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Coral Clinical Systems

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TECHNICAL SERIES

CK and its isoenzymes

The time tested biomarker for diagnosis and monitoring of MI

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Foreword

Coral Cinical Systems is a part of the innovative **TULIP** Group of companies based at Goa, India.

Coral manufactures reagents and kits for general pathological analysis. **Crest Biosystems,** a division of **Coral,** manufactures liquid stable reagents and kits for Clinical Biochemistry. The product range includes most enzymes, substrates and the largest range of kits for the estimation of metals & ions.

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 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** Group has created a strong knowledge base. **Tulip** Group believes that in the knowledge-based society of the 21st 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.

INTRODUCTION

Myocardial Infarction (MI) is the gross necrosis of the myocardium due to interruption of blood supply to the area.

In over 90% of patients with acute MI, an acute thrombus, often associated with plaque rupture, occludes the arteries (usually it is already partially obstructed by an atherosclerotic plaque) that supply the damaged area. Altered platelet function induced by endothelial change in the atherosclerotic plaque presumably contributes to thrombogenesis. Spontaneous thrombolysis occurs in about two thirds of the patients so that, 24hrs later, thrombotic occlusion is found in only about 30% of the patients.

MI is rarely caused by arterial embolization for example in mitral or aortic stenosis, infective endocarditis, and marantic endocarditis. MI has been reported in patients with coronary spasm and otherwise normal coronary arteries. Cocaine causes intense coronary arterial spasm, and users may present with cocaine-induced angina or MI. Autopsy studies and coronary angiography have shown that cocaine-induced coronary thrombosis may occur in normal coronary arteries or be superimposed on pre-existing atheroma.

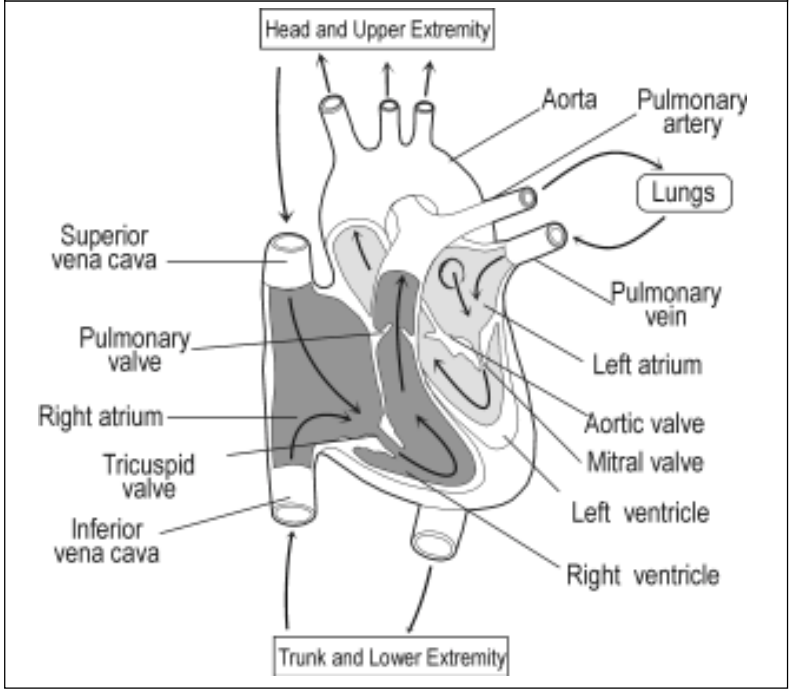
MI is predominantly a disease of the left ventricle (LV), but damage may extend into the right ventricle (RV) or the atria. RV infarction usually results from occlusion of the right coronary or a dominant left circumflex artery and is characterized by high RV filling pressure, often with severe tricuspid regurgitation and reduced cardiac output. Some degree of RV dysfunction occurs in about half of patients with an inferior-posterior infarction, producing hemodynamic abnormality in 10 to 15%. RV dysfunction should be considered in any patient with inferior-posterior infarction and elevated jugular venous pressure with hypotension or shock.

The ability of the heart to continue functioning as a pump relates directly to the extent of myocardial damage. Patients who die of cardiogenic shock usually have an infarct, or a combination of scar and new infarct, of about 50% of LV mass. Anterior infarcts tend to be larger and have

a worse prognosis than inferior-posterior infarcts. They are usually due to occlusion in the left coronary arterial tree, especially the anterior descending artery, whereas inferior-posterior infarcts reflect right coronary occlusion or occlusion of a dominant left circumflex artery.

Transmural infarcts involve the whole thickness of myocardium from epicardium to endocardium and are usually characterized by abnormal Q waves on ECG. Nontransmural or subendocardial infarcts do not extend through the ventricular wall and cause only ST segment and T-wave abnormalities. Subendocardial infarcts usually involve the inner one third of the myocardium where wall tension is highest and myocardial blood flow is most vulnerable to circulatory changes. They may also follow prolonged hypotension. Because the transmural depth of necrosis cannot be precisely determined clinically, infarcts are better classified by ECG as Q wave and non-Q wave.

The Human Heart



CLINICAL PRESENTATION

It is accepted that the term MI reflects a loss of cardiac myocytes (necrosis) caused by prolonged ischemia. Ischemia is the result of a perfusion-dependent imbalance between supply and demand. Ischemia in a clinical setting can be identified from the patient's history and from the ECG. Possible ischemic symptoms include chest, epigastric, arm, wrist or jaw discomfort with exertion or at rest. The discomfort associated with acute MI usually lasts at least 20 min, but may be shorter in duration. The discomfort may develop in the central or left chest and then radiate to the arm, jaw, back or shoulder. The discomfort is usually not sharp or highly localized and may be associated with dyspnea, diaphoresis, nausea, vomiting or light-headedness. The discomfort can develop in the epigastrium, which is often confused with indigestion, arm, shoulder, wrist, jaw or back, without occurring in the chest, but such a pattern is atypical. The discomfort is not affected by moving the muscles of the region where the discomfort is localized, nor is it worsened by deep inspiration. The discomfort is not positional in nature. Symptoms can also include unexplained nausea and vomiting, persistent shortness of breath secondary to left ventricular failure and unexplained weak-ness, dizziness, lightheadedness or syncope, or a combination of these. These symptoms may be noted in association with chest discomfort or they may occur in the absence of chest symptoms. Although many patients have symptoms such as those just described, these complaints may go unrecognized or may be erroneously labeled as another disease entity, such as indigestion or a viral syndrome. Myocardial necrosis may also occur without symptoms.

DIAGNOSIS OF MI

Electrocardiogram (ECG)

An electrocardiogram is usually the first diagnostic test performed. Diagnostic specificity is approximately 100 percent, and a positive tracing, signalled by an elevated ST segment, essentially confirms a diagnosis of AMI. The diagnostic sensitivity, however, has been estimated to range from only 63-82 %. ECG tracings are therefore indeterminate in a substantial fraction of MI patients. One of the major advantages of ECG is that it allows assessment of most nonischemic causes of acute chest pain, such as perimyocarditis, valvular heart disease (aortic stenosis), pulmonary embolism and aortopathies (aortic dissection).

This sensitivity limitation necessitates reliance on biochemical serum cardiac markers for triaging patients with indeterminate ECGs.

Imaging

Imaging techniques have been used to assist in:

- Ruling out or confirming the presence of acute infarction or ischemia in the Emergency Departments
- Identifying nonischemic conditions causing chest pain
- Defining short- and long-term prognosis
- Identifying mechanical complications of acute infarction

The rationale of imaging using echocardiographic or nuclear techniques in patients suspected of having acute ischemia is that ischemia results in regional myocardial hypoperfusion, leading to a cascade of events that can include myocardial dysfunction and ultimately cell death. Some imaging methods are –

- Cross-sectional Echocardiography
- Radionuclide 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. The accuracy of the studies is high when interpreted by skilled observers. These studies also provide simultaneous information on myocardial perfusion and function.

Biomarkers are more sensitive, more specific and less costly than imaging techniques for the diagnosis of myocardial necrosis. Injury involving >20% of myocardial wall thickness is required before a segmental wall motion abnormality can be detected by Echocardiography. In general, >10 g of myocardial tissue must be injured before a radionuclide perfusion defect can be resolved. Neither technique can distinguish ischemia from infarction.

Thus the sensitivity limitation in ECG, requirement of expensive equipment and skilled personnel for imaging, necessitates reliance on biochemical serum cardiac markers for routine diagnosis and follow-up of MI.

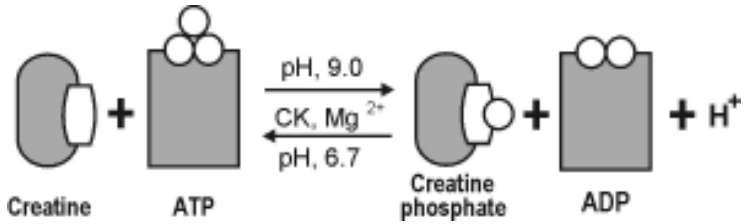
Biochemical markers of myocardial necrosis

Myocardial necrosis results in and can be recognized by the appearance in the blood of different markers released into the circulation due to the damaged myocytes: Creatine Kinase (CK), Myoglobin, cardiac Troponin T and Troponin I, Lactate dehydrogenase (LDH), SGOT as well as many others. Myocardial infarction is diagnosed when blood levels of sensitive and specific biomarkers are increased in the clinical setting of acute ischemia. These biomarkers reflect myocardial damage but do not indicate its mechanism. Thus, an elevated value in the absence of clinical evidence of ischemia should prompt a search for other causes of cardiac damage, such as myocarditis.

CK and its isoenzymes – The time-tested biomarker

Creatine kinase (CK), also called as creatine phosphokinase (CPK), catalyses the reversible phosphorylation of creatine by ATP, as shown in the following equation:

Catalytic action of Creatine Kinase



The optimum pH values for the forward and reverse reactions are 9.0 and 6.7 respectively. The reverse reaction is 2-6 times faster than the forward reaction, depending upon the reaction conditions. Mg^{2+} is the activating ion. The optimal concentration range of Mg^{2+} is quite narrow, and excess Mg^{2+} is inhibitory. Several metal ions and other agents inhibit CK enzyme activity. Some common inhibitors are shown in following figure:

Some common inhibitors of CK catalytic reaction

• Mn^{2+} ions	• Excess ADP	• Iodide
• Zn^{2+} ions	• Citrate	• Bromide
• Ca^{2+} ions	• Fluoride	• Chloride
• Cu^{2+} ions	• Nitrate	• Malonate
• Iodoacetate	• Sulphate	• L-thyroxine
• Sulfhydryl-binding reagents	• Adenylate Kinase enzyme	• Urate

Diagnostic applications of CK and its isoenzymes

- Suspected cardiac muscle disease in patients with clinical and ECG signs typical of myocardial infarction.
- In patients in whom an intervention is contraindicated.
- For evaluation of thrombolytic therapy.
- For risk stratifications in patients with angina.
- Myocarditis.
- Suspected skeletal muscle damage.
- Monitoring cardiac and skeletal muscle disease.

Special findings

Serum CK is particularly valuable for the following reasons:

- Serial total CK has sensitivity close to 100% early in course of Myocardial Infarction (MI).
- It allows early diagnosis because increased levels appear within 3-6 hours after onset of MI and reaches its peak at 24-36 hours.
- It is more sensitive indicator than other enzymes because an increased CK level shows a larger amplitude of change (6-12 times normal).

Isoforms of CK

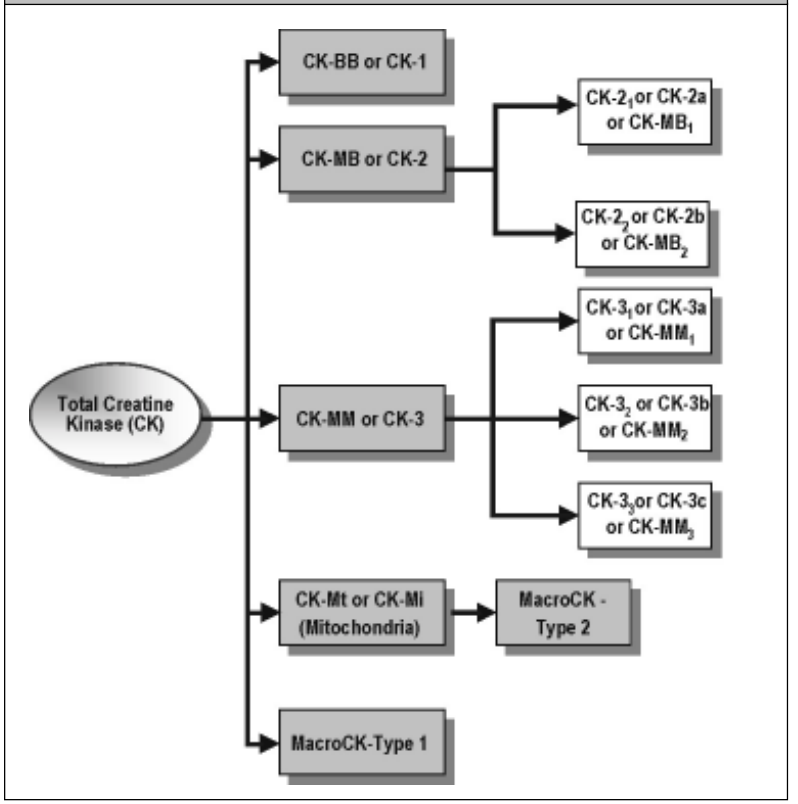
CK is a dimer composed of two subunits, each with a molecular weight of about 40000 Daltons. These subunits – B (brain) and M (muscle), are the products of the loci on chromosomes 14 and 19 respectively. Only three different pairs of subunits exist – CK-BB, CK-MB and CK-MM. Although CK-MB is not an isoenzyme in the true sense but a hybrid molecule consisting of one CK-M and one CK-B subunit, this term is, nevertheless, widely used.

There exists a fourth form of CK that differs from the others based on both immunologic and electrophoretic mobility. This isoenzyme, CK-Mt (or CK-Mi), is located between the inner and the outer membranes

of mitochondria. Its structure is determined by a locus on chromosome 15. Unlike other subunits, no hybridisation (e.g CK-MB) between subunits of CK-Mt has been found.

CK activity may also be found in macromolecular form – macro CK. Macro-CK is sometimes found in hospitalised patients in small proportions. It exists in two forms MacroCK-Type 1 and MacroCK-Type 2. MacroCK-Type 1 is a complex of CK-BB+IgG or CK-MM+IgA. It is associated with gastrointestinal diseases, adenoma, carcinoma, myocardial / vascular diseases and other life-threatening conditions. It often occurs in women older than 50 yrs.

Isoforms of Creatine Kinase



MacroCK-Type 2 is oligomeric mitochondrial CK. It is found predominantly in adults who are severely ill with malignancies / liver disease or in children with myocardial disease.
Both the M and B subunits have a C-terminal lysine residue, but only the former can be hydrolysed by the action of carboxypeptidases normally present in blood. Based on this hydrolysis, several isoforms are possible with different nomenclatures.

Biochemistry and physiology

The two enzymes CK and Adenylate kinase (AK) play a decisive role in the synthesis of ATP, the immediate energy source of the muscle, the CNS and many proliferating tissues. CK is involved in providing energy for the muscle in two ways. In mitochondria, the site of energy production, CK-Mt catalyses the synthesis of Creatine phosphate (CrP) from ATP. The energy-rich CrP is carried in the CrP shuttle from the mitochondria to the cytoplasm where it is converted back into ATP by CK at the sites of energy consumption. Some of the intracellular CK is present in free form, some attached to the respective cell structure. After release of CK into the blood (post-injury/infarct/damage), the enzyme undergoes the so-called postsynthetic modifications producing the forms known as CK-variants. Variants with normal molecularmasses of about 80kDA occur when carboxypeptidase successively removes the C-terminal lysine of both M-chains. The tissue form CK-MM_3 becomes first CK-MM_2 and finally CK-MM_1. The analogous action on the tissue form CK-MB_2 results in CK-MB_1.
As described earlier, variants with higher molecular mass > 200kDA can occur when CK is bound by specific immunoglobulins (macro CK type 1) or when CK-Mt occurs in preferred oligomeric form (macro CK type 2).
The total CK activity measurable in serum is composed of the activities of the cytoplasmic dimeric isoenzymes CK-MM, CK-MB, CK-BB and their post-synthetically modified forms and of the activities of the macro

CK. In healthy individuals the total activity consists mainly of CK-MM while the other CK isoenzymes and variants are only present in trace amounts or are undetectable. If the total activity of CK or of a particular isoenzyme is increased, the isoenzyme pattern can provide information on the underlying organ damage.

Tissue distribution of CK and its isoenzymes

Tissue	CK activity U/gm wet Weight	CK - MM %	CK - MB %	CK - BB %
Skeletal muscle	2500-3000	95-99	1-5	<0.1
Myocardium	500-700			
-Normal		90	5	
-Pathologically altered		70-80	20-30	
Brain	200-300			100
Gastrointestinal tract	120-150			100
Bladder	85			100
Uterus				
-Non Pregnant	165			100
-Pregnant	245		6	94
Placenta	250	19	1	80
Prostate gland	85			100
Lung	15	0-20		80-100

CK activity- SKELETAL MUSCLE DISEASE

Serum CK activity is greatly elevated, at some time during the course of the disease in all types of muscular dystrophy like Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD). Levels up to 50 times the upper limit of normal may be observed.
In progressive muscular dystrophies like DMD/BMD, serum CK activity is the highest at 7-10 yrs of age and falls, as the patient gets older. 50-80% of asymptomatic female carriers of DMD show a 3-6-fold rise in

CK activity, but values may be normal if specimens are obtained after patients have experienced a period of physical inactivity.

High to very high values of CK are noted in viral myositis, polymyositis, acute rhabdomyolysis and malignant hyperthermia. Hence attention needs to be paid when interpreting the results with the clinical findings.

Fructose-bisphosphate aldolase (FBPA) is also present in high concentrations in skeletal muscle and thus could be used to support the conclusions reached from the assay of CK. However, FBPA is also present in many other tissues, though not at such high concentrations. Particularly important in this respect is the high FBPA content of red blood cells. It is therefore essential that a sample of serum to be assayed for CK does not contain lysed red blood cells i.e., the serum should be free from haemolysis.

Apparently, CK-MB replaces part of the CK-MM form in the muscles, resulting in impaired storage of Creatine Phospahte in the muscle.In dystrophies and myopathies, usually only CK-MM is present in serum; but if total CK activity is high, some CK-MB can also be detected (<6%) because diseased skeletal muscle may contain a significant proportion of this isoenzyme owing to the phenomenon of fetal reversion, in which fetal pattern of protein synthesis reappear.

In polymyositis, CK-MM isoform pattern correlates well with the course of the disease. Total CK activity can also be elevated by direct trauma to muscle, including IM injections and surgical interventions, hypo or hyperthermia seizures, neuroleptic malignant syndrome, sepsis, exposure to certain drugs and chronic endocrinopathies.

Activity of Total CK &CK-MB as a result of ACUTE skeletal muscle damage

Disease		Assessment
Acute damage (general)		• Total CK can increase to well above 5000U/L (37^{0}C) in proportion to the damage • The CK-MB activity is < 3% of the total CK • CK-MB concentration shows the same behaviour
Physical activity	**Work / Sport**	• All strenuous exercises increases CK activity. • There is no limit which permits a distinction between a normal reaction and pathological muscle damage. • High activity is observed after some forms of exercise • (Like running downhill) and rhabdomyolysis • Excessive training (like marathon runners) leads to CK-MB activity > 3%
	Diseases	Increased CK activity is observed in diseases like tetany, cramps, Parkinson's disease, cough attacks, status astmaticus, psychosis and delirium tremens.
Intramuscular injections		• Depending upon the substance, volume, concentration and osmolality, IM injections increases CK activity and can interfere with diagnosis of infarction. • Some drugs chlorpromazine, diazepam and promethazine are known to cause muscle damage that causes a rise in
Surgery, Trauma, arterial embolism		• Surgery or trauma (also burns, electrical injuries and pressure necroses) leads to an increase in CK activity proportional to the severity of damage. • CK-MB increase after chest injuries is thought to indicate myocardial damage
Polymyositis, viral myositis,		In acute and sub acute myositides, the CK activity > 20000U/L (37^{0}C)
Medications and drugs of abuse. chemicals		• No dynamic changes in CK activity observed with pharmacological doses • Drugs of intoxication (abuse) like amphetamines, barbiturates, heroin etc. • In extreme cases of rhabdomyolysis or malignant hyperthermia CK activities > 20000 U/L (37^{0}C) are found

Activity of Total CK &CK-MB as a result of CHRONIC skeletal muscle damage	
Disease	**Assessment**
Chronic damage (general)	• CK is elevated depending upon the severity of the desease • CK-MB activity may be >10% of the total CK due to isoenzyme synthesis of the chronically stressed muscle. Thus an elevated CK-MB, in this case, is not due to myocardial involvement.
Muscular dystrophy - DMD and BMD	• Disease with X-Linked recessive inheritance and extreme CK activities > 25000 U/L (37 ^{0}C), particularly in the initial phase. • The CK-MB activity is also increased. This does not confirm myocardial involvement. • In other type of dystrophies like limb-girdle type, ocular type, myotonic dystrophy, CK activities are sometimes within reference range and rarely > 2000 U/L (37 ^{0}C)
Myotonia congenita, Glycogenosis type V, myasthenia gravis, spinal muscular atrophies	These primary myopathies also have CK activities which are within the reference range or only slightly increased depending on the phase of the disease.
Polymyositis,dermatomyositis, ocular myositis.	The CK activities in the chronic forms of myositis are considerably lower than in the acute forms. Activities within the reference are also possible.
Secondary myopathies	Secondary increase in CK activity may be seen in endocrine diseases, intoxications, seizure disorders, paralysis, Lupus erythematosus, multiple sclerosis and taking certain drugs. The CK activity returns to the reference range with improvement in underlying conditions.
Medications and drugs of abuse.	• Pharmacological doses of anti-arrhythmic drugs, beta-blockers, potassium lowering drugs, Lithium, halothane etc. lead to increased CK activity. • Prednisone and related steroids and chemotheraphy agents lead to lower CK activity.

CK activity- HEART DISEASE

Total CK activity shows an increase following MI in which it is increased earlier than other enzymes, beginning at 4-6hrs, peaking on an average at 24hrs and returning to normal within 2-3 days. The area under the peak and slope of the initial rise are proportional to the size of the infarct. False negatives are rare and are usually due to technical errors. False positives may occur, and for accurate diagnosis of MI CK-MB activity should be measured. If the Total CK- activity is raised and CK-MB contributes more than 6% of the activity then MI is considered highly probable.

After MI the maximum CK activity rarely exceeds 7500U/L (37°C). Higher CK activities suggest concomitant skeletal muscle damage. Early reperfusion is marked by a particularly rapid rise and early peak of the CK-MB. A characteristic second peak of CK-MB indicates a second trauma or reinfarction.

As CK-MB also occurs in skeletal muscles, it is, therefore, not absolutely cardiospecific; a diagnostic strategy for careful preselection of patients based on clinical criteria is necessary. It is also necessary, that, the **ratio of total CK to CK-MB activities** be measured. This helps in differential diagnosis of MI and skeletal muscle damage. If this is done, the clinical questions can be answered in most cases.

Activity of Total CK and CK-MB as a result of CARDIAC muscle damage

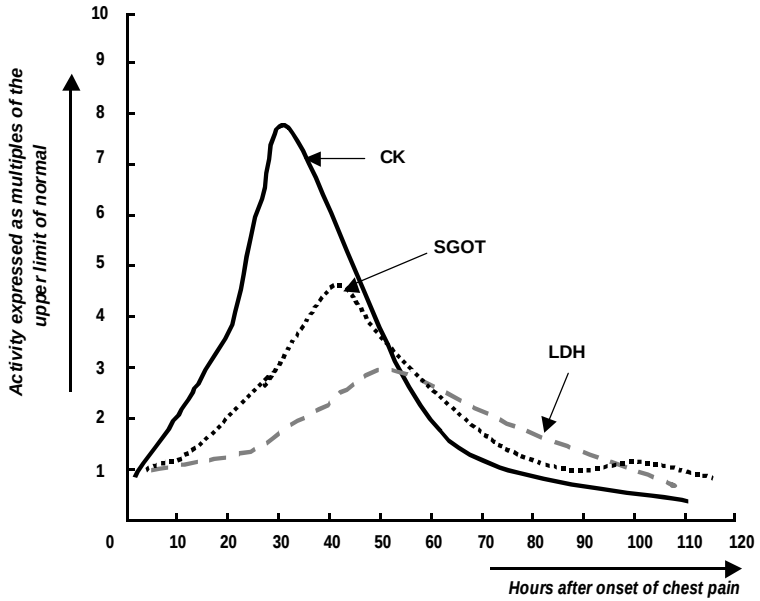
Disease	Assessment
Acute myocardial damage	• The CK increases in proportion to the damage; the CK - MB activity is more than 6% of the total CK. • Total CK activities > 7500U/L (37^{0}C) indicate additional sleletal muscle trauma, e.g. as a result of resuscitation. • CK-MB activities > 25% after immunoinhibition require determination of CK variants.
Myocarditis, Endocarditis and Pericardities	Elevated total CK and CK-MB Levels are typical, particularly in myocarditis; activity-time curves resembling infarction are possible.
Angina pectoris	• No increases in CK-MB are observed in statle angina with immunoinhibition method; unstable angina can show small increases in CK-MB concentration, which are within the reference interval. • Uncomplicated cardiac catheterisation and coronary angiography do not lead to significant increase in CK-MB. • Resuscitation, defibrillation and chest trauma lead to increased CK activities, which correspond to the extent of skeletal muscle damage; if there is myocardial involvement the CK-MB increases.
Diagnostic and therapeutic interventions, trauma	• Surgical procedures involving the myocardium release CK-MB. • CK-MB activities > 50 U/L (37^{0}C) after coronary artery bypass grafting suggest infarction. • Valve replacement and transplantation lead to increases in CK and CK-MB • Stress tests (exercise etc) do not lead to pathological concentrations.
Tachycardia, heart failure and valve defects	These conditions do not usually lead to a significant increase in activity.

CLINICAL ENZYMOLOGY IN MI – A BRIEF ANALYSIS

Enzymological methods have been particularly useful in providing supporting evidence for myocardial infarction (MI) and also monitoring the course of the infarct.

In MI, a coronary artery becomes obstructed and this leads to irreversible damage and necrosis of the heart tissue. Enzymes are released from the necrotic tissue into the plasma; the three enzymes most commonly assayed are creatine kinase (CK), aspartate aminotransferase (SGOT) and lactate dehydrogenase (LDH). Each enzyme shows a different time course for release into the plasma and subsequent disappearance. These differences depend upon the con-

Typical changes in CK, SGOT and LDH activities following MI



centration of each present in the cardiac tissue, their rate of release and their subsequent rate of clearance or degradation. Their **half-life** in the plasma is:

- CK: ≈ 15h
- SGOT: ≈ 17h
- LDH: ≈ 110h

CK is the earliest to be detectable, rising 4-6h after onset of pain, reaching a peak at 24-36h and then rapidly declining. SGOT reaches a peak between 48-60h and LDH between 48-72h, the latter declines more slowly than the former. The prognosis and progress can be assessed by the increase in the activities of these enzymes. CK, which does not occur in the liver unlike SGOT and LDH, can therefore be used to differentiate heart disease from liver disease. Thus, CK can be used in the early detection and prognosis of myocardial infarction.

Comparison of sensitivity and specificity of various test in MI

TEST	SENSITIVITY %	SPECIFICITY %
ECG	63 - 84	100
Increased SGOT	89 - 97	48 - 88
Increased LDH	87	88
LDH1 >LDH2 (on 3rd day after chest pain)	61-90	94-99
Increased Total CK	93 -100	57- 88
Increased CK-MB	94 -100	93 -100

Determination of CK and CK-MB activity alone is not suitable for assessment of the risk of MI. It can be particularly useful in interpretation, if its estimation is accompanied by determination of the isoenzyme (CK-MB), serial tests (enzyme-time curves), or ECG under

exercise testing. The CK-MB results can be interpreted on the basis of the 6% rule, a limit of 12U/L or preferably the dynamic activity time curve of an enzymogram.

The 6% Rule: The decision criterion is an increase in the total CK activity to > 100 U/L (25°C) within the diagnostic time window and a simultaneous increase in CK-MB activity. A CK-MB fraction more than 6% of the total activity is regarded as diagnostic for MI. A fraction less than 6% indicates skeletal muscle damage. The clinical specificity of this rule is high as the number of false positive results caused by the presence of extracardiac CK-MB is small. This high clinical specificity is of course obtained at the expense of some loss of sensitivity in the detection of smaller infarctions.

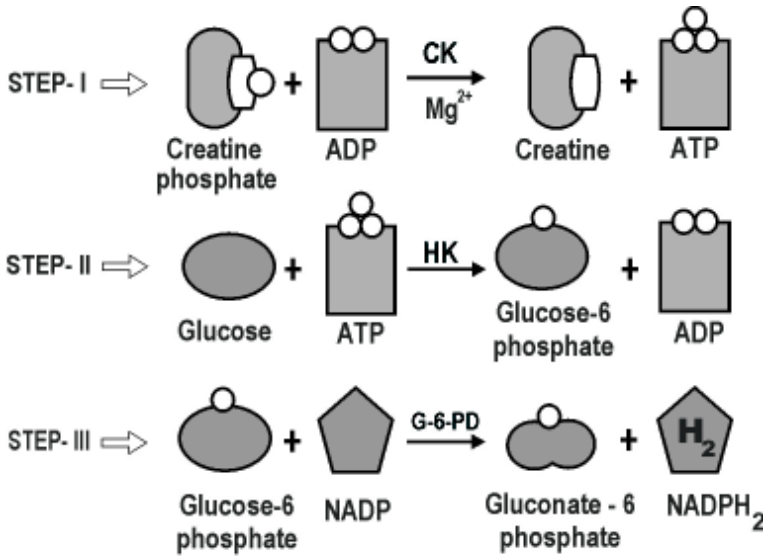
The 12U/L limit: The decision criterion is – increased total CK activity during diagnostic time window and a simultaneous increase in CK-MB activity to > 10U/L (25°C) / 15U/L (30°C) / 24U/L (37°C). This is equivalent to 12U/L CK-B activity (25°C). This strategy is characterised by a higher clinical sensitivity, which also permits identification of small infarctions. This high diagnostic sensitivity is obtained at the expense of some loss of specificity if the prevalence of infarction is low.

Dynamic activity-time curves (cardioenzymogram): If an acute MI is suspected serial determination of total CK and CK-MB activity are carried out so that the changes in the enzyme activities observed in this dynamic condition can be taken into account in the diagnosis and monitoring of treatment. This strategy is characterised by high levels of diagnostic sensitivity, specificity and flexibility as regards to medical and logistic requirements.

METHOD OF DETERMINATION – Total CK

The methodology recommended by International Federation of Clinical Chemistry (IFCC) is a photometric rate assay. The reaction conditions are so robust that the measurements can be performed without problems at reaction temperatures of 37°C, 30°C or 25°C under optimal reaction conditions. The principle of the IFCC method can be depicted as per the following equation

The IFCC principle of Photometric rate assay for CK



CK: Creatine Kinase
HK: Hexokinase
G-6PD: Glucose 6-phosphate dehydrogenase

METHOD OF DETERMINATION – CK isoenzymes

At present only determination of the isoenzyme CK-MB is of clinical significance.

Immunoinhibition method

This is currently the most economical, practical and widely used method in clinical laboratories worldwide. The theoretical basis for the clinical application of the Immunoinhibition method is the assumption that only CK-MM and CK-MB are released into the blood stream after muscle damage. The reagent used contain 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 activity of the dimeric CK-MB molecule is measured, multiplication by a factor of 2 gives the CK-MB activity in the specimen.

Isoenzyme electrophoresis method

The CK isoenzymes are separated on cellulose acetate strips or agarose gel and the activity bands then visualised fluorometrically using the total CK reagent. The fluorescence of the $NAPH_2$ formed in the reaction permits quantitative analysis.

This method is useful only to exactly differentiate between isoenzymes. It is labour intensive, complicated and hence expensive. Moreover, the interference due to AK makes the interpretation difficult. Not practical for routine use in a clinical laboratory.

Immunoassay for determination of CK-MB mass (concentration)

Here CK-MB is determined with a combination of CK-B and CK-M specific antibodies or with CK-MB specific antibodies. The first generation assays use the double antibody technique with CK-M and CK-B specific antibodies. The second-generation assays use CK-MB specific monoclonal antibodies, in some cases, in combination with CK-M and CK-B specific antibodies. The assay format may be EIA, Fluoroscent immunoassay, Luminiscent immunoassay, radio immunoassay etc.

Interferences have been described in these assays too.

- Apart from interference typical of immunoassays (like HAMA-human anti-mouse antibody), specific interference by high concentrations of CK-MM and CK-BB or by CK-B binding autoantibodies has been demonstrated.
- Lack of a universal standardisation has influenced the non-comparability of results.
- The data on reference ranges, decision limit and specific activities are therefore not uniform but vary from reagent-to-reagent.

CRITICAL DETERMINANTS OF CK AND CK-MB REAGENT SYSTEM DESIGN

Appropriate activator

During storage, CK is rapidly inactivated by the oxidation of the sulphydryl groups at the active site of the enzyme. The need to reactivate the enzyme has been demonstrated and the various sulphydryl reagents like cysteine, dithiothreitol, glutathione, 2-mercaptoethanol and N- acetylcysteine have been used as an activator in the reagent. But:

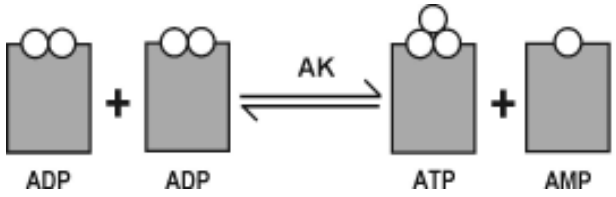
- Cysteine deteriorates rapidly in solution.
- Dithiothreitol causes turbidity by precipitation of albumin.
- Glutathione is unstable since it allows interference by glutathione reductase released from red cells in haemolysed specimens.
- 2-mercaptoethanol cause turbidity and its decomposition products inhibits CK.

N- acetylcysteine (NAC) suffers none of these shortcomings and is accepted to be the best thiol (sulphydryl) activator for maintenance and reactivation of CK activity in serum. The optimum concentration of NAC is of utmost importance. A small imbalance in its concentration may cause free cysteine to be released. This may cause the reagent system to behave erroneously.

Adenylate Kinase (AK) interference

AK is present in many tissues and cells including skeletal and cardiac muscle, liver, brain, and erythrocytes. The enzyme is also present in normal serum and may be markedly raised in haemolysed specimens. Adenylate kinase deficiency in the erythrocytes, a rare genetic disorder, is associated with haemolytic anaemia. AK catalyses the reaction as shown in figure.

Adenylate Kinase catalytic action



ATP : Adenosine Tri-phosphate
ADP : Adenosine Di-phosphate
AMP : Adenosine Mono-Phosphate

The ATP generated in this catalytic action of AK enters the reaction of the CK assay and leads to an apparent increase or false positive values of CK.

AK activity can be inhibited by adding:

- Fluoride or
- AMP or
- Diadenosine pentaphosphate (DAPP)

Although Fluoride is an effective inhibitor, it may form insoluable magnesium fluoride with magnesium ion in CK assay. AMP alone can competitively inhibit product formation ($2ADP \rightleftharpoons ATP+AMP$). Thus, to adequately inhibit AK from red cell and muscle and to ensure better results, an **optimised blend** of AMP and DAPP should be used. DAPP competitively inhibits the AK of muscle and erythrocytes while AMP inhibits AK derived from liver and kidney.

Fildes and Harris (1966) found electrophoretic variation in red cells

and defined 3 phenotypes, designated AK1, AK2-1 and AK2. In India, in majority of the populations the AK1 gene occurs in high frequencies, approaching 90% and above. Thus, this demographic pattern also indicates that, in India, to get accurate results, a CK assay systems should contain optimised blend of AK inhibitor.

Cation inhibitor

CK is readily inhibited by polyvalent cations such as Ca^{2+}. This can be prevented by addition of EDTA. EDTA also helps in the protection of the thiol reagent from oxidation by metal ions.

Anti CK-M antibody used in Immunoinhibition method

The anti CK-M antibodies quantitatively inhibit all M subunits of the sample within the incubation time. These antibodies should have high specificity and corresponding affinity.

Since, the specificity of the antibody used as anti-CK-M reagent is of utmost importance, a brief discussion on antibody structure would be worthwhile.

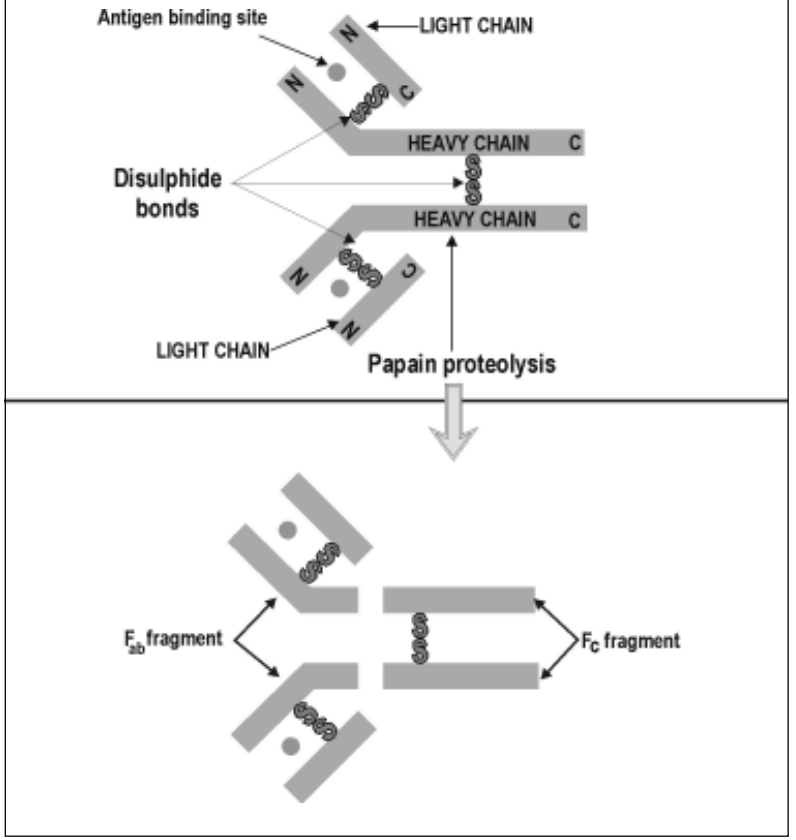
The antibody molecule is made up of two identical heavy and two identical light chains held together by interchain disulphide bonds. These chains can be separated by reduction of the S-S bonds and acidifications. The exposed hinge region is extended in structure due to high proline content and therefore vulnerable to proteolytic attack. The molecule is split by papain to yield two identical fragments, each with a single combining site for antigen, called F_{ab} fragment (Fragment antigen binding). A third fragment, known as F_c fragment, is also formed. The F_c fragment lacks the ability to bind to antigens.

The F_{ab} fragment of the "Y–shaped" antibody carries the paratopes, the binding sites that bind to the epitopes on the CK-M antigen. In an intact antibody molecule F_{ab} is present along with the F_c fragment.

Because the F_c fragment binds to complements, certain bacterial

proteins (like staphylococcal Protein A) and other non-specific molecules such as heterophillic antibodies, the reagent for inhibition should preferably be devoid of the F_c fragment. Since, the F_{ab} fragment is specific with high antigen binding capacity, to achieve adequate inhibition of CK-M activity without interference, a reagent system should preferably use the purified F_{ab} fragment in the anti-CK-M reagent.

Antibody model showing proteolytic fragments



Liquid reagent system

In the CK /CK-MB assay, the calculations to arrive at the results involve a multiplication step with a relatively high value of factor - over 6000 for CK-MB and over 8000 for total CK. This means a small change in the optical density (OD) would result in a significant change in the final results of CK /CK-MB activities.

In a dry powder–based assay system, the reagent powder is reconstituted with deionised water or buffer to make the working reagent. This reconstituted reagent may contain suspended microparticles, though apparently, it may seem to be **optically clean**. These microparticles are heterogeneous molecular aggregates derived from the lyophilised reagent. During the course of photometric measurement, the passage of light through the solution cause "excitation" of these microparticles leading to erroneous OD readings. This error is compounded by multiplication with the high factor. Consequently, the final results of CK /CK-MB activities may show unexpected variation leading to erroneous/inadequate clinical decisions.

This problem can be solved by filtration of the reconstituted reagent through a 0.22 micron sterile filter. But, in a routine clinical laboratory this may not be a practical option. Thus, the best option is to use ready-made liquid reagent system available with some manufacturers. These reagents are optically clean and do not cause the interference from suspended microparticles.

OTHER BIOMARKERS OF MI

Myoglobin

Myoglobin, a relatively small (17.8 kDa) heme protein that transports oxygen within muscle cells, constitutes about 2 percent of muscle protein in both skeletal and cardiac muscle. Thus, the marker is not cardiospecific. Because of its low molecular weight, myoglobin is rapidly released into the circulation and is the first marker to exhibit rising levels after an AMI. The advantages of myoglobin in the early diagnosis of MI are the high early sensitivity and the possibility of rapidly assessing the success of thrombolytic therapy.

The biologic half-life of myoglobin is extremely short (10-20 min) vis-à-vis CK (approx.15 hrs) and CK-MB (approx. 12 hrs). Thus the diagnostic window available is small compared to CK or CK-MB.

Cardiac Troponin-I (cTnI)

Troponin is a complex consisting of three single-chain polypeptides:

- Troponin-I, which prevents muscle contraction in the absence of calcium
- Troponin-T, which connects the troponin complex to tropomyosin
- Troponin-C, which binds calcium

Together with tropomyosin and under the influence of calcium, troponin regulates muscle contraction. The cardiac muscle-specific isoform cTnI (24 kDa) exhibits approximately 60 percent homology with the skeletal isoforms (sTnI), and has a unique 31 amino acid extension of the N-terminus. After myocardial infarction, elevated cTnI levels appear within 3 to 6 hours. Levels peak within 14 to 20 hours, and return to normal after 5 to 7 days. Cardiac Troponin - I is 100% cardiospecific. Unfortunately, the standardization of cardiac troponin - I assays is problematic. Up to 20-fold variation of serum cTnI mass determina-

tions may be observed for a given patient sample when measured by different assay systems. As a result, significant ambiguity often exists in the clinical interpretation of serum cTnI concentrations. Recent efforts have been directed toward the biochemical standardization of cTnI assays. However, the heterogeneous nature and biochemical complexity of the serum forms of cTnI and differences of the epitope recognition by the various methods have hindered the harmonization of serum cTnI assays.

GLOSSARY OF TERMS

Angina pectoris

Angina pectoris is the medical term used to describe chest pain caused by poor blood flow to certain areas of the heart muscle. Often, the name is shortened to angina.

Angiography

It is a description of blood vessels and lyphatics. The relative inaccessibility of the blood vessels and lyphatics for physical examination requires investigation with other means, primarily radiologic examinations that include lung scintigraphy, conventional or digital subtraction pulmonary angiography (DSPA), pulmonary computed tomography angiography (PCTA), and pulmonary magnetic resonance angiography (PMRA).

Aortic stenosis

Aortic stenosis (AS) is the obstruction of blood flow across the aortic valve. AS has several etiologies: congenital unicuspid or bicuspid valve, rheumatic fever, and degenerative calcific changes of the valve.

Arterial embolism

Embolism is the obstruction of a blood vessel by foreign substances or a blood clot. If the blood vessel concerned is an artery it is called arterial embolism. Obstruction in the pulmonary artery is called pulmonary embolism (PE). In PE the blood clot is in the lung. PE is usually caused by an embolus from thrombosis in lower extremities, pelvis, arms, or heart. When a clot forms in the legs or arms, it is referred to as a deep venous thrombosis (DVT).

Atheroma

Fatty degeneration or thickening of the walls of the larger arteries occurring in atherosclerosis.

Atria

A chamber or cavity communicating with another structure.

Defibrillation

Fibrillation is a small, local, involuntary, muscular contraction, due to spontaneous activation of single muscle cells or muscle fibers. Defibrillation is the termination of atrial or ventricular fibrillation, usually by electroshock.

Delirium tremens

Delirium tremens (DTs) is a severe manifestation of alcohol withdrawal. Pearson first described it in 1813 as an acute psychosis following abstinence from alcohol. Although it only occurs in a relatively small number of patients who undergo alcohol withdrawal, it can be fatal. DT is a medical emergency that requires prompt recognition and treatment.

Diaphoresis

Profuse sweating.

Dyspnea
Air hunger resulting in laboured or difficult breathing, sometimes accompanied by pain.
Endocarditis
Endocarditis refers to an infection of a heart valve. Although several different types of organisms can cause endocarditis, it is usually caused by a bacterial infection.
Endocardium
Serous lining membrane of inner surface and cavities of the heart. It is continuous with the intima or interior coat of arteries.
Epicardium
The inner or visceral layer of the pericardium, which forms a serous membrane forming the outermost layer of the wall of the heart.
Epigastrium
Region over the pit of the stomach.
Glycogenosis type V
Also known as McArdle disease. It is caused by a deficiency of myophosphorylase (alpha-1,4-glucan orthophosphate glycosyl transferase), which normally initiates glycogen breakdown by removing 1,4-glucosyl groups from the glycogen molecule with release of glucose-1-phosphate.
Malignant hyperthermia
Malignant hyperthermia (MH) is a genetic disease, which may result in rhabdomyolysis. In this disease, episodes of hyperthermia and rhabdomyolysis are triggered by exposure to volatile anaesthetics such as halothane, or succinylcholine, a depolarising muscle relaxant. MH appears to be an autosomal dominant condition.
Marantic endocarditis
Endocarditis is the inflammation of the lining membrane of the heart. It is usually confined to the external lining of the valve, sometimes to the lining membrane of its chambers. May be due to invasion of microorganisms or an abnormal immunological reaction. Marantic endocarditis, also known as Nonbacterial Thrombotic Endocarditis (NBTE), is the endocarditis due to non-bacterial causes.
Muscular Dystrophy
Muscular dystrophy (MD) is a collective group of inherited noninflammatory but progressive muscle disorders without a central or peripheral nerve abnormality. The disease affects the muscles, with definite fiber degeneration but without evidence for morphologic aberrations. Dystrophin was found to be at the root of the disorder. Defects in the genetic code for this 427-kDa-muscle protein resulted in the various manifestations commonly associated with MD.
Myasthenia gravis
Myasthenia gravis (MG) is an acquired autoimmune disorder characterized

clinically by weakness of skeletal muscles and fatigability on exertion. Thomas Willis reported the first clinical description in 1672.
Myocarditis
Myocarditis is an inflammatory disease of the myocardium with a wide range of clinical presentation, from subtle to devastating. It usually manifests in an otherwise healthy person and can result in rapidly progressive (and often fatal) heart failure and arrhythmia.
Myocardium
The middle layer of the walls of the heart composed of cardiac muscle.
Myocytes
Muscular tissue cells.
Myositis
Inflammation of muscle tissue especially voluntary muscles.
Myotonia congenita
A benign disease characterised by tonic spasms of the muscles induced by voluntary movements; usually congenital and transmitted from one generation to another.
Ocular myositis
Inflammation of muscle tissues of the eye.
Parkinson's disease
Parkinson disease (PD) is a progressive neurodegenerative disorder associated with a loss of dopaminergic nigrostriatal neurons. It is named after James Parkinson, the English physician who described the shaking palsy in 1817.
Pericarditis
Pericarditis is the inflammation of the lining of the heart. Pericarditis is a commonly found syndrome in the hospital setting, particularly in the coronary care unit. It most often affects men aged between 20-50 yrs, usually following respiratory infections.
Polymyositis
Polymyositis (PM) is an inflammatory muscle disease of unknown etiology. PM, dermatomyositis (DM), and inclusion body myositis are the major members of a group of syndromes called the idiopathic inflammatory myopathies. In much of the literature, PM and DM are grouped as one disease.
Regurgitation
A backward flowing, as in the return of solids or fluids from stomach or the backward flow of blood through a defective heart valve.
Resuscitation
Restoration to life of one apparently dead. Also may be used for cardiopulmonary resuscitation (CPR). CPR is the re-establishing of heart and lung action by artificial ventilation and closed-chest cardiac massage, after cardiac arrest or apparent sudden death resulting from electric shock,

drowning, respiratory arrest, and other causes.

Rhabdomyolysis

Rhabdomyolysis is the breakdown of muscle fibers with leakage of potentially toxic cellular contents into the systemic circulation. The final common pathway of rhabdomyolysis may be a disturbance in myocyte calcium homeostasis.

Spinal muscular atrophy

The spinal muscular atrophies (SMAs) are a clinically and genetically heterogeneous group of disorders. They are characterized by primary degeneration of the anterior horn cells of the spinal cord and often of the bulbar motor nuclei without evidence of primary peripheral nerve or long-tract involvement.

Status asthmaticus

Status asthmaticus is a medical emergency in which asthma symptoms are refractory to initial bronchodilator therapy in the emergency department. Patients complain of chest tightness, rapidly progressive shortness of breath, dry cough, and wheezing. Typically, patients present a few days after the onset of a viral respiratory illness or following exposure to a potent allergen or irritant.

Syncope

Syncope (fainting) is a sudden fall of blood pressure resulting in loss of consciousness.

Tachycardia

Abnormally rapid heart rate.

Tetany

A syndrome manifested by sharp flexion of the wrist and ankle joints, muscle twitching, cramps and convulsions due to abnormal calcium metabolism and occurring in parathyroid hypofunction, vitamin D deficiency, alkalosis and as a result of alkaline salts.

Thrombogenesis

The formation of a blood clot.

Transmural

Across the wall of an organ or structure, as in transmural myocardial thrombosis, in which the tissue in the entire thickness of the portion of the cardiac wall is affected.

Triage

Triage is a French word that literally means, “to separate”. Medical Triage refers to the classification of patients or casualties to determine priority of need and proper place of treatment.

Viral myositis

Viral myositis is an acute, subacute, or chronic infection of skeletal muscle. Once considered a tropical disease, it is now seen in temperate climates

as well, particularly with the emergence of HIV infection. In addition to HIV, other viruses, bacteria, mycobacteria, fungi, and parasites can cause myositis.

References and Suggested Reading

1. Alpert and Thygesen, et al. Myocardial Infarction Redefined. Journal of the American College of Cardiology. 36(3): 959–69. 2000.
2. Laurino Joseph P. Review: Troponin I: an Update on Clinical Utility and Method Standardization. Ann Clin Lab Sci 30(4): 412-421.2000.
3. Nicholas C. Price and Lewis Stevens. Fundamentals of Enzymology. Second edition. Oxford Science Publications. 1998.
4. Lothar Thomas. Clinical Laboratory Diagnostics - Use and assessment of Clinical Laboratory Results. TH-Books. 1998.
5. Guyton AC and Hall JE. Textbook of Medical Physiology. 9th edition. WB Saunders & Co. 1996.
6. Varley’s Practical Clinical Chemistry. 6th edition. 1996.
7. TeitzTextbook of Clinical Chemistry. Second Edition, WB Saunders publishing. 1994.
8. Singh KS et al. The Biological Variation in Indian Populations. National series Volume X. Anthropological Survey of India. Oxford University Press. 1994.
9. Thomas CL. Taber’s cyclopedic medical dictionary. 17th edition. Jaypee Brothers Medical Publishers. 1993.
10. Wallach J. Interpretation of Diagnostic Tests. A synopsis of Laboratory medicine. Fifth edition. 1992.
11. Roitt IM. Essential Immunology. 6th edition. Blackwell Scientific Pub.1988
12. Dorlands Pocket Medical Dictionary. 23rd Edition, 1983.Oxford and IBH publishing Co.
13. The Merck Manual of Diagnosis and Therapy. Section 16. Cardiovascular Disorders. Chapter 202. Coronary Artery Disease.