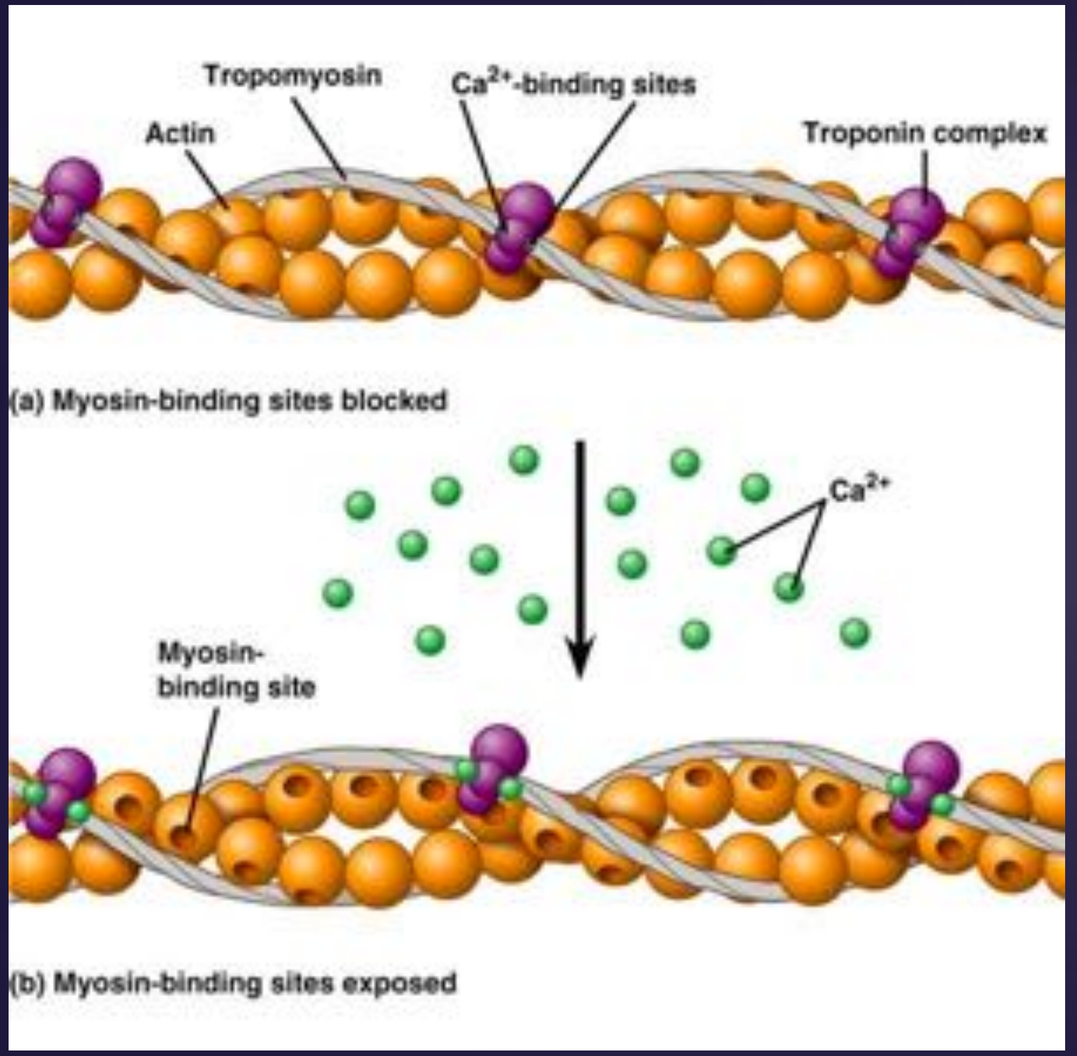
Maleficent Troponins

Charles Bruen, MD

Preview

- Structure and Function of Troponin
- Troponin Biochemistry
- Troponin Assays
- Defining Myocardial Infarction
- Troponin as diagnostic tool

cTn Structure and Function



Biochemistry: TnT

- ~40 kDa (288 aa)
- 3 isoforms
  - Cardiac, Fast & Slow twitch skeletal muscles
  - 3 pairs of genes, well consevered.
- cTnT 4 versions of post-translation modification
  - Embryonic

Biochemistry: TnT

- ~120-125 aa residue homology (56-58%)
- No cTnT found in skeletal muscle
- Human fetal exon present with complete homology for an exon in cTnT and sTnT caused initial confusion
- 94% cTnT part of contractile apparatus
  - 6% free cytosol

Cellular Release

- Infarction/necrosis — Cell death and irreversible
- ? Ischemia
- Released as
  - Whole free cTnT
  - Complexes cTnI-cTNT and cTnI-cTnT-cTnC
  - Fragments

Celular Release Mechanisms

- Apoptosis / Autophagy
- Normal myocyte turnover (40% over lifetime)
- Cellular release of proteolytic degradation products
- Increased cell wall permeability
- Formation and release of blebs

Circulation of TnT

- Half-life 120 min
- Prolonged detection
  - Continued release from myofibrillar pool as contractile apparatus within the cell undergoes degradation during necrosis

Biohemistry: cTnI

- ~24 kDa (210 aa)
- Same three isoforms
- cTnI has 40% overlap between isoforms
- 2 conformational phosphorylation sites
  - Changes protein interaction and detection Ab

Biochemistry: cTnI

- 2-8% free in cytosol
- Circulates as fragments, complexes, free
- Pure cTnI highly degraded
- Central part protected by cTnC, but cTnC competes for epitopes

Troponin Assays

- Developed in 1987 by Cummings
- Protein ELISA measurement as opposed to CK-MB enzymatic
- cTn very stable in samples at RT, 4deg, and multiple freeze thaw cycles
- Variability for serum, EDTA, heparin which is compensated for by analyzers

Detection

- Test variability mainly due to epitopes used
- Difficulties
  - Prostranslation modifications, oxidation, phosphorylation, degradation, compelxes
  - Heterophile, HAMA Ab

ELISA Detection

- Fragment antigen-binding (FAB) Mab recognize epitodes 5 residues apart in central molecule
  - Capture antibody M7: 125-131
  - Detection Ab M11.7 136-147
  - Cardiospeciic sequences with no fetal homology

Detection Epitopes

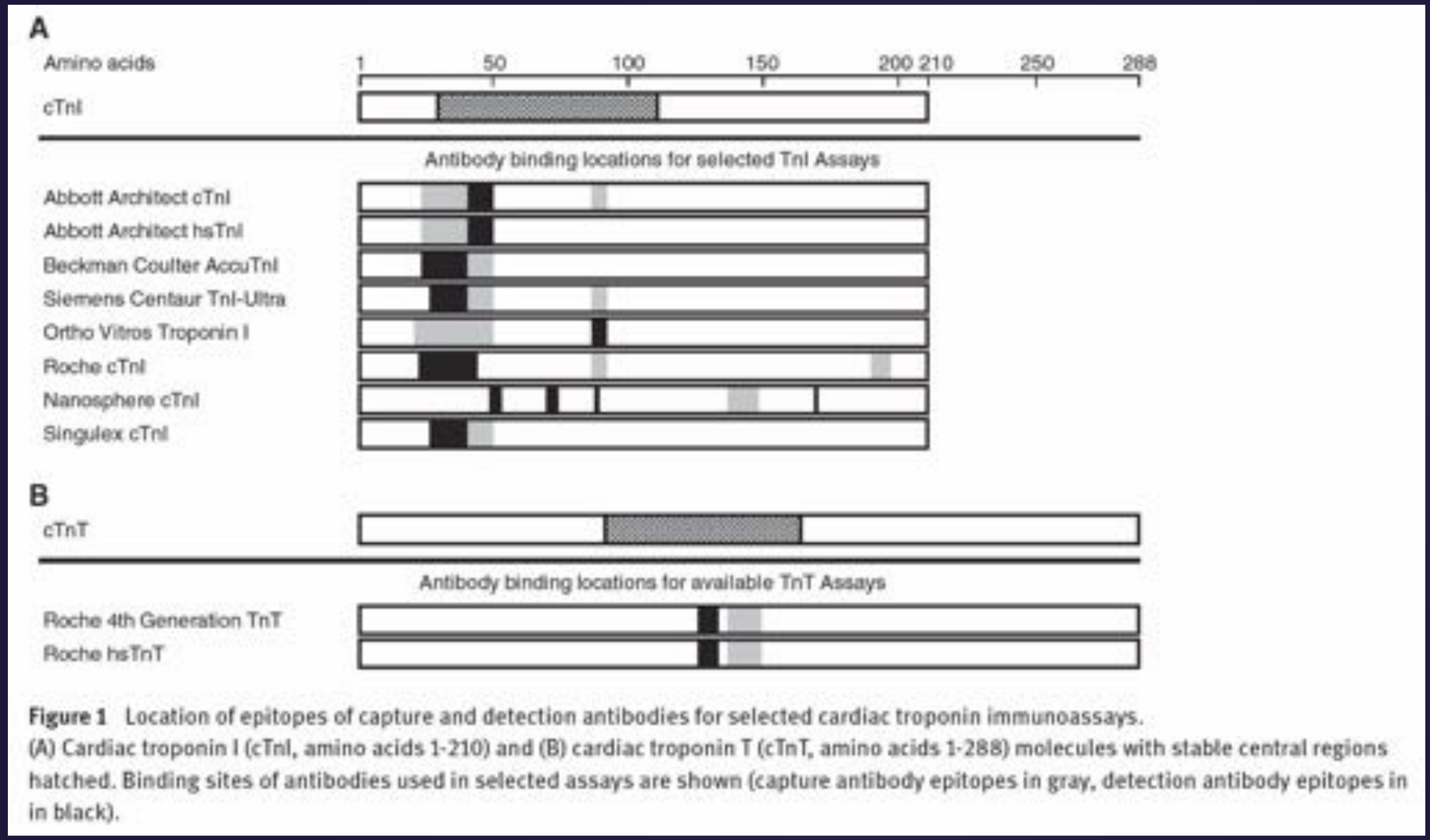


Figure 1 Location of epitopes of capture and detection antibodies for selected cardiac troponin immunoassays.
(A) Cardiac troponin I (cTnI, amino acids 1-210) and (B) cardiac troponin T (cTnT, amino acids 1-288) molecules with stable central regions hatched. Binding sites of antibodies used in selected assays are shown (capture antibody epitopes in gray, detection antibody epitopes in in black).

Jarlim 2014.

Detection Limits

- HyTest
- Have tested hundreds of Mab
- No signle Ab pair adequate
- Advocates 2+2 concept
- ?Mab directed at cTnI-cTnC complex
- Change from ELISA -> Unimolecule flow cytometry

Assay Naming

- Nomenclature is confusion
- Variations used
  - Sensitive, High-performance, Highly sensitivie, High-sensitive, Ultrasensitive, Novel highly sensitivie

What is High Sensitivity

- Sensitivity refers to analytical sensitivity
  - Characteristic of Assay
- 2 criteria
  - Measureable concentrations above LoD for 50% (preferabbly 95%) of healthy individuals
  - CV <10% at 99th percentile value
- All measurements in ng/L (Can get very high values)

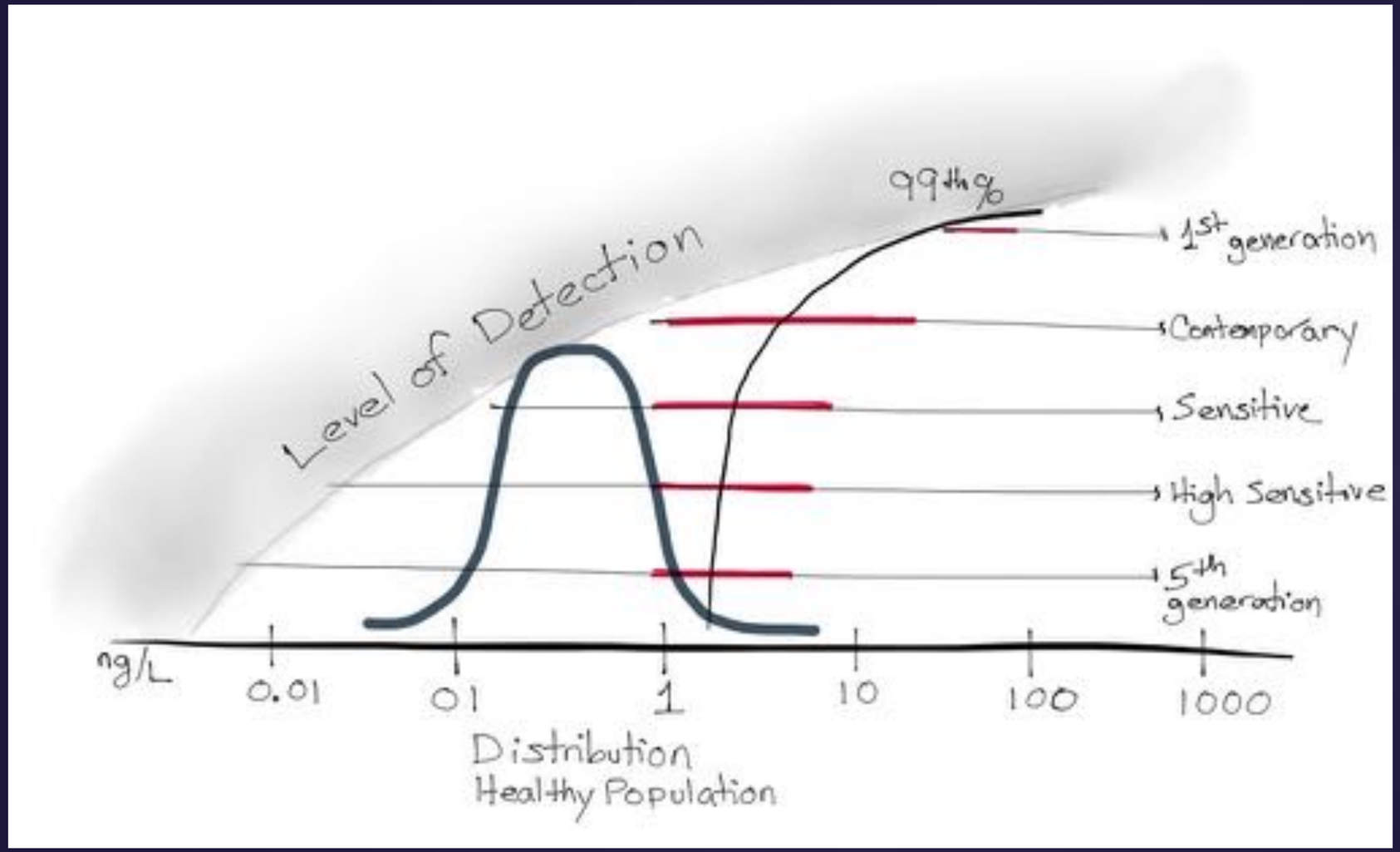
Coefficient of Variation

- Imprecision at 99th Percentile
- Assumption is that lower will help detect serial changes
- Most contemporary are ~20%
- All hs are much better (CV10% at order of magnitude less)

Assay Generations (IFCC)

	Generation	Criteria (CV / LOD)
Contemporary	2nd	
Contemporary sensitive	3rd	10%CV <99th %
High sensitive	4th	Detectable in >50%
Ultrasensitive	5th	Detectable in >95%

Assay Generations



Acheiving High Sensitivty

- M11.7 reengineered to have human IgG C1 to produce mouse-human chimeric detection Ab (limits HAMA)
- Sample volume increased from 15 to 650 uL
- Increased ruthernium concentration
- Buffer optimization to reduce background signal
- Calibrated against recombinant E.coli cTn E.coli

Specific hs-cTn Assays

Table 2. Analytical characteristics of hs cardiac troponin assays.

Company/ platform/assay	Cardiac troponin concentration at: LoD,[a] ng/L	99th Percentile, ng/L (CV)[b]	10% CV concentration, ng/L	Amino acid residues of epitopes recognized by capture (C) and detection (D) MAbs
hs-cTnI				
Abbott ARCHITECT[c]	1.2	16 (5.6%)	3.0	C: 24–40; D: 41–49
Beckman Access[c]	2–3	8.6 (10%)	8.6	C: 41–49; D: 24–40
Nanosphere MTP[c]	0.2	2.8 (9.5%)	0.5	C: 136–147; D: MAb PA1010
Singulex Erenna[c]	0.09	10.1 (9.0%)	0.88	C: 41–49; D: 27–41
Siemens Vista[c]	0.5	9 (5.0%)	3	C: 30–35; D: 41–56, 171–190
hs-cTnT				
Roche Elecsys[d]	5.0	14 (8%)	13	C: 136–147; D: 125–131

[a] LoD, limit of detection; MTP, microtiter plate.
[b] CV at the 99th percentile.
[c] Under development and not available for commercial use.
[d] Available for use worldwide but not cleared by the US Food and Drug Administration for use in the US.

Apple Clin Chem 2012;58:1.

Harmonization of Assays

- Difficult to achieve because companies use various epitopes
- Trade secrets / Competitive Advantage Limitat competition

99th Percentile

- 99th% determined by your "healthy population"
- Little consistency in manufacturer methods
- No standard

Healthy Populations

- Significant age and sex dependent
- Options
  - Apparently health
  - Age matched to hospital populations

Determining healthy

- Options
  - Interview with questions of hospitalizations, medications
  - Obtaining information on cardiovascular, renal disease, diabetes
  - Physical, ECG, echo
- Can change from 14 to 35 ng/L

HCMC Working Proposal

- Two age groups
  - <30yo and >30yo (median 60-65)
- Inclusion include history, BP, BNP
- Equal sex and mix of diverse racial/ethnic mix
- 300-500 individuals for statisical validity

Biologic Variability

- Something we have never been able to dertmine before
- cTnI: 2.2 ng/L
- cTnT 4.9 ng/L
- Differs 32-70% daily

After all that, at least, we have a great test

...well maybe

Major Trends

- Angiography
- Markers: CK-MB -> Troponin
- Pharmaceuticals
  - Lovenox, ReoPro, Integrilin, Plavix, Ticlid.
- Reperfusion -> thrombolytics, PTCA, PCI
- Pope article

Injury, Ischemia, Infarction

- Injury -- cell death marked by troponin elevation
- Ischemia -- inadequate perfusion to meet metabolic needs

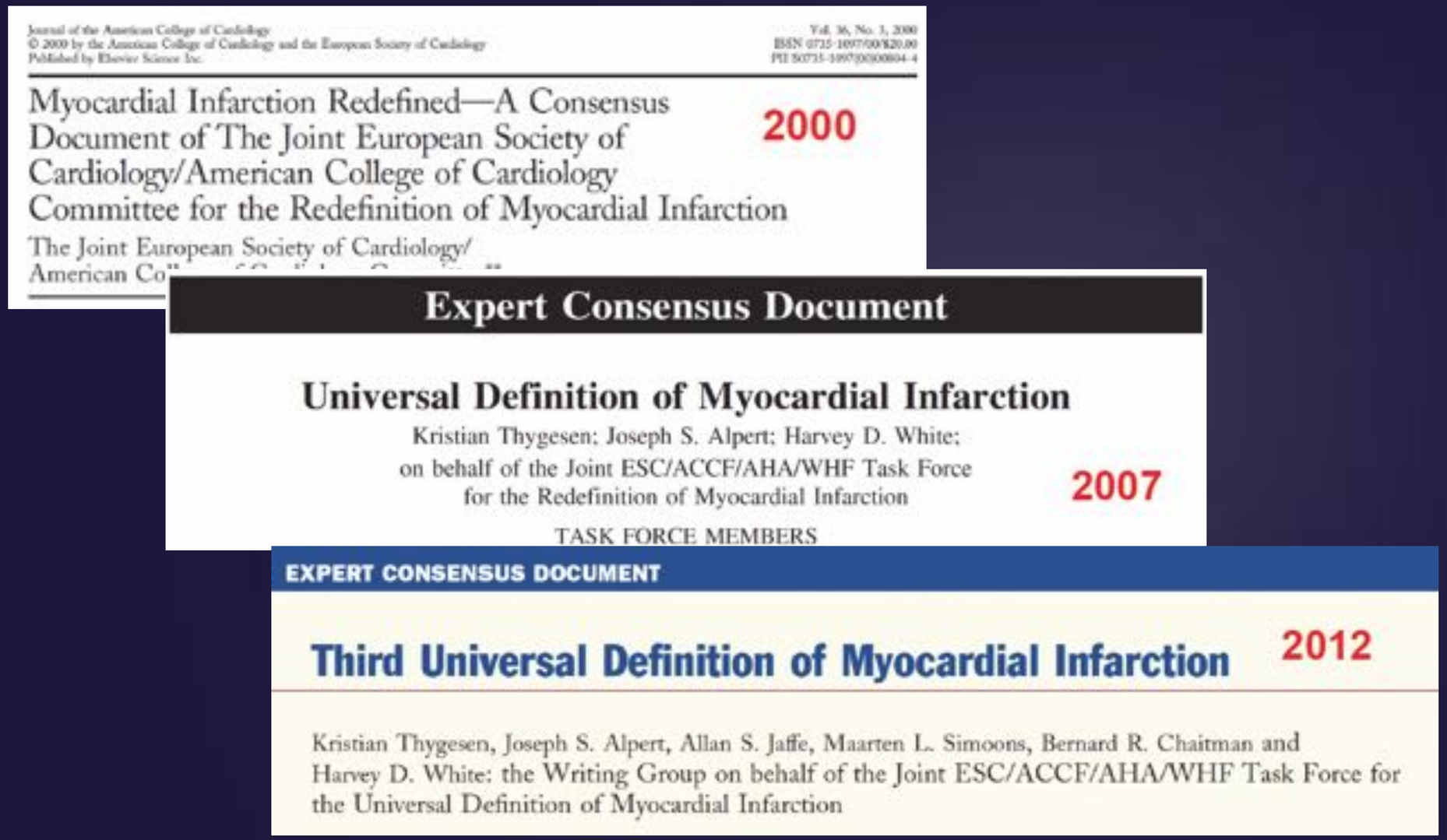
Injury, Ischemia, Infarction

- Infarction -- Cell death due to prolonged ischemia
- Ischemia ~20min
- Complete necrosis ->2-4hr depending on collateral circulation, persistent/ intermittent perfusion, perconditioning, oxygen demand

Injury, Ischemia, Infarction

- Scarring 5-6 weeks
- All are an ACUTE process, as compared to baseline elevated troponin levels

Defining MI



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Myocardial Infarction Redefined—A Consensus Document of The Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction

2000

The Joint European Society of Cardiology/
American Co

Expert Consensus Document

Universal Definition of Myocardial Infarction

Kristian Thygesen; Joseph S. Alpert; Harvey D. White;
on behalf of the Joint ESC/ACCF/AHA/WHF Task Force
for the Redefinition of Myocardial Infarction

2007

TASK FORCE MEMBERS

EXPERT CONSENSUS DOCUMENT

Third Universal Definition of Myocardial Infarction

2012

Kristian Thygesen, Joseph S. Alpert, Allan S. Jaffe, Maarten L. Simoons, Bernard R. Chaitman and Harvey D. White: the Writing Group on behalf of the Joint ESC/ACCF/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction

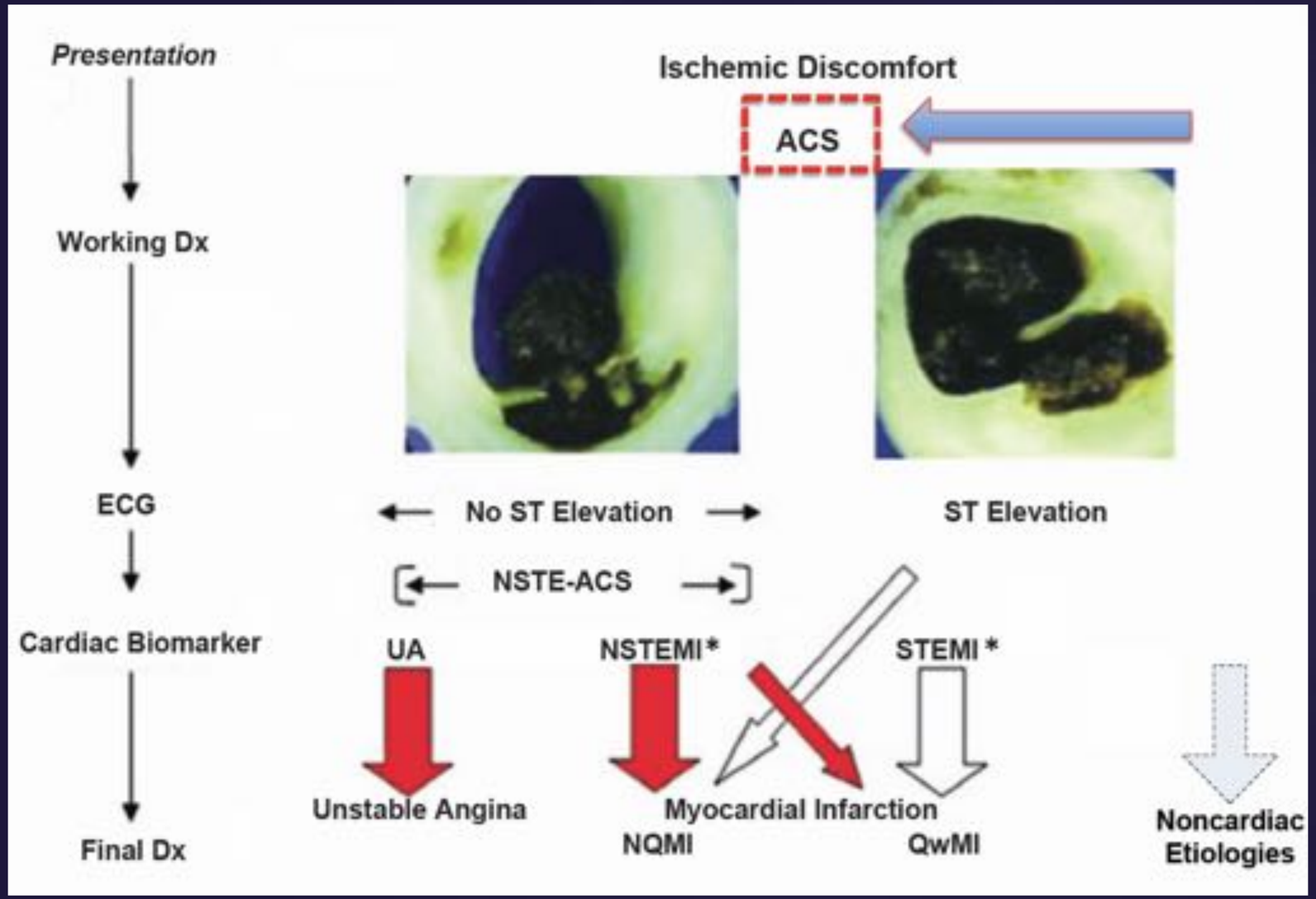
Universal Definition

- Rise and/or fall of cardiac biomarkers (cTn) with ≥1 value >99th percentile URL
- PLUS at least 1 more:
  - Ischemic symptoms
  - New or presumed new significant ST-segment or T-wave changes or new LBBB or development of pathologic Q waves
  - Imaging evidence of new loss of viable myocardium such as RWMA
  - Identification of intracoronary thrombus on angiography or autopsy

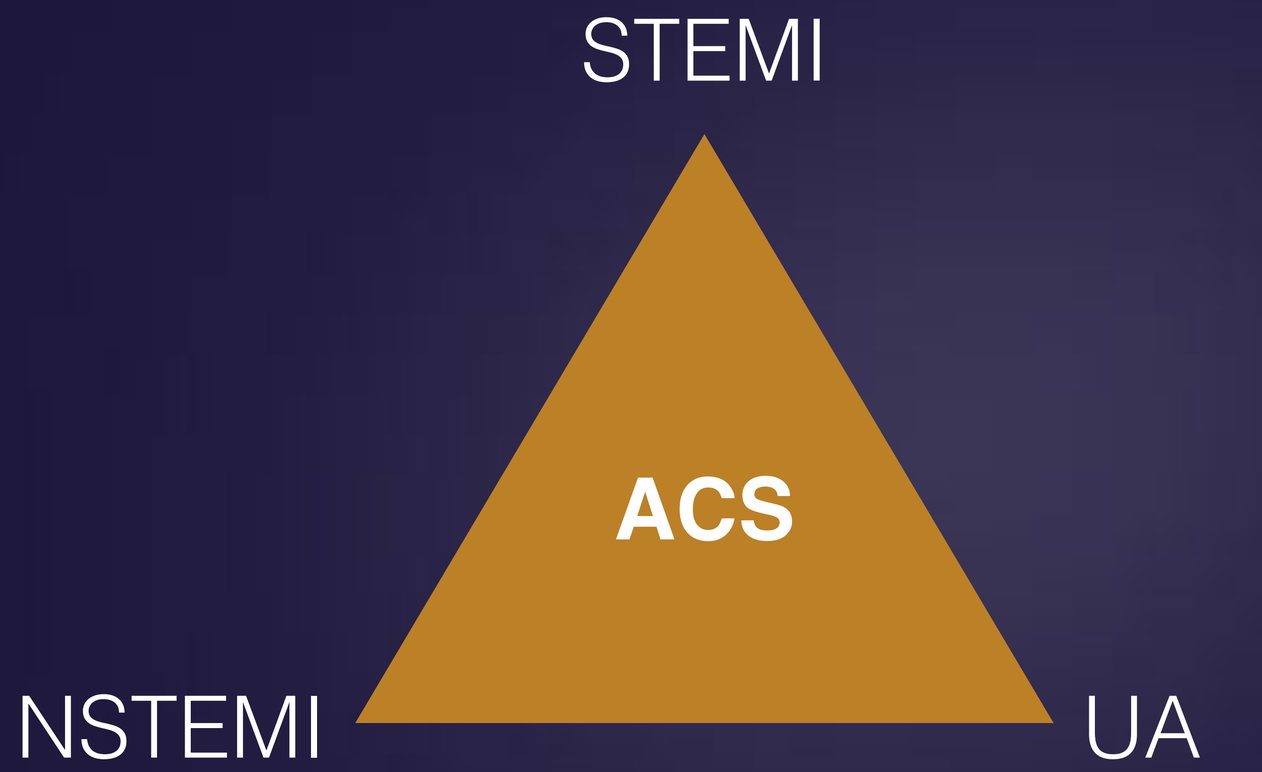
MI Subtypes

Type 1	Spontaneous MI (Atherosclerotic plaque rupture, >1x URL)
Type 2	Secondary to ischemic imbalance (>1x URL)
Type 3	MI resulting in death when biomarker values are unavailable
Type 4	Related to stent PCI (>5x URL)
Type 5	Related to CAB (>10x URL)

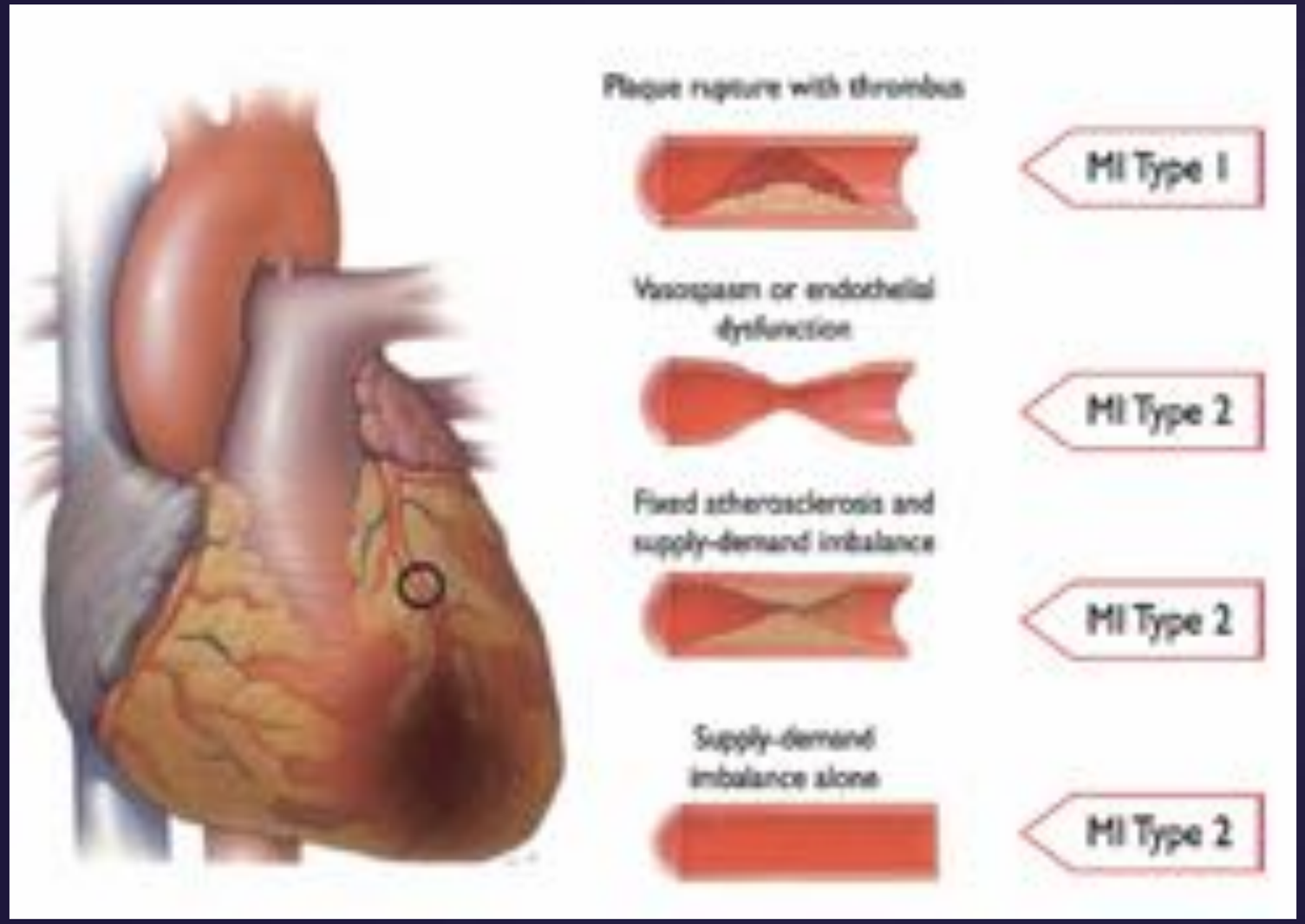
ACS


Presentation
Working Dx
ECG
Cardiac Biomarker
Final Dx
Ischemic Discomfort
ACS
No ST Elevation
ST Elevation
NSTE-ACS
UA
NSTEMI*
STEMI*
Unstable Angina
Myocardial Infarction
NQMI
QwMI
Noncardiac Etiologies

MI does not ACS



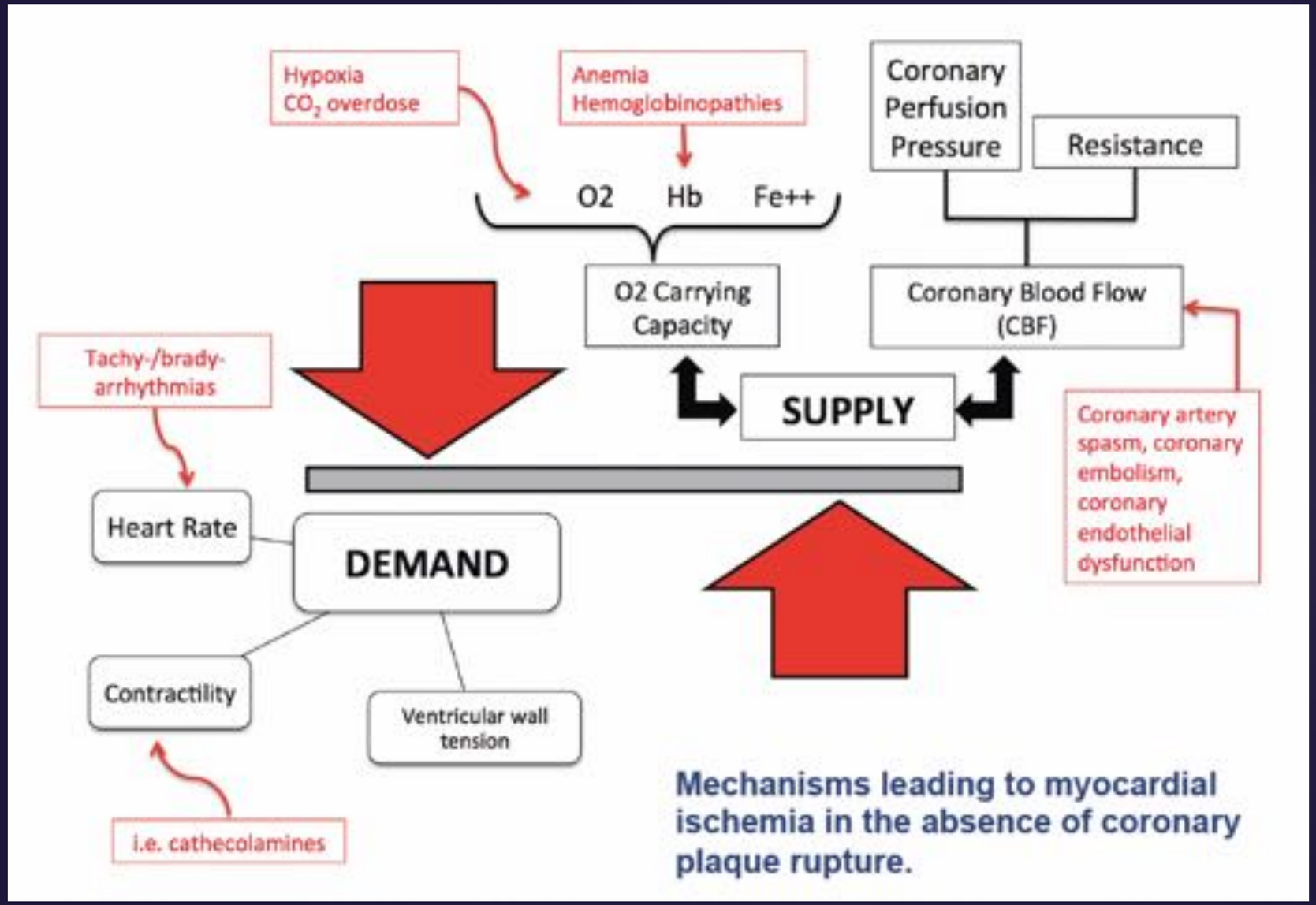
MI Type 1 vs Type 2



ECG Changes

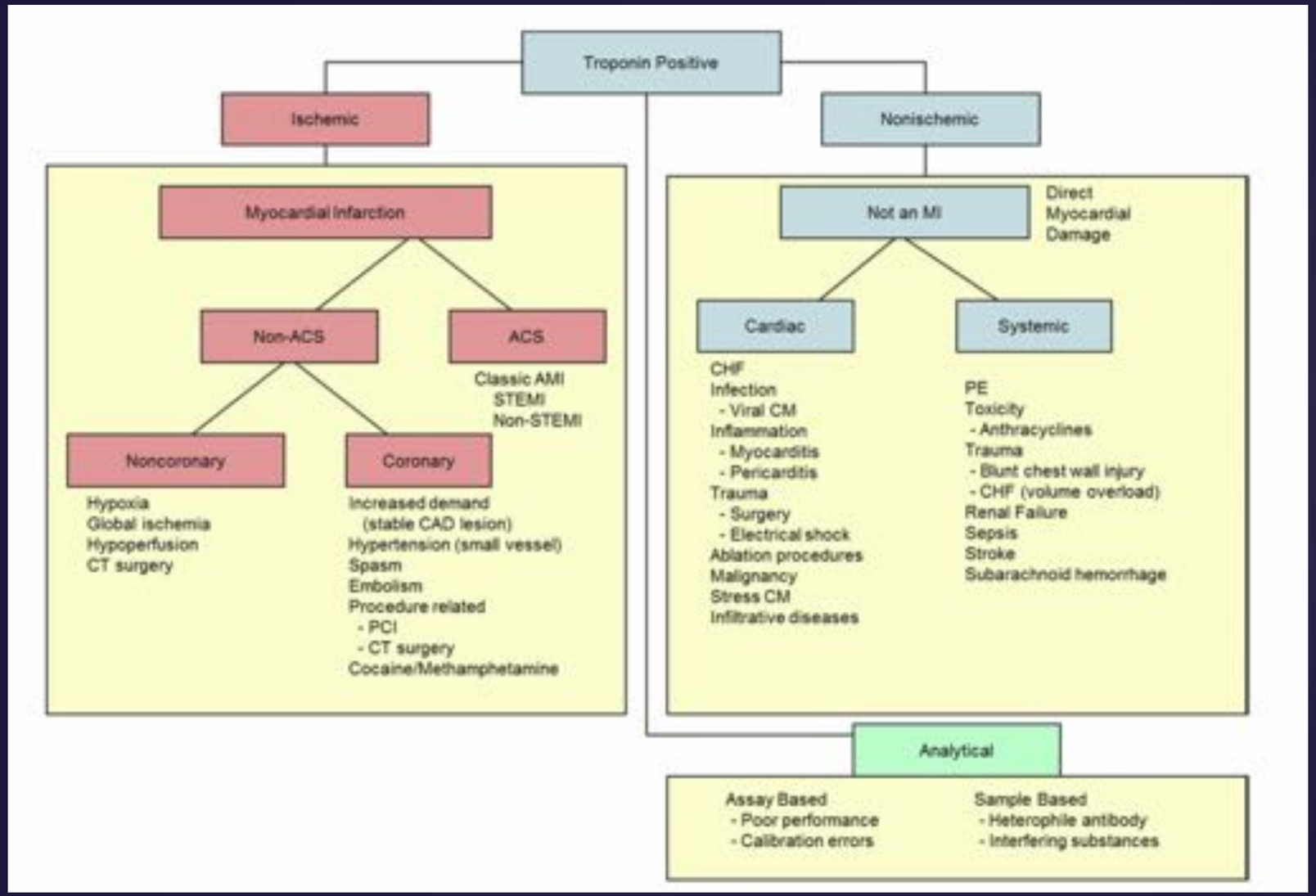
- NSTEMI/STEMI
- Application to T2 unclear
- Clinically intended to guide reperfusion therapy in T1 (ACS)

Supply/Demand Physiology

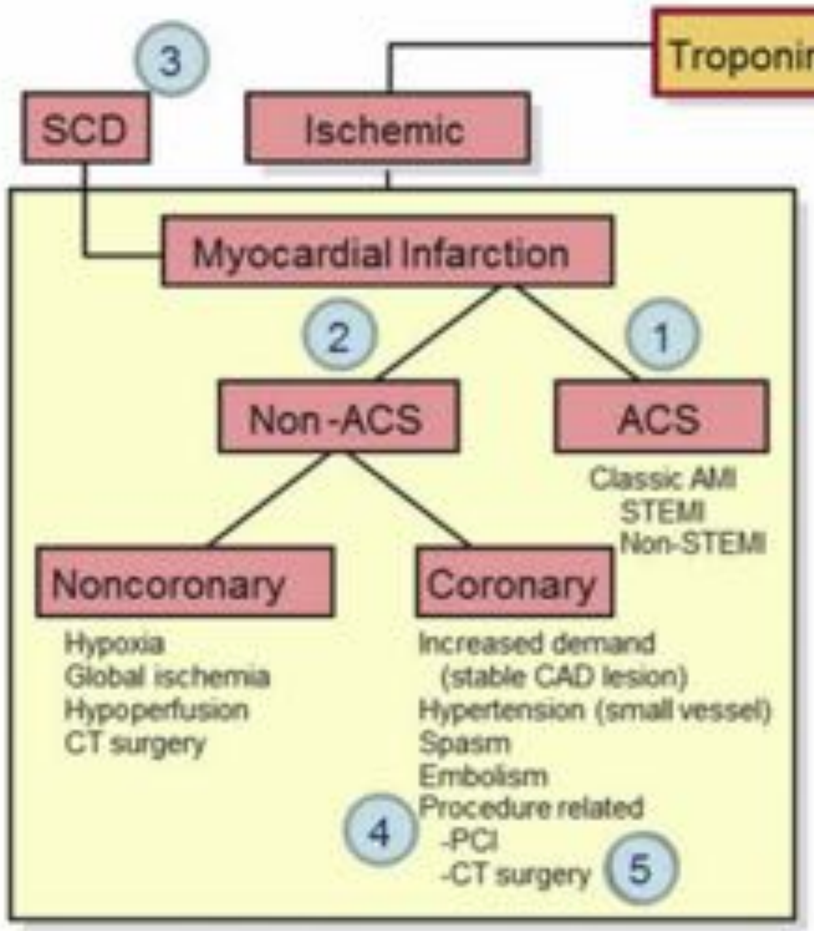


Injury related to primary myocardial ischaemia
Plaque rupture
Intraluminal coronary artery thrombus formation
Injury related to supply/demand imbalance of myocardial ischaemia
Tachy-/brady-arrhythmias
Aortic dissection or severe aortic valve disease
Hypertrophic cardiomyopathy
Cardiogenic, hypovolaemic, or septic shock
Severe respiratory failure
Severe anaemia
Hypertension with or without LVH
Coronary spasm
Coronary embolism or vasculitis
Coronary endothelial dysfunction without significant CAD
Injury not related to myocardial ischaemia
Cardiac contusion, surgery, ablation, pacing, or defibrillator shocks
Rhabdomyolysis with cardiac involvement
Myocarditis
Cardiotoxic agents, e.g. anthracyclines, herceptin
Multifactorial or indeterminate myocardial injury
Heart failure
Stress (Takotsubo) cardiomyopathy
Severe pulmonary embolism or pulmonary hypertension
Sepsis and critically ill patients
Renal failure
Severe acute neurological diseases, e.g. stroke, subarachnoid haemorrhage
Infiltrative diseases, e.g. amyloidosis, sarcoidosis
Strenuous exercise

Conceptual Model



Conceptual Model & MI Types



Type 1: Ischemic myocardial necrosis secondary to plaque rupture (ACS)

Type 2: Ischemic myocardial necrosis not due to ACS (e.g., supply/demand mismatch, coronary spasm, embolism, ↑ or ↓ BP, anemia, arrhythmia)

Type 3: Sudden cardiac death

Type 4: Procedure related

a) Secondary to PCI

b) From stent thrombosis

Type 5: CABG related

T1 vs T2 vs Nonischemic

- Can represent a challenge, especially in setting of known CAD
- Clinical History
- ECGs
- Identification of imbalance
- Troponin kinetics
- Exact T2 diagnosis criteria are difficult given multifactorial nature
  - Strict cutoffs wont work, HR, BP, pO2

Diagnoses

- “Non-ST-segement myocardial infarction”
- “Type 2 Myocardial Infarction secondary to severe hypoxemic respiratory failure”
- “Nonischemic myocardial injury with necrosis likely multifactorial including severe sepsis”

Application of Troponins

- What is the relationship between elevated troponin (myocardial Injury) as a marker of myocardial necrosis and clinical significance attached to it
- In early studies, ~25% had +cTn but negative CKMB
  - ? more sensitive or FP
  - Level depedent adverse outcome risk for troponin group

Application of Troponins

- Elevation does not provide ANY etiology information
- Clinical context is everything
- Analytical vs clinical Sensitivity
  - Most studies show a higher diagnostic accuracy of hs-cTn assays for the early diagnosis of AMI
- Diagnosis vs prediction of risk
- Clinical and cost implications of FP and FN

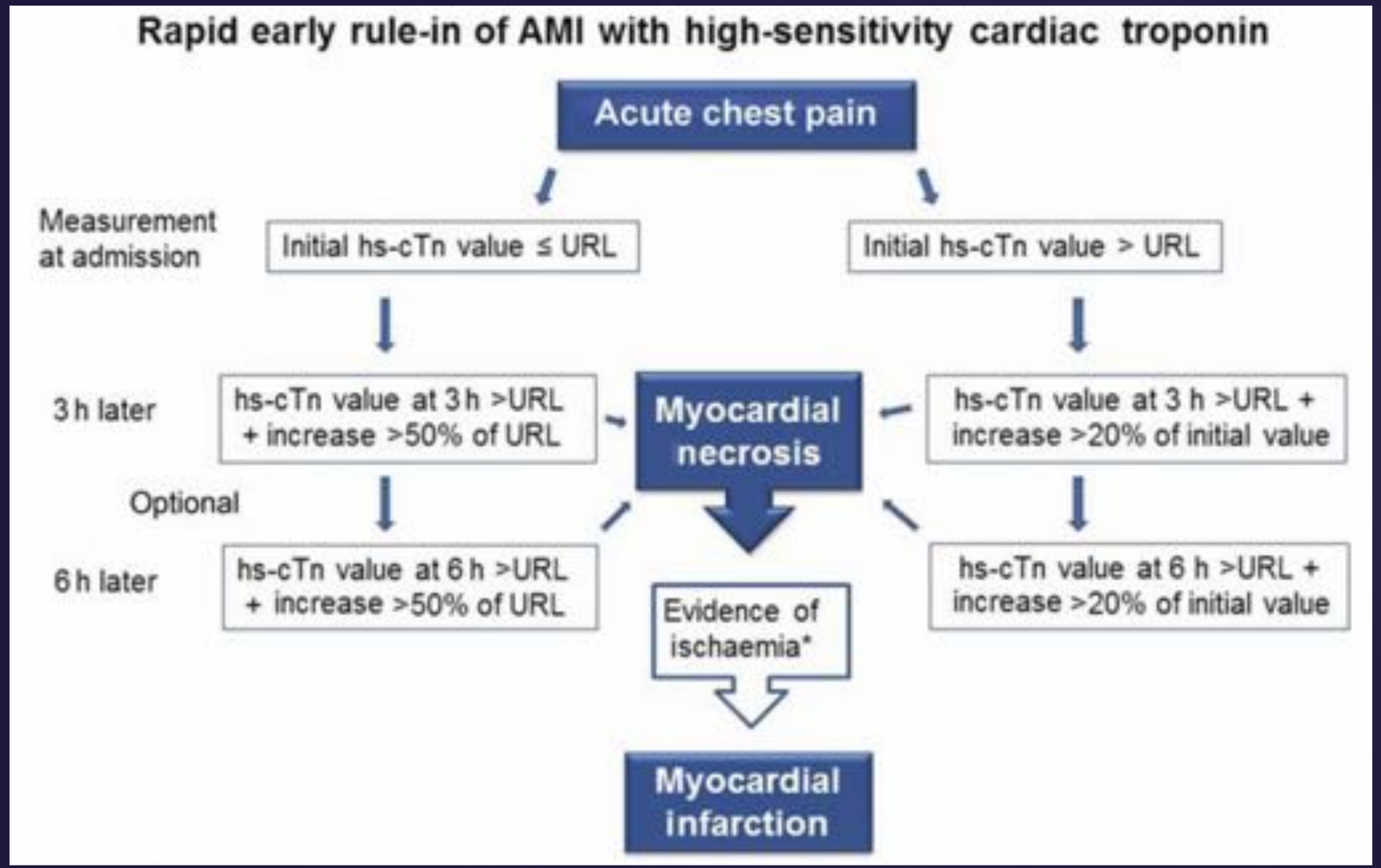
Rise/Fall of Troponins

- Elevations have cleary indentified increased risk for adverse outcomes compard to CKMB regardless of etiology
- But what cutoff?
  - Lower the they looked the kept seeing incrased risk
- Rise-fall coincides with myocardial injury and necrosis as opposed to baseline troponin levels which indicate programmed cell death (autophagy, apoptosis)
- Detection of rise/fall is essential in diagnosis of acute MI

Rise/Fall of Troponins

- Helps distinguish b/w AMI and baseline elevaion
- Absolute vs Relative
- Sensitivity vs Specificity
- Timing

AMI Alogirthm

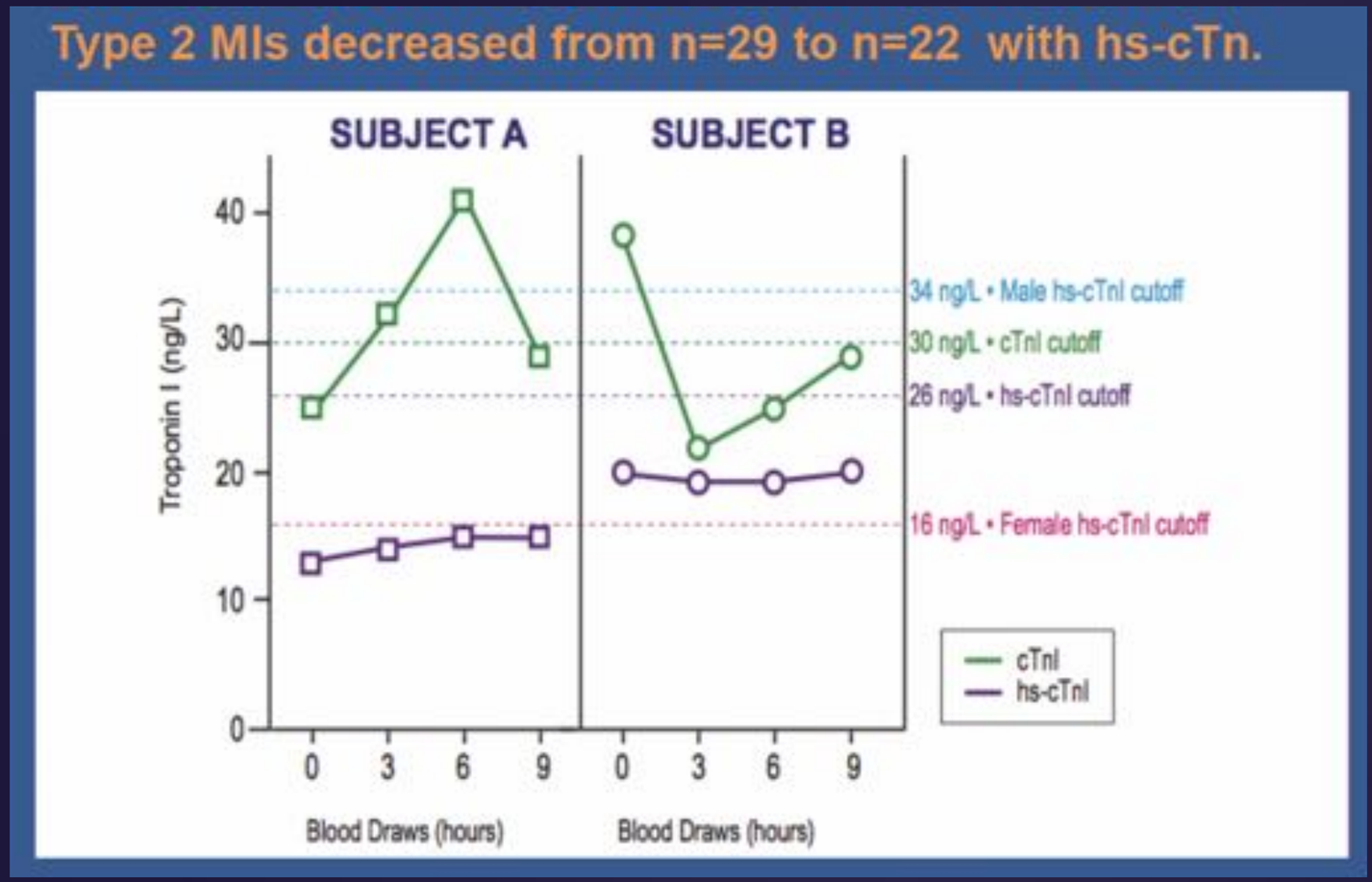


T1MI and T2MI with hs-cTnI

hs-cTn and Type 2 MI

Adjudication Method by Assay	99th percentile URL (ng/L)	MI n (%)	Type 1 n (%)	Type 2 n (%)
MIs adjudicated using **contemporary assay**	30	43 (14%)	14 (4.5%)	29 (9.4%)
MIs adjudicated using **hs-cTnI**	26	33 (11%)	11 (3.5%)	22 (7.1%)
MIs adjudicated using **hs-cTnI with GS cutoffs**	F:16; M:34	32 (10%)	10 (3.2%)	22 (7.1%)

Why Decrease in Dx



UTROPIA

- Clinical Trial Reg No. NCT02060760
- Hypothesis: High sensitivity cTnI assays will have improved diagnostic accuracy for type 1 MI compared to contemporary cTnI assays.
- Primary objective of the study is to determine the performance of a high sensitivity cardiac troponin I (hs-cTnI) assay compared to a contemporary cTnI assay for the diagnostic accuracy of type 1 acute myocardial infarction (AMI).
- Prospective observational
- Abbott ARCHITECT hs-cTnI
- Universal and gender specific cutoffs

UTROPIA

- Enrollment complete and all blood analyzed
- Currently cases are being adjudicated

Take home points

- Supply/demand mismatch (T2MI) should be diagnosed when there is evidence of myocardial necrosis in a clinical setting consistent with an acute imbalance without plaque rupture, in which there is a rise/fall of cTn with at least 1 value >99th percentil, plus 1 other MI criteria accoridng to Universal Definition.
- Report altered variable with supply/demand balance
- If one cannot readily identify clear alternature that would alter supply/demand balance, be cautious before labeling T2MI

Take home points

- If T1 and T2 distinctionis equivocal, consider consultation before empirically initiating usual T1 therapies, largerly because of risks asscoauted with such therapies
- In angiograhically normal patients with an MI diagnosis, careful clinical judgement should be made to clarify if a patient should dx and treated as T1, particularly if no clear alternate s/d mismatch is identified. Further evaluation to clarify the etiology of myocardial necrosis should be considered (cMRI)
- Ampong pts with T2, in which the index event appears to be related to underlying undiagnosed CAD, further evaluation for CAD or structural heart disease should be considered.

References

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- Jarolim et al. Clin Chem Lab Med 2014. High sensitivity cardiac troponin assays in the clinical laboratories.
- Newby et al. JACC 2012;60(23). ACCF 2012 Expert Consensus Document on Practical Clinical Considerations in the Interpretation of Troponin Elevations.