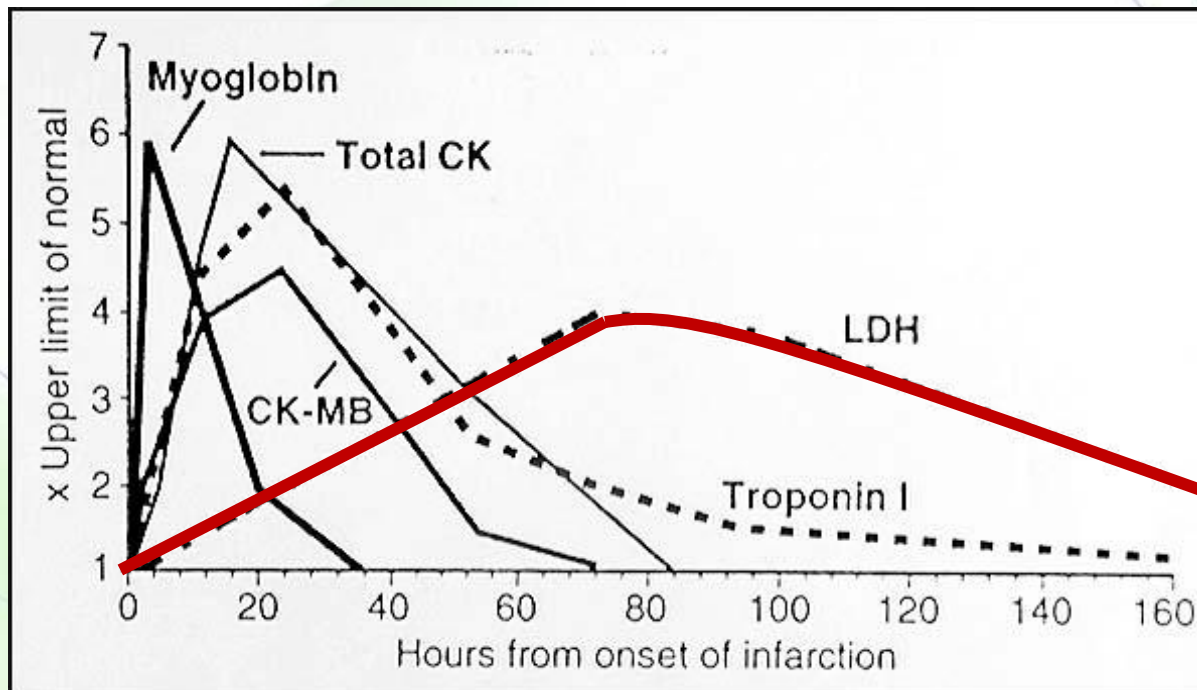**Lactate dehydrogenase**

Aspartate aminotransferase
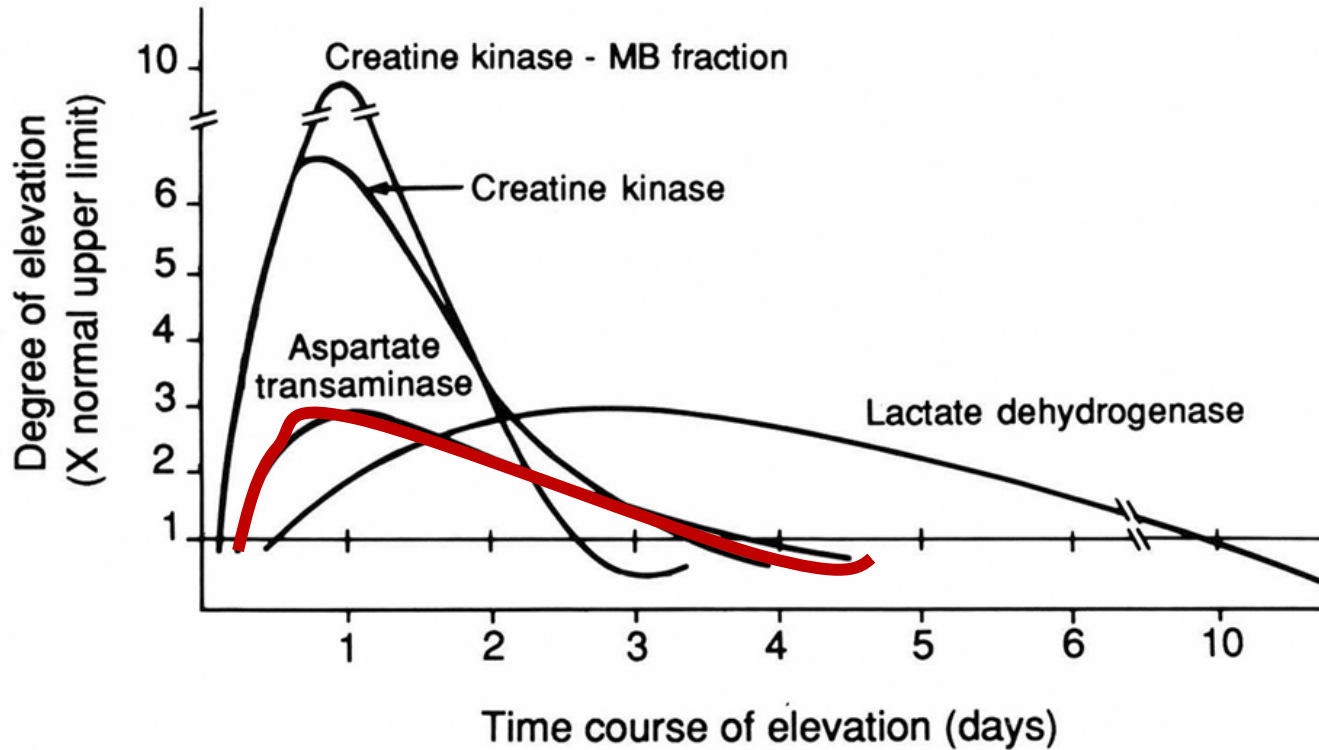
Time course of myocardial enzymes appearing in the blood after myocardial infarction

Lactate dehydrogenase in MI

- Rises in 12 to 24 hours following MI,
- Peaks in 2 to 3 days
- Gradually disappearing in 5 to 14 days

Tissue distribution of LDH

Isoenzyme	Composition	Present in
LDH1	(H_4)	Myocardium, RBC, kidney
LDH2	(H_3M_1)	Myocardium, RBC, serum, kidney
LDH3	(H_2M_2)	Kidney, Skeletal muscle
LDH4	(H_1M_3)	Kidney, Skeletal muscle
LDH5	(M_4)	Skeletal muscle, Liver

Normal
LD1:LD2 = 0.5-0.75

MI
LD1:LD2 > 1

LD isoenzyme electrophoresis (normal)

LD-2 > LD-1 > LD-3 > LD-4 > LD-5

LD-2
LD-1
LD-3
LD-4
LD-5
Cathode (-)
Anode (+)

LD isoenzyme electrophoresis (abnormal)

LD-1
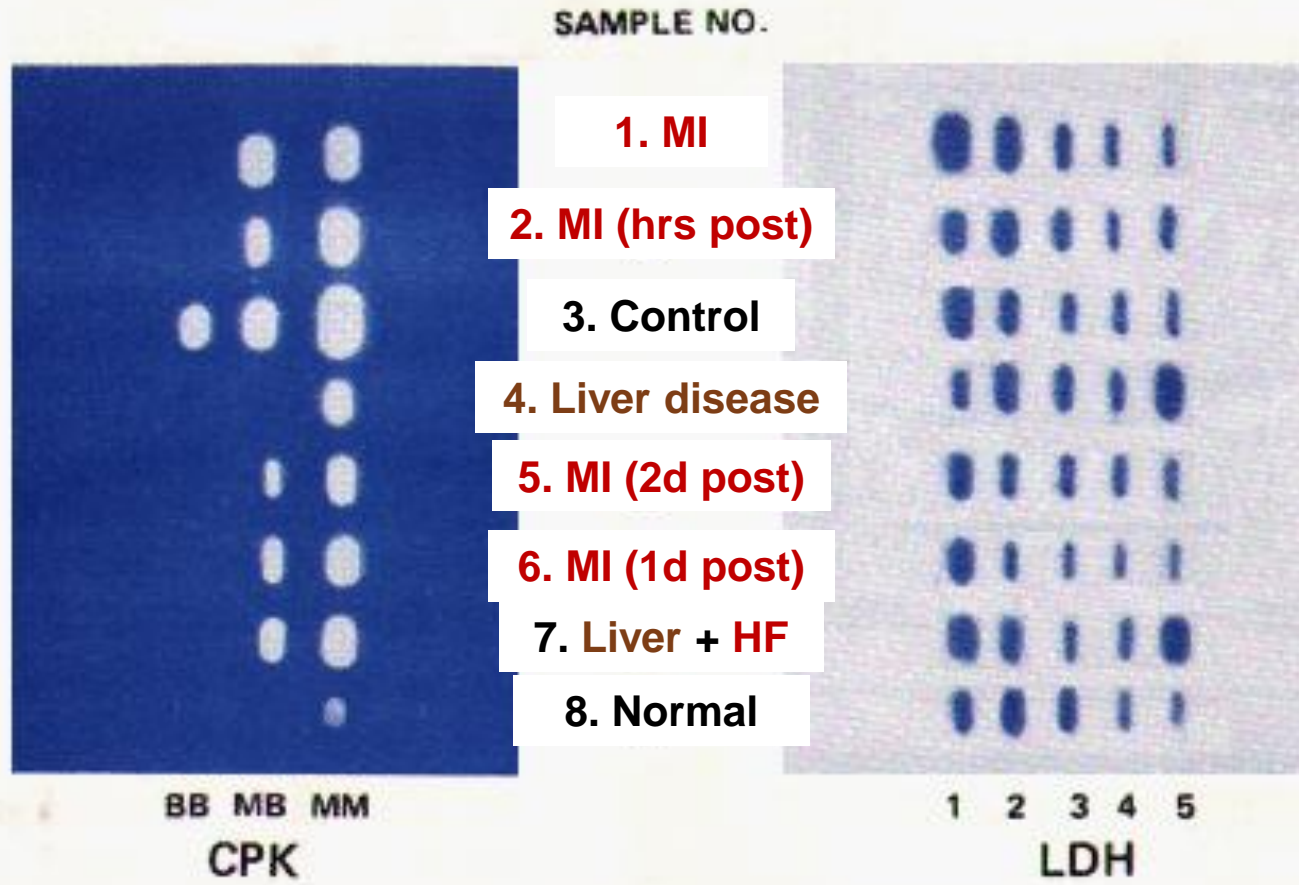LD-2
LD-1 > LD-2
LD-3
LD-4
LD-5
Cathode (-)
Anode (+)
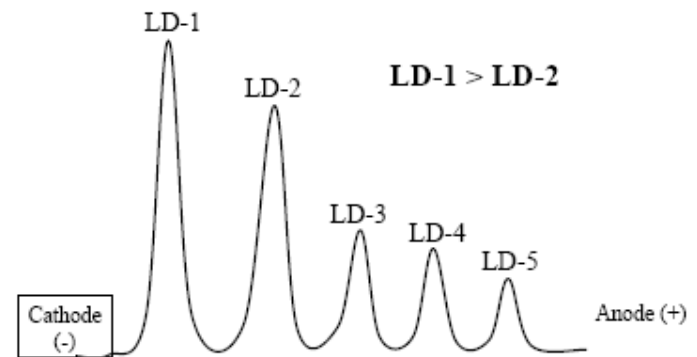
- Hemolysis
- Megoblastic & Pernicious Anemia
- Renal Cortex Infarction
- Testicular Germ Cell Tumors
- Small Cell Lung Carcinoma
- Adenocarcinoma of the Ovary
- Acute Coronary Insufficiency (Unstable Angina)
- Exercise Induced Myocardial Ischemia
- Polymyositis
- Muscular Dystrophies
- Well Trained Athletes
- Rhabdomyolysis

Example

Correspondence Between CPK and LDH Isoenzyme Patterns

SAMPLE NO.

1. MI
2. MI (hrs post)
3. Control
4. Liver disease
5. MI (2d post)
6. MI (1d post)
7. Liver + HF
8. Normal

BB MB MM
CPK

1 2 3 4 5
LDH

Interpretation

- **Sample #3** represent results for a control
- **Sample #8** results are from a normal specimen.
- **Sample# 1** MI patient. The specimen was collected at a time when the activity of both LDH and CK were elevated. Note the LDH flip and the high relative activity of the MB isoenzyme.
- **Sample# 2** MI patient who experienced chest pain only several hours previously. Total CK is significantly elevated with a high relative MB isoenzyme activity.
- **Sample# 6** MI patient (the 1st day post MI); CK activity is definitely elevated with a high relative MB isoenzyme activity and the LDH flip is evident.
- **Sample# 5** MI patient (2 days post MI) so that CK has almost returned to normal activity and the LDH flip is definite.
- **Sample# 7** MI patient with complications of heart failure and passive liver congestion or the patient was involved in an accident as a consequence of the MI, and suffered a crushing muscle injury.
- **Sample# 4** a patient with liver disease. Although the LDH isoenzyme pattern is indistinguishable from muscle disease or injury, the absence of at least a trace of CK-MB isoenzyme is inconsistent with the muscle CPK isoenzyme distribution as is the apparently normal total activity.
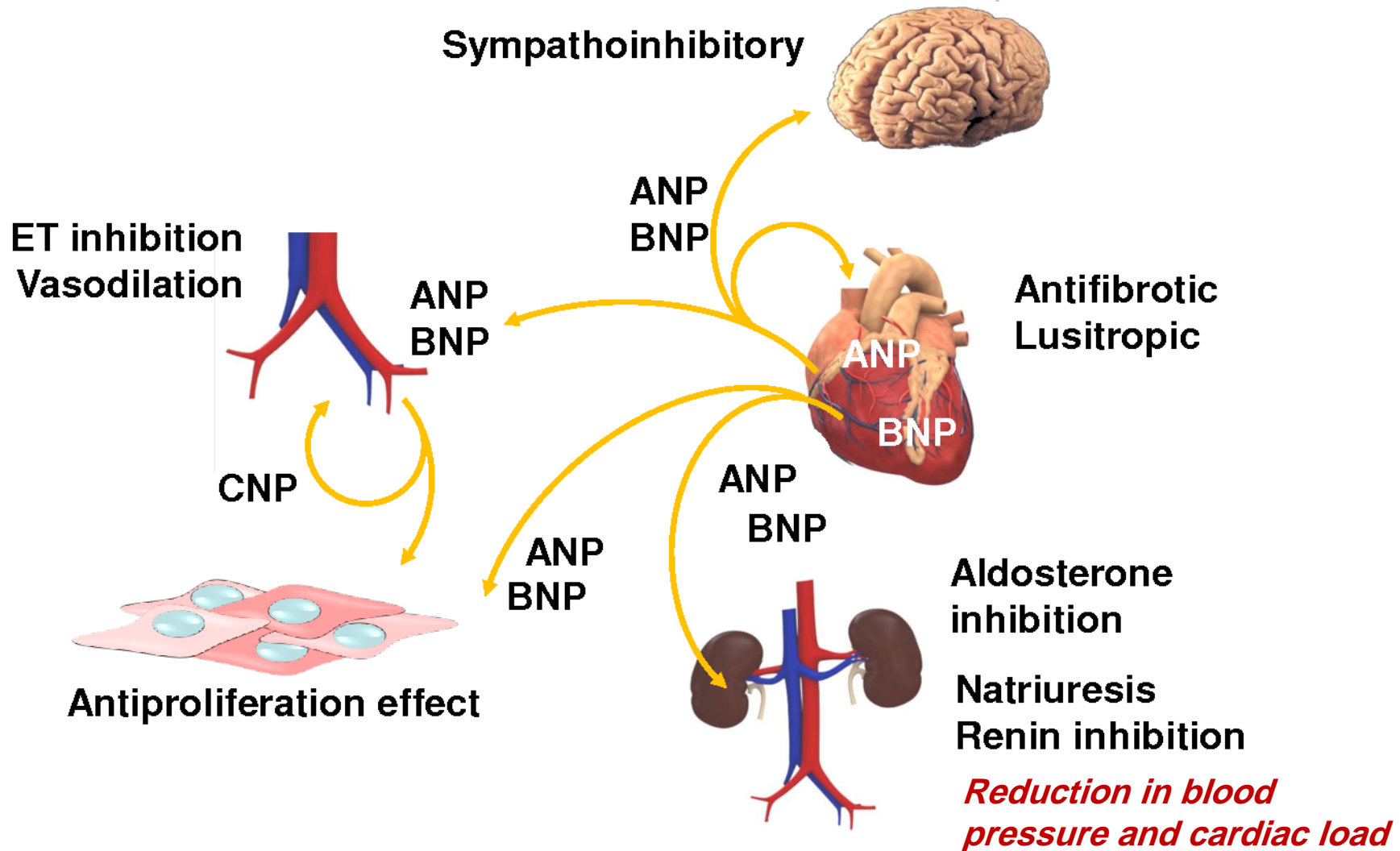
Future markers

Natriuretic peptides

- Atrial natriuretic peptide (ANP)
- B type natriuretic peptide (BNP)
- C type natriuretic peptide
- D type natriuretic peptide

All hormones function in the homeostasis of sodium and water retention

The Natriuretic Peptide System

Sympathoinhibitory

ANP
BNP

ET inhibition
Vasodilation

ANP
BNP

ANP
BNP

ANP

BNP

Antifibrotic
Lusitropic

CNP

ANP
BNP

ANP
BNP

Antiproliferation effect

Aldosterone
inhibition

Natriuresis
Renin inhibition

*Reduction in blood
pressure and cardiac load*

Adapted from Burnett JC Jr. *J Hypertens*. 1999;17(Suppl 1):S37–43.

Synthesis and secretion of BNP

- B type natriuretic peptide is produced constantly by the cardiac muscle cells.

- It is initially made as a prohormone called proBNP, which is hydrolyzed into an N terminal portion (NTproBNP) and BNP.

- An increase in BNP or NTproBNP will occur when the heart is stretched due to volume overload.
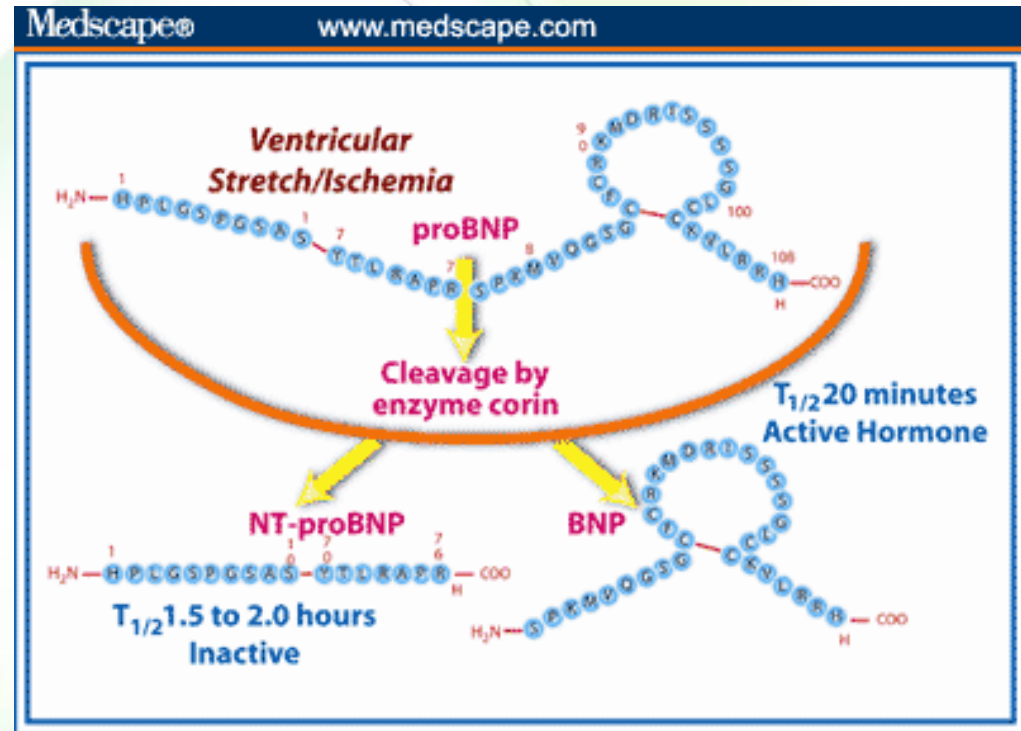
Clinical utilization

- A prognostic indicator of death, heart failure, risk prediction of AMI recurrence (Higher BNP suggests higher chance of AMI recurrence)

- it is useful in risk stratification

- It may also guide treatment

- But…
  - Issue related to cut off.
  - Gender and age differences (higher in women and increasing age).
 - The assays lack precision.
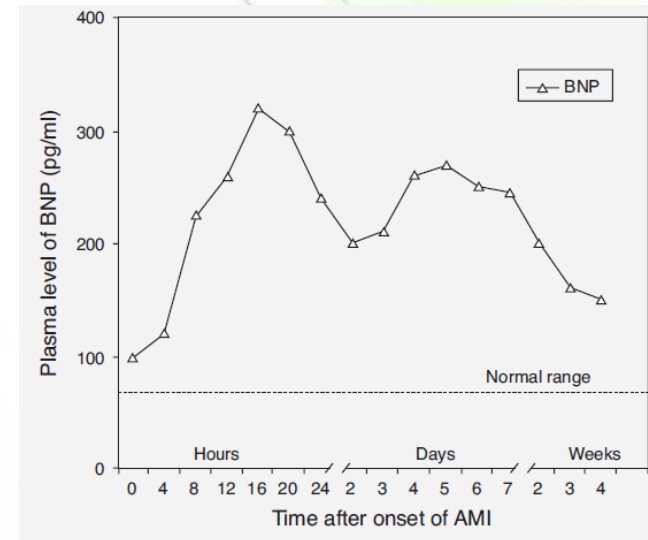
BNP and Mortality
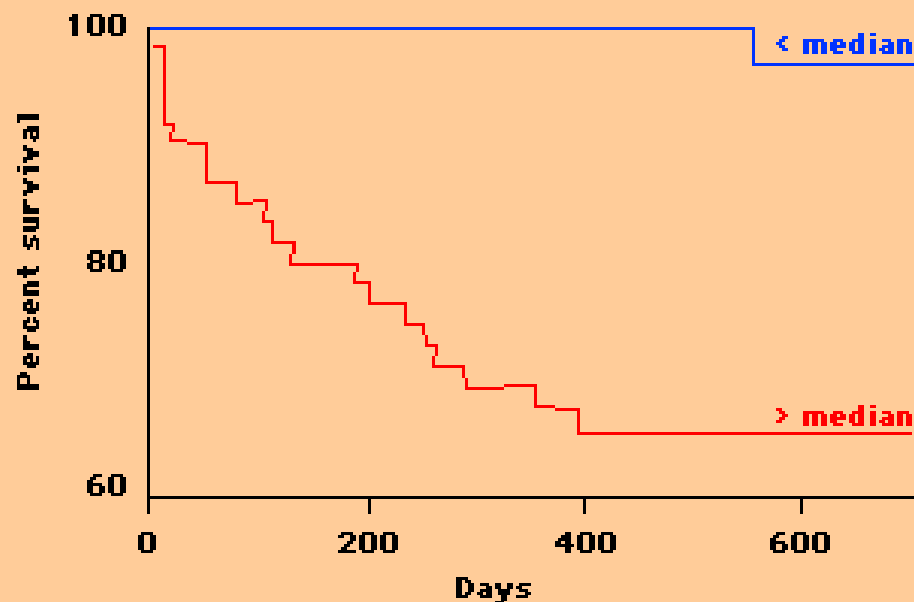
N-BNP predicts survival after acute MI Kaplan-Meier survival curves of 121 patients with an acute myocardial infarction show that patients with serum concentrations of N-BNP that are above the group median of 122 pmol/L had a poorer survival compared to those with N-PNP concentrations below the median (P<0.00001). (Redrawn from Richards, AM, Nicholes, MG, Yandle, TG, et al. Circulation 1998; 97:1921.)

- Heart and brain tissue
  - Because of the blood–brain barrier, GP-BB can be heart muscle specific
- A rapid rise in blood levels can be seen in myocardial infarction and unstable angina.
- GP-BB elevated 1–3 hours after process of ischemia.
  - Early diagnosis in acute coronary syndrome.
- High specificity and sensitivity

Heart-type fatty acid binding protein (H-FABP)
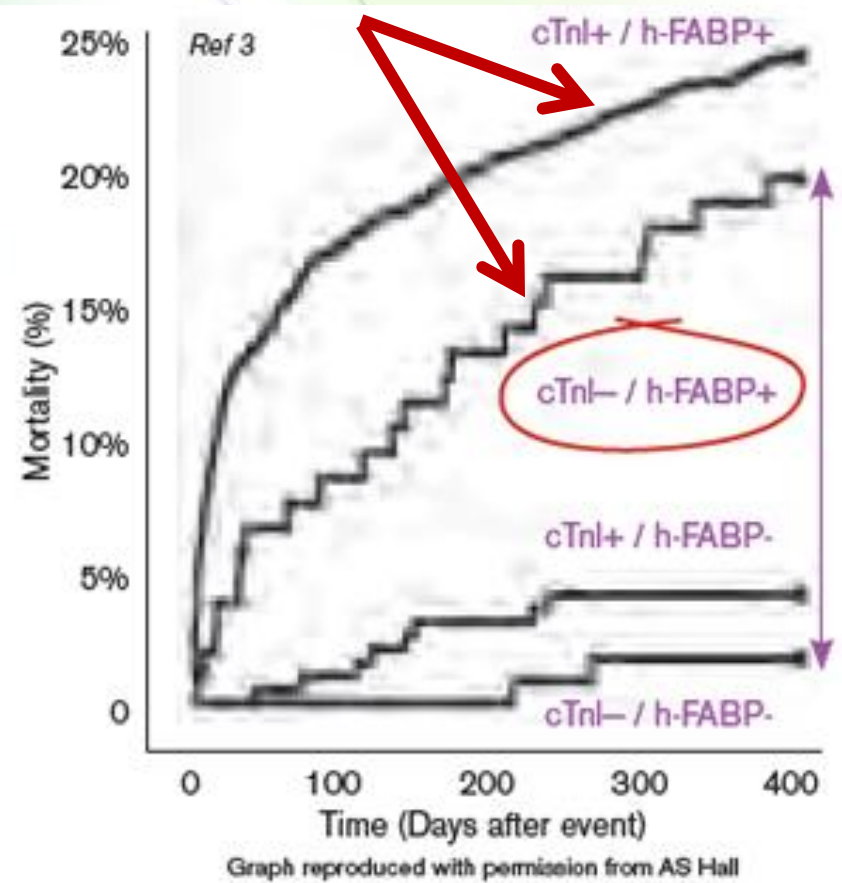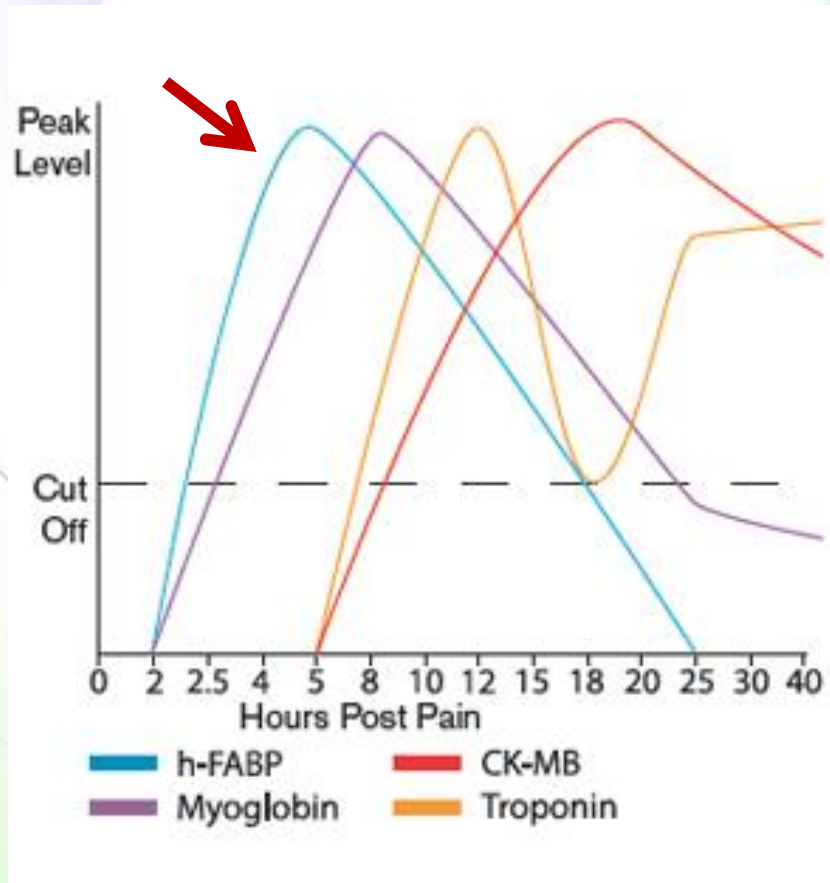
Not heart-specific, but can identify patients at high-risk
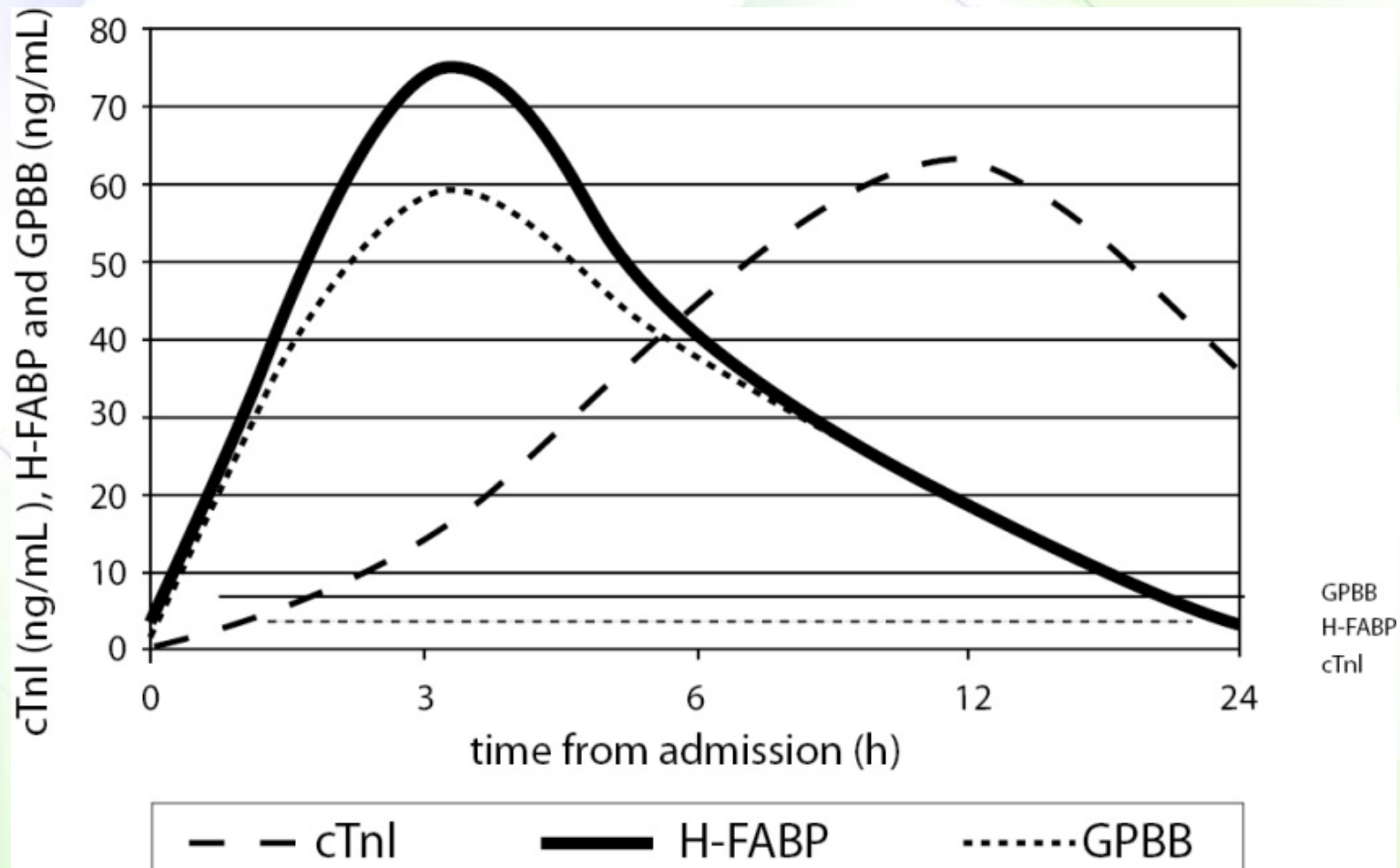
C-Reactive Protein

- Pentameric structure consisting of five 23-kDa identical subunits
- Produced primarily in hepatocytes
- Plasma levels can increase rapidly to 1000x baseline levels in response to acute inflammation
- "Positive acute phase reactant"
- Elevated levels predictive of:
    - Long-term risk of first MI
    - Ischemic stroke
    - All-cause mortality
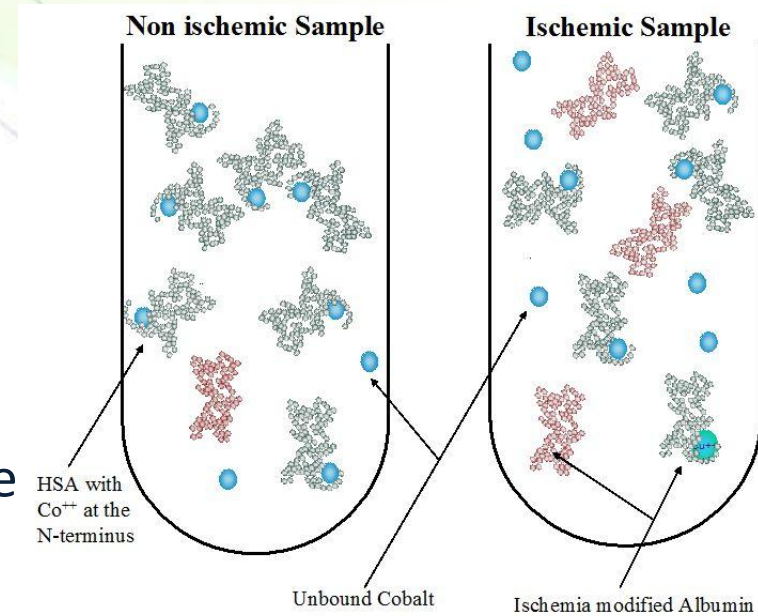
Limitations to CRP in Screening

- Low specificity
- Gender and racial differences exist
- Affected by physiologic conditions , lifestyle behaviors (smoking, obesity, exercise, and alcohol use), and drugs
- Clinical value??
  - No evidence that lowering CRP levels decreases CV risk
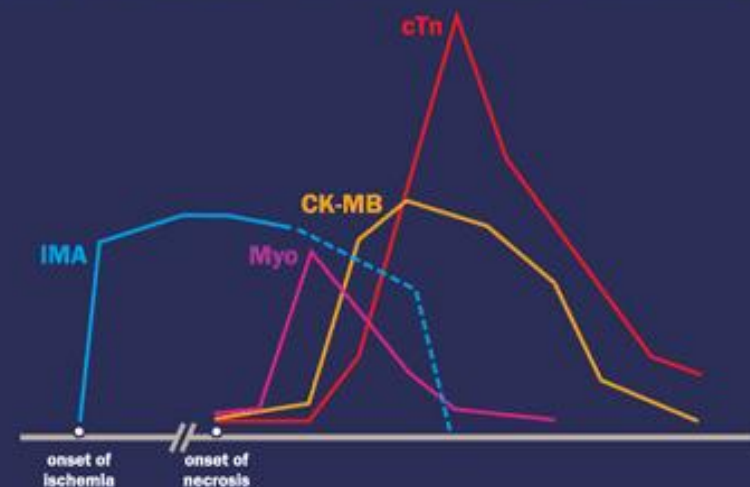
Ischemia-Modified Albumin (IMA)

- Under conditions of ischemia, albumin undergoes a conformational change, so that it can no longer bind to transitional metals such as copper or cobalt
  - Albumin cobalt binding (ACB) test
- Using the albumin cobalt binding test, the proportion of albumin modified by ischemia can be estimated
  - But, low specificity and sensitivity

**A predictor of long-term outcome in patients with acute myocardial infarction**

Non ischemic Sample   Ischemic Sample

HSA with Co++ at the N-terminus

Unbound Cobalt   Ischemia modified Albumin

IMA is a marker of ischemia, not necrosis

cTn
CK-MB
IMA
Myo

onset of ischemia   onset of necrosis

Myeloperoxidase (MPO)

- MPO appears to participate in the initiation and progression of plaque formation.

- Elevated early after ACS.

- Appears to identify patients with ACS earlier than biomarkers like troponin and CK-MB.

- Also appears to provide risk stratification for patients who are troponin negative.

    - For patients who present with chest pain and negative troponin levels are at increased risk for readmission if MPO is elevated.

Note
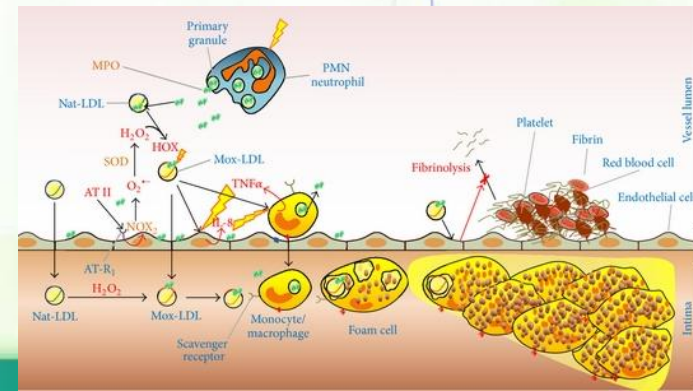
"Despite the multitude of cardiac biomarkers in production and under investigation, none have convincingly demonstrated their incremental utility beyond that of cTn."

SJ Aldous

The Future of Cardiac Biomarkers

- Many experts are advocating the move towards a *multimarker strategy* for the purposes of diagnosis, prognosis, and treatment design

Why do we need multiple Markers?

- No single ideal marker exists for ACS
- Complicated diseases are not likely to be associated with single markers
- Multiple markers define disease categories
- Multi-marker panels can aid in differential diagnosis

"Conversely, multi-marker assessment has been shown to be associated with <u>higher</u> Emergency Department, coronary care and cardiac intervention costs but…[it] has <u>not</u> been shown to reduce overall costs despite reducing admissions."

SJ Aldous

International Journal of Cardiology 164 (2013) 282–294