Biochemical Markers in Cardiac Injury

Dr Sami Saeed

Assistant Professor/Consultant Chemical Pathologist

Foundation University Medical College

Fauji Foundation Hospital

Rawalpindi

Email: drsami@comsats.net.pk

OUTLINE

- Classification of lab tests useful in cardiac disease
- Biochemical markers in Acute Coronary Syndromes (ACS)
- Redefinition of Myocardial Infarction (MI)
- Biochemical markers in the assessment of cardiac function
- Biochemical monitoring of cardiovascular risk factors
- Future developments in the assessment of cardiovascular disease
- Laboratory considerations in the choice of markers of myocardial damage
- Protocol for the use of biochemical markers in the patient with chest pain

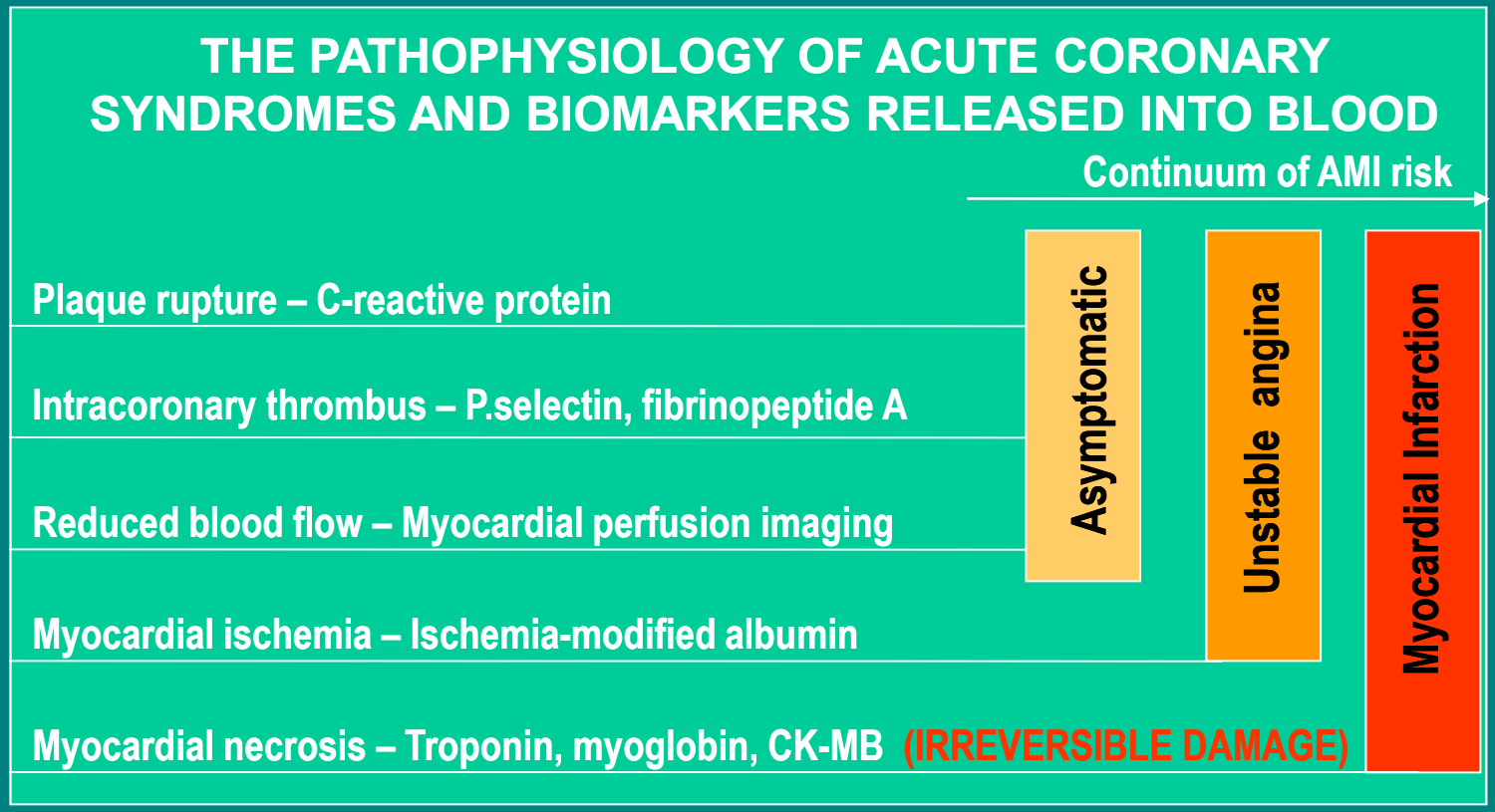
CLASSIFICATION OF LABORATORY TESTS IN CARDIAC DISEASE

- Markers of cardiac tissue damage
- Markers of myocardial function
- Cardiovascular risk factor markers
- Genetic analysis for candidate genes or risk factors

HISTORICAL CRITERIA FOR DIAGNOSIS OF MI (WHO, 1974)

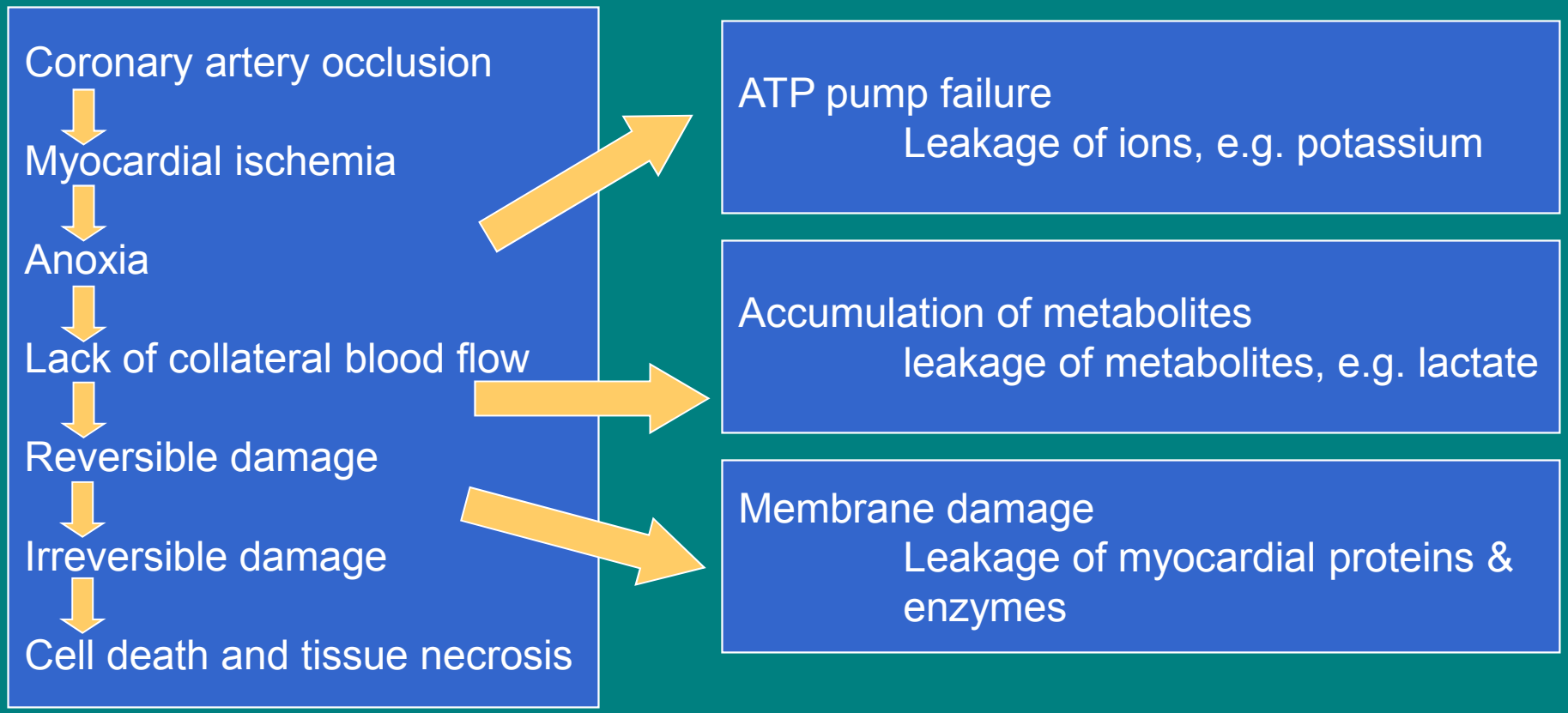
- Triad of criteria
- Diagnosis requires Two of:
  - Severe & prolonged chest pain
  - Unequivocal ECG changes consistent with acute MI
  - Elevated serum cardiac **enzymes**

PATHOPHYSIOLOGY OF MYOCARDIAL INFARCTION

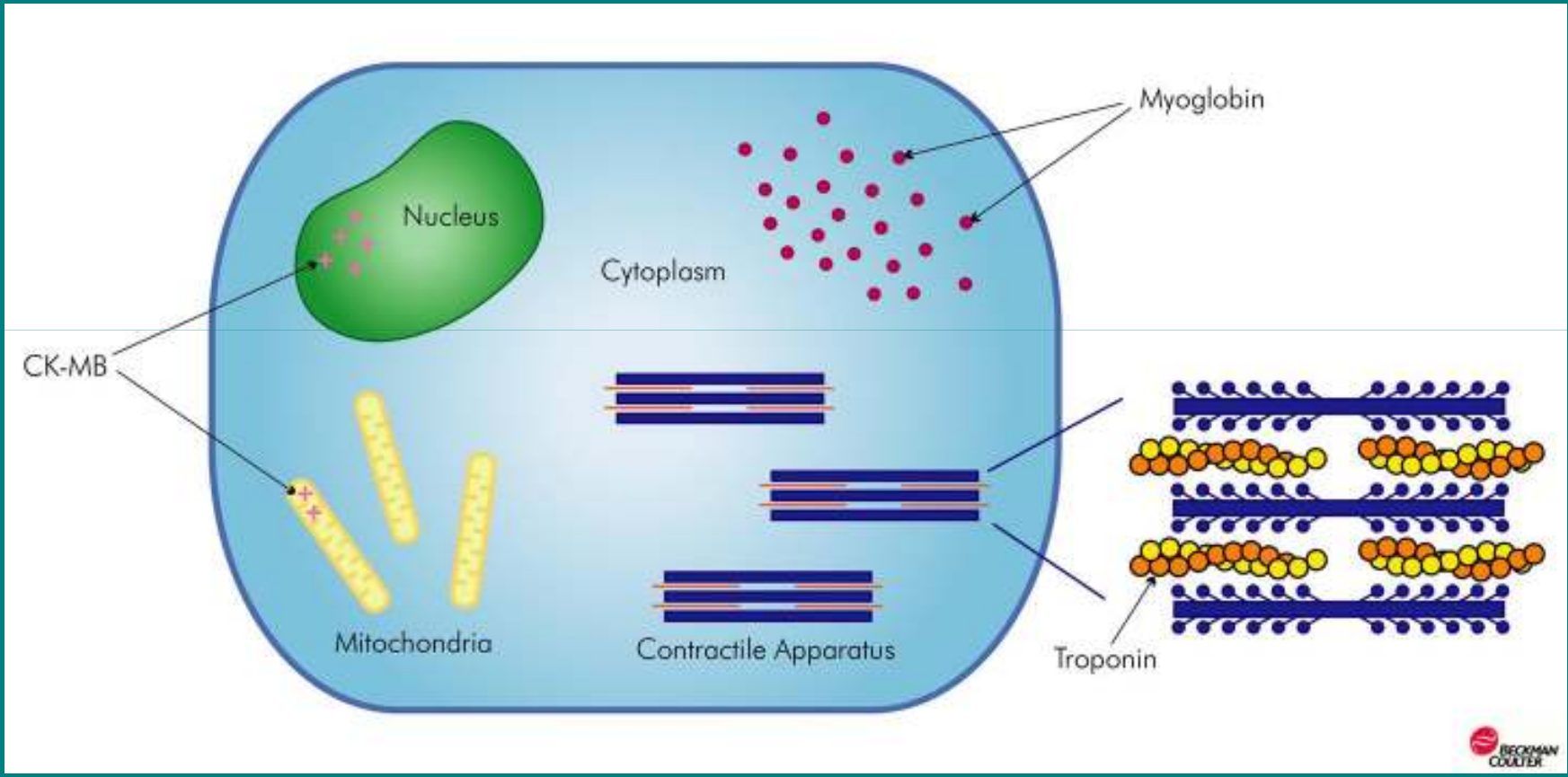


- Acute coronary syndromes are due to an acute or sub acute primary reduction of myocardial oxygen supply provoked by disruption of an atherosclerotic plaque associated with inflammation, thrombosis, vasoconstriction and microembolization.
- Finite process. (>4-6 h for necrosis to develop).

PATHOPHYSIOLOGY OF ACUTE CORONARY SYNDROMES & BIOMARKER RELEASE INTO THE CIRCULATION

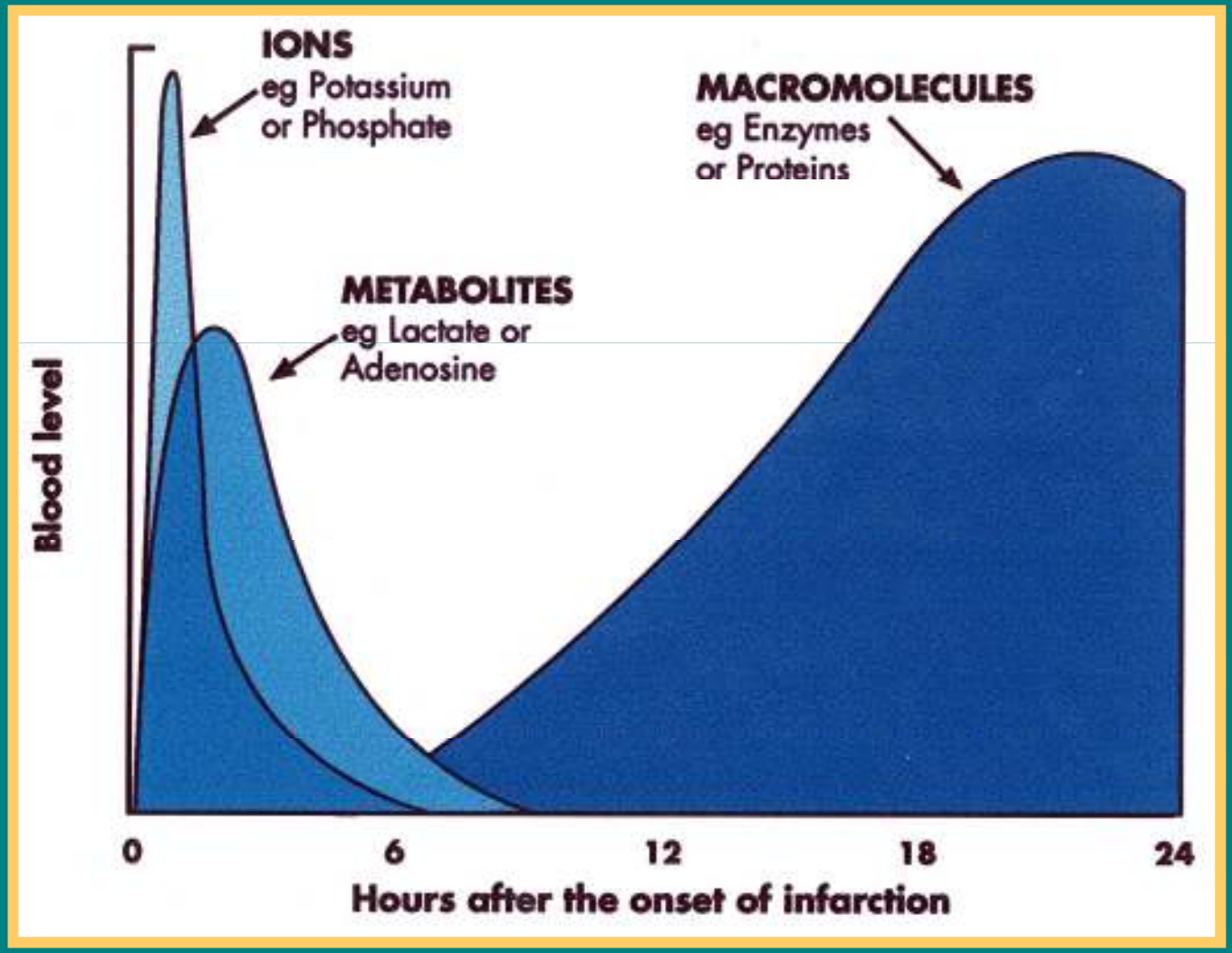


CARDIAC MUSCLE CELL



Size and subcellular distribution of myocardial proteins determines time course of biomarker appearance in the general circulation

RELEASE KINETICS OF MYOCARDIAL CELL CONSTITUENTS

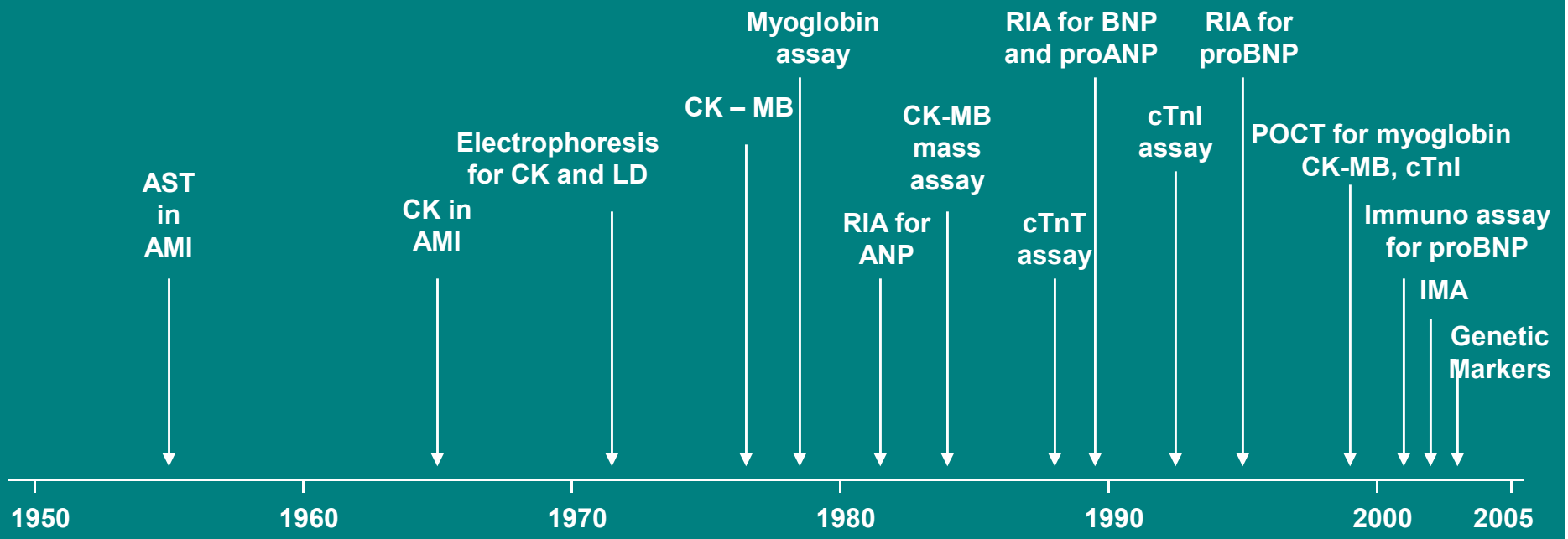


BIOCHEMICAL CARDIAC MARKERS

WHAT ARE CARDIAC MARKERS?

- Located in the myocardium
- Released in cardiac injury
  - Myocardial infarction
  - Non-Q-wave infarction
  - Unstable angina pectoris
  - Other conditions affecting cardiac muscle (trauma, cardiac surgery, myocarditis etc.)
- Can be measured in blood samples

TIME LINE OF MARKERS OF MYOCARDIAC DAMAGE & FUNCTION



Timeline history of assay methods for markers of cardiac tissue damage and myocardial function.

AST: aspartate aminotransferase

CK: creatine kinase

LD: lactate dehyydrogenase

cTn: cardiac-specific troponin

ANP: atrial natriuretic peptide

BNP: brain natriuretic peptide

POCT: point-of-care testing

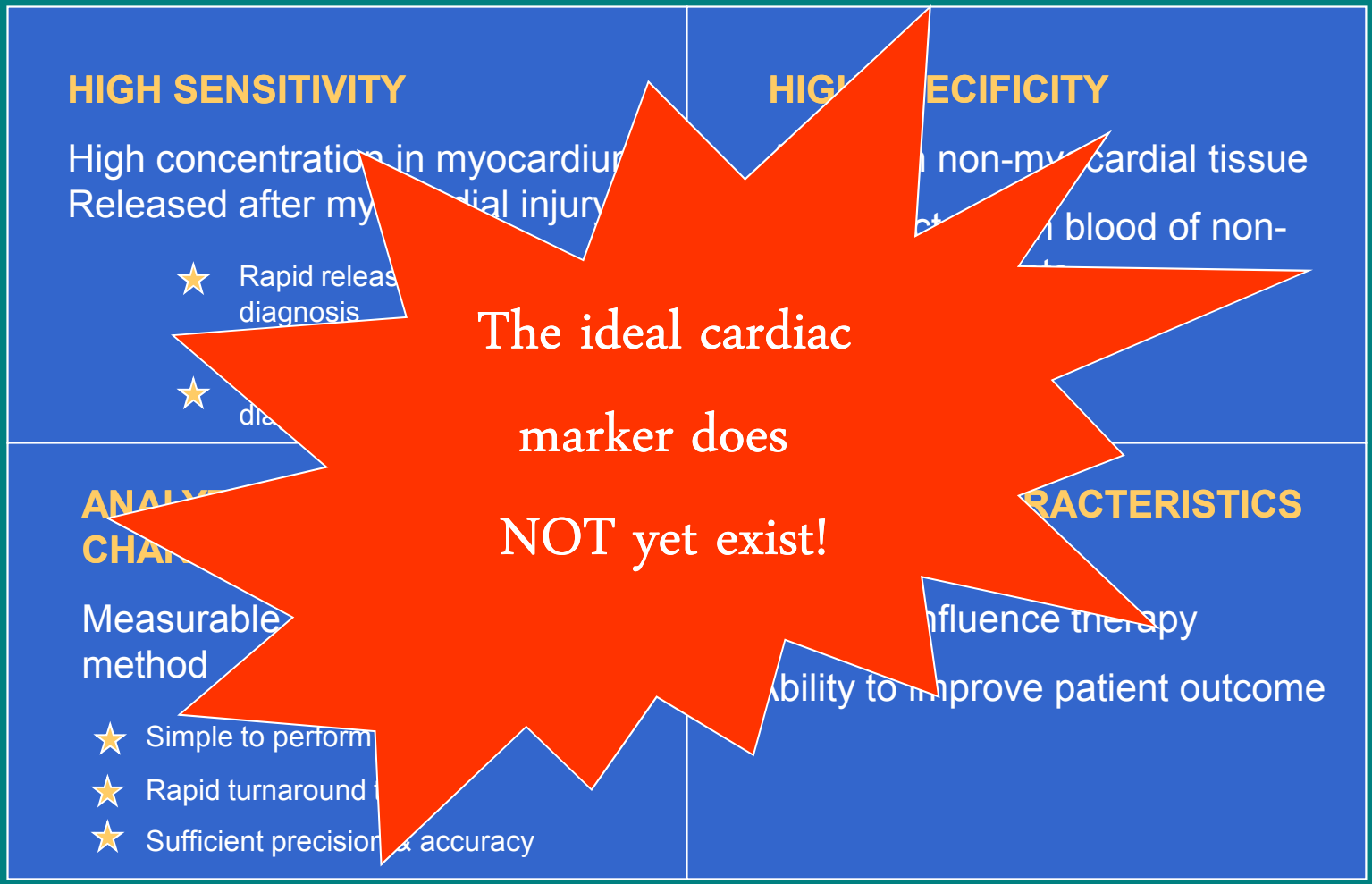
IMA: ischaemia-modified albumin

QUESTIONS ANSWERED BY CARDIAC MARKERS

- Rule in/out an acute MI
- Confirm an old MI (several days)
- Monitor the success of thrombolytic therapy
- Risk stratification of patients with unstable angina pectoris

N.B. Risk stratification in apparently healthy persons is **not** done with cardiac markers, but by measurement and assessment of cardiac risk factors

THE IDEAL CARDIAC MARKER



BIOCHEMICAL MARKERS IN MYOCARDIAL ISCHAEMIA / NECROSIS

IN:

- CK-MB (mass)
- c.Troponins (I or T)
- Myoglobin

OUT:

- AST activity
- LDH activity
- LDH isoenzymes

FUTURE:

- Ischaemia Modified Albumin
- Glycogen Phosphorylase BB
- Fatty Acid binding Protein
- CK-MB activity
- CK-Isoenzymes
- ?CK-Total

“CARDIAC ENZYMES”

are

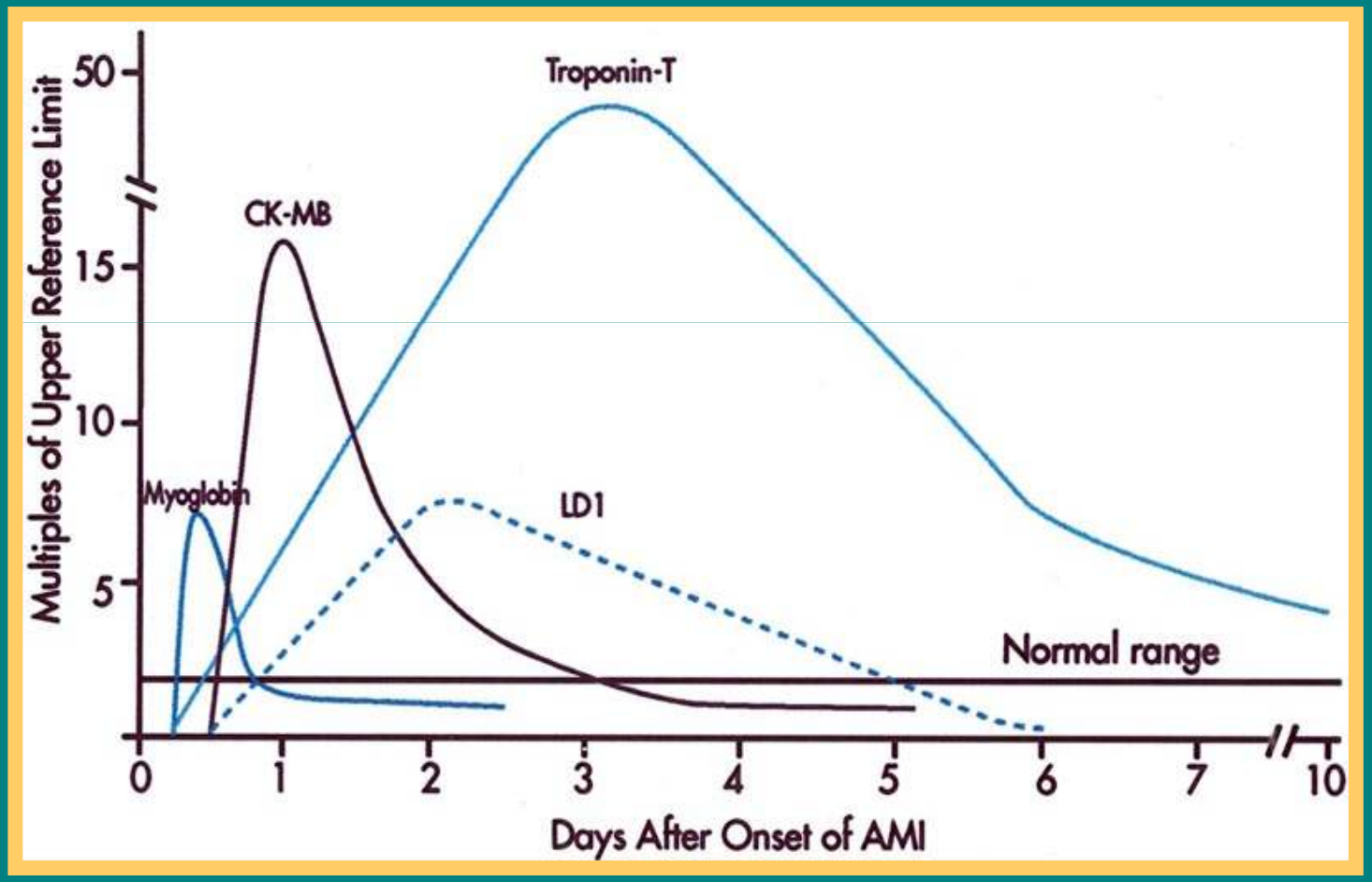
Obsolete!

KINETICS OF CARDIAC MARKERS AFTER AMI

MARKER	DETECTION	PEAK	DISAPPEARANCE
Myoglobin	1 – 4 h	6 – 7 h	24 h
CK-MB mass	3 – 12 h	12 – 18 h	2 – 3 days
Total CK	4 – 8 h	12 – 30 h	3 – 4 days
cTnT	4 – 12 h	12 – 48 h	5 – 15 days
cTnI	4 – 12 h	12 – 24 h	5 – 7 days
IMA (ischaemia)	**few minutes**	**2 – 4 h**	**6 hours**

These values represent averages.

BIOCHEMICAL MARKERS IN AMI:
RELEASE, PEAK AND DURATION OF ELEVATION



CREATINE KINASE

NORMAL VALUES:

Vary according to –

- age
- sex
- race
- physical condition
- muscle mass

PATHOLOGICAL INCREASES:

- Myocardial infarction or injury
- Skeletal muscle injury or disease
- Hypothyroidism
- IM injections
- Generalised convulsions
- Cerebral injury
- Malignant hyperpyrexia
- Prolonged hypothermia

CREATINE KINASE: CK-MB

- CK-MB is the most cardiac-specific CK isoenzyme
- Proportion of CK-MB varies in skeletal & cardiac muscle
- In normal population CK-MB < 6% Tot CK
- Sensitive marker with rapid rise & fall
- More specific than Tot CK but has limitations
- "Gold standard" biochemical marker for ~ 2 decades
- "There is no place for measurement of CK-MB by electrophoretic or immunoinhibition methods in the 21st century laboratory"

  Only CK-MB$_{mass}$ should be measured

$CK\text{-}MB_{mass}$ RELATIVE INDEX (%RI)

% RI = ($CK\text{-}MB_{mass}$ / Tot CK activity) x 100

- Increased RI suggests myocardial origin
- Not absolute – lack of $CK\text{-}MB_{mass}$ assay standardisation and tissue variability
- RI > 3 – 6 % with Tot CK activity elevated (preferably > 2x URR limit) suggests myocardial necrosis

NEW GENERATION CARDIAC MARKERS

- **Myoglobin**
  - Currently earliest marker
  - Like total CK it is by no means cardio-specific

Sensitivity

- **Troponins**
  - Kinetics comparable with total CK and CK-MB
  - Cardio-specific

Specificity

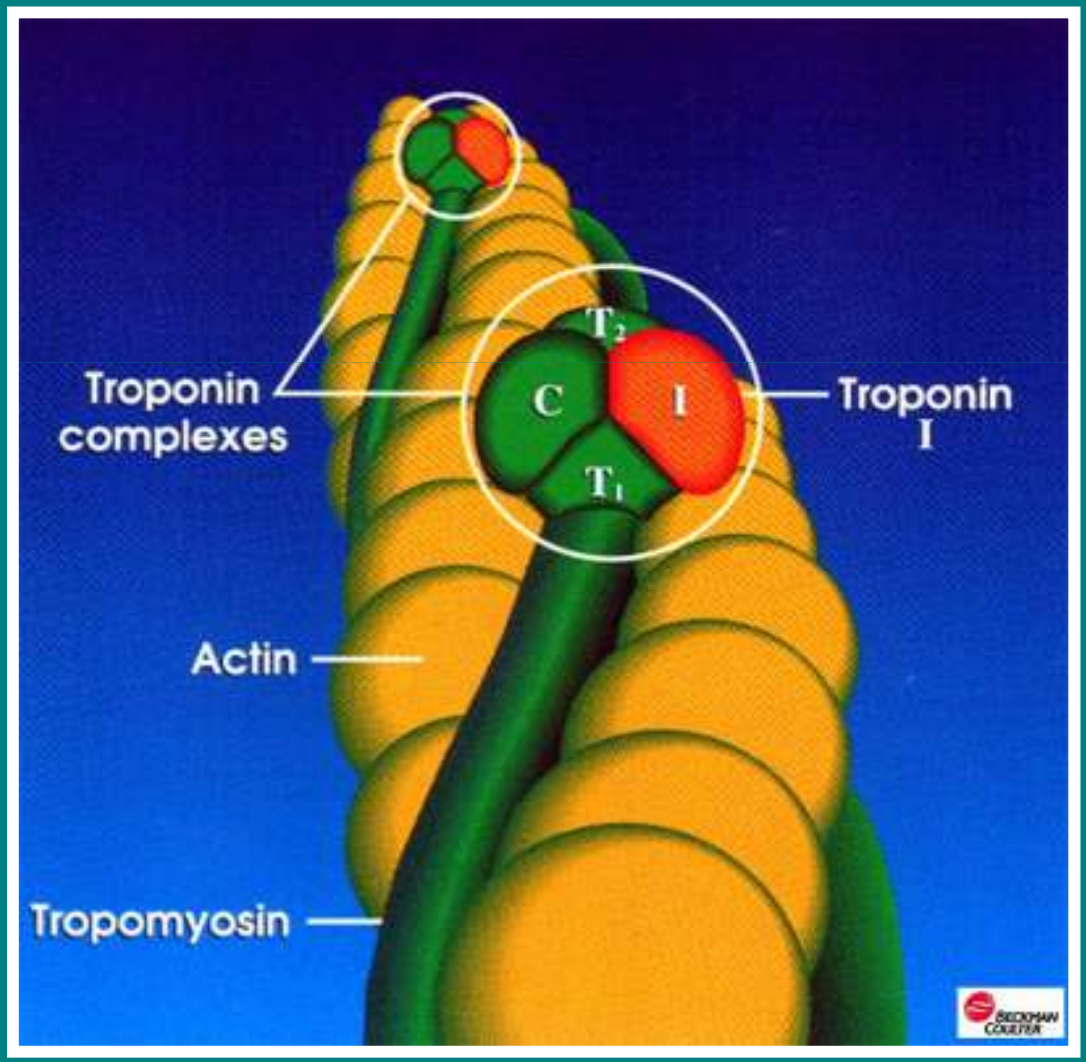
MYOGLOBIN (Mb)

- Low MW protein
- Skeletal & cardiac muscle Mb identical
- Serum levels increase within 2h of muscle damage
- Peak at 6 – 9h
- Normal by 24 – 36h
- Excellent NEGATIVE predictor of myocardial injury
  - 2 samples 2 – 4 hours apart with no rise in levels virtually excludes AMI
- Rapid, quantitative serum immunoassays

CARDIAC TROPONINS

- Striated and cardiac muscle filaments consist of:
  - Actin
  - Myosin
  - Troponin regulatory complex
- Troponin consists of 3 sub-units TnC, TnT & TnI
  - TnT MW = 37 000
  - TnI MW = 24 000
- A fraction of total troponin is found free dissolved in the cytosol
- TnT & TnI sub-units of skeletal & myocardial troponin are sufficiently different for antisera to differentiate between two tissue forms

THE TROPONIN REGULATORY COMPLEX



TROPONIN SUMMARY

- Regulatory complex of striated muscle contraction
- Early release ex cytosolic pool
- Prolonged release due degradation of myofilaments
- Distinct skeletal & myocardial muscle forms
- High specificity for myocardial injury
- Sensitive to minor myocardial damage

NATIONAL ACADEMY OF CLINICAL BIOCHEMISTRY (NACB) RECOMMENDATIONS FOR CARDIAC MARKERS IN CAD (1999)

- Rule in/out of AMI *cannot* be made on the basis of data from a *single* blood sample

  Serial determinations recommended

- Use of two markers:
  - Early marker (rising 2-4hr after pain onset)

    Myoglobin

  - Definitive marker (rising 4-6hr after pain onset)
    - High sensitivity and specificity
    - Remains abnormal several days

    cardiac Troponins

NACB RECOMMENDED SAMPLING FREQUENCY

MARKER	ADMISSION	2 – 4 h	6 – 9 h	12 – 24 h
EARLY				
Myoglobin (< 6 hrs)	X	X	X	
LATE				
Troponin (> 6 hrs)	X	X	X	X

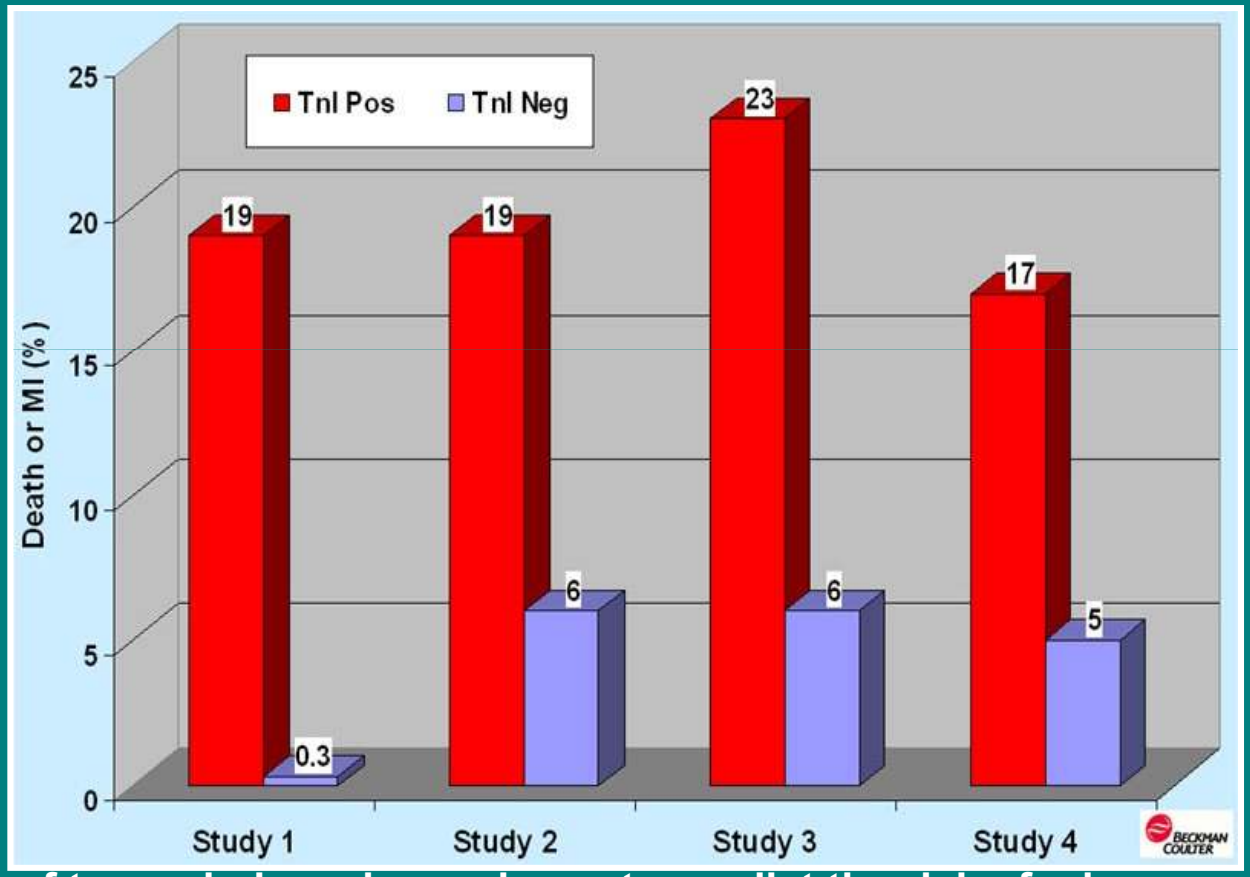
BIOCHEMICAL MARKERS IN ACS UNSTABLE ANGINA PECTORIS (UA)

- Characterised by chest pain at rest
- ? Caused by disruption of liquid-filled atherosclerotic plaque with platelet aggregation & thrombus formation
- Variable degree of ischaemia resulting in reversible or irreversible injury
- Non-occlusive plaques may produce sufficient ischaemia for release of low molecular weight markers
- cTnI & cTnT are often elevated in patients with unstable angina pectoris without additional clinical signs (ECG) or classical laboratory signs of acute MI (elevated CK-MB)
- These patients have a very high risk of cardiac events

BIOCHEMICAL MARKERS IN ACS
RISK STRATIFICATION IN UA

- Several studies have investigated the role of TnT/I in risk stratification of unstable angina (UA)
- Of importance is that UA patients with elevated Tn showed same incidence of cardiac death or AMI at 6 months as did patients with pre-existing AMI ($\pm$ 15%)
- Risk of AMI in UA patients with normal Tn was $\pm$ 4 %.
- Angina – a spectrum of disease rather than a single entity?
- Irreversible minor myocardial injury detected by TnT/I may stratify UA patients as high risk for progression to AMI

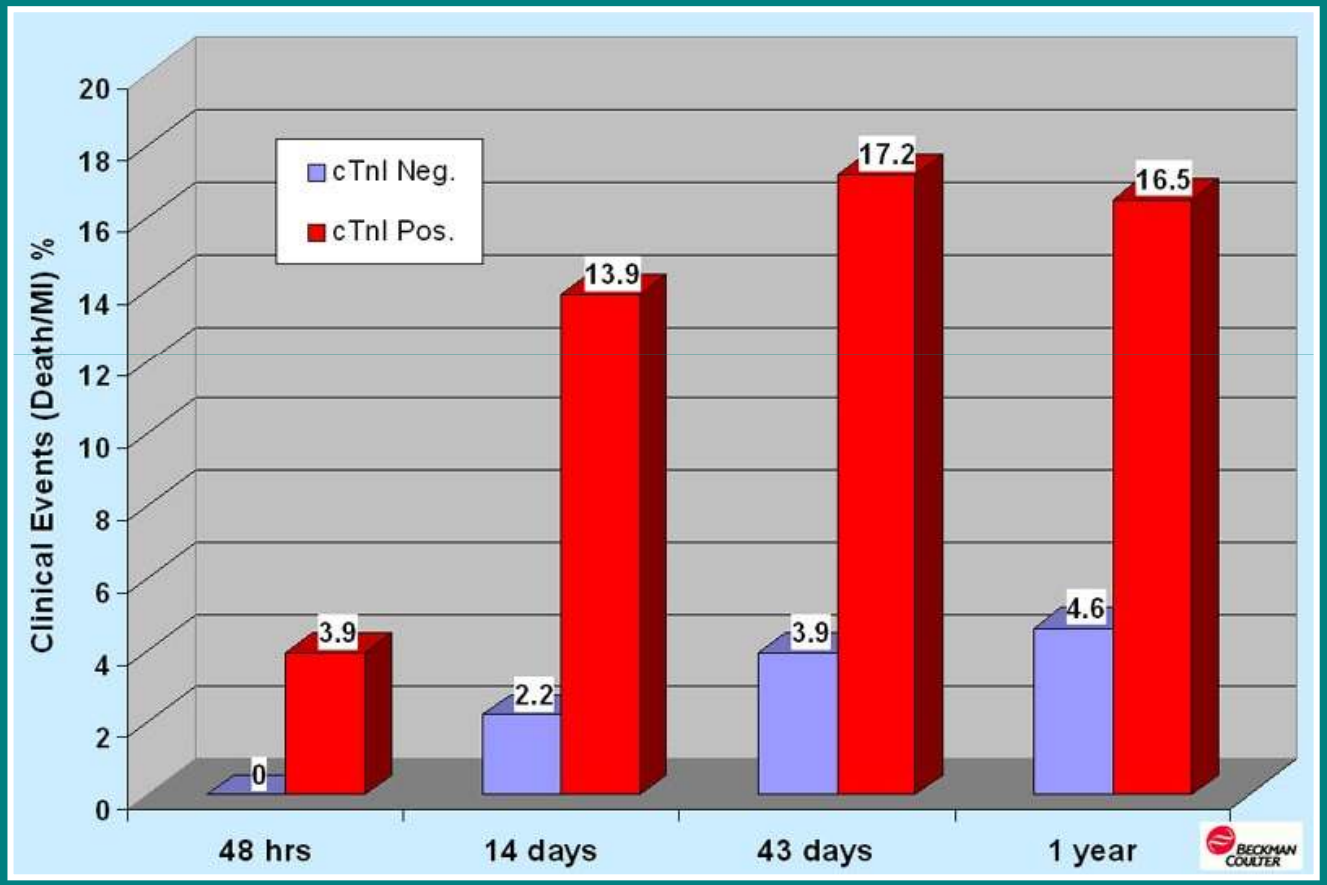
INCIDENCE OF DEATH OR MI IN ACS PATIENTS



Baseline levels of troponin have been shown to predict the risk of adverse cardiac events in patients with non-ST elevation ACS

From: NEJM 1997;337:1648 (Study 1);JACC 1998;32:8 (Study 2); Circulation 1997;95:2053 (Study 3); Am J Cardiol 2002;89:1035 (Study 4).

CLINICAL OUTCOME AT DIFFERENT FOLLOW-UP PERIODS



The prognostic information of an elevated cTnI upon presentation is maintained over time.

From: JACC 2000;36:1812 and Am J Cardiol 2002;89:1035

CARDIAC TROPONINS IN UNSTABLE ANGINA PECTORIS (UA)

QUESTION:

- Does an elevated Troponin level in the absence of other signs reflect irreversible myocardial damage?

  - Epidemiological studies
  - Animal experiments
  - Clinical trials
  - Sensitive imaging techniques

Say YES!

MI must be REDEFINED!

REVISED DEFINITION OF MI

- 2000 – Consensus Document of Joint European Society of Cardiology & American College of Cardiologists Committee for the Redefinition of Myocardial Infarction

  - JACC 2000; 36 : 959 – 967
  - Eur Heart J 2000; 21 : 1502 – 1513
  - Clin Chem 2001; 47 (3) : 382 – 392

THE ESC/ACC CONSENSUS DOCUMENT: "MI REDEFINED"

- "MI is diagnosed when blood levels of sensitive and specific **biomarkers** such as **cardiac troponins** and CKMB **are increased in the clinical setting of cardiac ischaemia**"
- "ECG changes such as ST segment elevation/depression, T wave inversion reflect myocardial ischemia but are not sufficient by themselves to define MI. **The final diagnosis depends on the detection of elevated levels of cardiac biomarkers.**
- Preferred marker is a cardiac Troponin (I or T)

THE ESC/ACC CONSENSUS DOCUMENT: "MI REDEFINED"

- If Troponins are not available, best alternative is CK-MB_{mass}
- Degree of elevation of the marker is related to clinical risk
- CK(total), AST & LDH (Cardiac Enzymes) should NOT be used!
- Combine early (myoglobin) & late (Troponins) markers
- Serial testing: admission, 6 – 9 h, 12 – 24 h
- Acceptable imprecision (CV) at the 99^{th} percentile for a Troponin assay defined as $\leq$ 10 %
- An elevated Troponin level in the absence of clinical evidence of ischaemia should prompt searching for other causes of cardiac damage

ESC/ACC MI REDEFINED

Revised Criteria: Acute/Evolving/ Recent MI

- Typical myocardial necrosis-associated rise & fall of Troponin or CK-MB_{mass}

PLUS

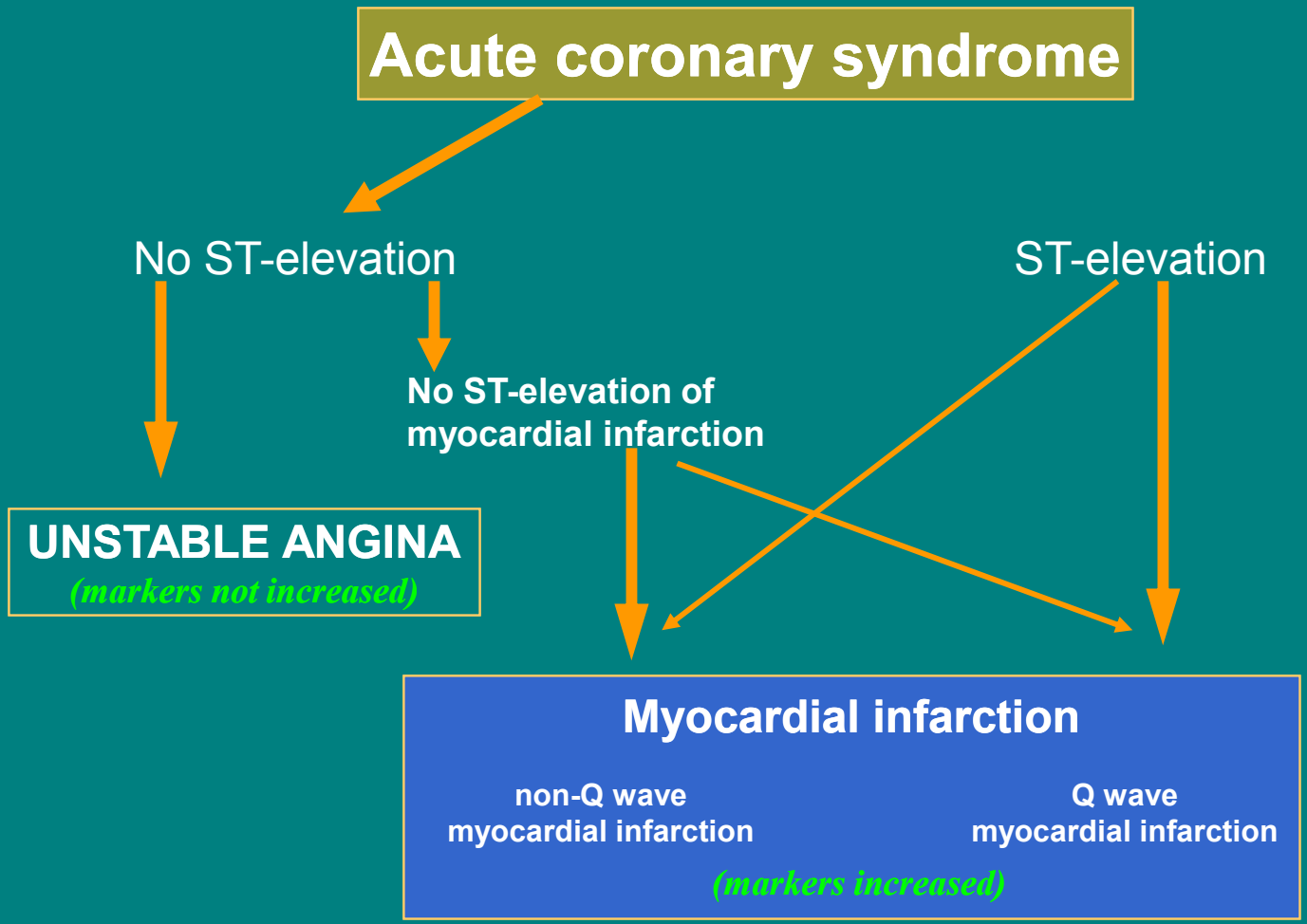
- One of:
  - Cardiac Ischaemia symptoms
  - Q waves on ECG
  - ST segment changes indicative of ischaemia
  - Coronary artery imaging (stenosis/obstruction)

OR Pathologic findings of an acute MI

NON-ISCHAEMIC CARDIAC INJURY:
CAUSES OF ELEVATED CARDIAC TROPONINS

- Congestive heart failure
- Hypertension with left ventricular hypertrophy
- Hemodynamic compromise, e.g. shock
- Right ventricular injury resulting from pulmonary embolism
- Myocarditis
- Cardiac trauma
- Mechanical injury (e.g. defibrillation)
- Myocardial toxins (e.g. 5-flurouracil)
- Elevated cTnI or cTnT in patients with end stage renal failure is associated with increased risk of cardiac death

ROLE OF CARDIAC MARKERS IN EVALUATION OF ACUTE CORONARY SYNDROMES:

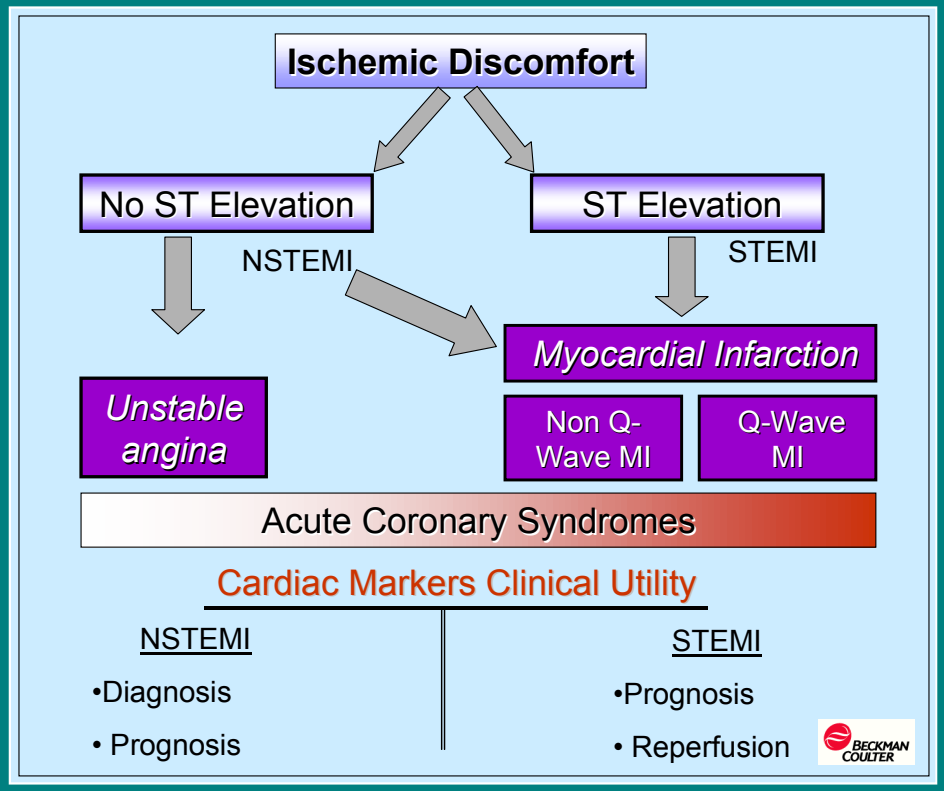


ROLE OF CARDIAC MARKERS IN EVALUATION OF ACUTE CORONARY SYNDROMES:

- Key role of cTnI & cTnT in MI diagnosis
- Upper reference limit (URL) at 99th percentile
- Any Troponin level > URL = Myocardial damage

  This identifies a new sub-group of high-risk, poor prognosis ACS patients
- Reason for myocardial injury needs to be determined in patients without clinical cardiac ischaemia
- ACS patients with even small elevations in cTroponin derive clinical benefit from early follow-up and appropriate therapy

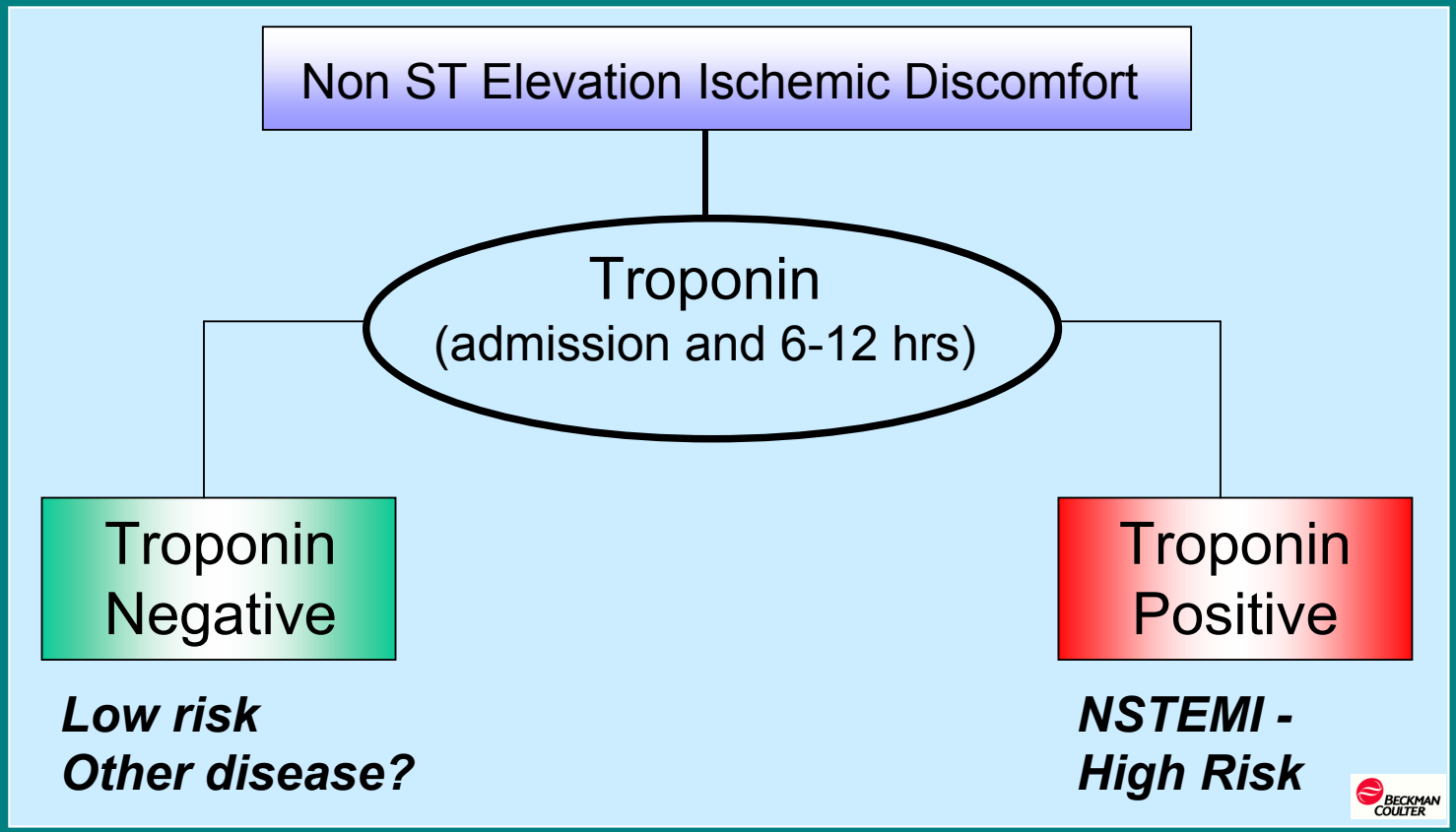
TROPONIN AND MI DIAGNOSIS



"It is estimated that about 30% of patients who present with chest pain without ST-segment elevation and would otherwise be diagnosed as having unstable angina because of a lack of CK-MB elevation actually have NSTEMI when assessed with cardiac-specific troponin assays"

From:JACC and Circulation 2002

PREDICTION OF RISK/PROGNOSIS



Troponin can be used to efficiently categorise patients into **high and low risk** groups for appropriate management pathways.

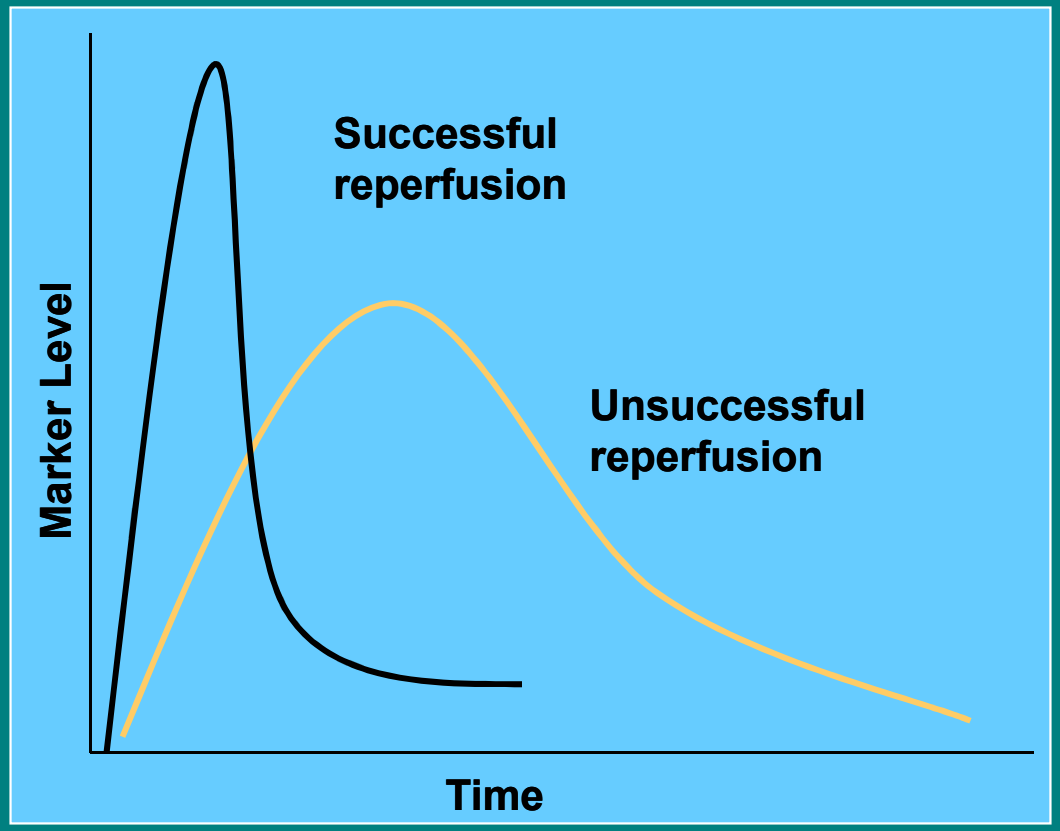
RISK STRATIFICATION IN ACS

- **Useful for:**
  - Selection of the site of care
    - Coronary care unit versus monitored step-down unit or outpatient setting
  - Selection of most appropriate therapeutic intervention
    - Aggressive versus conservative therapy

BIOCHEMICAL MARKERS IN ACS CLINICAL DECISION POINTS

- Unstable Angina
- AMI
- Infarct size
- Prognosis
- Thrombolysis and Reperfusion
- Peri-operative infarcts
- Coronary surgery complications
- Transplant rejection

BIOCHEMICAL MARKERS IN AMI
ASSESSMENT OF REPERFUSION



- "Washout" phenomenon – enzymes & proteins have direct vascular access when occluded coronary circulation becomes patent
- Peak concentrations earlier & at higher levels if reperfusion successful

Due to short plasma half life ($t_{½}$ = 10 min) Myoglobin is considered the best re-perfusion marker

BIOCHEMICAL MARKERS IN ACS CURRENT RECOMMENDATIONS

AMI – Routine diagnosis	Troponins (CK-MB_{mass})
Retrospective diagnosis	Troponins
Skeletal muscle pathology	Troponins
Reinfarction	Mb, CK-MB_{mass}
Reperfusion	Mb, Tn, CK-Mb_{mass}
Infarct size	Troponins
Risk stratification in UA	Troponins

ISCHAEMIA-MODIFIED ALBUMIN (IMA)

- Serum albumin is altered by free radicals released from ischaemic tissue
- Angioplasty studies show that albumin is modified within minutes of the onset of ischaemia.
- IMA levels rise rapidly, remain elevated for 2-4 h + return to baseline within 6h
- Clinically may detect reversible myocardial ischaemic damage
- Not specific (elevated in stroke, some neoplasms, hepatic cirrhosis, end-stage renal disease)
- Thus potential value is as a **negative predictor**
- Spectrophotometric assay for IMA adapted for automated clinical chemistry analysers
- FDA approved as a rule-out marker in low risk ACS patients (2003)

BIOCHEMICAL MARKERS IN ACS

OTHER MARKERS CURRENTLY UNDER INVESTIGATION

- Free fatty acids
- Fibrin peptide A
- Fatty acid binding protein
- Glycogen phosphorylase BB

BIOCHEMICAL MARKERS OF MYOCARDIAL FUNCTION

CARDIAC NATRIURETIC PEPTIDES:

(ANP, BNP & pro-peptide forms)

- Family of peptides secreted by cardiac atria (+ ventricles) with potent diuretic, natriuretic & vascular smooth muscle relaxing activity
- Levels of these neuro-hormonal factors can be measured in blood
- Clinical usefulness (especially BNP/N-terminal pro-BNP)
  - Detection of LV dysfunction
  - Screening for heart disease
  - Differential diagnosis of dyspnea
  - Stratification of CCF patients

SOME COMMON DISEASES IN WHICH PLASMA CARDIAC NATRIURETIC PEPTIDES HAVE BEEN FOUND TO BE ALTERED, COMPARED TO HEALTHY SUBJECTS

	DISEASES	ANP/BNP LEVELS
a)	**Cardiac diseases**	
	Heart failure	Greatly increased
	AMI (first 2 – 3 days)	Greatly increased
	Essential hypertension with CMP	Increased
b)	**Pulmonary diseases**	
	Acute dyspnea	Increased
	Obstructive pulmonary disease	Increased
c)	**Endocrine & metabolic diseases**	
	Hyperthyroidism	Increased
	Hypothyroidism	Decreased
	Cushing's syndrome	Increased
	Primary aldosteronism	Increased
	Addison's disease	Normal or increased
	Diabetes mellitus	Normal or increased
d)	**Liver cirrhosis with ascites**	Increased
e)	**Renal failure (acute or chronic)**	Greatly increased

AMI = acute myocardial infarction; CMP = cardiomyopathy with left ventricular hypertrophy

CARDIOVASCULAR RISK FACTORS

ESTABLISHED RISK FACTORS	EVIDENCE
Raised serum low density lipoprotein cholesterol	++
Decreased serum high density lipoprotein cholesterol	++
Smoking	++
High Blood pressure	++
Increased plasma glucose concentrations	+
Physical inactivity	+
Obesity	+
Advanced age	+
EMERGING RISK FACTORS	
Inflammatory Markers	
Sensitive C-reactive protein	+
Interleukins	+
Serum amyloid A	+
Pregnancy-associated plasma protein A	?
Chronic infection (*Chlamydia pneumoniae, Helicobacter pylori, etc)*	*?*
Procoagulant Markers	
Plasma Homocysteine	+
Tissue plasminogen activator	+
Plasminogen activator inhibitor	+
Lipoprotein A	+
Process Markers	
Fibrinogen	+
D-dimer	?
Coronary artery calcification	?

++ Clear evidence, and modification of the risk factor decreases the risk of cardiovascular disease

+ Clear evidence, but less clear whether modification of the risk factor decreases the risk of cardiovascular disease

? Risk factor under scrutiny

GENETIC ANALYSIS OF CANDIDATE GENES OR RISK FACTORS FOR CARDIOVASCULAR DISEASE

- Recent explosion of genetic analysis & micro-array technology
- Common cardiovascular diseases are polygenic. Multiple susceptibility loci interact with lifestyle & environment
- Single gene defects may account for some of the cardiomyopathies, inherited cardiac arrhythmias
- Possible genetic cardiovascular risk factors under assessment
- Technology is still complex & expensive but is developing very rapidly

LABORATORY CONSIDERATIONS IN THE CHOICE OF CARDIAC MARKERS

- Instrumentation should allow rapid & reliable measurement of Troponin, Myoglobin & CK-MB_{mass}
- Good Troponin tests should be heparinate (plasma) compatible. Plasma specimens preferred for cardiac markers to improve turn-around time of results
- Choice of Troponin cut-off level:
  - For our TnT assay we use a cut-off 0.1 ng/mL
- To achieve comparability with the less sensitive CK-MB method, a TnI cut-off of 0.4 ng/mL would have to be used

GUIDELINES: USE OF CARDIAC MARKERS IN PATIENTS WITH CHEST PAIN

GUIDELINES: USE OF CARDIAC MARKERS IN PATIENTS WITH CHEST PAIN

- Serial sampling is critical for accurate diagnosis
- Do NOT discharge patients on the basis of negative results on a single (admission) specimen
- If onset of chest pain >9-12 h before admission only Troponin is necessary
- CK-MB_{mass} is most useful in assessing a recent vs an older MI or to confirm reinfarction (occurs in 17% of AMI's). Repeat CK-MB_{mass} if chest pain recurs in AMI patients
- Use Heparin tube (plasma) specimens to improve cardiac marker TAT

GUIDELINES: USE OF CARDIAC MARKERS IN PATIENTS WITH CHEST PAIN

- **Mb, CK-MB$_{mass}$, Troponin** **POSITIVE**
  - **AMI**
- **Mb ONLY** **POSITIVE**
  - **Possible early infarction or skeletal muscle injury**
  - Repeat markers
  - (NB importance of Mb is as a **Negative Predictor**)
- **Mb + CK-MB** **POSITIVE**
  - **Probable early infarction**
  - Repeat markers
  - A rising CK-MB or increased CK-MB$_{mass}$ RI → AMI

GUIDELINES: USE OF CARDIAC MARKERS IN PATIENTS WITH CHEST PAIN

- **TnI ≤ 0.06 ng/mL OR TnT ≤ 0.03 ng/mL on two specimens > 6 hours apart**
  - **Unstable Angina**

- **Troponin I > 0.06 OR TnT > 0.1 ng/mL**

  (TnT levels > 0.03 and ≤ 0.1 ng/mL are equivocal and should be repeated)
  - **? High risk ACS(AMI) or non-ischaemic myocardial damage** depending on clinical cardiac ischaemia
  - These patients require follow-up!!

- **Troponin I > 0.4 ng/mL**
  - **"traditional" AMI**

NON-ISCHAEMIC CAUSES OF CARDIAC TROPONIN ELEVATION

- Myocarditis / Pericarditis
- Heart failure (including acute pulmonary oedema)
- Hypertension
- Hypotension (especially if associated with cardiac arrhythmias)
- Critically-ill patients (NB diabetics)
- Hypothyroidism
- Cardiac trauma
- Chemotherapy-induced myocardial toxicity
- Heart transplant rejection

GUIDELINES: USE OF CARDIAC MARKERS IN PATIENTS WITH CHEST PAIN

FOR ASSESSMENT OF:

Reperfusion	Mb, CK-MB_{mass}
Intra- or post-operative AMI	Troponin
MI after percutaneous coronary artery intervention	Troponin (↑ in 30 - 40 % patients) CK-MB (↑ in 5 - 30 % patients) (compare with baseline or use 5-15 fold higher cut-off level)
Reinfarction	Serial CK-MB_{mass} determinations

SUMMARY

- “Cardiac Enzymes” are obsolete
- Medical & laboratory progress has required a redefinition of Myocardial Infarction
- Cardiac Troponins & Myoglobin now play a pivotal role in the diagnosis of AMI
- Cardiac Troponins play an important role in the risk stratification of ACS patients
- Elevated Troponin levels in patients without ECG changes & with normal CK-MB levels may identify patients at increased risk of cardiac events

SUMMARY

- Elevated Troponins in the absence of clinical signs of ischaemic heart disease require consideration of other causes of cardiac injury
- Need for rapid TAT & reliable cardiac markers
- Additional roles for cardiac markers in:
  - Reperfusion monitoring
  - Infarct size/prognosis
  - Intra/post-operative MI (non-cardiac/cardiac surgery)
- Evolving laboratory role in the evaluation of cardiac disease particularly in the areas of cardiac dysfunction & general biochemical or genetic risk factors

Thank You!