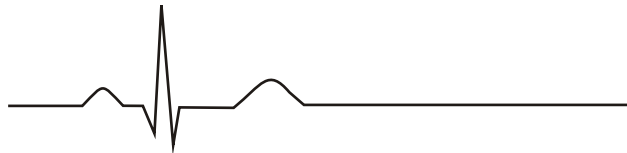


**N**

**TECHNICAL SERIES**

## **Cardiac Troponin I (cTnI)**

New generation cardiac marker of choice



...Setting trends

## Foreword

**Zephyr Biomedicals** is a part of the innovative **TULIP** Group of companies based at Goa, India.

The group's commitment in building products of international standards, through indigenous R&D has accorded the company virtual leadership in most product segments in the Indian marketplace. Its state-of-art manufacturing facility conforms to the strictest FDA (India) and GMP regulations. In its efforts to build world-class Quality products, the group has recently received the ISO 9001(2000) certification from TUV. It is this commitment to Quality, which has given the group international acclaim.

The Group products are now exported to over 45 countries globally with an ever-increasing user base. With decades of experience in *in-vitro* diagnostics (IVD), **TULIP** has created a strong knowledge base. **TULIP** believes that in the knowledge-based society of the 21<sup>st</sup> century, regular upgradation of knowledge is essential not only for better diagnosis and patient care, but also to improve the overall quality of life.

Publishing of **Technical Series** is one such initiative to make available to the Laboratory professionals and clinicians updated knowledge that is vital for them to set trends in their day-to-day practice.



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## Introduction

Coronary artery disease (CAD) is the most important cause of morbidity and mortality in the industrialized world. In western countries approximately 15 million people are affected by heart failure. The W.H.O.-MONICA project on myocardial infarction and coronary death in 38 populations from 21 countries in 4 continents revealed that with regards to both morbidity and mortality significant regional differences exist. The risk of coronary artery disease is significantly higher in Northern Europe than in Central and Southern Europe. This is the so-called '**French Paradox**' (low rate of coronary artery disease with high caloric nutrition), which can be best explained by differences in drinking, eating patterns and genetic factors. The risk of coronary artery disease is especially high in Eastern Europe, whereas Canada, USA and Australia show a midlevel risk. Also the risk of CAD is comparatively higher in males as compared to females.

In the more recently reported SHARE study the overall prevalence of coronary artery disease was 10.7% among South Asians as against 4.6% in Europeans and 1.7% in Chinese population. Projections based on global burden of disease estimate that by year 2020, the burden of atherothrombotic cardiovascular disease in India would surpass that in any other region in the world.

Hence during preselection of patients for further cardiological examinations sensitive and specific laboratory tests can play an important role in diagnosing acute and chronic heart diseases.

### **Triaging of patients with or without AMI - A diagnostic challenge**

According to W.H.O. criteria, diagnosis of Acute Myocardial infarction (AMI) is based on the detection of at least two out of three infarction specific findings:

- Chest pain > 20 minutes, resistant to nitro derivatives.
- Infarction specific ECG changes (ST segment elevation, development of abnormal Q wave) in at least two leads of the standard 12 lead ECG within the same vascular area.
- Serial enzyme changes (cardiac markers) with initial rise and subsequent reduction in level of concentration.

The triaging of patients presenting in emergency department is the major diagnostic challenge the physicians face today.

If an ECG reveals ST segment elevation or abnormal Q wave, the probability of acute myocardial infarction is high and further management is well established.

However, the sensitivity of ECG may be as low as 50% and even less in patients with an evolving myocardial infarction i.e. in Non-ST segment elevated myocardial infarction and Non-Q wave myocardial infarction. Though these patients may present with chest pain because of the absence of diagnostically specific ECG changes they could be discharged undiagnosed.

On the other hand, many patients with symptoms of chest pain suffer from severe unstable angina pectoris. Also in these patients ECG changes may be less specific and fail to provide conclusive diagnostic information.

Besides a subset of patients with absence of chest pain but with myocardial infarction i.e. silent infarction is especially common in diabetic patients. With advancing age the course of AMI is often atypical (without chest pain) but presenting with shortness of breath.

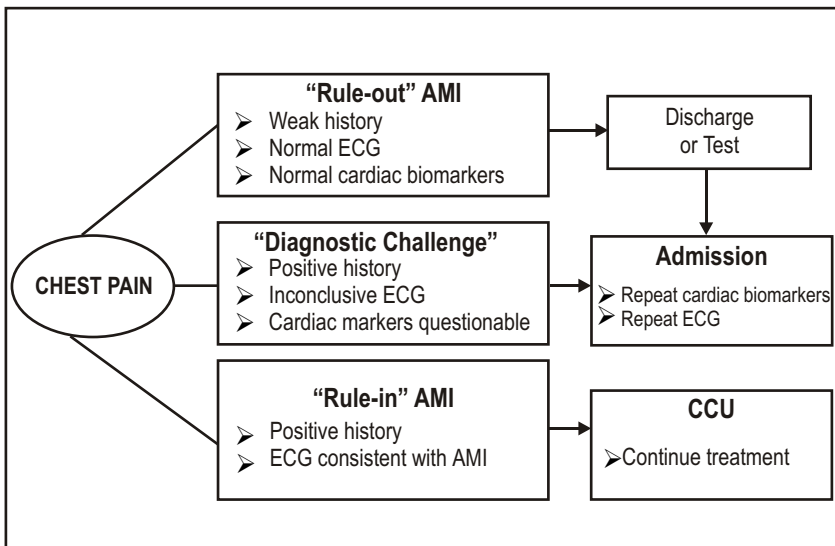


Fig.1:Triaging of patients with chest pain-a diagnostic challenge

## Importance of accurate triaging

Coronary ischaemia is the root cause of acute myocardial infarction, hence early and reliable detection of myocardial ischaemia is a prerequisite for appropriate triage decision in emergency room so as to initiate the right therapy. It has been observed that only 10-15% of patients presenting in emergency room with the cardinal symptom of chest pain develop acute myocardial infarction. **Hence early diagnosis of AMI has to be so sensitive that all suitable patients with myocardial infarction can be treated with thrombolytic therapy and yet so specific that patients with chest pain but without myocardial infarction are not unnecessarily exposed to the risks of such therapy.** This is the most important decision the clinicians have to take when the patients present in the emergency rooms.

## Role of cardiac markers

Following are the important applications of cardiac markers for management of patients with acute coronary syndrome:

- Confirm the diagnosis of AMI in the presence of diagnostically specific ECG changes.
- Diagnosis of AMI in the absence of unequivocal ECG changes (NSTEMI and Non-Q wave MI).
- Identification of high-risk patients with unstable angina pectoris in the absence of unequivocal ECG changes.
- For monitoring patients with AMI undergoing thrombolytic therapy i.e. success of therapy for reperfusion.

From a clinical point of view, an ideal cardiac marker that detects myocardial injury should satisfy the following properties:

- It should be present in myocardium in high concentration and absent in other tissues thereby ensuring high cardiac specificity.
- It should be released rapidly in blood stream after myocardial injury, so as to achieve optimal sensitivity in early phase after the onset of myocardial injury.
- It should remain abnormal for several days thereby offering wide diagnostic window time.
- It should be assayed with a rapid turnaround time.

## Cardiac Markers

### **Creatine Kinase (CK) / Creatine Kinase MB (CK-MB) activity**

Three different isoenzymes of CK exist namely CK-MM, CK-MB and CK-BB. Skeletal muscle approximately consists of CK-MM (97-99%) and CK-MB (1-3%). The cardiac muscle approximately contains CK-MM (95%) and CK-MB (5%). CK-BB is found primarily in brain and contributes very little to the total CK level.

After myocardial infarction CK and CK-MB levels rise after 2-6 hours; peak levels are observed at 12-24 hours. CK returns to normal levels after 3-4 days where as CK-MB because of shorter half-life returns to normal level after 2-3 days.

The calculation of CK-MB/CK ratio improves the specificity of CK-MB for acute myocardial infarction in patients accompanying with skeletal muscle damage. CK-MB returns to normal levels within 2-3 days after myocardial infarction, hence it is useful in detecting reinfarction.

#### **Limitation:**

Though CK-MB/CK ratio improves specificity of CK-MB, however small myocardial necrosis may be missed (unstable angina pectoris may show the presence of micro infarcts / minor myocardial injury).

### **CK-MB isoforms**

In an attempt to improve sensitivity of CK-MB, high voltage electrophoresis technique was developed to separate CK-MB into its two isoforms: CK-MB<sub>2</sub> and CK-MB<sub>1</sub>. In serum of healthy individuals CK-MB<sub>2</sub>/CK-MB<sub>1</sub> ratio of approximately 1 is present. The reference range of this ratio is 1.5. A higher ratio indicates acute myocardial infarction.

#### **Limitation:**

- This technique is labour intensive.
- Requires high level of technical skill.
- Long delay in reporting of results.

### **CK-MB mass**

Here CK-MB is detected immunologically by using a combination of CK-B and CK-M specific monoclonal antibodies or with CK-MB specific monoclonal antibodies.

**Limitation:**

- Interference in these assays is observed because of CK-MM, CK-BB, and CK-B autoantibodies.

**CK-MB immunoinhibition method**

The theoretical basis for the clinical application of immunoinhibition method is the assumption that only CK-MM and CK-MB are released into the blood stream after muscle damage. The reagent contains anti CK-M antibodies, which completely inhibit all CK-M activity i.e. both M subunits in CK-MM and the single M subunit in CK-MB. The remaining non CK-M activity corresponding to the CK-B activity of CK-MB is measured. Since only CK-B of the dimeric CK-MB molecule is measured, multiplication by a factor of 2 gives the CK-MB activity in the specimen.

**Limitation:**

- In case of macro CK, which contains no CK-M subunits immunoinhibition cannot take place.

**Common limitations of CK-MB assays:**

- As CK-MB is also present in skeletal muscle it is not absolutely specific to cardiac muscle damage.
- Evaluation of CK-MB levels may present problems in conditions such as extensive skeletal muscle injury with small infarction, chronic skeletal muscle injury and myocardial infarction after coronary artery bypass graft.
- Determination of CK and CK-MB activity alone is not suitable for assessment of risk in patients with unstable angina pectoris (minor myocardial damage).

**Lactate Dehydrogenase**

Lactate Dehydrogenase is also an enzyme released by ischaemic heart muscle. Out of the 5 isoenzymes only two of them LD1 and LD2 are useful in the diagnosis of AMI. Usually in normal healthy individuals the amount of LD2 in blood is higher than LD1 but patients with AMI show more of LD1 than LD2.

**Limitation:**

- LD1 and LD2 are not cardiospecific markers
- Elevated levels of LD1 and LD2 are observed in leukemia, renal and hemolytic diseases.



## Myoglobin

Myoglobin, the oxygen binding haem protein constitutes about 2% in both skeletal and cardiac muscle. The low molecular weight of Myoglobin (17.8 kDa) facilitates its rapid release in circulation and is the first marker to exhibit rising levels after AMI. The advantages of Myoglobin in early diagnosis of myocardial infarction are its high early sensitivity and the possibility of rapidly assessing the success of thrombolytic therapy.

### Limitation:

- Since Myoglobin is also present in skeletal muscle it is not a cardiospecific marker.
- The extremely short biological half life (10-20 minutes) restricts the usage of Myoglobin to detect unstable angina pectoris (minor myocardial injury or micro infarcts).

Cardiac Marker	M.Wt (kDa)	Half life (hours)	Increase (hours)	Peak* (hours)	Normalization (days)
LD - 1	135	110	6 - 12	48 - 144	7 - 14
CK	86	17	3 - 12	12 - 24	3 - 4
CK-MB	86	13	3 - 12	12 - 24	2 - 3
CK-MB mass	86	13	2 - 6	12 - 24	3
Myoglobin	17.8	0.25	2 - 6	6 - 12	1

\* Strongly dependent on the timing of reperfusion of the infarct-related blood vessel

**Fig.2: Typical characteristics of cardiac markers**

Cardiac Marker	Hours after onset of pain		
	0 - 2	3 - 4	5 - 6
CK activity	15	35	70
CK-MB activity	10	25	55
CK-MB mass	30	70	90
CK-MB isoform ratio	25	60	90
Myoglobin	35	80	95

**Fig.3: Average diagnostic sensitivities (%) of cardiac markers during early phase of AMI**

## **Cardiac Troponins (cTn)-emerging cardiac marker of choice**

Limitations of existing cardiac markers led to the search for markers uniquely expressed by the myocardium. The cardiac troponins T and I (cTnT and cTnI) have excellent sensitivity and specificity and are superior to CK-MB in indicating minor myocardial injury.

The advent of cardiac Troponins T and I, unarguably the most sensitive and specific markers encompass all the requirements that physicians and laboratorians require for accurate triaging and better risk stratification of patients with acute coronary syndrome.

### **Role of Troponins in muscle contraction**

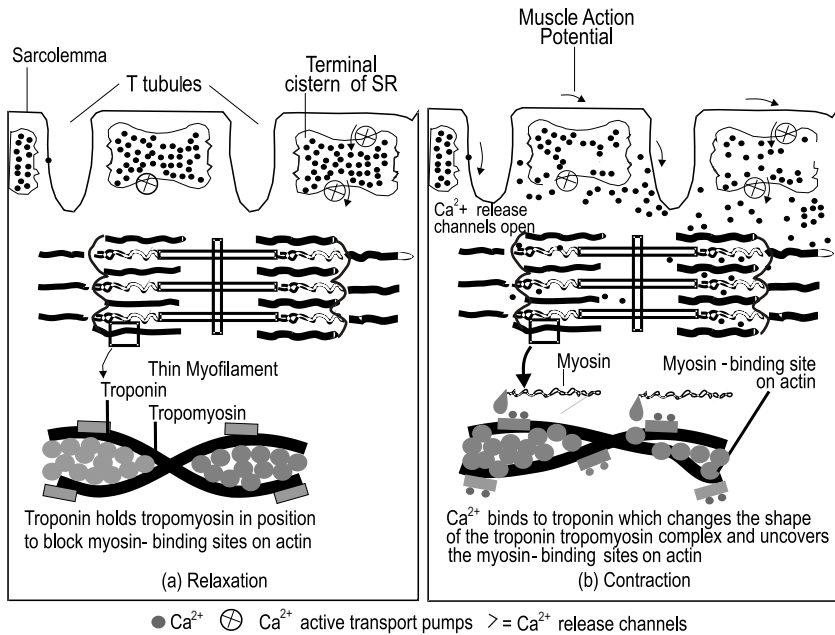
The contractile apparatus of striated muscle fiber is composed of thick and thin filaments. The thick filament is composed mainly of myosin. Actin, tropomyosin and Troponin comprise of thin filament. Muscle contraction occurs when thick and thin filament slide past each other. The interaction between thick and thin filament is regulated by Troponin complex found on thin filaments. The Troponin complex is composed of three protein subunits: Troponin I (TnI), Troponin T (TnT) and Troponin C (TnC). The calcium-mediated contraction of striated muscle (fast-skeletal, slow-skeletal and cardiac muscle) is regulated by the Troponin complex. Contraction of smooth muscle is regulated by calmodulin (intracellular protein that combines with calcium and is involved in smooth muscle contraction).

Troponins are proteins that are integral to the functioning of striated muscle. They exist as a complex with actin and tropomyosin on thin filament of the contractile apparatus. The Troponin complex consists of three protein subunits:

- Troponin C, binds with calcium and regulates activation of thin filaments during contraction.
- Troponin T, binds the Troponin complex to tropomyosin.
- Troponin I, prevents the contraction of muscle in the absence of calcium and Troponin C.

During the functioning of the contractile apparatus depolarization of muscle leads to intracellular release of calcium, which binds with Troponin C. A conformational change occurs in Troponin-Tropomyosin complex in such a way that actin molecules can then interact with myosin, resulting in muscle contraction.

## Cardiac Troponin I



**Fig.4 : Diagram of muscle contraction depicting Troponin involvement**

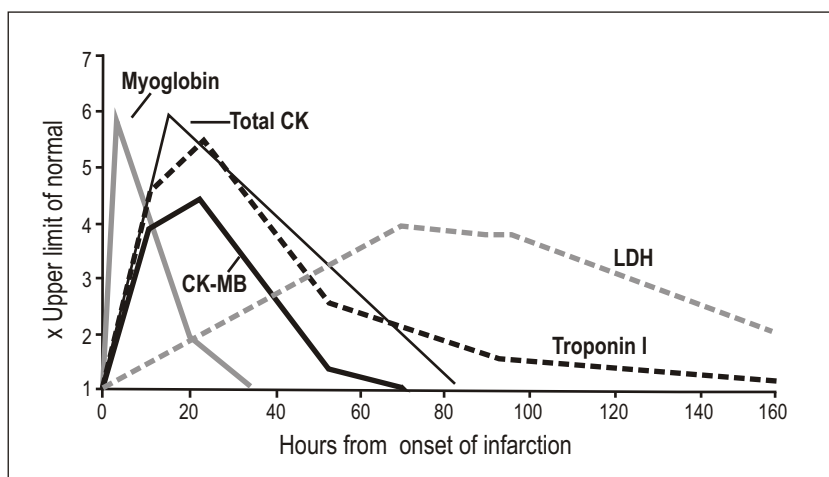
### Types of Cardiac Troponins

- Troponin C exists as two isoforms, fast and slow. The fast isoform is found only in skeletal muscle, but the slow isoform is found both in skeletal and cardiac muscles. The molecular weight of cardiac isoform (cTnC) is 18 kDa.
- Troponin T is also found in fast and slow skeletal muscle, cardiac muscle. Troponin T present in skeletal muscle exists as a slightly different subform. The cardiac isoform (cTnT) has a molecular weight of 37 kDa.
- Three isoforms of Troponin I have been identified, one each in fast and slow skeletal muscles and one isoform in cardiac muscle. The cardiac isoform of Troponin I (cTnI) has a molecular weight of 22.5kDa. cTnI has an extra 30 amino acid sequence at the N terminal portion of molecule making it absolutely specific to cardiac muscle. cTnI is mostly bound to contractile apparatus in myocardium, but about 8% is found free in cytoplasm.

Cardiac Troponin	M.Wt. (kDa)	Half Life (hours)	Increase (hours)	Peak* (hours)	Normalization (days)
cTnI	22.5	2 - 4	3 - 8	12 - 24	7 - 10
cTnT	37	2 - 4	3 - 8	12 - 96	7 - 14

\* Strongly dependent on the timing of reperfusion of the infarct-related blood vessel

**Fig.5: Characteristics of cardiac troponin (I and T) in AMI**



**Fig.6: Graphical representation-Levels of cardiac markers in AMI**

### **Cardiac Troponins (cTnI and cTnT) - sensitivity & specificity**

Any damage or injury to myocardial cells results in the release of cardiac Troponins into the circulation. Concentration of cTnI and cTnT in the circulation initially increases with the number of hours after the onset of chest pain and decreases as the enzymes are cleared from the circulation. The most important take home message is that sensitivity of cardiac Troponin tests, like any other cardiac marker is dependant on the number of hours after the onset of chest pain.

Internationally a lot of scientific research work has been done to evaluate important parameters of sensitivity, specificity and predictive values of cTnI and cTnT in clinical settings.

The comparative data and evaluation report along with the study defined are summarized below.

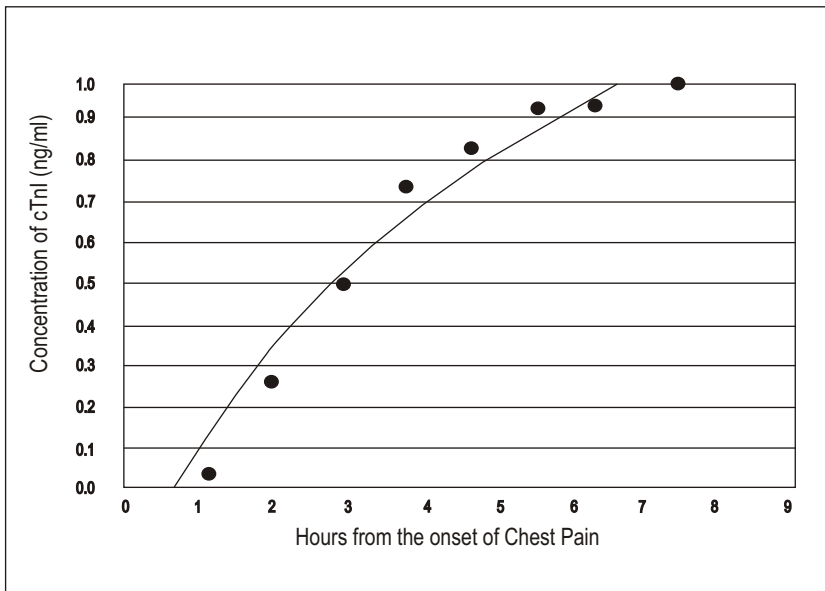
- Sensitivity: Proportion of patients with AMI with abnormal cardiac Troponin test results.
- Specificity: Proportion of patients without AMI with normal cardiac Troponin test results.
- The positive and negative likelihood ratios were calculated using the following equations,
- Positive likelihood ratio= $\text{Sensitivity}/(100-\text{Specificity})$
- Negative likelihood ratio= $(100-\text{Sensitivity})/\text{Specificity}$

The positive and negative likelihood ratios correspond to the clinical concepts of ruling in and ruling out disease. A higher positive likelihood ratio means that a test result is better for ruling in the disease when test is positive. A lower negative likelihood ratio means that a test result is better for ruling out disease when the test result is negative.

Summary of data for cardiac Troponin T and I tests for diagnosing AMI					
	Hours from Onset of Chest Pain	Sensitivity	Specificity	LR+	LR-
Cardiac Troponin T > 0.1ng/ml	1	0.47	0.87	3.7	0.6
	2	0.53	0.87	3.9	0.5
	3	0.58	0.86	4.1	0.5
	4	0.64	0.85	4.2	0.4
	6	0.74	0.83	4.4	0.3
Cardiac Troponin I > 0.1ng/ml	1	0.13	0.95	2.7	0.9
	2	0.34	0.95	6.8	0.7
	3	0.52	0.95	10	0.5
	4	0.67	0.95	13	0.34
	5	0.80	0.95	16	0.23
	6	0.90	0.95	18	0.1

Adapted from 'The Journal of Family Practice', 2000, 49:550-556

**Fig.7: Summary of data for cardiacTroponin T and I Tests for Diagnosing AMI**



**Fig.8: Sensitivity of cTnI viz a viz number of hours of onset of chest pain in AMI**

**Inferences from the study:**

- Sensitivity of cardiac Troponin tests (cTnI and cTnT) is dependant on the number of hours since the onset of chest pain.
- **cTnI appears to be better at ruling in MI than cTnT. A positive cTnT value is only moderately useful at ruling in AMI at 6 hours from the onset of chest pain where as cTnI values appears to be very useful at ruling in AMI at 6 hours from onset of chest pain.**
- cTnI and cTnT are very useful at ruling out AMI when the value is negative at 10 or more hours from the onset of chest pain.
- A normal cTnT or cTnI level at 8 or more hours after the onset of chest pain is strong evidence against the presence of AMI.
- Abnormal values of cTnT and cTnI at 8 or more hours after the onset of chest pain are moderately strong evidence in favor of presence of AMI.

### **cTnI versus cTnT**

The debate continues as to which of the two, cTnI or cTnT is better for management of patients with acute coronary syndromes. Since assays of cTnT were commercially available few years before cTnI, more peer-reviewed publications on the clinical utility of cTnT might have appeared in the past.

However, recent studies have questioned the diagnostic specificity of cTnT assays in patients with myocardial injury and chronic renal failure, muscular dystrophies and skeletal muscle damage. cTnI indeed scores over cTnT in the specificity aspect because cTnI is the only Troponin I expressed in myocardial cells during postnatal development. cTnI is not expressed in normal skeletal muscle at any time including, during postnatal development.

Thus cTnI determination promises higher diagnostic efficacy because of the following unique characteristics,

- Wide diagnostic window time with early appearance and prolonged presence in circulation.
- Allows detection of minor myocardial injury because cTnI levels is almost absent in normal healthy individuals.
- No cross reactivity with skeletal muscle isoforms
- Virtually absent in skeletal muscle tissue.

### **Inferences from international reports highlighting the superior diagnostic efficacy of cTnI**

- cTnI values of less than 0.4 ng/ml are associated with a 42 day mortality of 1% and this risk increases progressively to a mortality of 7.5% at values of 9.0 ng/ml or more. Patients presenting with cardiac chest pain and ECG changes can be classified as Troponin positive or negative acute coronary syndromes, with consequent prognostic and therapeutic implications<sup>1</sup>.

**-BMJ, 2002; 34.**

- The current study demonstrates that ACS patients who have increased cTnI measured on a point of care whole blood assay show a significant increase in risk over 30-180 days for all cause death, cardiac death and cardiac events in the presence or absence of ST-elevation. These findings add to the evidence based metaanalyses which demonstrates that increased cTnI predicts the risk of adverse outcomes in ACS<sup>1</sup>.

**- Clinical Chemistry, 2002; 48.**

- 'Cardiac Troponin T, but not cardiac Troponin I, has been found to be re expressed in skeletal muscles of patients with renal failure and muscular dystrophy. cTnI appears to be more specific than cTnT, with discordant results more often being cTnT(+) /cTnI(-). cTnI is more specific for cardiac injury in settings of renal and muscle disease.'  
**- The Journal of Emergency Medicine, Vol.23, Jan. 2002.**
- 'In patients with clinically documented acute coronary syndrome who are treated with glycoprotein IIb/IIIa inhibitors, even small elevations in cTnI identify high risk patients who derive a large clinical benefit from an early invasive strategy'.  
**- JAMA, Vol. 286 No.19, November 21, 2001.**
- 'The prognostic power of cTnI testing in combination with ECG improves efficiency of low risk patient management and improved patient risk stratification. This study adds to the evidence favoring cTnI evaluation as part of the management of acute coronary syndromes'.  
**- Q. J. Med. 2001; 94.**
- 'In the management of acute coronary syndromes and acute MI in clinical practice, cTnI is comparable in diagnostic and prognostic efficacy to cTnT. In renal impairment even against second generation cTnT assays, cTnI is superior'.  
**- Heart, 2000; 83.**
- 'Early studies have questioned the clinical specificity of cTnT assays in patients with chronic renal failure. With the development of second generation assay for cTnT, the frequency of positive results in these patients is lower than first generation, although still higher than for cTnI'.  
**- Clinical Chemistry, 45 No.7, 1999.**
- 'The goal of this prospective study was to assess whether cTnI could replace CK-MB mass as the serum biomarker for detection of AMI. Findings have strongly supported our clinical implementation of cTnI, replacing CK-MB mass as the preferred marker for detection of AMI'.  
**- American Heart Journal, 1999 Feb.; 137 (2).**
- 'The first generation of cTnT assay lacked absolute specificity for the cardiac isoform and allowed interference by skeletal muscle Troponin T. However, cTnT is still detectable in many cases of end stage renal failure, to a lesser extent than first generation but to an extent far more frequent than cTnI'.  
**- Clinical Chemistry, 44:7, 1998.**



➤ 'The cTnI determination is expected to reveal absolute myocardial specificity because cTnI is not expressed in fetal and healthy or diseased adult human skeletal muscle tissue, promising no false positive test results in patients with substantial skeletal muscle damage or renal failure. In conclusion the use of cTnI rapid device could improve efficacy and safety of decision making in patients with chest pain that might produce more cost effective use of intensive care facilities'.

**- Clinical Chemistry, 44, No.9, 1998.**

➤ 'Questions about the cardiac specificity of cTnT remain. Some subjects with musculoskeletal or renal disease have elevated levels of cTnT, thus this marker may not be as sensitive as cTnI for detection of myocardial injury'.

**- American Journal of Critical Care, Vol.7, No.6, Nov.1998.**

➤ 'cTnI is part of a new generation of biochemical markers that provide an additional clinical tool for assessment of acute coronary syndromes, a term that describes the continuum of myocardial injury ranging from angina, or so called reversible ischaemia to Q-wave MI and definite tissue necrosis. Studies have indicated that cTnI is a more specific marker in cases involving skeletal muscle injury and renal failure. Therefore cTnI may have an important role in real time strategies for evaluating acute coronary syndrome patients, an area that has been of intense interest, discussion and study over recent years'.

**- Clinical Chemistry, 44:1, 1998.**

➤ 'Routine use of cTnI bed side test in the emergency room improves decision making and is highly cost effective. cTnI values provide additional prognostic information over and above the level of critical illness in patients presenting to the emergency room'.

**- CAP, February 1998.**

➤ 'The slightly higher sensitivity of cTnI test as compared with the cTnT may be related to different release kinetics. The findings of false positive results for cTnT but not cTnI, in patients with renal failure may, however, represent a true difference between the two test'.

**- The New England Journal of Medicine, Dec.4, 1997**

➤ 'In conclusion, the results of our prospective study provide evidence that cTnI is an indicator of adverse outcome in patients with severe unstable angina. Our results are keeping in mind with the recent retrospective analysis of patients enrolled in the TIMI III B study. The use of cTnI in the immediate triage of patients with unstable angina appears warranted to identify those at greater risk for cardiac events'.

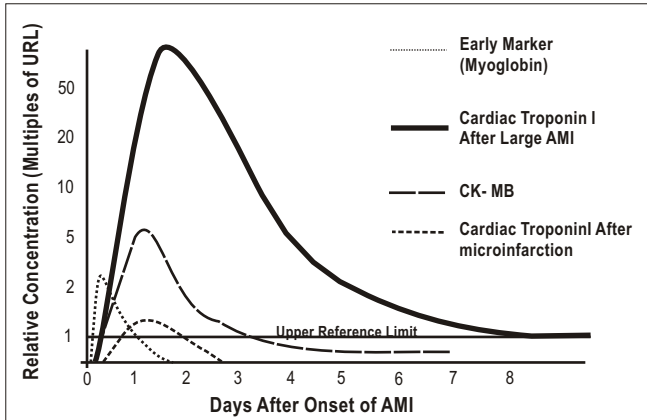
**- Circulation, 1997; 95.**

- 'The study compared the diagnostic accuracy of measurement of serum cTnI with CK-MB mass in patients with minor myocardial injury whose measured total CK activity did not exceed twice the upper reference limit. The clinical sensitivity of myocardial injury for cTnI was 100% compared with 81.8% for CK-MB. Thus, cTnI was more sensitive than CK-MB mass for detection of myocardial injury in patients with small increases of total CK'.  
**- Clinical Chemistry, 1997; 43.**
  
- 'cTnI was as sensitive and specific for AMI as was CK-MB in ED patients who presented within 24 hours of symptom onset. However, cTnI was more sensitive in patients who presented at 24 hours after symptom onset'.  
**- Academic Emergency Medicine, Vol.4, 1997.**
  
- 'cTnI could replace CK-MB and would facilitate the rapid and effective triage of patients with chest pain in the emergency department'.  
**- American Journal of Clinical Pathology, 1997 Nov., 108 (5).**
  
- 'In patients with acute coronary syndromes, cardiac Troponin I levels provide useful prognostic information and permit early identification of patients with an increased risk of death'.  
**- The New England Journal of Medicine, Vol. 335, No.18, 1996.**
  
- 'Elevations of cTnI are highly specific for myocardial injury. Use of cTnI should facilitate distinguishing whether elevations of CK-MB are due to myocardial or skeletal muscle injury'.  
**- Circulation, Vol.88, 1993.**

### **cTnI - Cut off levels**

The National academy of Clinical Biochemistry (NACB), USA and International Federation of Clinical Chemistry (IFCC), Germany have recommended the use of two decision cut off limits for cardiac Troponins. A low limit that establishes the presence of myocardial injury and a high limit that establishes injury to the extent that qualifies as AMI.

Based on literature data and clinical assessments cTnI levels greater than 0.1 ng/ml places a patient with unstable angina in the high-risk category for short-term risk of death or non-fatal MI. The cut off for the definition of AMI is taken to be greater than 1.2 ng/ml. Thus cTnI levels with a cut off of 0.1 ng/ml identify patients at higher risk for very early adverse outcomes.



**Fig.9: Graphical representation - two decision cut off limits of cTnI**

### cTnI - Standardization

In 1998 the American Association for Clinical Chemistry (AACC) appointed a troponin I Standardization Subcommittee to address the critical need for standardization between different cardiac Troponin I tests. Working with diagnostic companies and National Institute of Standards (NIST) this group was performing complex technical studies that involved cardiac Troponin I calibration materials from several sources to help improve the clinical utility of this very important test by identifying a single reference material that all assays can use to assign cardiac troponin I values.

AACC Troponin I Standardization Subcommittee recently conducted a second round robin study. Liquid native Troponin I-C-T complex from Hytest has been selected as committee's choice of cTnI reference standard. The Standard Reference material - 2921, issued in April, 2004 is now available widely for assay standardization.

### Applications of cTnI test

Following are the important applications of cTnI test in management of patients with acute coronary syndrome:

- Patient with ECG specific findings and elevated cTnI test confirms the diagnosis of AMI.
- An elevated cTnI level is of immense diagnostic value in patients with symptoms of chest pain, absence of diagnostically significant ECG change and normal CK-MB level. cTnI test value  $\geq 1$  ng/ml classifies these patients under NSTEMI. A negative cTnI test classifies the patient as UAP.

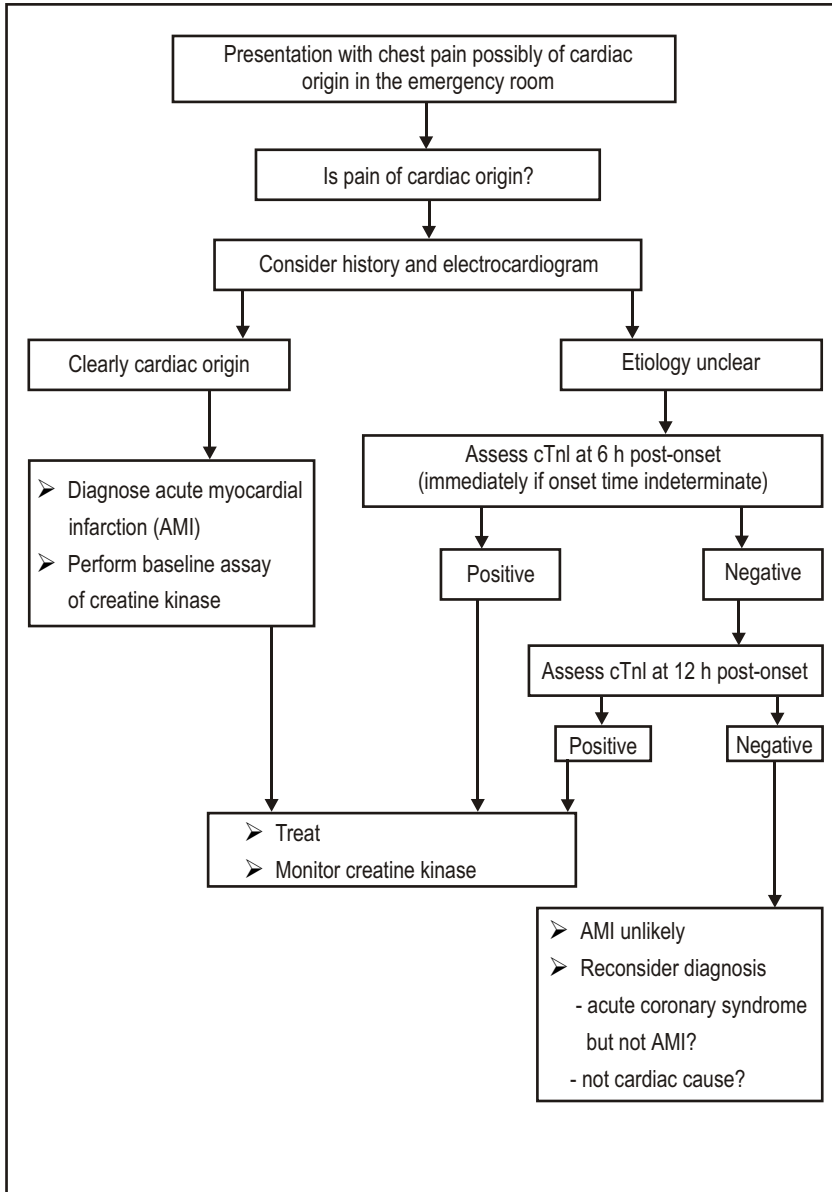
- Also elevated cTnI value provides prognostic value in identifying patients with unstable angina pectoris. Symptoms of chest pain, absence of diagnostically significant ECG change, normal CK-MB level and cTnI value >0.1 ng/ml are classified under UAP. If the test is negative retesting probably at 12 hours after post onset of chest pain is important to rule out diagnosis of acute coronary syndrome.
- Patients presenting with chest pain after trauma or surgery and elevated CK-MB assay value (to rule out true elevation of CK-MB).
- Patients presenting with chest pain 2 to 6 days prior to admission may have sustained acute myocardial infarction but CK-MB would have returned to normal levels.

#### **Superior diagnostic efficacy of cTnI over CK-MB in detecting microinfarcts**

Patients with normal CK-MB levels and elevated cTnI levels could be attributed probably to the low sensitivity and specificity of CK-MB in detecting micro infarction. Since CK-MB is present in skeletal muscle and normal healthy individuals, diagnostic cut off values are typically set above the upper limit of reference range for CK-MB assay. Cardiac troponin I is not present in normal healthy individuals and is approximately 13 times more abundant in myocardium than CK-MB on a weight basis. Hence the signal to noise ratio (increased sensitivity) associated with cTnI is more favorable for detection of micro infarction (NSTEMI and UAP).

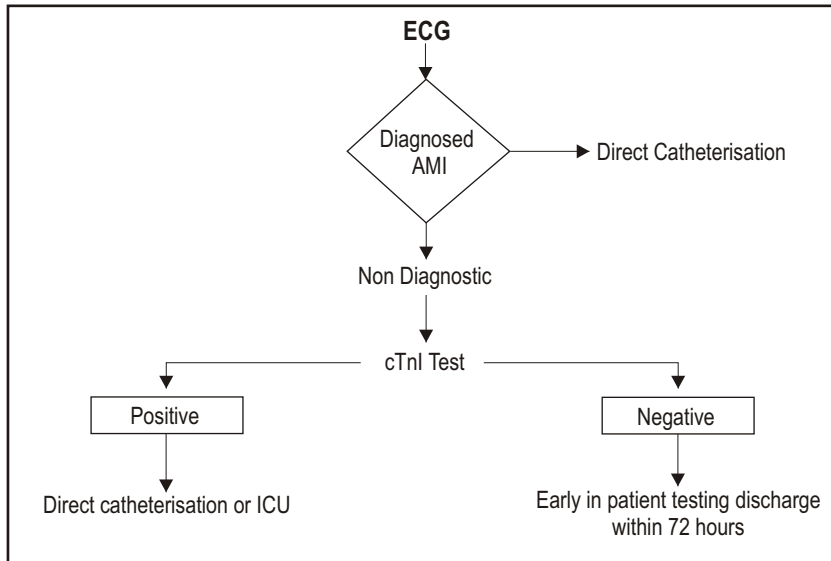
#### **Limitations**

- cTnI levels remain elevated for about 7 days hence for serial monitoring of patients undergoing thrombolytic therapy cardiac markers such as CK-MB and Myoglobin may be used for successful reperfusion. Also in cases of reinfarction markers with shorter half-life such as CK-MB should be used for accurate diagnosis.
- Since cardiac Troponins (both cTnI and cTnT) are sensitive markers for myocardial damage, they are detected in many other cardiac conditions such as acute pericarditis, acute myocarditis, congestive heart failure (CHF), perioperative myocardial infarction and cardiac contusion.

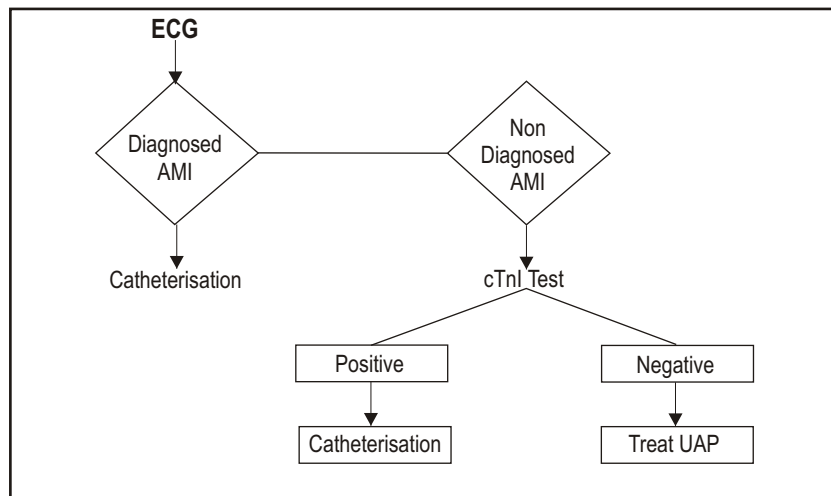


(Adapted from: Troponin testing: an audit in three metropolitan hospitals, Richard X Davey, MJA, Vol.179, 21 July 2003)

**Fig.10 :Protocol for use of cardiac troponin I test**



**Fig. 11: Clinically stable patient admitted for UAP/ rule out AMI**



**Fig. 12: Patient with chest pain: UAP or AMI?**

### Critical parameters for correct usage of cTnI test

Rising levels of cardiac markers are dependant on time elapsed since the onset of myocardial necrosis. This is true regardless of whichever cardiac marker being used for ruling in and ruling out AMI. Since patients present at varying times for testing following the onset of chest pain in a cardiac event, **it is necessary to perform sequential testing of cTnI levels for optimal diagnostic accuracy.**

Following are the important points to be considered regarding cTnI test when triaging patients for acute coronary syndrome:

- First test must be done preferably within onset of symptoms at baseline or within 3 hours of onset of chest pain along with ECG test. A single cTnI negative test within 3 hours cannot safely rule out AMI.
- If the first test is negative, second test for cTnI must be done at 6 hours from the onset of chest pain accompanied by ECG test and if negative should be repeated between 6-12 hours after the onset of chest pain. A third test at 8-9 hours after onset of chest pain represents the best time for assessment of cTnI level and is the most predictive for ruling in or ruling out acute coronary syndrome.
- If 6 hour cTnI test and ECG is normal it is unlikely that the patient will have an adverse outcome in the next 30 days (1% chance).
- An elevated level of cTnI (positive cTnI test) in patients with normal ECG, but with UAP or NSTEMI or Non Q wave MI identifies patient group at greater risk of death.
- Any elevated level of cTnI is indicative of an increased short-term risk of death or nonfatal myocardial infarction.
- The half life of cTnI is approximately 2-4 hours hence samples should be tested preferably immediately. In case of delay in testing the samples may be tested within 2 hours of blood collection.

## ANNEXURE-I

### The heart and how it works

The normal human heart is a strong muscular pump little larger than a fist. Each day an average heart beats 1,00,000 times and pumps about 2000 gallons of blood. In a 70-year lifetime, an average human heart beats more than 2.5 billion times.

The heart pumps blood continuously through the circulatory system. The circulatory system is the network of elastic blood vessels that carries blood throughout the body. The circulatory system comprises of heart, arteries, arterioles, veins and capillaries. The arteries are the blood vessels which carry oxygen and nutrient rich blood to all parts of the body. The veins and capillaries are the blood vessels that carry oxygen and nutrient depleted blood back to heart and lungs. If all these blood vessels were laid end to end they would extend for about 60,000 miles.

The circulating blood brings oxygen and nutrients to all the body's organ and tissues, including the heart. It also picks up waste products from the body's cells. These waste products are removed as they are filtered through the kidney, liver and lungs.

### Structure of heart

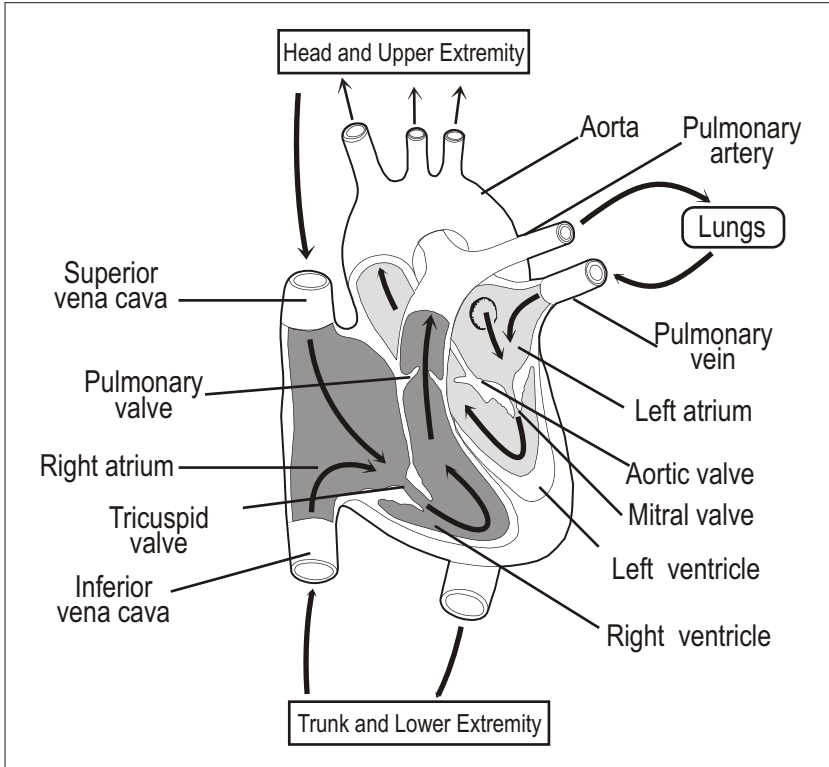
Heart is a hollow, muscular, contractile organ, and the center of circulatory system. It provides the propulsive force for circulating blood throughout the vascular system. The heart wall is composed of three layers, the outer epicardium, a serous layer, the middle myocardium, composed of cardiac muscle, and the inner endocardium, a layer that lines the four chambers of the heart and covers the valves. The heart is enclosed in a fibrous sac, the pericardium. The space between pericardium and the epicardium is the pericardial cavity.

Heart has four chambers through which blood is pumped. The upper two are the right and left atria. The lower two are right and left ventricles. Four valves open and close only in one direction when the heart beats.

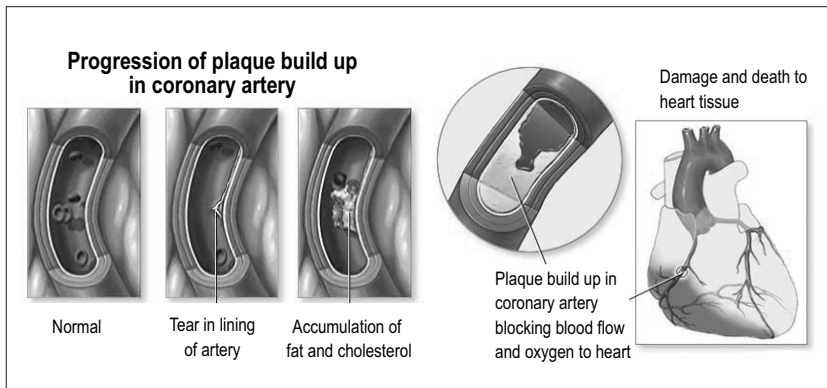
- The tricuspid valve is between the right atrium and right ventricle.
- The pulmonary valve is between the right ventricle and the pulmonary artery.
- The mitral valve is between the left atrium and left ventricle.
- The aortic valve is between the left ventricle and aorta.



Each valve has a set of flaps, also known as leaflets or cusps. The mitral valve has two flaps. The other valves have three flaps. Under normal conditions, these valves allow blood flow in one direction. Blood flow occurs only when there is a difference in pressure across the valves that cause them to open.



**Fig.1: Structure of heart**



**Fig.2: Depiction of Atherosclerosis**

### **Atherosclerosis - Risk factors**

Epidemiological studies indicate the following risk factors that potentiate atherosclerosis,

- Hyperlipidaemia
- Hypertension
- Cigarette habituation
- Diabetes mellitus
- Age
- Sex

### **Signs and symptoms associated with atherosclerosis**

The signs and symptoms of atherosclerosis are highly variable, but mainly present as follows:

- Unstable angina pectoris
- Acute Myocardial Infarction
- Transient ischaemic attack
- Stroke
- Peripheral vascular disease
- Mesenteric angina
- Abdominal aortic aneurysm
- Atheroembolism

## ANNEXURE-II

### **Atherosclerosis: major cause of cardio vascular disease**

Atherosclerosis comes from the Greek word athero (meaning gruel or paste) and sclerosis (meaning hardness). The inner lining of blood vessels namely the arteries contains deposits of fatty substances, cholesterol, cellular waste products and calcium. This build up is known as plaque.

Plaque formation results in luminal obstruction, abnormalities of blood flow, diminished oxygen supply to target organs.

Atherosclerosis begins with damage to innermost layer of blood vessels namely the endothelium. The probable cause of endothelial injury includes oxidized LDL cholesterol, by products of cigarette smoking, hyperglycemia and hyperhomocystinaemia.

Circulating monocytes infiltrate the intima of the vessel wall and the tissue macrophages act as scavenger cells forming the characteristic foam cell of early atherosclerosis. These activated macrophages produce numerous factors that are injurious to endothelium.

Injury to endothelium leads to increased platelet adhesion, increased tissue factor release, increased plasminogen activator inhibitor, decreased plasminogen activator, decreased thrombomodulin and alterations in heparan sulfate. Thus the sequence of events results in procoagulant milieu and enhanced thrombus or clot formation.

Atherosclerotic plaques characteristically occur in regions of branching and marked curvature at areas of geometric irregularity and where blood undergoes sudden changes in velocity and direction of flow. Decreased shear stress and turbulence may promote atherogenesis at these important sites within the coronary arteries, the major branches of the thoracic and abdominal aorta and vessels of lower extremities of our body.

Generally plaques are static, but they can also become unstable and rupture. Those that rupture can initiate thrombus formation and can totally block blood flow (occlusion) in the artery. A blood clot that breaks off and travels to another part of the body is known as emboli.

If a clot blocks a blood vessel, that supplies blood to heart it causes heart attack. If it blocks a blood vessel supplying blood to brain, it causes stroke. And if blood supply to the arms or legs is reduced, it can cause gangrene.

#### **Fig4.: Risk factors and associated signs in Atherosclerosis**

##### **Angina pectoris**

Angina pectoris is the medical term for chest pain or discomfort due to coronary heart disease. Angina is a symptom of condition known as myocardial ischaemia. It occurs when the heart muscle (myocardium) is deprived of required amount of blood it needs for performing normal function. Myocardial ischaemia (insufficient blood supply to myocardium) occurs due to narrowing or occlusion (blockage) of one or more arteries that supply blood to heart.

Typical angina is characterized by uncomfortable pressure, fullness, squeezing pain in center of chest. The discomfort also may be felt in the neck, jaw, and shoulder.

Angina is a sign that someone is at high risk of heart attack, cardiac arrest and sudden cardiac death.

##### **Stable angina pectoris**

People with stable angina pectoris have episodes of chest pain that are usually predictable. The chest pain episode occurs with exertion or mental or emotional stress. Normally the chest discomfort is relieved with rest and/or sublingual nitroglycerin administration.

### **Unstable angina pectoris**

People with unstable angina pectoris have unexpected chest pain that usually occurs while even at rest. The discomfort may be more severe and prolonged than typical angina.

People with unstable angina pectoris should be treated as an emergency because they are at increased risk for acute myocardial infarction, severe cardiac arrhythmias and cardiac arrest leading to sudden death.

### **Variant angina pectoris (prinzmetal angina pectoris)**

Unlike typical angina, it nearly always occurs when a person is at rest. It does not follow a period of physical exertion or emotional stress. Attacks can be very painful and usually occur between midnight and 8 a.m. morning.

### **Ischaemic Heart Disease**

Ischaemia is a condition where the flow of blood, and therefore oxygen, to a part of body is restricted. Cardiac ischaemia refers to lack of blood flow and oxygen to heart muscle.

Ischaemic heart disease refers to heart problems caused by narrowing down of arteries that supply blood to heart. When arteries are narrowed, less blood and oxygen reaches the heart. This condition is also known as coronary artery disease or coronary heart disease.

### **Acute Myocardial infarction**

Acute myocardial infarction is defined as death or necrosis of myocardial cells. It is the end diagnosis of myocardial ischaemia. Myocardial infarction occurs when myocardial ischaemia exceeds a critical threshold and overwhelms myocardial cellular repair mechanisms that are designed to maintain normal operating function.

Critical myocardial ischaemia may occur as a result of increased myocardial metabolic demand, decreased supply of oxygen and nutrients to myocardium via the coronary circulation. An interruption in the supply of myocardial oxygen and nutrients occurs when a thrombus is superimposed on an unstable atherosclerotic plaque and results in coronary occlusion. Conditions associated with increased myocardial metabolic demand include extremes of physical exertion, severe hypertension and severe aortic valve stenosis.

## **Mechanism of Myocardial damage**

The severity of myocardial infarction is dependant on three factors:

- Level of occlusion in coronary artery
- Length of time of occlusion
- Presence or absence of collateral circulation

Generally, more proximal the coronary occlusion, more extensive is the amount of myocardial necrosis. Larger the myocardial infarct, greater is the chance of death due to mechanical complication. Longer the time period of vessel occlusion, greater the chances of irreversible myocardial damage distal to the occlusion.

The death of myocardial cells first occurs in the area of myocardium that is most distal to arterial blood supply, the endocardium. As the duration of occlusion increases, the area of myocardial cell death enlarges, extending from the endocardium to the myocardium ultimately to the epicardium. Thus the extent of myocardial cell death defines the magnitude of myocardial infarction.

The human body often creates small blood vessels known as collaterals to help compensate for reduced blood flow. Collateral vessels normally are not open and normal healthy individuals do show the presence of collateral vessels but in microscopic form. But in people suffering from coronary artery disease or any other blood vessel disease these collateral vessels grow and enlarge. When a collateral vessel enlarges, it allows flow of blood from an open artery to either an adjacent artery or further downstream on the same artery. Thus collateral vessels grow and form a detour around a blocked blood vessel.

## **Types of Myocardial Infarction**

Myocardial infarction can be subcategorized on the basis of anatomic, morphologic and diagnostic clinical information.

From an anatomic or morphologic standpoint, the two types of myocardial infarction are as follows,

### **Transmural Myocardial infarction:**

In transmural myocardial infarction the ischaemic necrosis affects muscle segment extending from the endocardium through the myocardium to epicardium.

### **Non-Transmural Myocardial infarction:**

In non-transmural myocardial infarction the area of ischaemic necrosis does not extend through the full thickness of myocardial wall segments. The area of ischaemic necrosis is limited to either endocardium or endocardium and myocardium. It is the endocardial and subendocardial zones of myocardial wall segment that are least perfused regions of heart and are most vulnerable to conditions of ischaemia.

An old sub classification of myocardial infarction based on clinical diagnostic criteria is determined by the presence or absence of Q wave on ECG. But a more accepted clinical diagnostic scheme based on ECG findings is the presence of ST segment elevation.

The presence of Q wave or ST segment elevation is associated with high early mortality and morbidity. However the absence of these two findings does not necessarily confer better long-term mortality and morbidity.

### **Signs and symptoms of AMI**

Acute myocardial infarction may have unique presentations in individual patients. The degree of symptoms range from none to sudden cardiac death. Asymptomatic myocardial infarction is not necessarily less severe than a symptomatic event, but patients who experience asymptomatic myocardial infarction are more likely to be diabetic. Despite the diverse presenting symptoms of myocardial infarction, there are some characteristic typical symptoms,

- Chest pain described as pressure sensation, fullness or squeezing in the midportion of thorax
- Chest pain radiating to jaw, teeth, shoulder, arm and back
- Associated dyspnoea or shortness of breath
- Associated epigastric discomfort with or without nausea and vomiting
- Associated diaphoresis or sweating
- Syncope or near syncope without other cause

Acute myocardial infarction may occur at any time of the day, but most appear to be clustered around the early hours of morning and are associated with demanding physical activity. Approximately 50% of patients have some warning symptoms prior to infarction.

## Diagnosis of Myocardial Infarction

W.H.O. criteria for diagnosis of AMI

Twenty years ago, W.H.O. defined the diagnosis of AMI as a triad, two of which atleast must be present for diagnosis,

- Typical history of severe and prolonged chest pain greater than 20 minutes resistant to nitroglycerin
- Unequivocal electrocardiographic changes, with ST segment elevation and development of abnormal Q wave.
- Serial enzyme changes (cardiac markers) with initial rise and subsequent fall of catalytic concentrations.

## Electrocardiogram (ECG)

ECG is usually the first diagnostic test performed. Diagnostic specificity is approximately 100%, and a positive tracing, signaled by an elevated ST segment, essentially confirms diagnosis of AMI. The diagnostic sensitivity however has been estimated to range from 63-82%.

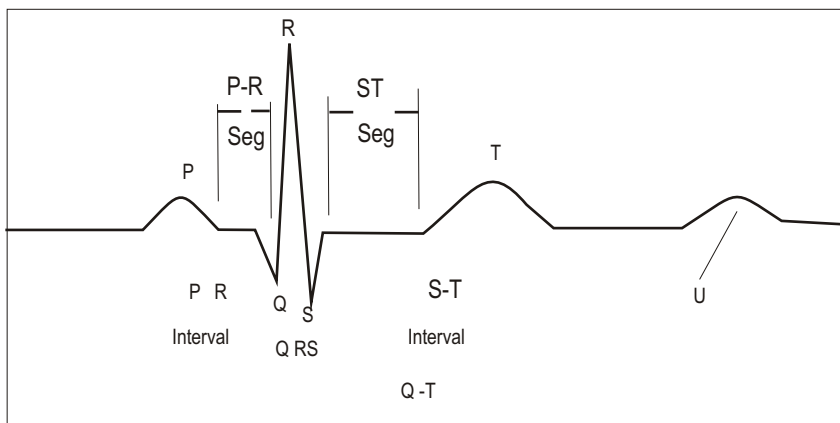
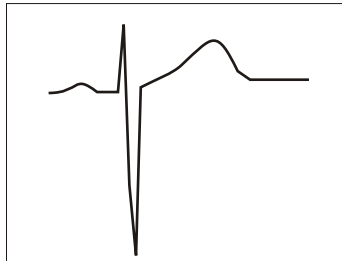


Fig.4: Normal ECG tracing





**Fig.5: ST segment elevation ECG tracing in AMI**

ECG tracings are indeterminate in a substantial fraction of patients with chest pain at rest but no ST segment elevation (Non ST segment elevation myocardial infarction - NSTEMI), severe unstable angina pectoris, non-Q wave myocardial infarction. Testing for elevated levels of serum cardiac markers that indicate myocardial necrosis when non Q-wave myocardial infarction or NSTEMI is present, usually makes the discrimination between these conditions.

### **Imaging**

Imaging techniques have been used to assist in:

- Ruling out or confirming the presence of acute infarction or ischaemia
- Identifying non ischaemic conditions causing chest pain
- Identifying mechanical complications of acute infarction
- Defining short term and long term prognosis

Imaging methods that are used:

- Cross sectional echocardiography
- Radio nuclide angiography
- Myocardial single photon emission computed tomographic (SPECT) perfusion imaging

Radionuclide techniques enable the physician to assess perfusion at the time of patient presentation. This can be performed with immediate tracer injection because image acquisition can be delayed for 60 to 90 minutes. Quantitative analysis is an advantage of this technique, but accuracy of the studies is high when interpreted by skilled observers.

Some of the limitations of imaging being,

- Requirement of expensive equipment and skilled personnel
- Injury involving greater than 20% of myocardial wall thickness is required before an abnormality can be detected. Also greater than 10 g of myocardial tissue must be injured before a radionuclide perfusion defect can be resolved.

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NOTES



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