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CARDIAC BIOMARKERS:TROPONINS

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- 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, pathologic processes or pharmacologic responses to a therapeutic intervention.
- CARDIAC MARKERS are substances released from heart muscle when it is damaged.

CHARACTERISTICS OF AN IDEAL CARDIAC MARKER

- HIGH CARDIAC SPECIFICITY
- EASY DIAGNOSIS
- IT SHOULD PLAY A DESIGNED ROLE IN THE MANAGEMENT OF CLINICAL SUBJECT
- REPRODUCIBLE
- EASY TO INTERPRET
- ACCURATE

MARKERS OF MYOCARDIAL NECROSIS

- MYOGLOBIN
- CREATINE KINASE-MB
- TROPONINS

CREATINE KINASE

- Creatine phosphokinase (CPK) is an enzyme found mainly in the heart, brain, and skeletal muscle which catalyses the conversion of creatine to phosphocreatine.
- CPK enzyme consists of 2 subunits. B type (brain type) and M type (muscle type) making 3 different isoenzymes- CPK-BB, CPK-MM, CPK- MB.
- Skeletal muscle has mainly CPK-MM (98%) , CPK-MB (1%)
- Myocardium has CPK –MM(70%) and CPK –MB (30%).

CPK-MB

- High specificity for cardiac tissue
- Begins to rise 4-6 hrs after the onset of infarction
- Peaks in 12 hrs.
- Returns to baseline within 36-48 hrs
- Helps to identify reinfarction if level normalises and rises again.

FALSE POSITIVE ELEVATIONS IN CPK-MB can be seen in-

- Significant skeletal muscle injury
- Cardiac injuries other than MI-

  blunt chest trauma

  defibrillation

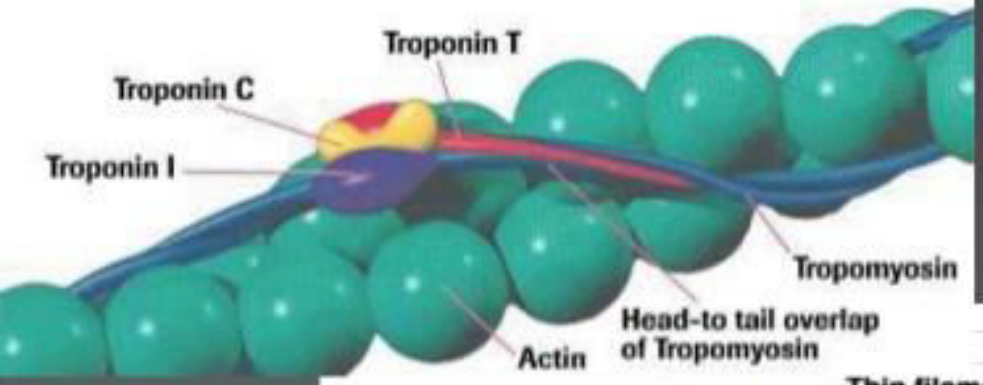
  cocaine abuse

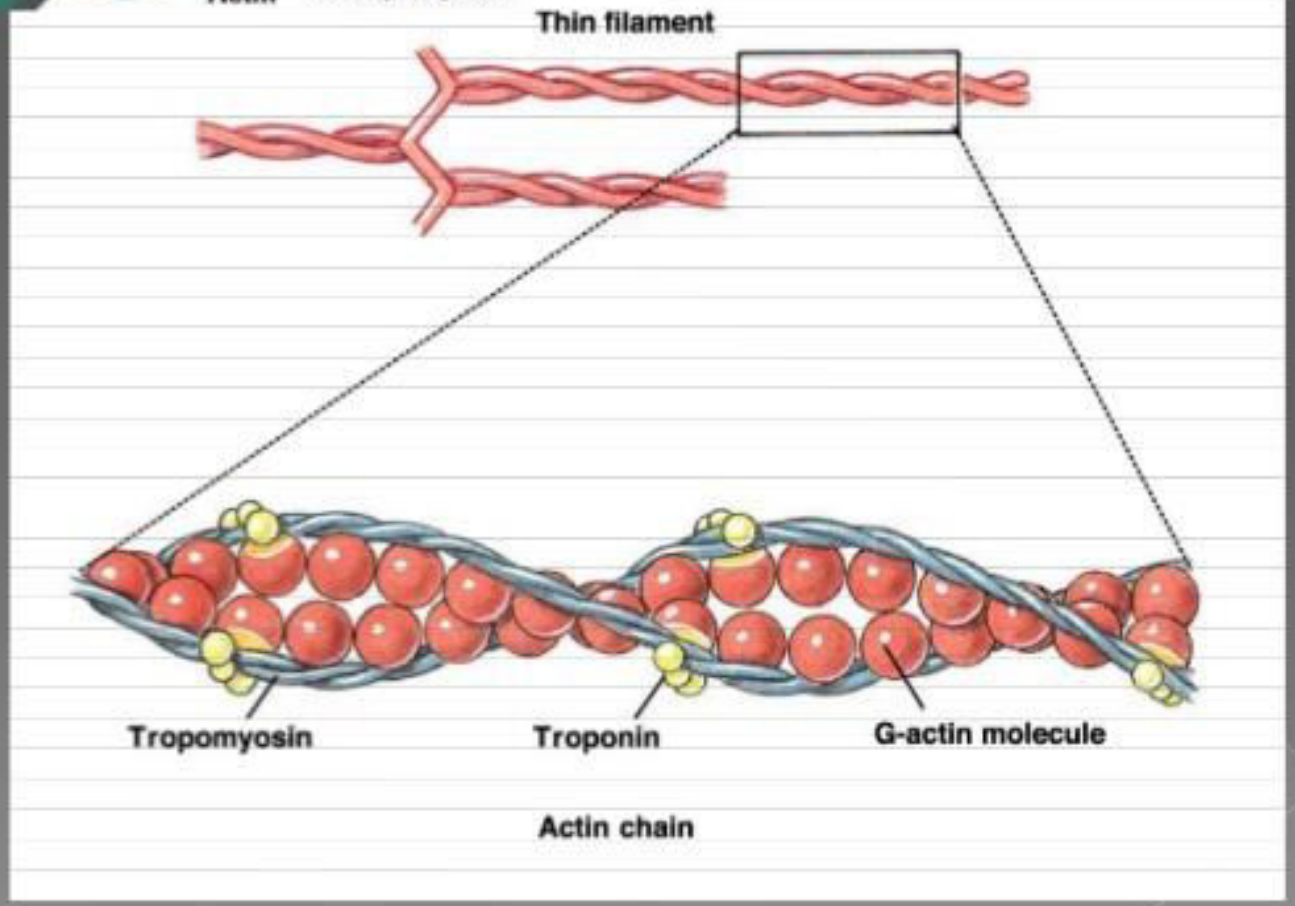
THE SEARCH FOR CARDIAC SPECIFICITY CONTINUES.....

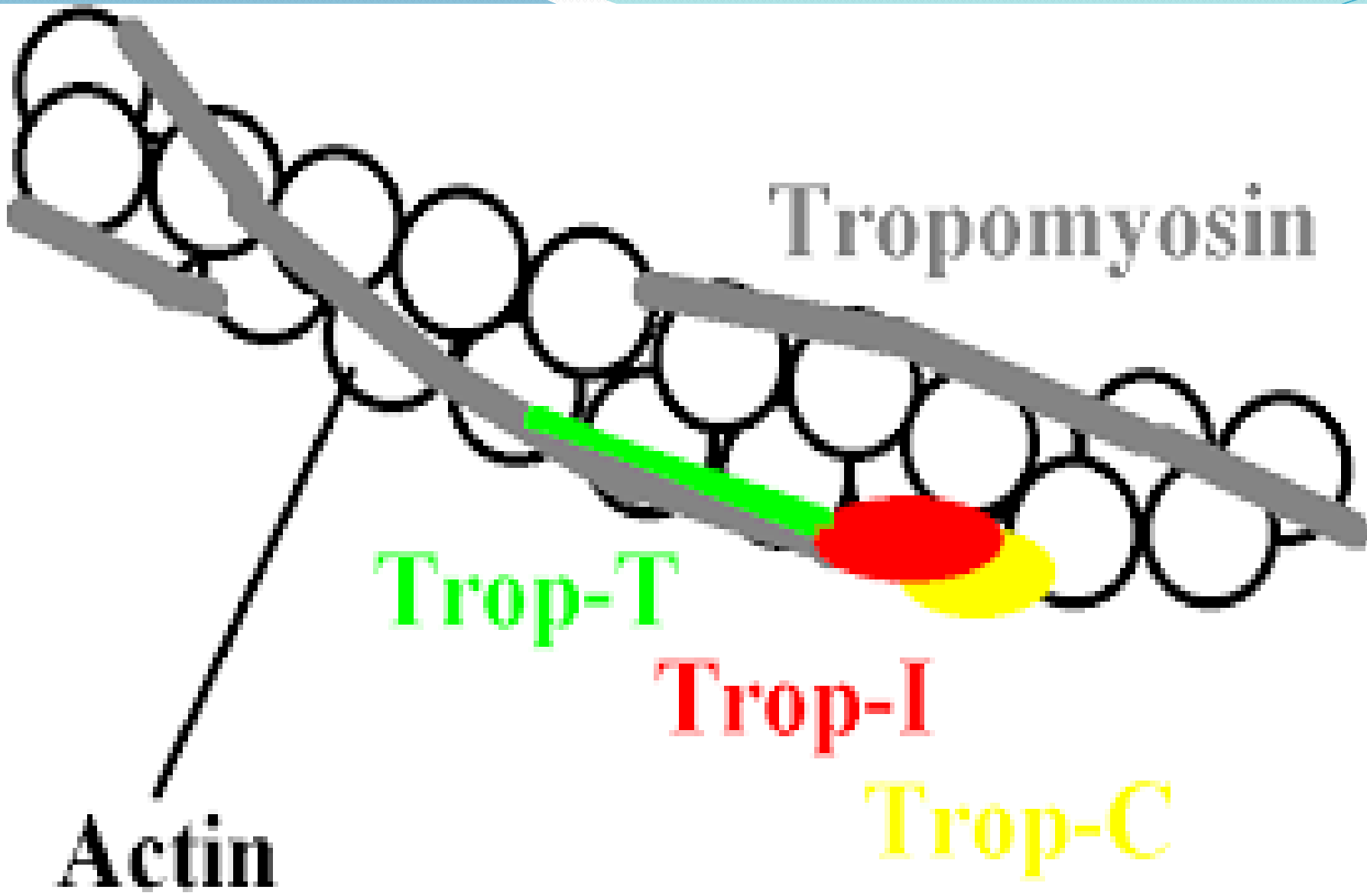
- Cardiac troponins are cardiac regulatory proteins that control the calcium mediated interaction between actin and myosin.
- Three types of troponins exist—troponin I, troponin T, and troponin C. Each subunit has a unique function: Troponin T binds the troponin components to tropomyosin
- Troponin I inhibits the interaction of myosin with actin
- Troponin C contains the binding sites for Ca^{2+} that helps initiate contraction.[1]

TROPONIN I

A regulatory protein released when cardiac cell necrosis occurs.






Tropomyosin
Trop-T
Trop-I
Trop-C
Actin

- Troponin C is not useful for diagnosing cardiac injury because the cardiac isoform is shared by skeletal muscle.
- Multiple cTnT isoforms are expressed in the human heart (predominantly cTnT3) whereas TnC and TnI are expressed as single isoforms.
- There are substantial differences between the amino acid sequences of the cardiac isoforms of TnI and TnT and other isoforms and it has been possible to produce highly specific monoclonal antibodies without cross reactivity with other non-cardiac forms.

TROPONIN I

- TROPONIN I is a cardiac muscle protein with a molecular wt of 24 kilo daltons.
- It has additional amino acid residue in its N terminal that does not exist on skeletal form
- Half life 2-4 hrs
- Serum increase within 2-8 hrs

TROPONIN T

- Cardiac troponin T is present in fetal skeletal muscle
- In healthy adult skeletal muscle cTnT is absent
- The gene for cTnT may be re-expressed in skeletal muscle disease.
- Biological half life and serum increase times are similar to trop I.

- *Clin Chem 1999:45:2129-35*

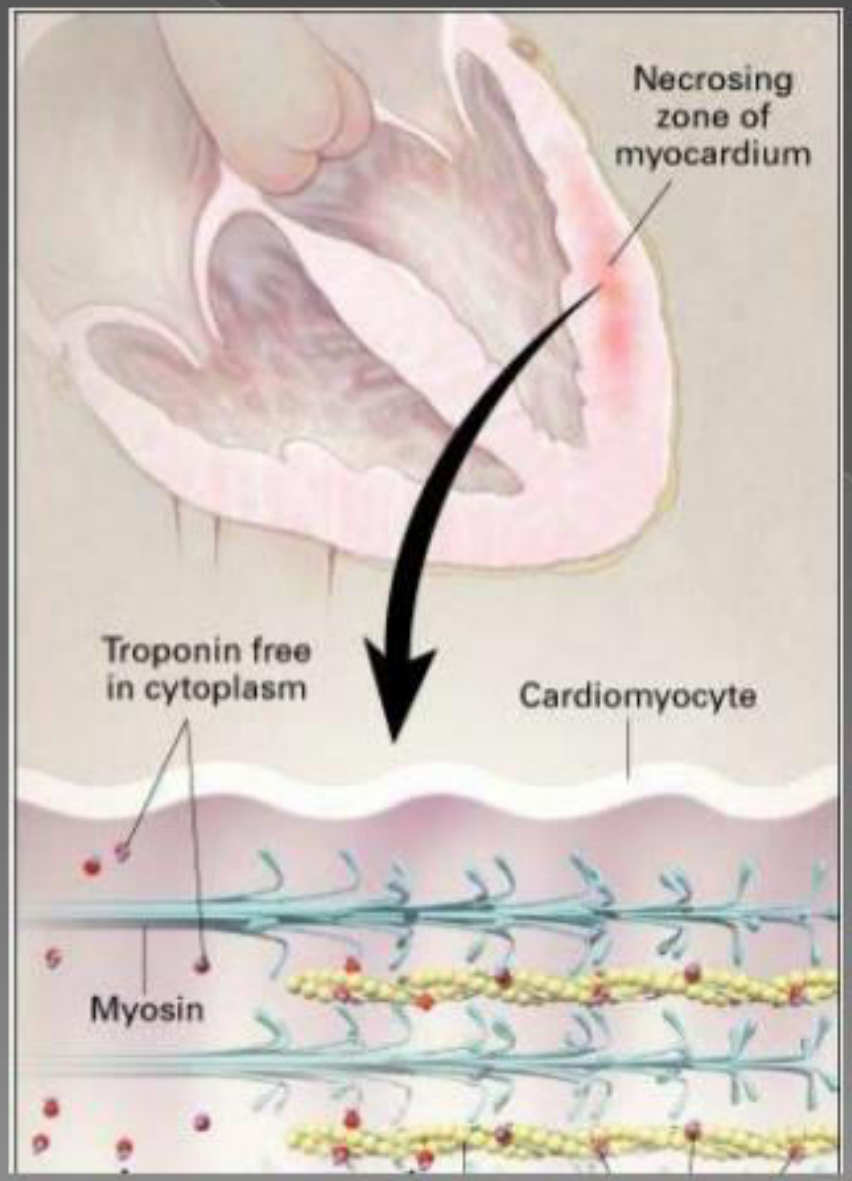
Troponin release in cardiac injury

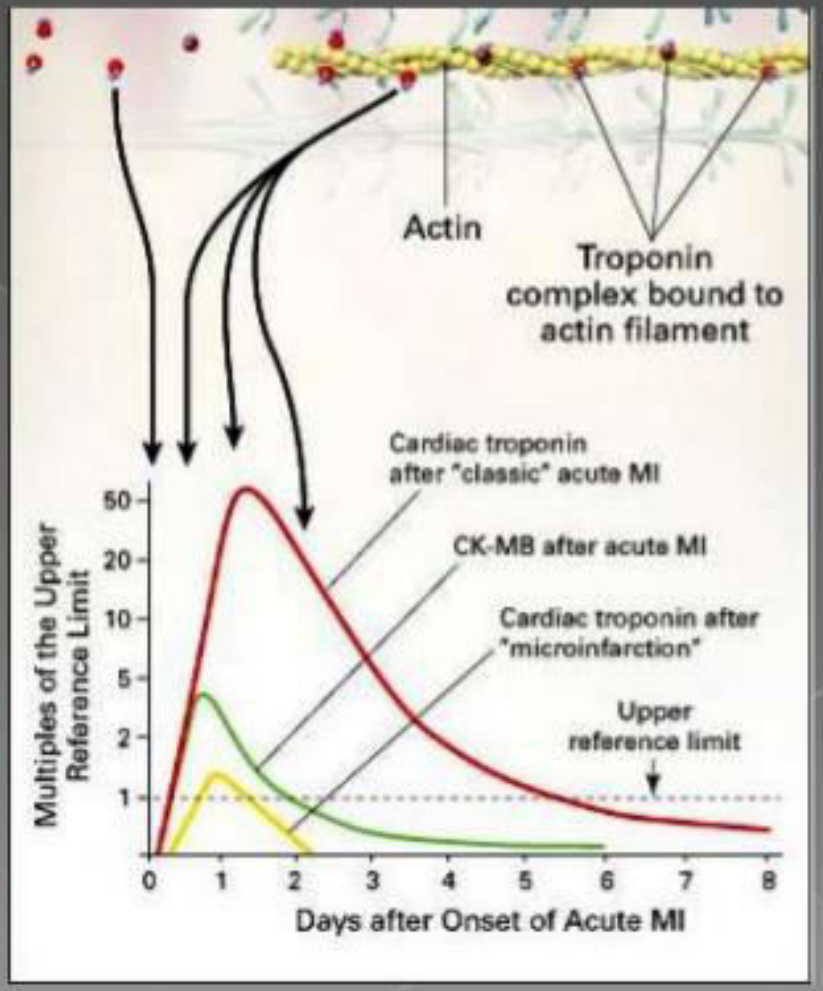
- It is proposed that there is a small cytosolic pool and a larger muscular pool of troponins. During cardiac injury, depending on the severity, troponins are released from both pools. An initial small elevation occurs when troponins are released from the cytosolic pool, when troponin molecules in the cytosol of cardiac muscle diffuse across the sarcolemma into the surrounding lymphatics and blood vessels, becoming detectable in blood. If the injury persists and necrosis progresses, further troponins are released from the muscular pool

PATTERN OF RISE AND FALL

- When measured with older generation assays, elevated troponin levels can be detected 4-12 hours after onset of myocardial injury, peaking at about 12-24 hours, followed by a gradual decline over several days(7-10 days trop I, 10-14 days trop T)
- Continuing breakdown of myofibrillary-bound complex explains the prolonged elevation of both troponins for up to 10 days after infarction. This accounts for increased detection of cardiac events using troponin and its increased sensitivity but can make diagnosis of reinfarction more difficult

Cardiac Troponin Release after MI





REVERSIBLE V/S IRREVERSIBLE INJURIES

- Reversible ischemia after exercise stress testing in humans has not resulted in cTn elevations. However, several studies have suggested that troponin I can be released in reversible ischemia. For example, **Feng et al**. showed in a porcine model of ischemic heart disease that reversible ischemia was associated with release of cTnI. The source of troponin is hypothesized to be from the free cytosolic pool leaking through a reversibly damaged myocyte membrane. This hypothesis is also supported by clinical observations of 2 protein release patterns in patients with unstable angina: an early transient pattern and a persistent pattern.

- *Feng J[1], Funk WD, Wang SS, et al. Science.1995 Sep 1;269(5228):1236-41.*

TROPONIN ASSAYS Collection and Panels

- The troponin sample assay relies on serum levels of troponin. The sample collected is whole blood through venipuncture. No special patient preparation is needed, and fasting is not required. Blood for the test can be drawn at any time of the day.
- Generally, troponin T requires sample collection in heparinized test tubes (green top), while EDTA (purple top) or heparinized tubes are used for troponin I.

- Most specimens require centrifugation prior to running the test in order to separate the serum from the cellular components.

- In general, for troponin T, the blood sample should not be frozen or refrigerated immediately, and it can be stored at room temperature for up to 8 hours for analyses.

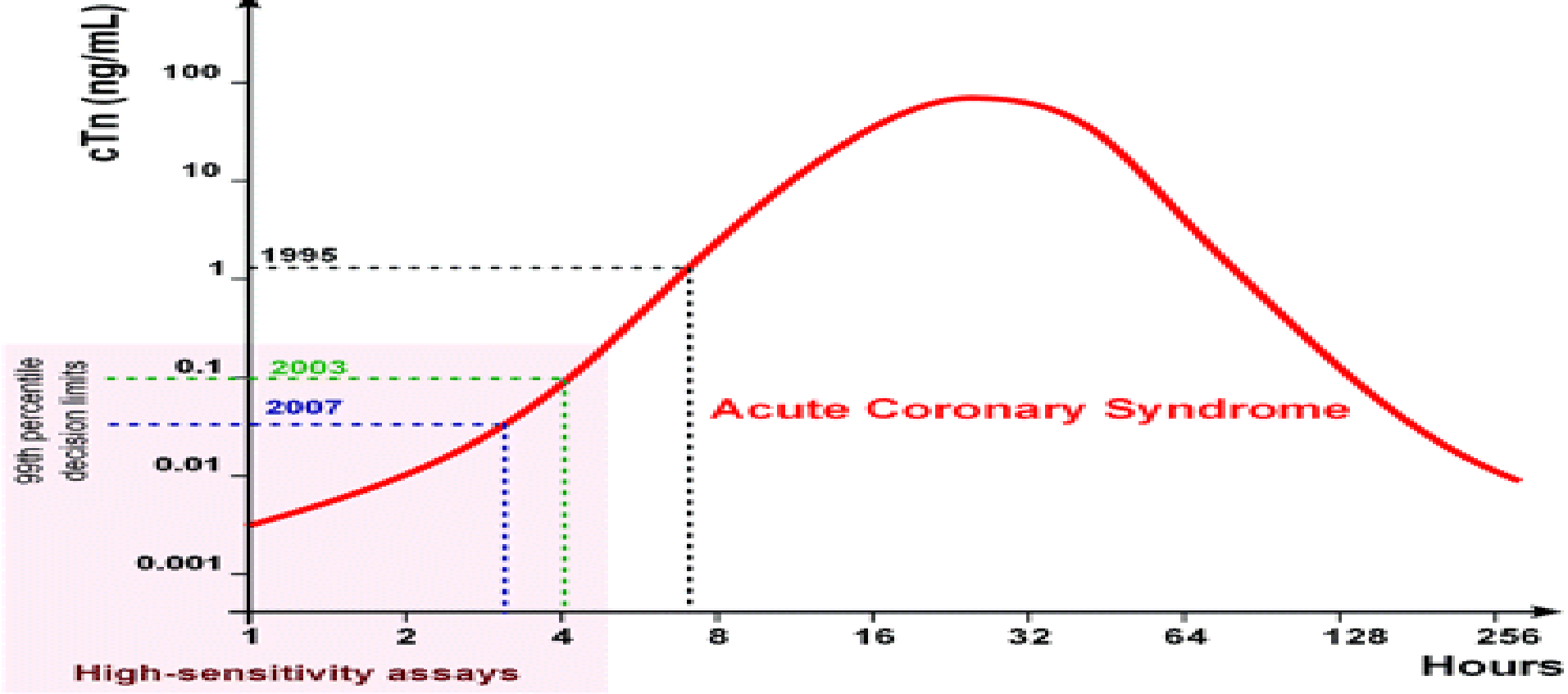
Troponin assays contd..

- Troponin levels are determined using enzyme-linked immunosorbent assays (ELISA)
- The difference in amino-acid sequences from skeletal muscle and cardiac troponin I and T has allowed production of antibodies specific for cardiac troponins in these assays.

- Rapid advances in immunoassay technologies and the international adoption of traceable troponin calibration standards have allowed manufacturers to develop and calibrate troponin assays with unprecedented analytic sensitivity and precision. Thus, a contemporary cTnI assay such as TnI-Ultra detects plasma cTn levels as low as 0.006 ng/mL with an assay range that spans 4 orders of magnitude (0.006–50 ng/mL).

- Similarly, the limit of detection of a contemporary cTnT assay (Elecsys TnT-hs, Roche Diagnostics;) is as low as 0.005 ng/mL

- Between 1995 and 2007, the limit of detection fell from 0.5 ng/mL for some cTn assays to 0.006 ng/mL for TnI-Ultra, an ≈100-fold improvement in analytic sensitivity



cTn Assay	Diagnostic cutoff	Implementation
TnI	≥ 1.5 ng/mL	1995
cTnI	> 0.10 ng/mL	2003
TnI-Ultra	> 0.04 ng/mL	2007

- hypothetical case of acute coronary syndrome is depicted with the earliest times of potential diagnosis corresponding to the diagnostic cutoffs of more sensitive cTn assays. The years correspond to the availability of the respective assays in the US market.

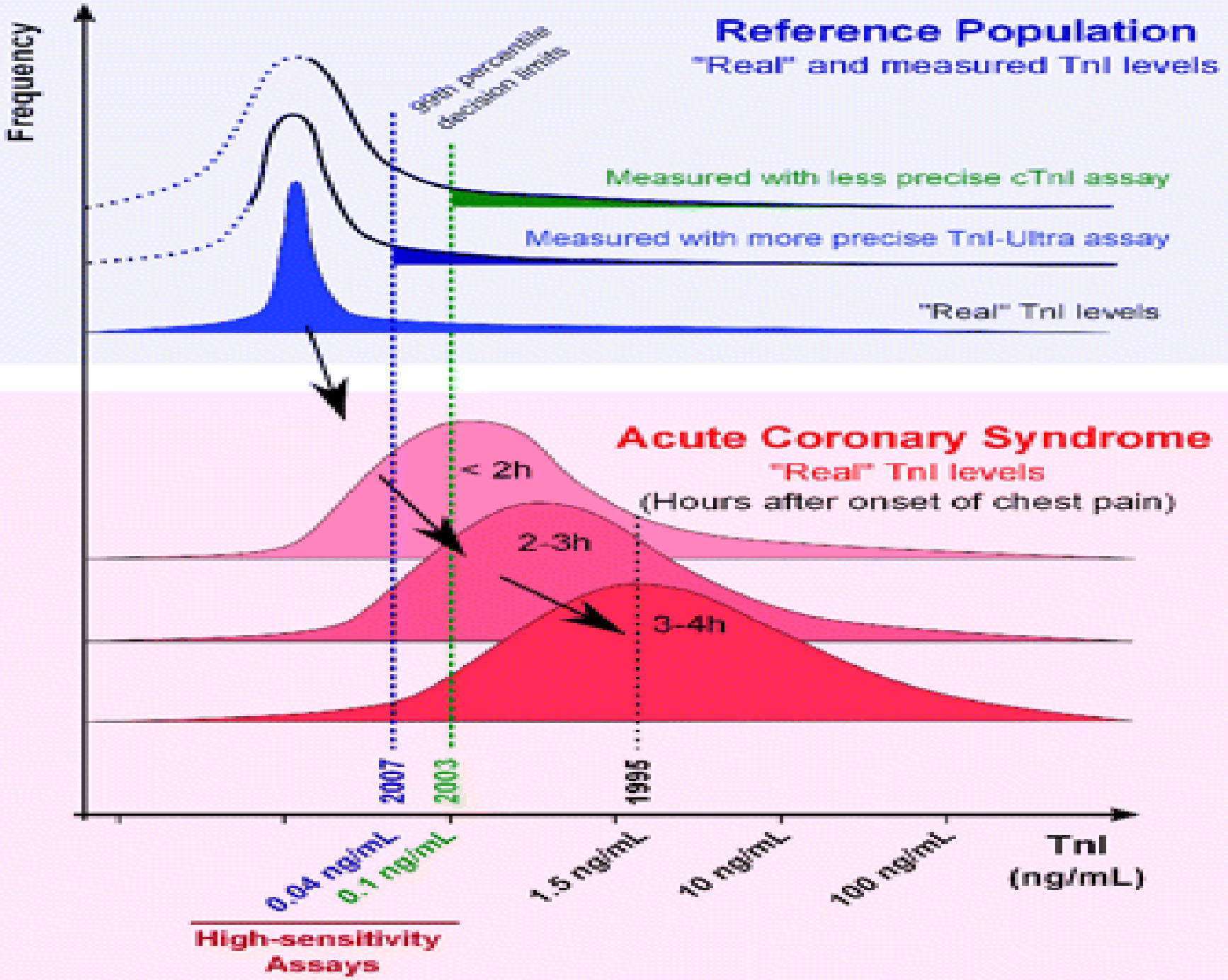
- Remarkably, the use of contemporary high-sensitivity cTn assays makes it possible to detect low levels of cTn even in plasma from healthy subjects. Indeed, high-sensitivity cTn assays are designated as such on the basis of their ability to detect cTns even in healthy individuals. The latest generation of high-sensitivity cTn assays can detect cTn in >95% of a reference population.The ability to detect cTns in healthy individuals made it imperative to define a clinical decision limit for cTn concentration, ie, a “positive” cTn result

What Is a Positive Troponin Result? The 99th Percentile Rule

- The National Academy of Clinical Biochemistry issued a guideline in 2007 that stated that “in the presence of a clinical history suggestive of ACS, the following is considered indicative of myocardial necrosis consistent with myocardial infarction:
- maximal concentration of cTn exceeding the 99th percentile of values (with optimal precision defined by total c.v. [coefficient of variation] <10%) for a reference control group on at least one occasion during the first 24 hours after the clinical event.

- This guideline provides the framework for determining the decision limit or a “positive” troponin result.

- Based on the 99th percentile rule, troponin decision limits of several high-sensitivity cTn assays can be set as low as 0.01 ng/mL
- This makes it possible to identify patients with ACS earlier, enabling earlier coronary intervention . However, while improving clinical sensitivity for the diagnosis of myocardial infarction, the increased analytic sensitivity has come at the cost of reduced specificity, thus presenting an additional diagnostic challenge for clinicians


Reference Population
"Real" and measured TnI levels
Frequency
99th percentile decision limits
Measured with less precise cTnI assay
Measured with more precise TnI-Ultra assay
"Real" TnI levels
Acute Coronary Syndrome
"Real" TnI levels
(Hours after onset of chest pain)
< 2h
2-3h
3-4h
2007
2003
1995
0.04 ng/mL
0.1 ng/mL
1.5 ng/mL
10 ng/mL
100 ng/mL
TnI
(ng/mL)
High-sensitivity
Assays

- Cardiac troponin I (cTnI) levels in a healthy reference population and in an acute coronary syndrome (ACS) population. Top, Frequency histograms of real TnI levels (blue filled) in healthy reference controls are shown, along with the distribution of the same TnI levels as measured with a less precise cTnI (green) and the more precise TnI-Ultra (blue) assay for comparison. In practice, the values below the assay detection threshold (dashed portions of the histogram plots) cannot be distinguished from one another. Note how the 99th percentile decision limits decrease with increased assay precision. Bottom, Hypothetical frequency histograms of cTnI concentrations in individuals with ACS <2, 2 to 3, or 3 to 4 hours after the onset of symptoms. The decision limits (dashed vertical lines) for the contemporary high-sensitivity cTnI assays are based on the 99th percentile in a healthy reference population. Note the impact of decreased diagnostic cutoffs of the newer cTnI assays on the fraction of acute myocardial infarctions diagnosed at earlier time intervals.

The Specificity of a Troponin Test for ACS

- The use of the 99th percentile cutoff for cTn positivity does not imply that 1% of the population suffers from myocardial damage.

- Rather, this cutoff is useful only when applied to patients with a high pretest probability of ACS.\
- A positive troponin in the setting of a low pretest probability for ACS may be suggestive but clearly is not indicative of a coronary event.

- Traditional wisdom, before the advent of high-sensitivity cTn assays, held that troponins do not appear in the circulation of individuals with a healthy myocardium.

- These levels used to be considered indicative of myocardial necrosis.
- However, with high-sensitivity troponin assays, circulating cTnT or cTnI can be found in the plasma as a result of transient ischemic or inflammatory myocardial injury.

Thus, elevated cTn may be detected in conditions other than ACS

- CARDIAC CAUSES:
- Cardiac contusion resulting from trauma
- Cardioversion
- Acute and Chronic heart failure
- Endomyocardial biopsy
- Aortic dissection
- HOCM
- Tacchyarrythmia
- bradyarrhythmia

- Apical ballooning syndrome
- Post–percutaneous coronary intervention
- Rhabdomyolysis with myocyte necrosis
- Myocarditis or endocarditis/pericarditis

NON CARDIAC CAUSES

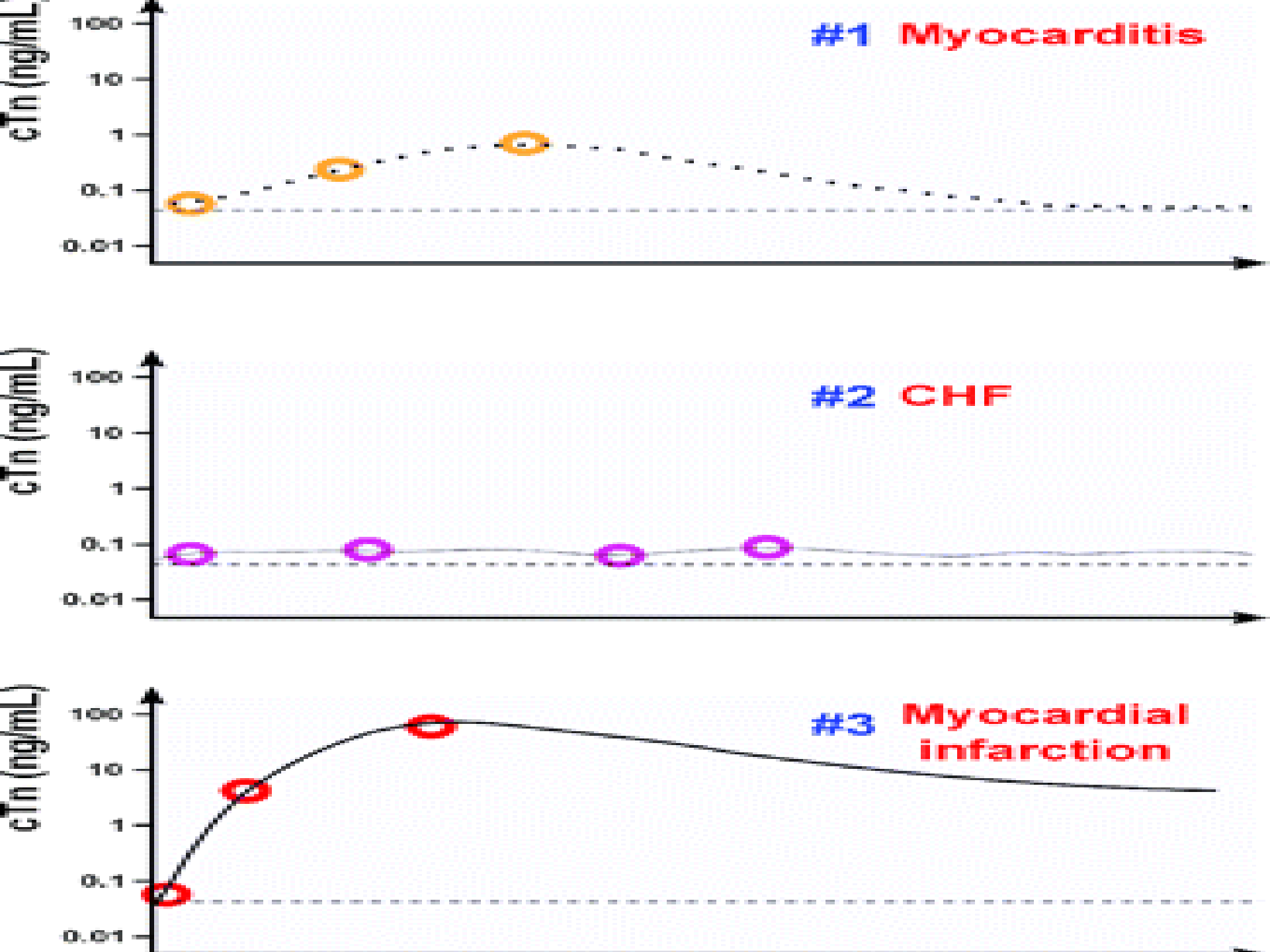
- Pulmonary embolism
- Severe pulmonary hypertension
- Renal failure
- Stroke, subarachnoid hemorrhage
- Infiltrative diseases eg; amyloidosis
- Sepsis
- Extensive burns
- Critical illness
- Extreme exertion

The Need for Serial Troponin Testing

In addition to the absolute level of cTn in plasma or serum above the decision limit, a critical component of the diagnosis of ACS is cTn kinetics.

- Although absolute cTn elevations are seen in multiple chronic cardiac and noncardiac conditions, a rise or fall in serial cTn levels strongly supports an acutely evolving cardiac injury such as, most commonly, acute myocardial infarction.

- Serial cTn testing helped establish final diagnoses in 3 patients. Patient 1 had a steady but relatively slow increase in cTnI with a peak value of 0.9 ng/mL. The findings of acute dilated cardiomyopathy and global ventricular dysfunction on echocardiography were consistent with a diagnosis of acute myocarditis.


cTn (ng/mL)
100
10
1
0.1
0.01
#1 Myocarditis
cTn (ng/mL)
100
10
1
0.1
0.01
#2 CHF
cTn (ng/mL)
100
10
1
0.1
0.01
#3 Myocardial infarction

- Patient 2 had modest cTn elevations fluctuating just above the decision limit in the 0.05 to 0.09 ng/mL range. She was diagnosed with acutely decompensated heart failure. Additional TnI testing did not provide evidence of ACS.

- TnI levels in patient 3 rose to a peak of 53 ng/mL within 24 hours. He was diagnosed with non–ST-segment–elevation myocardial infarction when the second cTn result of 6.3 ng/mL was obtained after 6 hours. The rapid, steep increase from the initial barely positive value of 0.06 ng/mL to the 6-hour value of 6.3 ng/mL illustrates that more frequent testing during the first several hours may be sufficient to detect a diagnostic rise in cTn levels that is eventually destined to increase by a few orders of magnitude such as the peak of 53 ng/mL in this patient.

- Previously, clinicians often had to wait an average of 6 hours with the lower-sensitivity, lower-precision cTn assays to see a conclusive increase in plasma cTn levels after the first troponin measurement, but today's high-sensitivity cTn tests that are separated by a mere 2 to 3 hours can be highly informative.
- Given the urgent need for early diagnosis of ACS and appropriate emergency intervention, as well as the ease of performing this relatively inexpensive assay, clinicians do not need to wait 6 to 8 hours before ordering a second troponin test to rule in ACS

- IT is recommended to collect a second specimen for cTn testing within 2 to 3 hours from the collection of the blood sample at presentation to help confirm the diagnosis of MI.

ROLE IN PROGNOSIS

- Cardiac troponins also have a role in establishing prognosis.
- With MI, any troponin level above the reference range is associated with an increased risk of adverse events in both the short- and long-term.
- It has also been shown that the magnitude of troponin level elevation correlates with risk of future cardiac events or death and aids the identification of patients with greater disease severity who may benefit from more aggressive

- In fact, the size of the infarcted area may be predicted based on peak cTnI levels or cTnT levels at 72 hours. In addition, elevations of cTn have prognostic significance in other forms of cardiovascular disease such as heart failure and pulmonary thromboembolism.


THANK YOU

Team Biomarkers welcomes you all to the next chapter – 7th International Conference on Biomarkers & Clinical Research scheduled for Nov 28-30, 2016 in Baltimore, USA

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