Cardiac Biomarkers in Acute Chest Pain

Dr. K. Shanthi Naidu,
Prof & HOD & Dept of Biochemistry,
CARE Hospitals,
Banjara Hills, Hyderabad.

Dr Maddury Jyotsna
Prof & HOU-IV , Dept of Cardiology,
Nizam's institute of medical sciences,
Panjagutta, Hyderabad-500082.

- **Cardiac biomarkers** are substances that are released into the blood when the heart is damaged or stressed.

The Ideal Biomarker

Then	Now
Sensitive and specific	Either highly sensitive (diagnosis) OR highly specific (treatment effect)
Reflects disease severity	Reflects abnormal Physiology/biochemistry
Correlates with prognosis	Prognosis is most meaningful if level is clinically actionable
Should aid in clinical decision making	Should be used as a basis for specific "biomarker guided-therapy"
Level should decrease following effective therapy	"Bio-monitoring" during treatment is an effective surrogate of improvement

Ischemia and reprefusion
ECG
GDF-15 ?

Acute myocardial damage
TnT, TnI, CK-MB

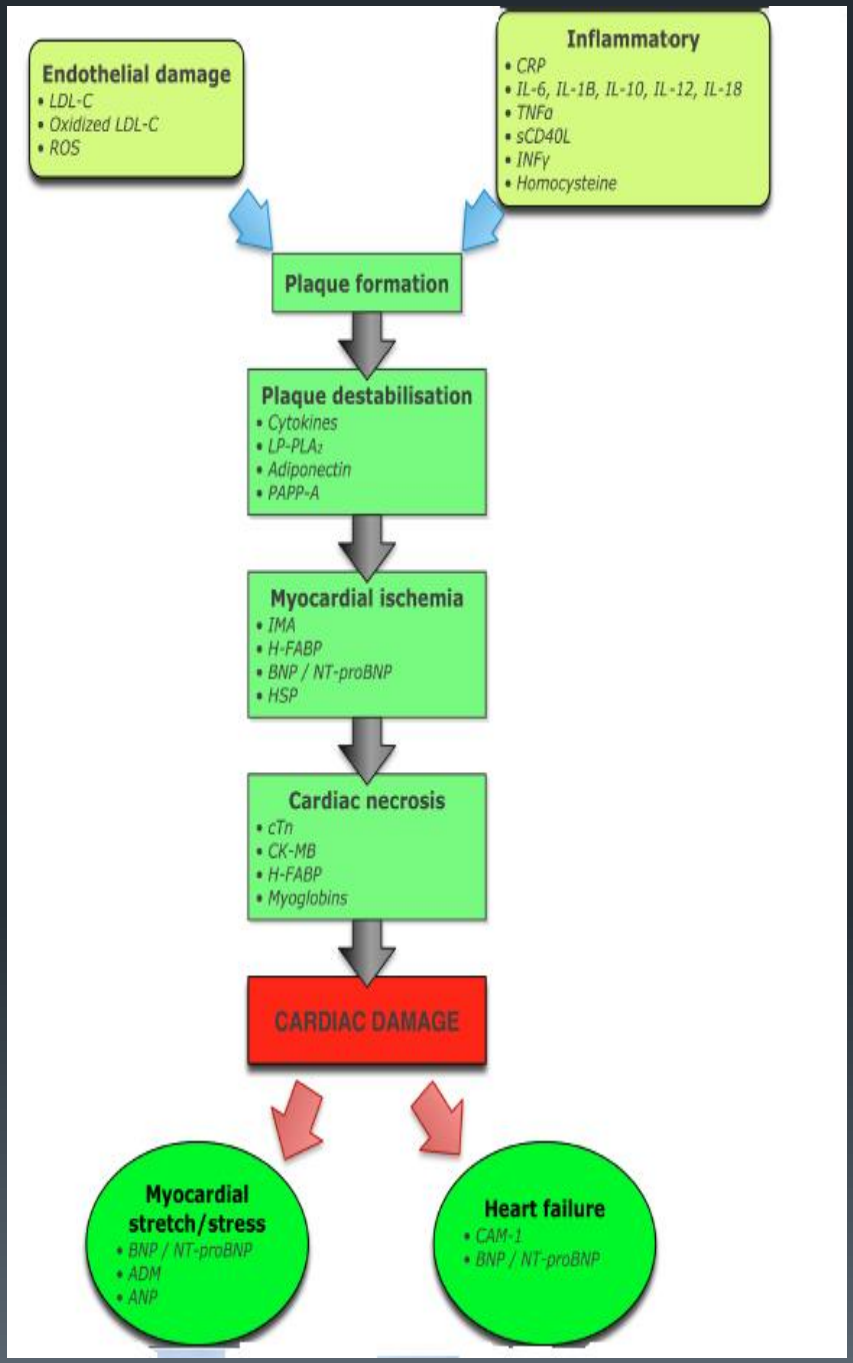
Clinical background

Renal Dysfunction
Crea Cl
Cystatin C

Inflammatory Activity
CRP, IL-6, Cd-40 lig

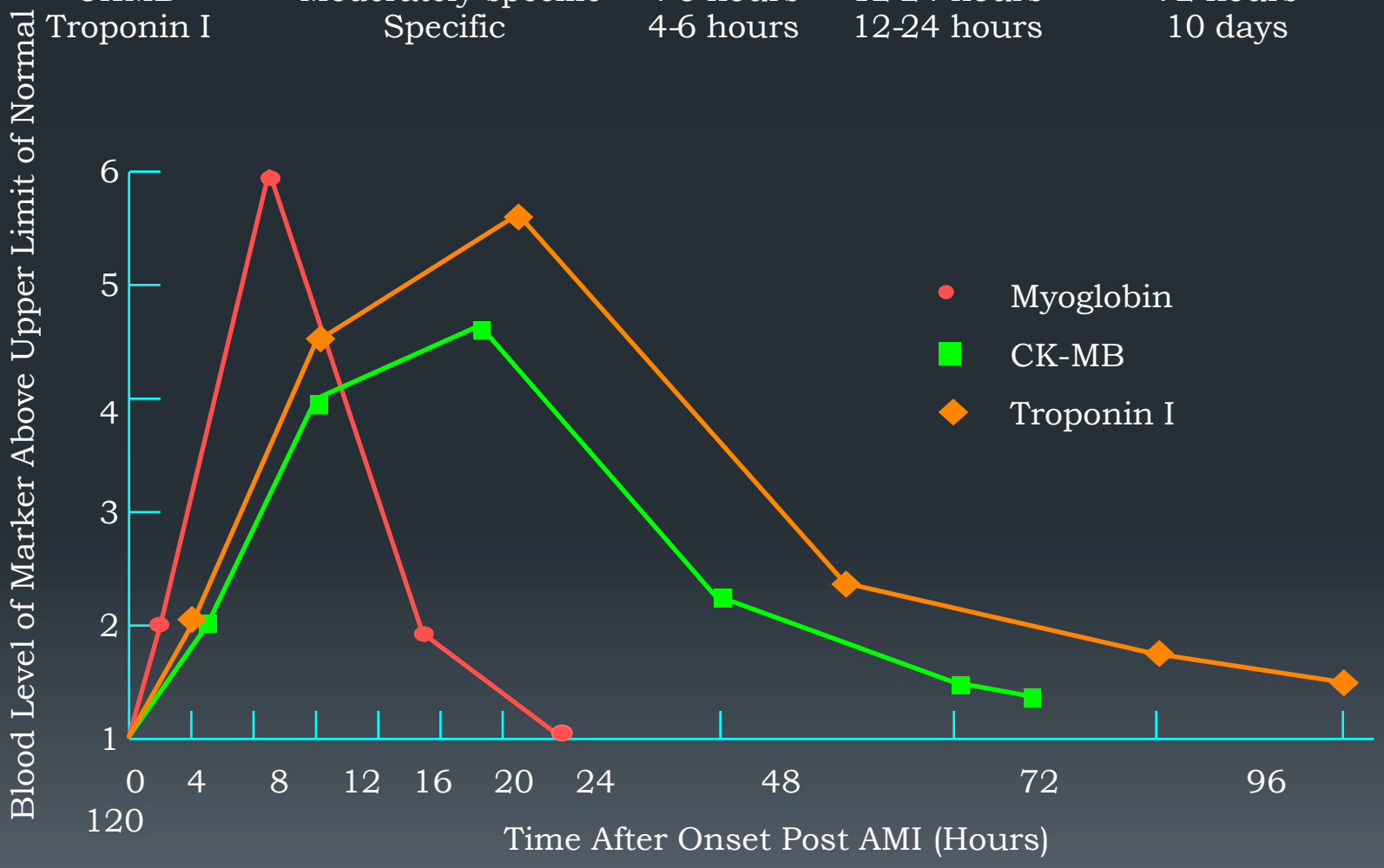
Left ventricular Dysfunction
BNP, NT-proBNP

Classification of Cardiac Biomarkers according to various stages during cardiac disease process



Cardiac Markers Release Kinetics

Cardiac Marker	Specificity	Appears At	Peaks At	Returns to Normal
Myoglobin	Non-specific	1-3 hours	9 hours	24 hours
CK-MB	Moderately specific	4-6 hours	12-24 hours	72 hours
Troponin I	Specific	4-6 hours	12-24 hours	10 days



Case capsule 1

- 54 yr old diabetic male, came to ED with chest pain for 15 minutes with normal ECG.
- Pt was hemodynamically stable.
- What biomarkers should be done ?

- Depends on time of arrival
- Within one hour – Myoglobin
- > 2hrs – CK MB/ Troponin

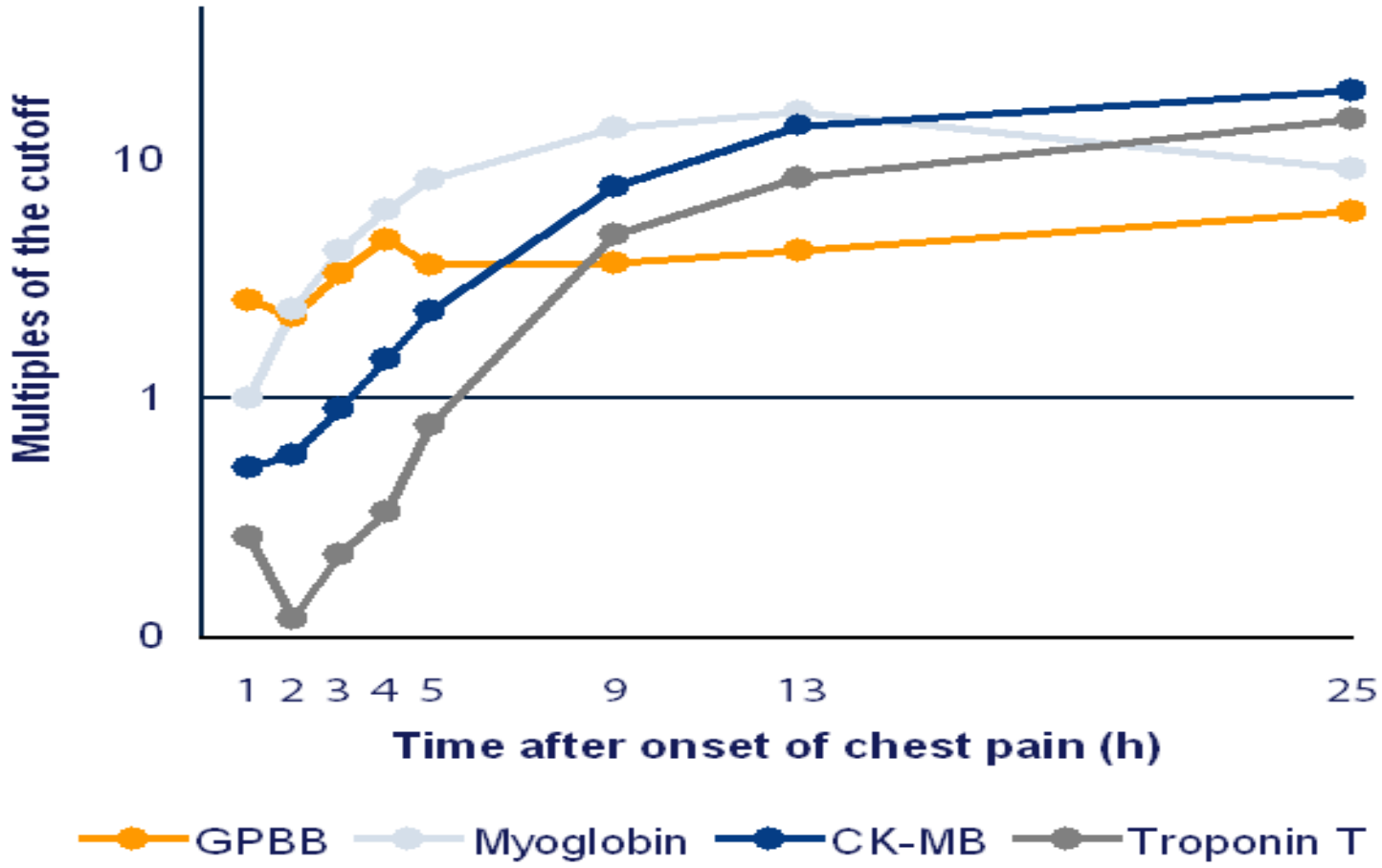
Myoglobin

- Small-size heme protein found in all tissues mainly assists in oxygen transport
- It is released from all damaged tissues
- Its level rises more rapidly than cTn and CK-MB.

- Released from damaged tissue within 1 hour

- Normal value: 17.4-105.7 ng/ml

- Timing:
  - Earliest Rise: 1-4 hrs
  - Peak 6-9 hrs
  - Return to normal: 12 hrs


Multiples of the cutoff
10
1
0
1 2 3 4 5
9
13
25
Time after onset of chest pain (h)
GPBB
Myoglobin
CK-MB
Troponin T

- The earliest expressions (≤30 min) were observed for connexin 43, JunB, and cytochrome c, followed by fibronectin (≤1 h), myoglobin (≤1 h), troponins I and T (≤1 h), TUNEL (≤1 h), and C5b-9 (≤2 h).
- Early markers for myocardial ischemia and sudden cardiac death. Int J Legal Med. 2016 Sep;130(5):1265-80.
- Continuous immunosensing of myoglobin in human serum as potential companion diagnostics technique. Biosens Bioelectron. 2014 Dec 15;62:234-41.

Hindawi Publishing Corporation
Journal of Biomarkers
Volume 2014, Article ID 624930, 9 pages
http://dx.doi.org/10.1155/2014/624930

Hindawi

Research Article

Diagnosis of Non-ST-Elevation Acute Coronary Syndrome by the Measurement of Heart-Type Fatty Acid Binding Protein in Serum: A Prospective Case Control Study

Priscilla Abraham Chandran,[1] Basharat Ara Wani,[1] Oruganti Sai Satish,[2] and Noorjahan Mohammed[1]

[1] *Department of Biochemistry, Nizam's Institute of Medical Sciences, Punjagutta, Hyderabad, Andhra Pradesh 500082, India*
[2] *Department of Cardiology, Nizam's Institute of Medical Sciences, Punjagutta, Hyderabad, Andhra Pradesh 500082, India*

Correspondence should be addressed to Priscilla Abraham Chandran; priscillachandran@yahoo.com

AIM

Since limited numbers of studies have been conducted, in this study the utility of H-FABP as candidate markers for diagnosis of NSTE-ACS.

EXCLUSION CRITERIA

- Chest pain > 8 hours duration
- non cardiac chest pain
- recent injuries
- renal failure

Laboratory Analysis

Serum H-FABP : Quantitative immunoturbidimetric method (Randox Laboratories, Ltd. Co., Antrim, United Kingdom)

cardiac Troponin T and Troponin I : Time resolved immunofluorescence method (AQT90 FLEX, Radiometer, Denmark).

CK-MB : Quantitative immunoturbidimetric method (Roche)

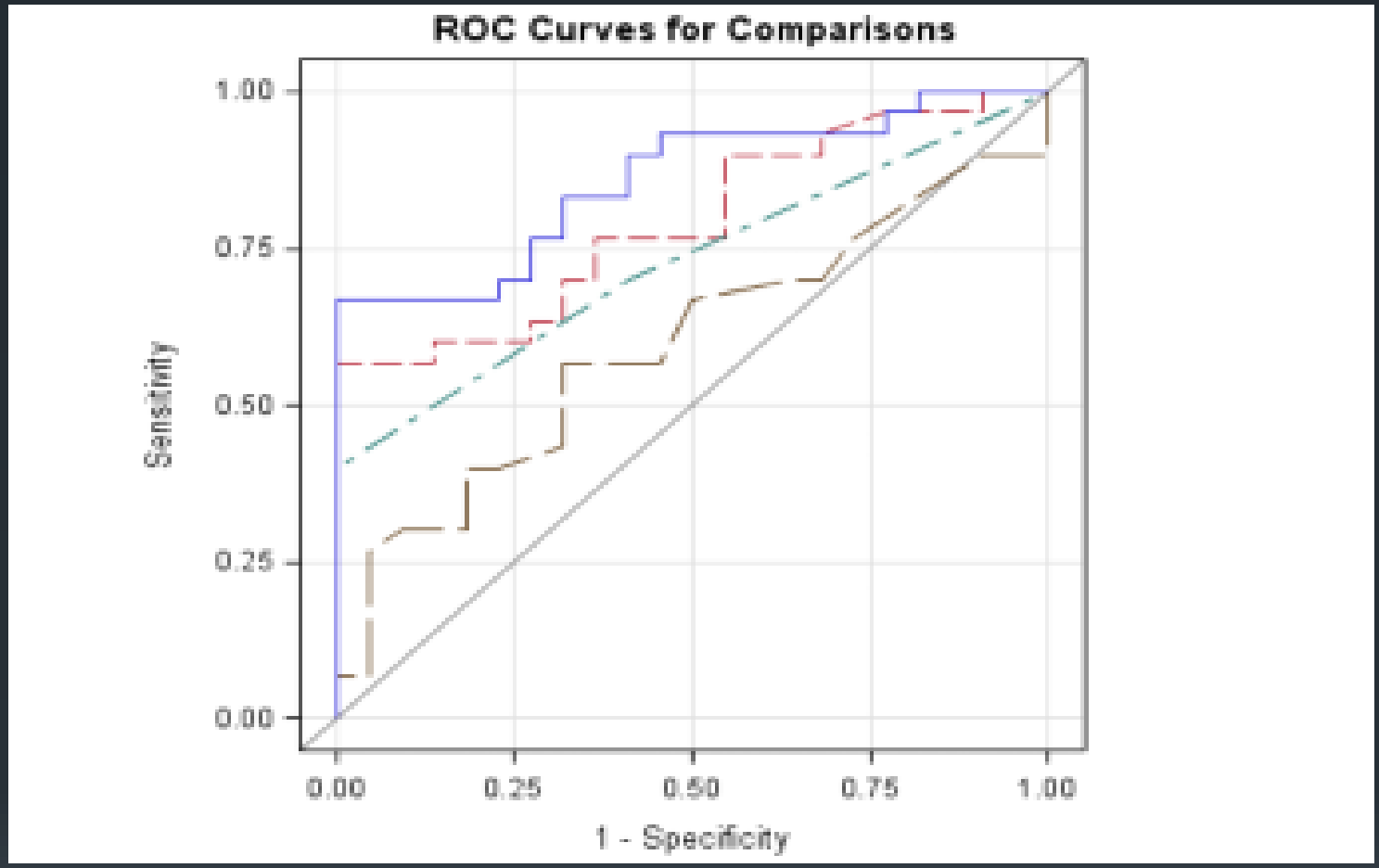
Results

TABLE 1: DEMOGRAPHIC VARIABLES AND Lab. PARAMETERS

DEMOGRAPHICS	CASES(n=30)	CONTROLS(n=22)	
SEX	M=20(66.67%), F=10(33.33%)	M=16,F=6	
AGE(yrs.)	57.7 ± 11.1	55.1 ± 5.1	
VARIABLE	**N**		
SMOKERS	7		
ALCOHOLICS	5		
HYPERTENSIVES	10		
DIABETICS	6		
Lab. PARAMETERS	**CASES(n=30)**	**CONTROLS(n=22)**	**P-VALUE***
TC(mg/dl)	181 ± 53.68	179.54 ± 39.34	0.89[a]
LDL-C(mg/dl)	105 ± 48.58	107.32 ± 30.59	0.84[a]
VLDL-C(mg/dl)	32.60 ± 13.77	39.68 ± 16.54	0.11[b]
TG(mg/dl)	162 ± 70.15	198.41 ± 82.68	0.11[b]
HDL-C(mg/dl)	45.43 ± 11.36	32.54 ± 6.72	<0.001[a]
RATIO(TC/HDL-C)	4.13 ± 1.19	5.64 ± 1.33	<0.001[b]

CARDIAC BIOMARKERS

CARDIAC BIOMARKER	CASES(N=30)	CONTROLS(N=22)	P VALUE
HFABP(ng/ml)	40.42±65.53	3.47±1.52	<0.001
cTnt(ng/L)	370±1007	8.59±0.5	<0.01
cTnI(µg/L)	0.18±0.35	0.008±0.0005	0.005



Model	0.859
CK-MB	0.59
cTnT	0.71
cTnI	0.727
H-FABP	0.79

Diagnostic Performance of Different Cardiac Biomarkers for NSTE-ACS

Variable	CK-MB	cTnT	cTnI	H-FABP
Sensitivity	40%	47%	41%	58%
Specificity	81%	100%	100%	100%
AUC	60	71	73	79
PPV	75%	100%	100%	100%
NPV	50%	58%	55%	63%
Cut-off	18IU/L	16ng/L	0.012µg/L	9.4ng/ml

DISCUSSION

- H-FABP was elevated (>9.4 ng/mL) in 17 of the 30 patients (57%). Among the cases, the mean level of H-FABP was 40 ng/mL, where as in controls it was 3.47ng/ml and the difference was statistically significant ($p < 0.001$).
- In a number of studies, H-FABP has been reported to be particularly sensitive within the first few hours after the onset of coronary occlusion and symptoms.
  - small molecular weight (15 kDa)
  - cytoplasmic unbound abundance
  - rapid release from damaged myocardial cells.
- Kathrukha et al. proved in their study that H-FABP levels elevate earlier than cTnI levels in patients with UA.

- ***LIMITATIONS OF THE STUDY***

- The sample size was small to allow for a generalization of the results. Hence, further larger studies are required to evaluate the diagnostic role of the novel biomarker.

- This work only studied the potential benefit from a single measurement of H-FABP at admission, sequential measurements were not performed.

- ***Take home message***

- In order to decrease the risk of falsely excluded patients with on-going AMI, a combined measurement of two biomarkers, an early one such as H-FABP and a later marker such as troponins may provide the optimum diagnostic performance.

Creatinine Kinase - MB

- Prior to cardiac Troponins marker of choice CK-MB isoenzyme
- Criterion – 2 serial elevations above diagnostic cut off/single result more than twice upper limit of normal
- Appearance 4 to 6 hours after symptom onset/normal 48 to 72 hours.
- Release kinetics assist in diagnosing re-infarction if rise follows decline.
- CK-MB isoforms

$$\text{Relative Index} = \frac{\text{CK-MB}}{\text{Total CK}} \times 100$$

- The relative index allows the distinction between increased total CK due to myocardial damage and that due to skeletal or neural damage.

Relative index calculated by ratio of CK-MB mass to Total CK assist in false positive elevations.

- The relative index is clinically useful when both CK and CKMB are increased.

CK-MB/CK relative index

A relative index exceeding 3 is indicative of AMI

Ratios between 3 and 5 represent a gray zone.

No definitive diagnosis can be established without serial determinations to detect a rise.

- Note that the diagnosis of acute MI must not be based on an elevated relative index alone, because 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.

Testing strategy

- The American College of Emergency Physicians (ACEP) recommends 3 different testing strategies for ruling out NSTEMI in the ED.
- Strategy 1 - is to use a single negative CK-MB, TnI, or TnT measured 8-12 hours after symptom onset.
- Strategy 2 - is to use negative myoglobin in conjunction with a negative CK-MB mass or negative TnI measured at baseline and at 90 minutes in patients presenting less than 8 hours after symptom onset.
- Strategy 3 - is to use a negative 2-hour delta CK-MB in conjunction with a negative 2-hour delta TnI in patients presenting less than 8 hours after symptom onset.

Testing strategy

- ACEP's recommendations on the use of delta CK-MB and delta TnI are based on determining the change in the level of TnI or CK-MB on samples drawn 2 hours apart.
- However, the delta TnI evaluation is partially based on the use of older TnI assays and outdated WHO acute MI cutoffs in a retrospective study.
- Therefore, ACEP's recommendation to use a delta TnI in conjunction with a delta CK-MB may not be generalizable to other commercially available Troponin assays.
- The 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.

Case 1 – follow up

- Immediate Trop I (POC) testing after arrival to ED was negative.
- But CK MB which was sent to lab came as elevated.
- How can we interpret this discordant Troponin and CK-MB results?

WHAT IS discordant Troponin and CK-MB results

- In the CRUSADE registry, a review of almost 30,000 patients revealed that discordant Troponin and CK-MB results occurred in 28% of patients. However, patients who were Troponin negative but CK-MB positive had in-hospital mortality rates that were not significantly increased from patients who were negative for both biomarkers.[30]
- Similarly, in a report of more than 10,000 patients with ACS from the multicenter GRACE registry, in-hospital mortality was highest when both Troponin and CK-MB were positive, intermediate in troponin-positive/CK-MB-negative patients, and lowest in patients in whom both markers were negative and in those who were troponin-negative/CK-MB-positive.[31] Thus, an isolated CK-MB elevation has limited prognostic value in patients with a non-ST elevation ACS.

Troponins

- Generally undetectable in healthy patients ??
- ? Sensitive assays available
- Absolute abnormal value – varies depending on the clinical setting
- Above 99th percentile of healthy population as cut off using an assay in the acceptable precision

Features of serum markers of acute myocardial infarction

			Sensitivity at:			
Marker	Time to appearance	Duration of elevation	6hr	12hr	Specificity	Comments
MB2 Isoform	2 – 6 hr	1 – 2 d	95%	98-100%	95%	Not widely available
Myoglobin	1 – 2 hr	< 1 d	85%	90%	80%	Slightly improved sensitivity early in AMI when added to troponin/CK-MB but not widely used due to low specificity
AMI = acute myocardial infarction; CK=creatine kinase						

Courtesy: Cecil Textbook of Medicine, 22nd ed., Chapter 69 St.Elevation Acute Myocardial Infarction and Complications of Myocardial Infarction

The recent developments in interpretation of Troponin

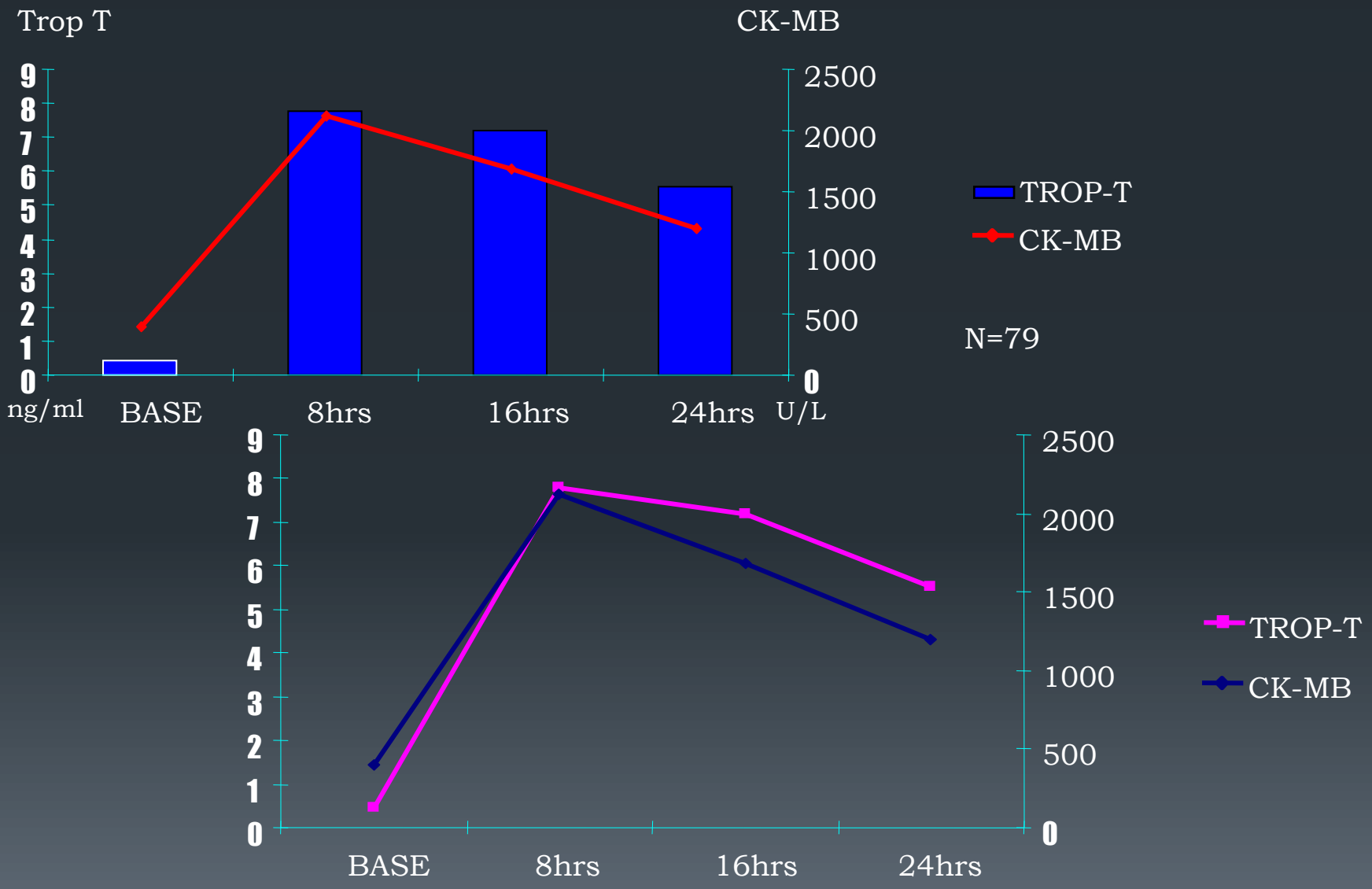
- Cardiac Troponin – shows our understanding is still evolving
- Improved sensitivity – Compared to prior markers – Utilization by Clinicians debatable ..Laboratory understanding of these sensitive values is deter mental to help the physician

Heterogeneity of cut off values – MIXED MESSAGE – in its usage, pushes in more individuals as AMI.. Not easy on the clinician, Hence the situation must be circumstance where the clinical signs, symptoms lead to a strong suspicion of AMI

Study from CARE hospital
Materials and Methods

- 100 patients were analysed for cardiac markers
- 79 were studied for CK-MB and Troponin T serially up to 48 hours
- 23 patients were compared with Troponin T and Troponin I
- 20 patients were compared with Troponin and NT proBNP
- Less than 15 were studied for myoglobin
- HsCRP was found not to be effective in analysis

Serial Measurement of Cardiac Markers after Chest pain



Study in our Laboratory – on changing over

50 samples were compared on Roche platform Elecsys 2010 between Troponin T and Hs Troponin T Assay showed – concordance of 98% and

Paired sample statistics p value 0.39

Inferring that the two assays did not differ significantly at 5% level

Validation between Elecsys 2010 and Cobas e601 for Hs Troponin T had concordance of 100%

Features of serum markers of acute myocardial infarction

			Sensitivity at:			
Marker	Time to appearance	Duration of elevation	6hr	12hr	Specificity	Comments
Troponin I	2 – 6 hr	5 – 10 d	75%	90-100%	98%	Generally regarded as a test of choice
Troponin T	2 – 6 hr	5 – 14 d	80%	95-100%	95%	A test of choice. Less specific than Troponin I (elevated in renal insufficiency)
CK-MB	3 – 6 hr	2 – 4 d	65%	95%	95%	Test of choice for recurrent angina once Troponin elevated

Sensitivity, specificity and precision of commercial Troponin assays:

- Vary considerably – due to
- Lack of standardization
- Use of different monoclonal antibodies
- Presence of modified Troponin in serum
- Variations in antibody
- Cross reactivity with degradation products

Study in our Laboratory – on changing over

75 samples were validated Roche platform Elecsys 2010 and Beckman Coulter Access for Hs Troponin T and Hs Troponin I

Assay showed – concordance of 89% and

Paired sample statistics p value 0.008

Inferring that the two variables of instrumentation and methodology differ significantly at 5 % level.

Validation between Elecsys 2010 and Cobas e601 for Hs Troponin T had concordance of 100%

Cobose601 and Access 2 for 20 samples showed concordance 90%.

TROPONINS

Troponins – released – in response to myocardial infarction – regardless of cause

Ischemia – most common cardiac muscle damage

Cytosolic pool small, muscular pool larger

Cardiac injury – severity – release from both pools injury

Initial small elevation – cytosolic pool

Diffuse across the sarcolemma in to the surrounding lymphatics and blood vessels and there by detectable in blood

If injury persists and necrosis progresses further Troponins are released from the muscular pool

TROPONINS

↑Levels of Troponin complex T & I

- Not present in serum unless-cardiac necrosis Cardiac specific
- Levels remain elevated from 3-14 days after MI, sensitivity high when other markers have

returned to normal

Adv: Delay in seeking medical advice

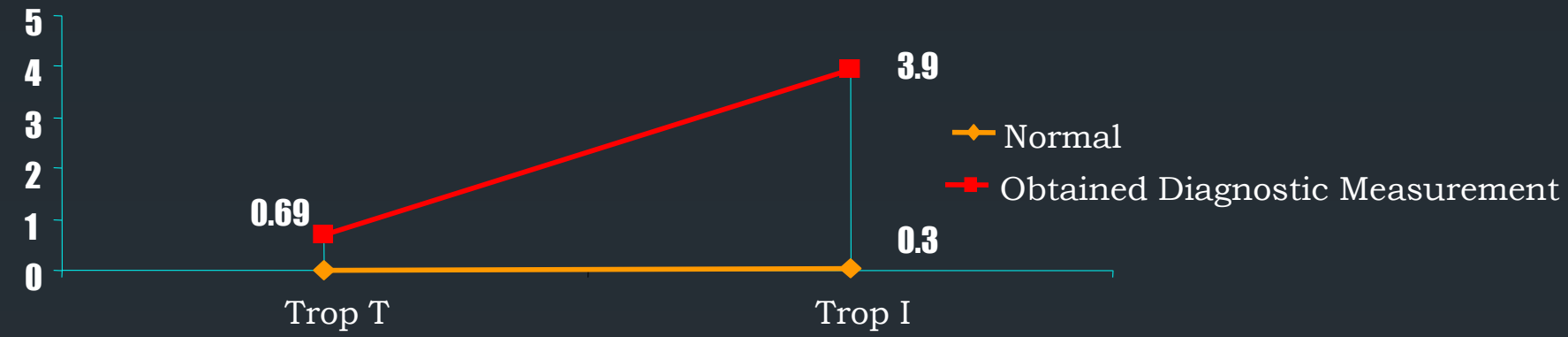
- Elevated levels are predictive of poor outcome in patients with acute coronary syndrome

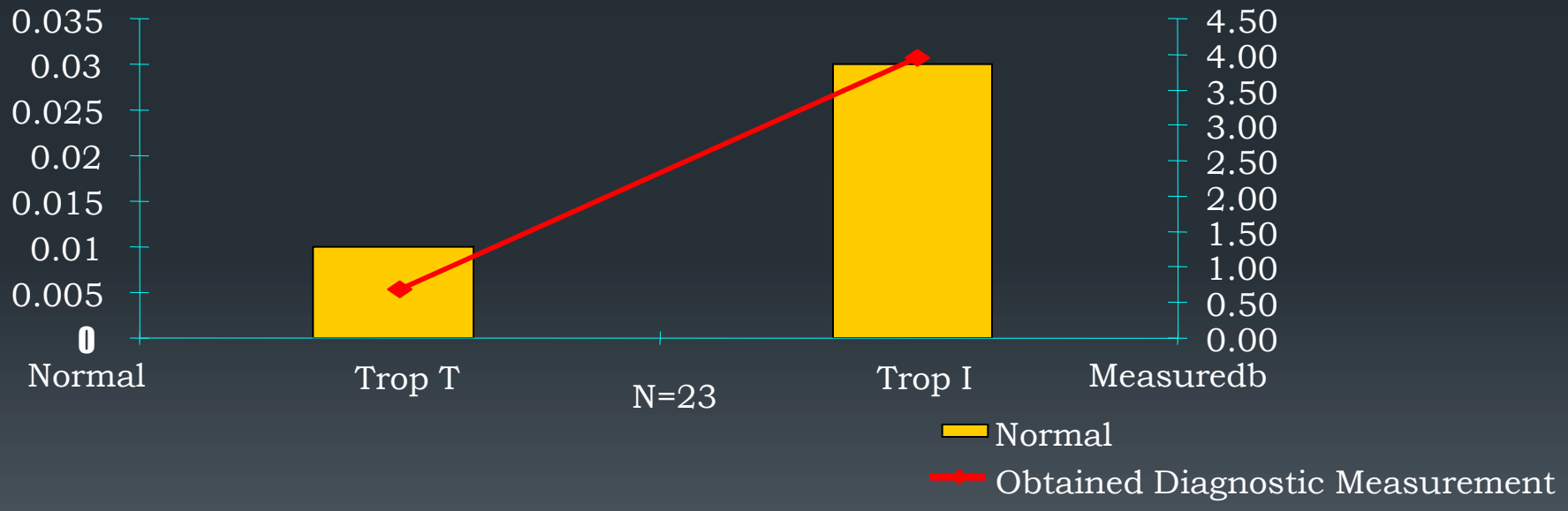
Disadvantage - Detection of Reinfarction

- Less sensitive in early stages of infarction

TROPONINS

? Ideal Marker

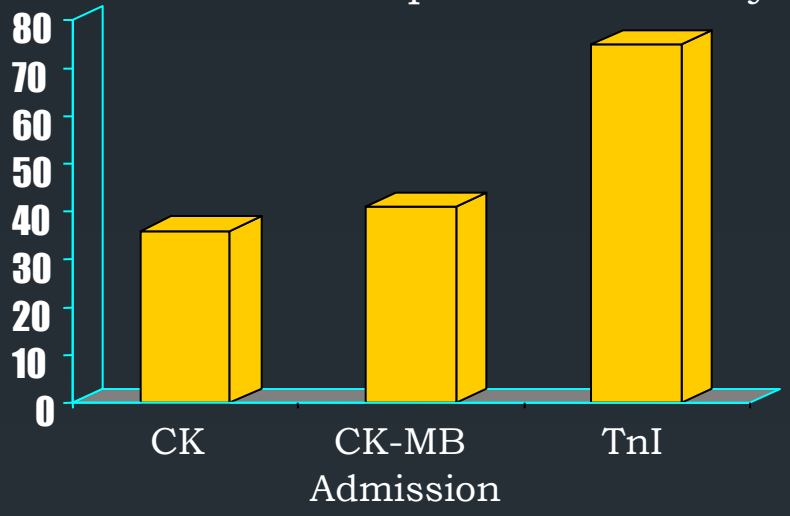


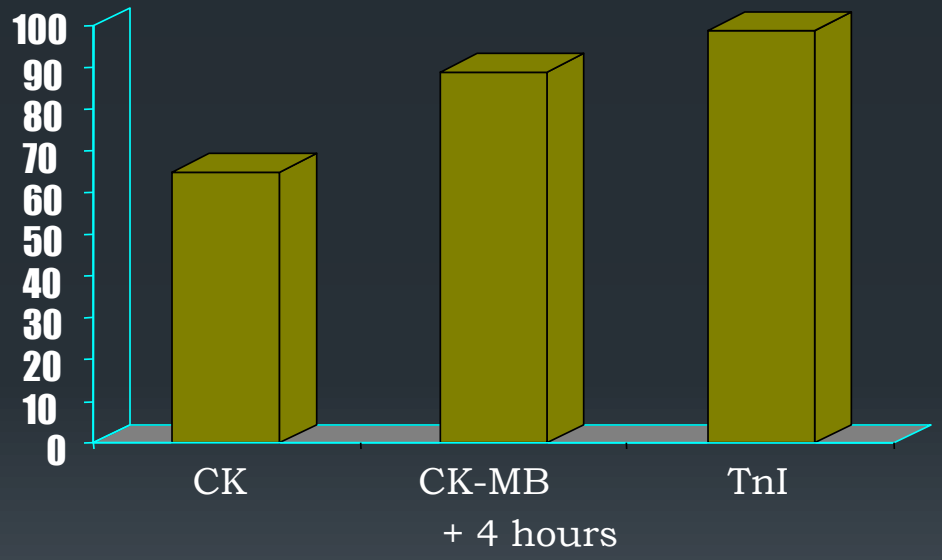


	Troponin T	Troponin I
Normal	< 0.01 ng/ml	0.03 ng/ml
Cut off for AMI	0.1 ng/ml	0.5 ng/ml

Percentage increase in routine Cardiac Markers emphasizing best alternative

Troponin: Sensitivity in the Emergency Department





Troponin I compared with CK activity and CK-MB concentration in patients with AMI on admission and 4 hours later.

Reference: Hamm, Christian W., M.D., Braunwald Heart Disease Update 3.

Concordance Report

Site: Care Hospital Banjara Hills

Randox System: Randox Imola/

Assay Name : H-FABP

Date: 08\03/2013

Reference System: Access/

Assay Name Ref: Troponin I

Concordance Report prepared by:

Sample Data

ID	Access Troponin I		Randox Imola H-FABP	
1	24.400		18.620	
2	0.140	0	7.840	0
3	45.210	0	12.540	0
4	0.570	0	15.310	0
5	37.400	0	56.460	0
6	11.090	0	3.860	0
7	6.870	0	123.000	0
8	0.530	0	16.740	0
9	3.520	0	112.800	0
10	9.230	0	6.410	0
11	3.480	0	15.380	0
12	47.620	0	26.210	0
13	0.060	0	18.820	0
14	25.500	0	8.400	0
15	15.500	0	17.880	0
16	23.320	0	123.000	0
17	95.000	0	123.000	0
18	71.600	0	98.200	0
19	0.510	0	18.850	0
20	18.200	0	84.260	0
21	60.120	0	123.000	0
22	0.460	0	18.860	0
23	0.720	0	12.860	0
24	3.270	0	18.200	0
25	12.750	0	18.020	0
26	2.070	0	19.860	0
27	25.500	0	66.380	0
28	1.510	0	25.940	0
29	40.010	0	123.000	0
30	50.000	0	123.000	0
31	5.010	0	12.860	0
32	2.290	0	18.860	0
33	2.810	0	46.200	0
34	0.390	0	9.860	0
35	0.010	0	3.020	0
36	0.020	0	3.700	0
37	0.010	0	5.290	0
38	0.020	0	3.950	0
39	0.010	0	4.020	0
40	0.010	0	3.020	0
41	0.001	0	4.000	0

Sample Data

ID	Access Troponin I		Randox Imola H-FABP	
		0		0

Randox Cut-Off 6.32

Reference Cut-Off 0.50

Discordant Result

Statistical Information:

	Access/Other	Randox
n	41	41
mean	15.774	38.329
min	0.001	3.020
max	95.000	123.000

	OLS	Adjusted
intercept	19.133	8.265
slope	1.217	1.906
r	0.638	

Concordance Table

Access/Troponin I

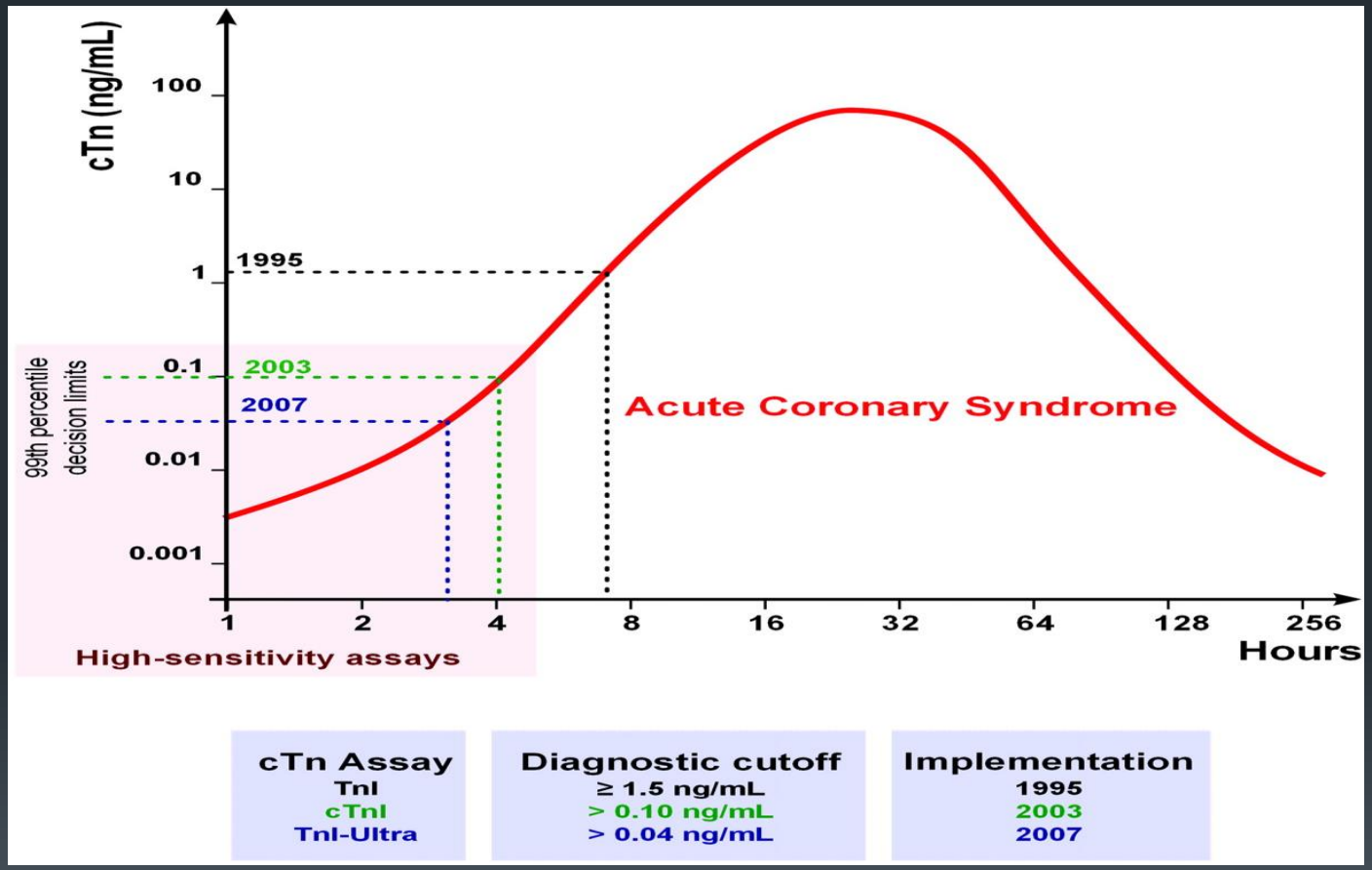
andox Imola/H-FAB	-	+
-	7	1
+	4	29

concordance: 88%

Count:	41

Date: _____ Randox Associate: _____

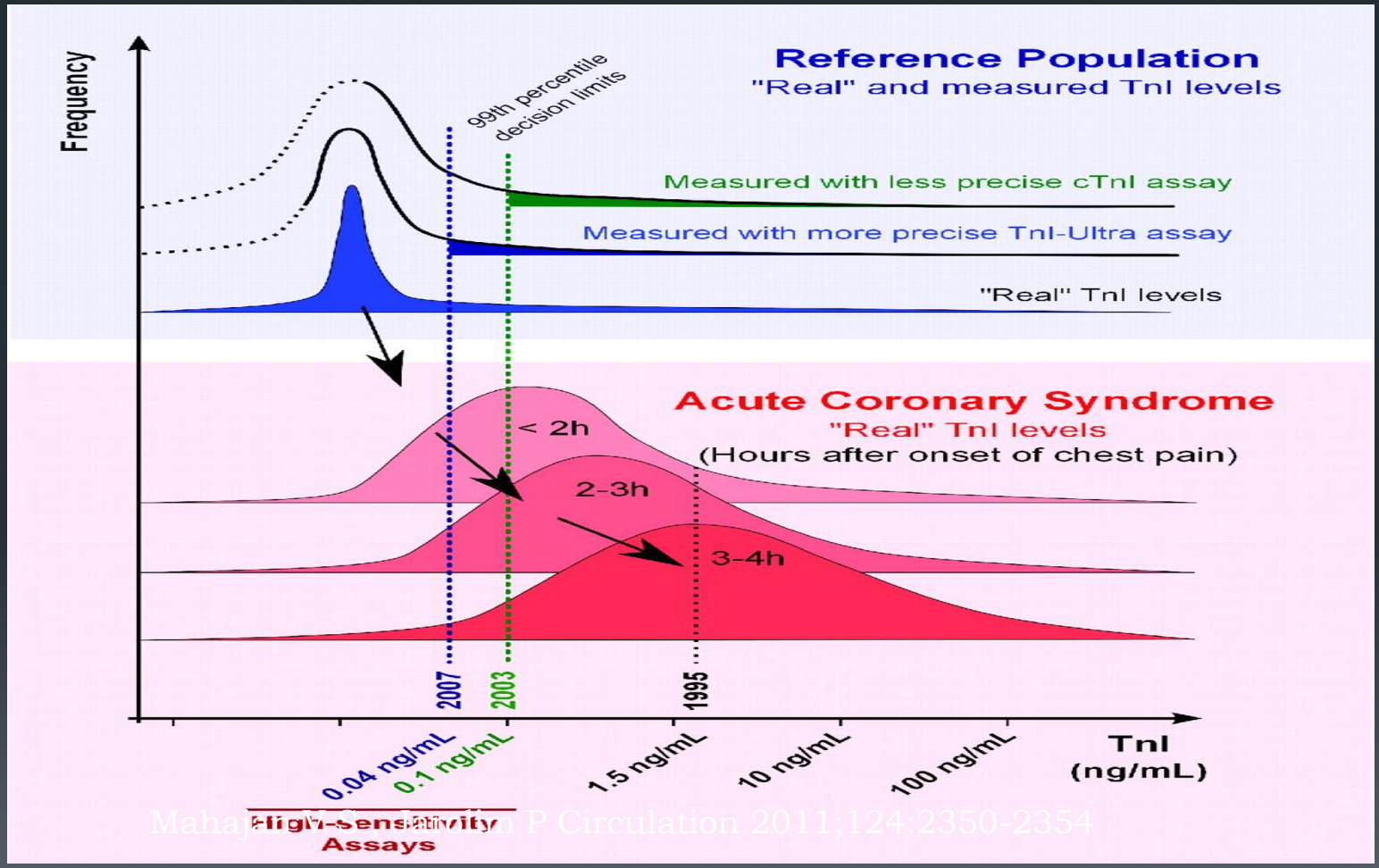
Evolution of the cardiac Troponin (cTn) assays and their diagnostic cut-offs.



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

Mahajan V S , Jarolim P Circulation 2011;124:2350-2354

Cardiac Troponin I (cTnI) levels in a healthy reference population and in an acute coronary syndrome (ACS) population.



Mahaffey S, Morrow P Circulation 2011;124:2350-2354

High sensitivity cardiac Troponin T (hs-cTnT) – as an isolated marker in ED for chest pain evaluation

- Troponin-only Manchester Acute Coronary Syndromes (T-MACS) decision aid: single biomarker re-derivation and external validation in three cohorts
- At the 'rule out' threshold, in the derivation set (n=703), T-MACS had 99.3% (95% CI 97.3% to 99.9%) negative predictive value (NPV) and 98.7% (95.3%-99.8%) sensitivity for ACS, 'ruling out' 37.7% patients (specificity 47.6%, positive predictive value (PPV) 34.0%). In the validation set (n=1459), T-MACS had 99.3% (98.3%-99.8%) NPV and 98.1% (95.2%-99.5%) sensitivity, 'ruling out' 40.4% (n=590) patients (specificity 47.0%, PPV 23.9%). T-MACS would 'rule in' 10.1% and 4.7% patients in the respective sets, of which 100.0% and 91.3% had ACS. C-statistics for the original and refined rules were similar (T-MACS 0.91 vs MACS 0.90 on validation).
- CONCLUSIONS:
- T-MACS could 'rule out' ACS in 40% of patients, while 'ruling in' 5% at highest risk using a single hs-cTnT measurement on arrival. Emerg Med J. 2016 Aug 26

Case capsule 2

- 63 years old female hypertensive patients with anterior STMI of 3 hours duration .
- No contraindications for thrombolysis.
- Hemodynamically stable.
- What biomarker we should use?
- Is there any difference in gender for biomarkers elevation timing and severity of elevation?

Are cardiac biomarkers are required for STMI?

- Note that cardiac markers are not necessary for the diagnosis of patients who present with ischemic chest pain and diagnostic ECGs with ST-segment elevation.

- Clinical Effect of Sex-Specific Cutoff Values of High-Sensitivity Cardiac Troponin T in Suspected Myocardial Infarction. *JAMA Cardiol* 2016;Sep 21:[Epub ahead of print].
- once using the uniform 99th percentile cutoff value level of 14 ng/L and once using sex-specific 99th percentile levels of hs-cTnT (women, 9 ng/L; men, 15.5 ng/L).
- The diagnosis in two women was upgraded from unstable angina to AMI, and the diagnosis in one man was downgraded from AMI to unstable angina. These diagnostic results were confirmed when using two alternative pairs of uniform and sex-specific cutoff values.
- Conclusions: The authors concluded that uniform 99th percentile should remain the standard of care when using hs-cTnT levels for the diagnosis of AMI.

Exceptions

- Simple markers can distinguish Takotsubo cardiomyopathy from ST segment elevation myocardial infarction. Int J Cardiol. 2016 Sep 15;219:417-20.
- The concentration of NTproBNP was greater in pts with TTC than STEMI (4702pg/ml vs 2138pg/ml). The concentration of TnI and CKMB mass was greater in the STEMI group than in the TTC group (TnI: 2.1ng/ml and CK MB mass: 9.5ng/ml in pts with TTC vs TnI: 19ng/ml and CK MB mass: 73.3ng/ml in pts with STEMI). The NTproBNP/TnI ratio and NTproBNP/CKMB mass ratio were, respectively, 2235.2 and 678.2 in pts with TTC and 81.6 and 27.5 in pts with STEMI ($p<0.001$). Moreover, the NTproBNP/EF ratio was also statistically significant (110.4 in TTC group and 39.4 in STEMI group).
- CONCLUSIONS:
- NTproBNP/TnI, NTproBNP/CKMB mass and NTproBNP/EF ratios can distinguish TTC from STEMI at an early stadium. The most accurate marker is the NTproBNP/TnI ratio.

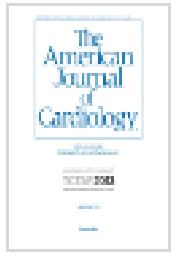
ELSEVIER

The American Journal of Cardiology

Volume 111, Issue 7, Supplement, 2 April 2013, Pages 67B

Eighteenth Annual Symposium — April 23-26, 2013

Interventional Vascular Therapeutics Angioplasty Summit-
Transcatheter Cardiovascular Therapeutics Asia Pacific Symposium



Poster Abstract

Toll Like Receptor 4 in Acute Myocardial Infarction

Maddury Jyotsna[1], Madhavapeddy Aditya[1], Janaswamy Vibhav Sri Narayana[2], Laxmi Laxmi[1]

Available online 20 March 2013

AS-138

Toll Like Receptor 4 in Acute Myocardial Infarction

- Background:

Previous studies on Toll-like receptor 4 (TLR 4), which are identified as central innate immune receptors, were done for local (at plaque rupture site) and systemic expression of TLR 4 from mononuclear concentrate (MNC) in acute myocardial infarction (AMI) pts. TLR inhibition is considered as a emerging new therapeutic modality for LV remodeling. We want to study difference of expression of TLR 4 on MNC and plasma in AMI pts. Even though, for TLR 4 protein detection in plasma requires higher concentration on the lymphocytes and to be secreted into plasma , but still, if plasma detected TLR 4 has same prognostification importance as from TLR 4 of MNC then, TLR 4 detection from plasma may be used at bed side.

Methods

- We recruited acute MI pts who presented with in 48 hrs of onset of chest pain.
- TLR4 estimation was done with TLR4 ELISA kit from Cusabio at the time of admission.
- Group 1 are AMI pts with TLR 4 measured from MNC (Ficoll paque method) and Group 2 are AMI pts with TLR 4 measured from plasma.
- In two groups of Controls (Control A are volunteers without known CAD and coronary risk factors and Control B are pts with obvious sepsis), TLR4 was estimated in both plasma and from MNC.
- According to that kit standards TLR 4 concentration < or = 0.03 ng/dl is considered as negative.
- Killips class, adverse events in hospitals (including recurrent angina, LVF, ventricular arrhythmias and death) and CPK levels were correlated with TLR 4 levels.

Table 1: AMI

Parameter	AMI		Controls	
	Group 1 (MNC)	Group 2(Plasma)	A(no CAD+RF)	B (Infection)
Number	26	14	15	5
TLR 4 pos.	14 (53.8%)	2(14.3%)	0	5(100%)

Table 2:

Parameter	Variable
Age (yrs)	**54.1 ± 11.6**
M:F	**12:8**
HTN	**21 (52.5%)**
DM	**10 (25%)**
Location of MI	
Inferior MI	**11 (27.5%)**
Anterior MI	**26 (65%)**
Extensive MI	**3 (7.5%)**
Average Killips class	**1.7 ± 0.9**
CPK levels	**1652.2 ± 1294.6 units/l**
Mean TLR 4	**0.7 ± 0.4 ng/dl**

Table 3:

Parameter	TLR Positive	TLR Negative	P value
Group 1			
Average Killips class	2.4 ± 0.8	1.17 ± o.4	< 0.001
Average CPK level	1760.4 ± 875.4	1994 ± 986.3	NS
Primary VT	1	1	NS
Death	1	0	0.05
Group 2			
Average Killips class	3 ± 1.4	1.3 ± 0.5	0.001
Average CPK level	1348 ± 439.3	1537 ± 45.7	NS
Primary VT	1	1	NS
Death	1	0	0.05

Conclusion

- In Group 1, & group 2 also there is no correlation to TLR 4 concentration and occurrence of primary VT and CPK levels but strong association with Killips class and death.

- Plasma TLR 4 detection was given same correlation with Killips class and dreaded prognostication of the patient (that is mortality) in AMI like the TLR 4 detected from MNC. Therefore, kit to design using plasma of the AMI pt may be useful after larger AMI patients study.

Case capsule 3

- 44 year old gentleman admitted with road traffic accident developed chest pain. What would be the preferred marker for diagnosing infarction
- Road traffic accident with rhadomyolysis
- Trop

How we can standerdize the TnT assay

- Only one manufacturer produces the TnT assay, and its 99th percentile cutoffs and the 10% CV are well established. However, up to 20-fold variation has occurred in results obtained with the multitude of commercial TnI assays currently available, each with their own 99th percentile upper reference limits and 10% CV levels.

How we can standerdize the TnT assay

- In the GUSTO IV study, a relatively insensitive point-of-care TnI assay was used to screen patients for study eligibility. In a subsequent study, the blood samples were reanalyzed using the 99th percentile cutoff of a far more sensitive central laboratory TnT assay. The more sensitive 99th percentile cutoff of this TnT assay identified an additional 96 (28%) of 337 patients with a positive TnT result but negative point-of-care TnI; these patients had higher rates of death or MI at 30 days.[14]
- In a similar reanalysis of the TACTICS-TIMI 18 trial, 3 different TnI cutoffs were compared on 1821 patients to evaluate the 30-day risk of death or MI: the 99th percentile, 10% CV, and the World Health Organization (WHO) acute MI cutoffs. (The WHO cutoffs define acute MI using CK-MB and report troponin levels as either a higher "acute MI level" or a lower "intermediate level" that is correlated with "leak" or "minor myocardial injury.")
- Using the 10% CV cutoff identified, an additional 12% more cases were identified relative to the WHO acute MI cutoff. The 99th percentile cutoff identified an additional 10% of cases relative to the 10% CV cutoff, as well as a 22% increase in the number of cases over the WHO acute MI cutoff. Nevertheless, the odds ratios for the adverse cardiac event rates of death or MI at 30 days were similar for all 3 cutoffs, suggesting that the lower cutoffs detected more patients with cardiovascular risk without sacrificing specificity.[

How we can standerdize the TnT assay

- The National Academy of Clinical Biochemistry (NACB) working with the ACC/ESC guidelines has recommended adoption of the 99th percentile upper reference limit as the recommended cutoff for a positive troponin result. Ideally, the precision of the assay at this cutoff level should be measured by a CV that is less than 10%.

- However, most TnI assays are imprecise at the 99th percentile reference limit.[17]Some have therefore recommended that the cutoff level be raised to the slightly higher 10% CV level instead of the 99th percentile reference limit to ensure adequate assay precision.

Is Point-of-care assays are available?

- NACB recommendations specify that cardiac markers be available on an immediate basis 24 h/d, 7 d/wk, with a turnaround time of 1 hour.[18] Point-of-care (POC) devices that provide rapid results should be considered in hospitals whose laboratories cannot meet these guidelines.

- POC assays for CK-MB, myoglobin, and the cardiac troponins TnI and TnT are available. Only qualitative TnT assays are available as POC tests, but both quantitative and qualitative POC TnI assays are currently marketed.

Point-of-care assays

- In a multicenter trial, the time to positivity was significantly faster for the POC device than for the local laboratory (2.5 h vs 3.4 h).[19]

- In another multicenter study, which evaluated the i-STAT POC TnI assay in comparison with the central laboratory in 2000 patients with suspected ACS, POC testing reduced the length of stay by approximately 25 minutes for patients who were discharged from the ED.[20, 21] The sensitivity of current POC assays coupled with the benefit of rapid turnaround time make the POC assays attractive clinical tools in the ED.

Point of care assays:

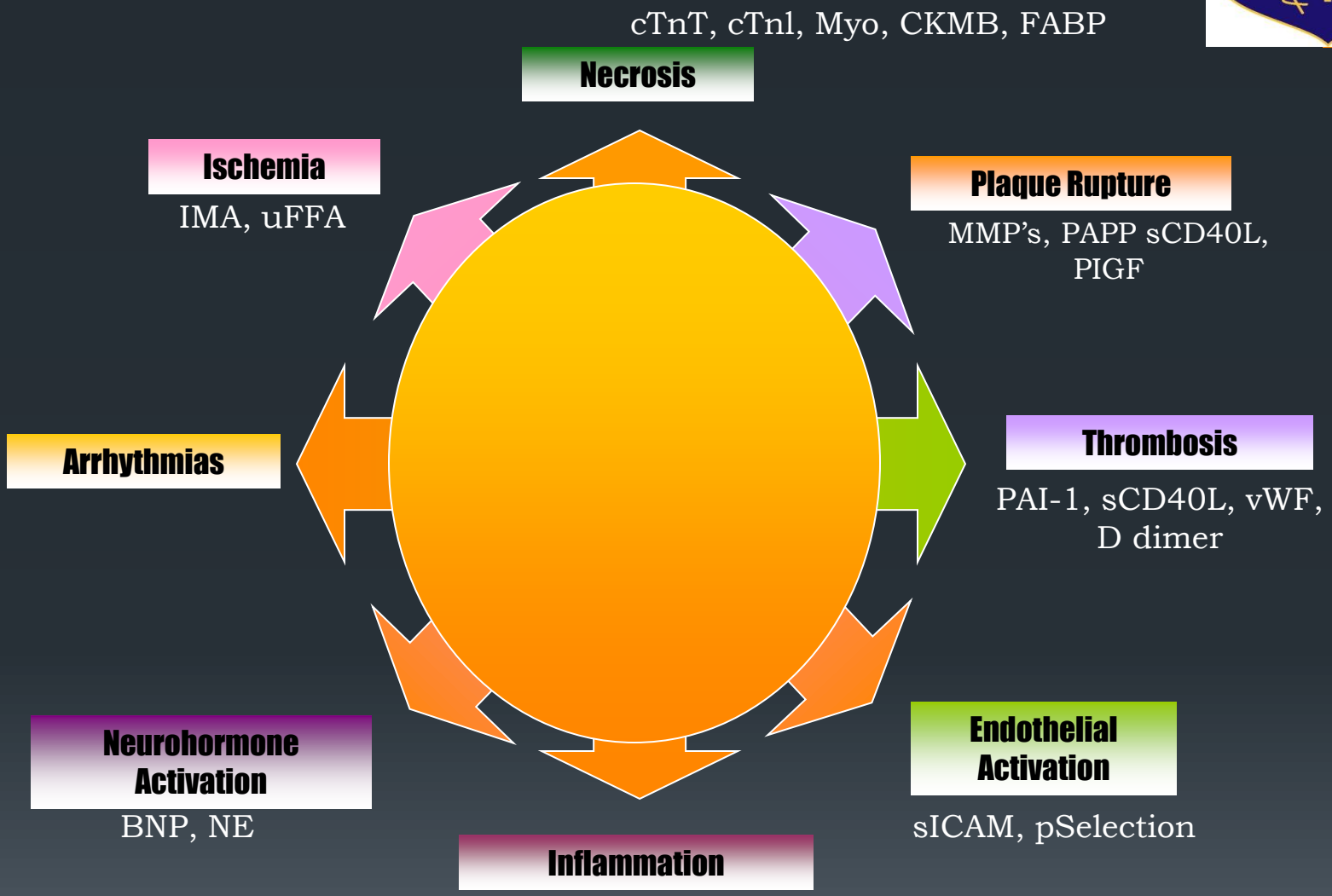
- NACB – Cardiac markers to be available 24 hrs/day, 7 d/week
- Ideal turn around time – 1 hour
- POC – when labs cannot meet this guidelines
- Qualitative TnT assay
- Qualitative and Quantitative for TnI assay

Ultrasensitive and low-volume point-of-care diagnostics are available for trop T testing?

- Sci Rep. 2016 Sep 16;6:33423. Ultrasensitive and low-volume point-of-care diagnostics on flexible strips - a study with cardiac Troponin biomarkers. Shanmugam NR1, Muthukumar S2, Prasad S1.
- A flexible, mechanically stable, and disposable electrochemical sensor platform for monitoring cardiac Troponin through the detection and quantification of cardiac Troponin-T (cTnT). They designed and fabricated nanostructured zinc oxide (ZnO) sensing electrodes on flexible porous polyimide substrates.

- Additive biomarkers in AMI

Potential Biomarker Targets in ACS



- Midregional fragment of the N-terminal of pro-ANP (MR-proANP) and 2 extracardiac biomarkers; the c-terminal provasopressin (copeptin) and the midregional portion of proadrenomedullin (MR-proADM).

Novel markers in ACS

✂ Cost effective

- Traditional markers – CK/CK-MB/cTnI/cTnT/ Myglobin
- PAPPP-A – Mettaloproteinate causes extracellular matrix degradation, activates insulin like growth factor IGF-1 (mediator of atherosclerosis)
- HsCRP – Acute phase/prothrombotic/specificity ?
- BNP – Neurohormonal activity, risk stratification
- IL-6 – Cytokine, indipendent marker for risk stratification
- Albumin – Cobalt binding ischemic modified albumin ?

Summary of studies using BNP or NTproBNP for risk stratification of AMI

Study Pop	N	Endpoints	Thresholds	Odds ratio or Hazard Ratio
ACS-TIMI16	2,525	Death (30 day, 10 months) HF(10 months) MI (10 months)	Quartiles BNP > 80 pg/ml	1, 3.8, 4.0, 5.8 Approximately 2.7 Approximately 2
AMI	70	Death (18 months)	Median (>59 pg/ml)	Approximately 2.5
AMI-CONSENSUS	131	Death (one year)	75th centile BNP 33.3 pmol/L	Approximately 1.36
ACS	609	Death	Median	2.4
NSTEACS	1,483	Death (in hosp) (180 days)	BNP > 586 pg/ml	(1.7) (1.67)
FRISC-II	2,019	Death	Top Tertile	4.1(invasive) vs 3.5(non-invasive)
TACTICS-TIMI18	1,676	Death (six months) HF (30 days)	BNP > 80 pg/ml	OR 3.3 OR 3.9
ACS	1,033	Death (30 day) (six months)	Quartiles	(2.24) (1.84)
AMI	473	Death	Median	OR 3.82

Novel markers in ACS

✂ Cost effective

- Glycogen Phosphorylase (GPBB)
  - Released early from injured Myocardial cells
  - Reflecting burst in Glycogenolysis associated with MI
  - Greater discriminating power than other markers
- Haemostatic
  - Fibrinopeptide A (FPA) - Ongoing Thrombin Activity
  - Thrombin – Antithrombin Complex(TAT) - Thrombin Generation
  - Prothrombin Fragmen 1.2(F1.2)- Ongoing Haemostatic Activation

Summary of studies using GDF-15 for risk stratification of AMI

Study Pop	N	Endpoints	Thresholds	Odds ratio or Hazard Ratio	Ref
GUSTOIV (NSTEACS)	2,081 + 429	Death (one year)	Tertiles < 1,200 ng/L, 1,200 to 1,800 ng/L > 1,800 ng/L	1.5%, 5%, 14.1%	[46]
FRISC-II (invasive vs conservative)	2,079	Death or MI (2 yrs)	Invasive at > 1,800 ng/L Invasive 1,200 to 1,800 ng/L Invasive < 1,200 or Trop -ve	HR 0.49 (risk reduction) HR 0.68 No benefit	[47]
ASSENT-2/plus (STEMI)	741	Death (1 yr)	Tertiles < 1,200, 1,200 to 1,800, > 1,800	2.1%, 5.0%, 14%	[48]
AMI	1,142	Death or HF (1.5 yr)	<1,470 ng/L, >1,470 ng/L	HR 1.77	[49]

Comprehensive Metabolomic Characterization of Coronary Artery Diseases.

- A total of 89 differential metabolites were identified. The altered metabolic pathways included reduced phospholipid catabolism, increased amino acid metabolism, increased short-chain acylcarnitines, decrease in tricarboxylic acid cycle, and less biosynthesis of primary bile acid.

- Plasma metabolomics are powerful for characterizing metabolic disturbances. Differences in small-molecule metabolites may reflect underlying CAD and serve as biomarkers for CAD progression.

- J Am Coll Cardiol. 2016 Sep 20;68(12):1281-93.

Novel markers in ACS

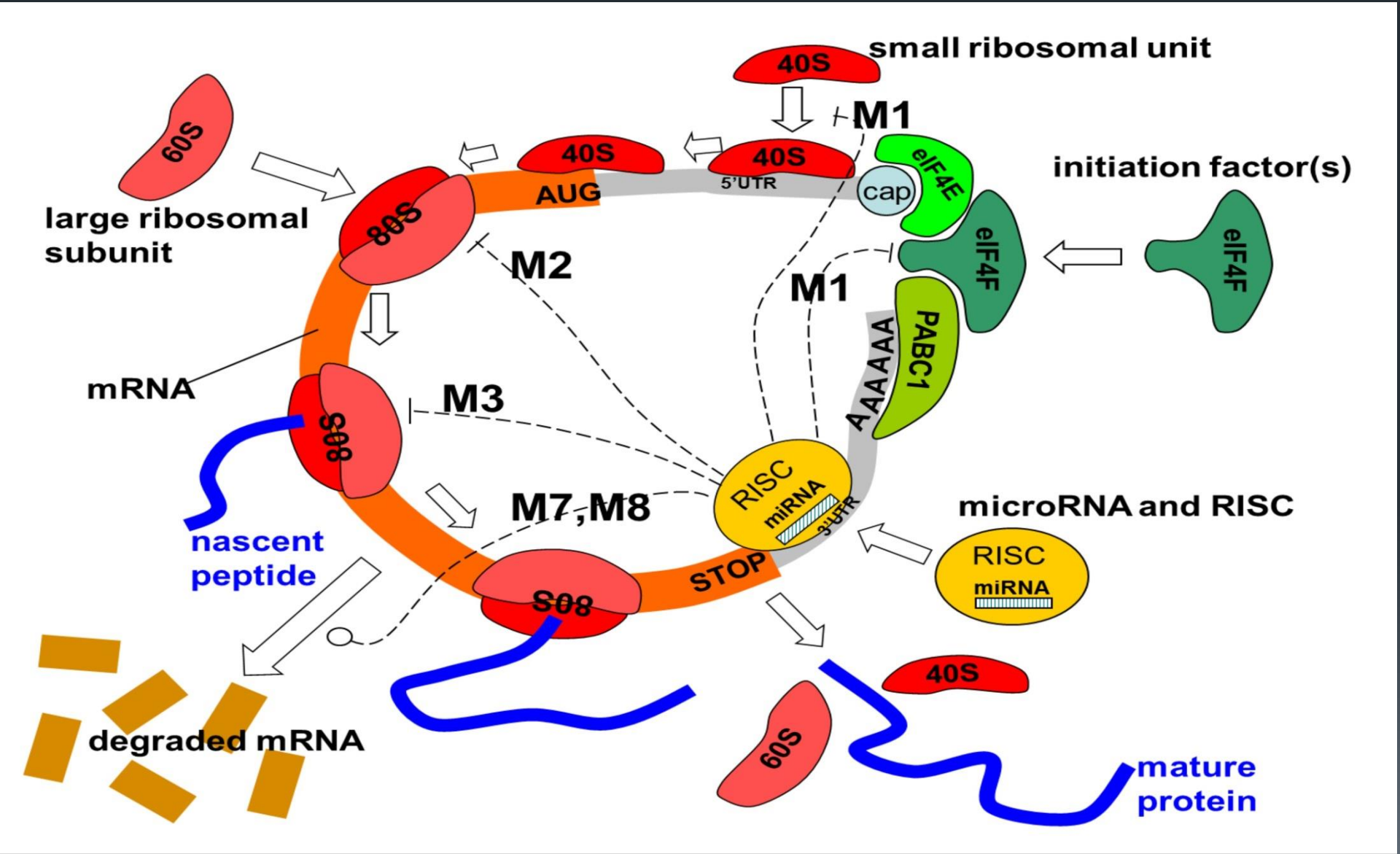
✂ Cost effective

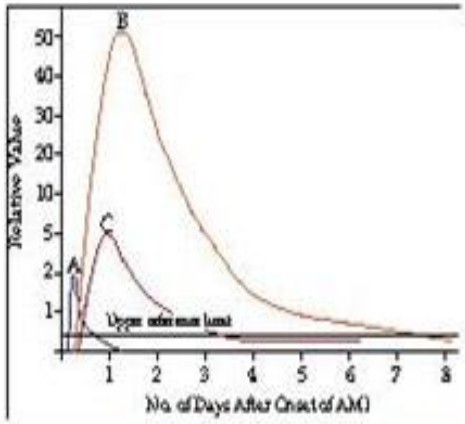
-Plasma D-Dimer - Activation of coagulation/ fibrinolysis

-S100 Protein- Levels at early & late ph

? Continuous Release after injury

? Future Marker

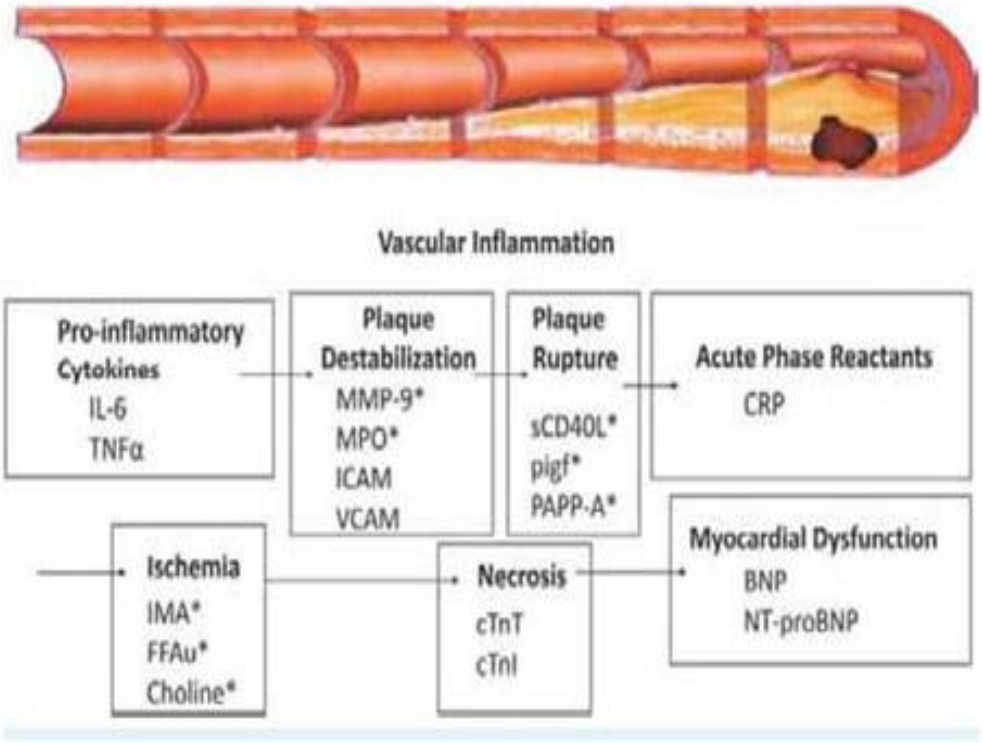

small ribosomal unit
40S
M1
60S
large ribosomal subunit
40S
40S
AUG
5'UTR
cap
eIF4E
initiation factor(s)
eIF4F
eIF4F
80S
M2
M1
mRNA
PABC1
AAAAA
M3
80S
RISC
miRNA
3'UTR
M7,M8
microRNA and RISC
RISC
miRNA
nascent peptide
STOP
S08
40S
degraded mRNA
60S
mature protein



Timing of release of cardiac biomarkers

Why Do We Need Biomarkers for Diagnosis?

ACS is a result of numerous pathophysiological events like



Ack. JAPI, Volume 63, June 2015

Advantages of troponins[13]

Feature of biomarker	Cardiac Troponin	Myoglobin	CK-MB
ACC/AHA guidelines' recommendation for cardiac markers	IB	IIB	IIB
Specific for myocardial injury	✓	X	X
Cardiac-specific expression	✓	X	X
Increased levels in blood hours after onset of MI	✓	✓	✓
Elevated levels in circulation for many days following MI	✓	X	X
2-hour delta value sensitive and specific for diagnosing MI	✓	X	X

Cardiac marker sampling frequency[2]

	Baseline	3-4 hr	6-9 hr	12-24 hr	> 24 hr
CK-MB isoform, myoglobin	✓	✓	✓		
CK-MB, TnI, TnT	✓	✓	✓	v (only if very high-risk)	
Late presenters					P

Hs Troponin – Cut off point of 99% percentile – highly sensitive for diagnosis of AMI, 2 hours after presentation

Ack. JAPI, Volume 63, June 2015

Questions in thought process

- Diagnosing acute MI using high sensitive Troponin
- How to use high sensitive cardiac troponin in acute cardiac insult
- Groups with subclinical Ischemic heart disease and slightly elevated troponin
- Studies of diagnostic performance study design influences sensitivity and specificity
- Age cut off - ? Elderly
- ?? Cardiac – Troponin – A marker of Myocardial necrosis and not a specific marker of AMI
- Constant values without diagnosis changes are likely to be marker of chronic heart disease

The 10 commandments of troponin

- Collaborate with the laboratory and the emergency department
- Understand some analytical considerations
- Make the diagnosis of AMI based on cTn and the clinical scenario
- Rule out myocardial infarction differently than ruling it in
- Use common sense to interpret elevations of cTn in patients who are critically ill
- Do not be intimidated by elevations in patients with renal failure
- Take the baseline value of cTn into account with percutaneous coronary intervention
- It takes multiple parameters to make the diagnosis of AMI following bypass surgery
- Do not forget drug toxicities as an aetiology for cTn elevations
- Be cautious with cTn elevations post-exercise

Heart 2011;97:940-946