Cardiac Biomarkers

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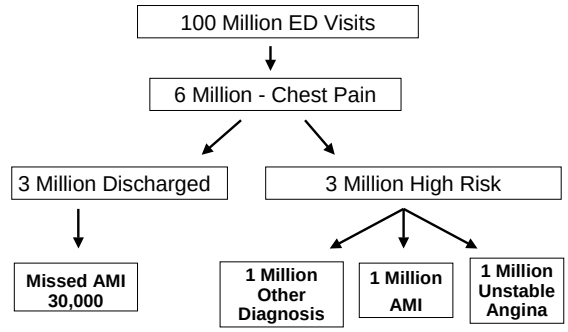
Learning Objectives

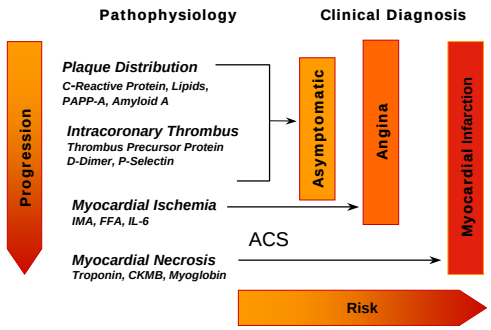
By the end of this session you will be able to:

- Discuss the biochemical markers that are used to detect myocardial infarction
- Discuss the sensitivity and specificity of CK-MB and the Troponins for the detection of myocardial infarction
- Describe the pitfalls associated with the immunochemical methods that are used to measure Troponin I

Annual ED Visits in the United States



Acute Coronary Syndrome



Diagnosis of AMI

- WHO Criteria
- Must meet 2 out of 3 criteria
  - Clinical presentation, chest pain >20 minutes
  - ECG changes
  - Positive serial cardiac enzymes

Diagnostic Challenges

AMI patients may present to the ED with non-diagnostic ECG ~ 50% of the time

Chest pain may be a dull pain, burning sensation or a sharp stabbing pain

~35% of patients do not have chest pain

50% have no history of MI or angina

Cardiac Biomarkers

- CK-MB
- Myoglobin
- Troponin I and T
- BNP
- NT ProBNP
- Ischemia Modified Albumin

Ideal Cardiac Marker for Myocardial Injury

- Found in high concentrations in myocardium
- Organ Specific – detected only in heart muscle
- Released rapidly and completely after the onset of pain
- Concentration is proportional to the extent of damage
- Remains elevated for several days
- Easy to measure
- Rapid turnaround time
- Cost effective

CKMB Biochemistry

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

Tissue Distribution Of CK & CK Isoenzymes

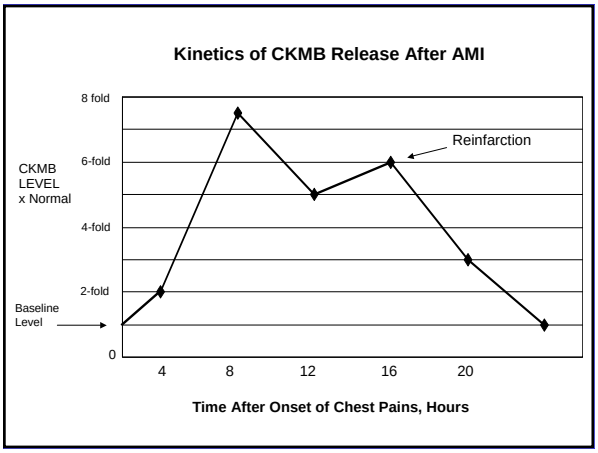
	Range of Total CK	Range of CK Isoenzymes (%)		
Tissue	U/gm tissue	MM	MB	BB
Skeletal Muscle	1080-3050	96-100	0-4	0
Heart Muscle	190-692	58-86	15-42	0-1
Brain	73-200	0	0	100
Bladder	162	0-2	0-6	92-100
Placenta	250	19	1	80
Colon	200	0-5	0-4	95-100
Ileum	175	0.3	0-4	93-100
Stomach	170	0-5	4	96
Diaphragm	140	96	4	22

CKMB Kinetics

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.



CKMB IN AMI

Advantages:

- Detects AMI 4-6 Hours After Chest Pain
- Methodology is Rapid and Automated
- Turnaround Time <20 Minutes
- CKMB was the gold standard for AMI detection in the 1980's

Limitations of CKMB in AMI

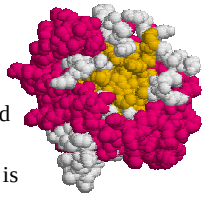
Elevated CKMB Levels can be observed in:

- Skeletal Muscle Involvement
- Duchenne Muscular Dystrophy
- Polymyositis
- Alcohol Myopathy
- Thermal or Electrical Burn Patients
- Carcinomas
- Colon, Lung, Prostate, Endometrial

Myoglobin

Biochemistry

- Myoglobin is a relatively small (17.8 kDa) globular protein
- It is a heme containing protein
- It constitutes about 2 percent of muscle protein in both skeletal and cardiac muscle
- The prime function of myoglobin is to store oxygen in muscle for release during oxygen deprivation



Myoglobin Molecule

Myoglobin

Present in Cardiac and Skeletal Muscle

Post AMI		Myoglobin	CKMB
Increase	Hrs	2-4	4-6
Peak	Hrs	5-9	10-24
Return to Normal	Hrs	24-36	36-76

Myoglobin in AMI

Advantages:

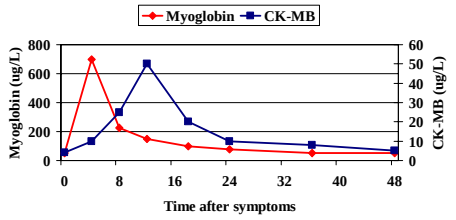
- Early Indicator of AMI
- Methodology – Automated
- Results Available in <60 Minutes
- Concentration is dependent upon the amount of cardiac damage

Limitations of Serum Myoglobin in AMI

Increased In:

- Exhaustive exercise
- Skeletal muscle damage
- Progressive Muscular Dystrophy
- Shock
- Renal Failure
- Following IM injection

Time course of Myoglobin and CK-MB after an AMI



Specificity of CK-MB Mass & Myoglobin In Noninfarct Patients with Chronic Renal Failure or Severe Polytrauma

Pathology & Markers	No. (%) of Positive Sera	Specificity %
Severe Polytrauma (24 Sera)		
CK-MB mass	14(58)	42
Myoglobin	21(88)	12
Chronic Renal Failure (49 Sera)		
CK-MB mass	4 (8)	92
Myoglobin	43(88)	12

Summary

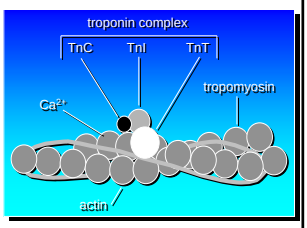
Myoglobin

- Excellent early marker for myocardial damage
- Skeletal muscle damage can also greatly increase serum levels

- CK-MB
- CK-MB exists as three molecular forms MM, MB, BB
- After myocardial infarction, elevated CK-MB levels appear within 4 to 8 hours, peaking within 9 to 30 hours, and levels return to normal after 48 to 72 hours
- The time to appearance of elevated levels is slower than that for myoglobin
- Although CK-MB is present at low levels in skeletal muscle, skeletal muscle damage can potentially lead to false-positive diagnosis of AMI

Troponin Characteristics

- **Troponin C (18 kd)**
- Calcium-binding subunit
- **Troponin I (26.5 kd)**
- Actomyosin-ATP-inhibiting subunit
- **Troponin T (39 kd)**
- Tropomyosin-binding subunit



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
- Troponin I and Troponin T are detected in heart muscle and are cardiac specific
- Circulating concentrations of cTnI and cTnT are very low
- cTnI and cTnT remain elevated for several days
- The false-positive CKMB results that are due to skeletal muscle involvement should be eliminated with use of the Troponin assays.

Troponin I and T

Cardiac Specific Marker

Post AMI		Troponin I	Troponin T	CKMB
Increase	Hrs	4-6	3-6	4-6
Peak	Hrs	14-24	10-24	10-24
Return to Normal	Days	5-7	6-10	2-3

Troponin I, CKMB & Myoglobin

192 Patients With Chest Pain
59 Had An AMI

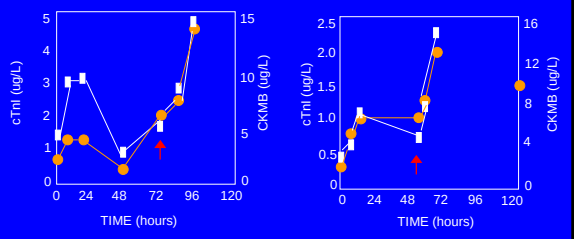
Clin Chem 45, 199-205, 1999

	Troponin I	CKMB	Myoglobin
		Sensitivity %	
<6 hr	65	78	75
6-24 hrs	72-93	78-80	73-75
		Specificity %	
<6 hr	100	91	74
6-24 hrs	94-97	82-86	68-82

Specificity of cTnl, CK-MB Mass & Myoglobin In Noninfarct Patients with Chronic Renal Failure or Severe Polytrauma

Pathology & Markers	No. (%) of Positive Sera	Specificity %
Severe Polytrauma (24 Sera)		
CK-MB mass	14(58)	42
Myoglobin	21(88)	12
cTnl	0 (0)	100
Chronic Renal Failure (49 Sera)		
CK-MB mass	4 (8)	92
Myoglobin	43(88)	12
cTnl	0 (0)	100

Troponin I and CKMB Profiles in Hospitalized MI Patients Who Experienced a Reinfarction



Arrow indicates reinfarction

Diagnostic Performance of Troponin I and Troponin T for AMI

	Sensitivity %		Specificity %	
	Troponin I	Troponin T	Troponin I	Troponin T
Admission	6	15	100	97
1 hr	25	38	100	96
2 hr	70	74	100	93
6 hr	96	97	99	93
12-24 hr	96	99	99	93

Troponin I Concentrations and Outcomes at 42 Days

1404 Patients with Unstable Angina or Non QMI
TNI Cutoff 0.4 ng/mL

TNI	Mortality
<0.4	1.0%
0.4 - <1.0	1.7%
1.0 - <2.0	3.4%
2.0 - <5.0	3.7%
5.0 - <9.0	6.0%
≥ 9.0	7.5%

NJEM 1996,335, 1342-9.

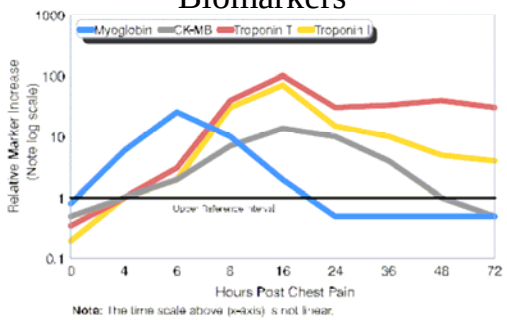
Troponin T Concentrations and Outcomes at 150 Days

976 Patients with Unstable Coronary Disease
TNT Cutoff ≥ 0.1 ng/mL

TNT	Mortality
<0.06	0%
0.0 - 0.62	2%
0.60 - 2.12	7%
>2.12	9%

Circulation, 1996, 93, 1651-7.

Time-course of Cardiac Biomarkers



Note: The time scale above (x-axis) is not linear.

Troponin I vs Troponin T

Troponin I	Troponin T
Multiple manufacturers	One manufacturer
Remains abnormal for 4-7 days	Remains abnormal for 6-10 days
Increases at 4-6 hours after AMI	Increases at 3-6 hours after AMI
Sensitivity: > 98% Specificity: > 98%	Sensitivity: > 98% Specificity: ~ 95%

Case Study 1

62 y.o. man presents to the Emergency Department with crushing chest pain of 2 hours duration. EKG shows S-T elevation and a new Q wave.

Time	Total CK (40-250 U/L)	CK-MB (< 3.0 ng/mL)	CK-MB Index (< 4.0%)	Troponin (< 0.2 ng/mL)
On Admission	120	2.1	1.8	< 0.20
6 hrs Later	380	19.6	5.2	2.0
12 hrs Later	550	42.2	7.7	10.0
24 hrs Later	250	10.5	4.1	20.0

INTERPRETATION
This man had an acute myocardial infarct.
The infarct is of moderate size as indicated by the magnitude of rise in the enzymes. It probably occurred around the time the chest pain began because the CK-MB and Troponin were normal on admission and peaked at 12 to 24 hours after the pain began. There is no evidence of reinfarction.

Case Study 2

62 y.o. man presents to the Emergency Department following a car accident. The patient "blacked out" while driving and now has chest pain as well as chest wall tenderness. EKG is normal.

Time	Total CK (40-250 U/L)	CK-MB (< 3.0 ng/mL)	CK-MB Index (< 4.0%)	Troponin (< 0.2 ng/mL)
24 hrs Later	10,000	150	1.5	<0.2

INTERPRETATION
Severe muscle damage will cause release of large amounts of total CK and smaller amounts of CK-MB. Skeletal muscle usually contains less than 1% MB. Increased total CK with a CK-MB index of less than 4.0 suggests muscle damage but does not entirely exclude an MI in the setting of extensive skeletal muscle damage. In these cases patients may have an MI and with a normal CK-MB Index. Troponin is normal and excludes the possibility of an MI.

Case Study 3

75 y.o. woman presents to the Emergency Department with vague left arm pain of 4 hours duration. EKG shows no specific S-T changes.

Time	Total CK (40-250 U/L)	CK-MB (< 3.0 ng/mL)	CK-MB Index (< 4.0%)	Troponin (< 0.2 ng/mL)
24 hrs Later	60	3.0	5.0	< 0.20

INTERPRETATION
The CK-MB is borderline but the CK-MB index is high. The low total CK is due to low skeletal muscle mass. The borderline CK-MB value and the elevated CK-MB index suggests that there was cardiac damage. *However the normal* Troponin and EKG indicates that there was no MI.

Reporting Cardiac Markers
Speed Counts

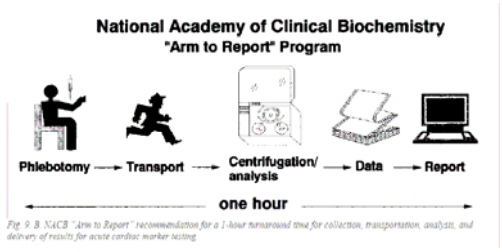


Fig. 9. B. NACB "Arm to Report" recommendation for a 1-hour turnaround time for collection, transportation, analysis, and delivery of results for acute cardiac marker testing.

www.NACB.org

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

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

Point-of-Care Troponin I & Troponin T

773 Patients

47 Patients Had an AMI	Troponin I Sensitivity %	Troponin T Sensitivity %	CKMB Sensitivity %
Admission	66	51	53
4 hrs	100	94	91

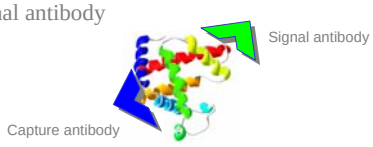
N Engl J Med 1999 , 337, 1648-83

CAP 2006 Proficiency Survey for Troponin I

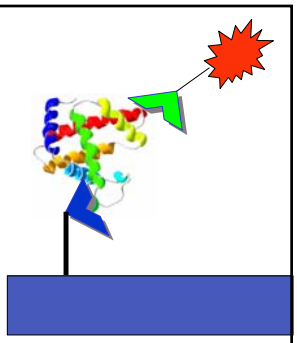
	# of Labs	Mean Troponin I Levels ng/mL
Centaur	298	28.6
Dimension	816	21.8
AXSYM	286	21.5
Stratus CS	121	14.9
Vitros ECI	185	13.8
ACCESS	614	9.5

Basics of a sandwich immunoassay

- Two antibodies to the analyte
- Capture antibody
- Signal antibody



- The capture antibody tethers the analyte to a support system
- The signal antibody generates a signal. The amount of signal is proportional to the amount of analyte
- Key point, you need both antibodies to bind to the analyte to get a signal



Forms of Troponin I in Blood

Troponin I Exists As:

- Binary - TNI-C complex
- Tertiary - TNI-C-T complex
- Fragments of Troponin I
- Free Troponin I
- Reduced and/or oxidized TnI forms
- Phosphorylated forms of TnI

Troponin T Exists As:

- Free TNT
- Binary - TNT-C complex
- Tertiary - TNT-I-C complex
- Fragments of Troponin T

Causes for Different cTnI Results

- Different calibration
  - 2-10 fold differences
- Different epitope recognition
  - Free and IC complex recognition
- Epitopes susceptibility to:
  - Proteolysis
  - Phosphorylation
  - Oxidation

Troponin Methods: *Lack of Standardization*

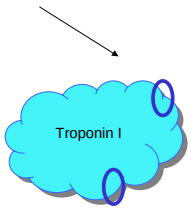
Platform	Pool A	Pool B	Pool C	Pool E	Pool G	Pool H
Abbott AxSYM	< 0.3	0.78	0.95	2.09	3.72	8.08
Bayer ACS 180	0.08	0.15	0.20	0.46	1.16	2.67
Bayer Centaur	0.18	0.30	0.37	0.71	1.56	3.51
Beckman Access	0.05	0.16	0.17	0.42	0.76	1.79
Dade RxL	< .04	0.13	0.15	0.46	0.79	2.33
Dade Stratus CS	0.05	0.21	0.21	0.61	0.96	2.72
DPC IMMULITE	0.13	0.32	0.45	0.72	1.66	2.28
OCD Vitros ECi	< .02	0.14	0.20	0.44	0.98	2.04
Roche Elecsys 1010*	0.03	0.03	0.03	0.06	0.15	0.29
Tosoh AIA-21	0.10	0.22	0.28	0.68	1.17	2.13

Panteghini, M. et al. Clin Chem. 2004;50(2):327-332

* Troponin T

Epitopes affect the assay

Pretend these are the epitopes for troponin I

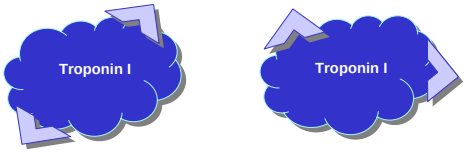


Epitopes affect the assay

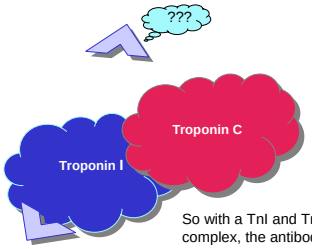
The antibodies recognize the epitopes and bind to them.

Antibody recognition and binding is critical for an immunoassay to pick the right molecule out of the serum

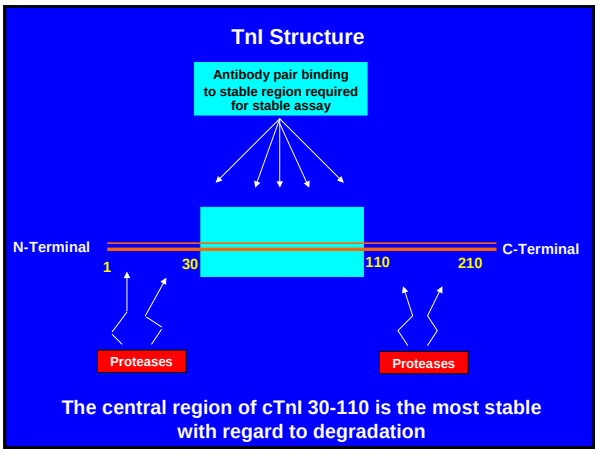
TnI



TnI Complex



So with a TnI and TnC complex, the antibody can't bind, so no recognition of these complexes



Summary
Troponin

- Cardiac troponin is the gold standard for detecting myocardial damage in patients with suspected ACS
- A normal Troponin level on admission, especially within 6 hours of the onset of chest pain does not exclude MI. Suggested testing protocol on admission and at 3 to 6 hours and at 6 to 12 hours
- Measure Troponin I levels using the same immunochemical method