Clinical Enzymology

Isoenzymes

- These are multiple molecular forms of an enzyme catalysing the same reaction
- They differ in physical and chemical properties like: structure, Km, Vmax, electrophoretic mobility and susceptibility to inhibitors.
- Isoenzymes are synthesised in different tissues , sometimes within a single cell but different organelle.

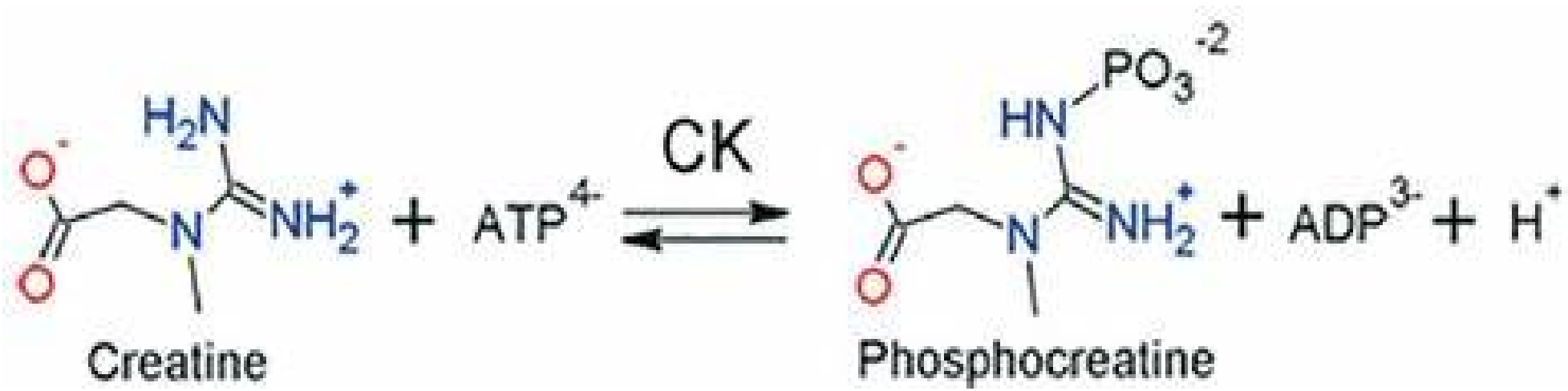
- Isoenzymes are oligomeric proteins.

- They have quarternary structure(two or more subunits)
- Isoenzymes are products of different genes.
- The genes may be located in different chromosomes e.g. salivary and pancreatic amylase.

- Isoenzyme estimation is used in diagnosis of organ dysfunction

Creatine Kinase

- **Catalyses phosphorylation of creatine**



- Normal levels: 15-100 U/L

- CK is a dimer consisting of 2 subunits : B(for brain) and M (Muscle)

- 3 isoenzymes exists: CKBB (1%) ,CKMB (5%) ,CKMM (80%).

Three Iso-enzymes can be separated by electrophoresis.

CPK-1 (also called CPK-BB) is found mostly in the brain & lungs.

CPK-2 (also called CPK-MB) is found mostly in the heart.

CPK-3 (also called CPK-MM) is found mostly in skeletal muscle.

Clinical significance

- In myocardial infarctions CK MB starts to rise within 3-6 hours of onset of infarction.

- It is useful for diagnosis of AMI

CPK & Muscle diseases

- CPK level is elevated in muscular dystrophy (500-1500U/L)
- CPK level is highly elevated in crush injury, fracture & acute cerebrovascular accidents.

- *Estimation of total CPK is employed in muscular dystrophies & CPK-MB isoenzyme is estimated in myocardial infarction.*

CK-Mi (Mitochondrial CK-Isoenzyme)

It is an atypical isoenzyme of CK

It is present bound to the exterior surface of inner mitochondrial membrane of muscle, liver & brain.

It is not present in normal serum.

Clinical significance

- It is present in serum when there is extensive tissue damage causing breakdown of mitochondrial & cell wall.

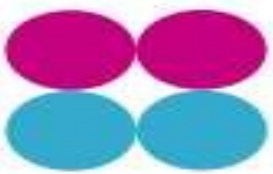
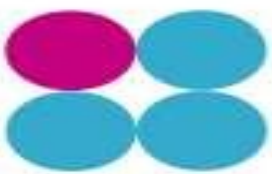
- Its presence in serum indicates cellular damage, seen in malignancies.

Lactate Dehydrogenase

- It catalyses conversion of lactate to pyruvate and vice versa
- Normal levels 100-200 U/L
- 4 subunits of 2 types : hence 5 isoenzymes
- M(muscle) and H (heart) are encoded by different genes

- These isoenzymes are separated by cellulose acetate electrophoresis at pH 8.6

LDH isoenzymes

Isoenzymes of lactate dehydrogenase	Highest levels found in the following:	Isoenzymes of lactate dehydrogenase	Highest levels found in the following:
		 H_2M_2 (LDH_3)	Brain, lung, white blood cells
 H_4 (LDH_1)	Heart, kidneys	 HM_3 (LDH_4)	Lung, skeletal muscle
 H_3M (LDH_2)	Red blood cells, heart, kidney, brain	 M_4 (LDH_5)	Skeletal muscle, liver

Isoenzyme name	Composition	Electrophoretic migration	Present in	Elevated in
LDH 1 Heat resistant	(H_4)	Fastest moving	Myocardium, RBC, kidney	myocardial infarction
LDH2 Heat resistant	(H_3M_1)		Myocardium, RBC, kidney	Kidney disease, megaloblastic anemia
LDH3	(H_2M_2)		brain	Leukemia, malignancy
LDH4 Heat labile	(H_1M_3)		Lung, spleen	Pulmonary infarction
LDH5 Heat labile Inhibited by urea	(M_4)	Slowest moving	Skeletal muscle, Liver	Skeletal muscle and liver diseases

Clinical significance

- In healthy individuals LDH2>LDH1
- This pattern is reversed in MI within 12-24 hours of the attack: flipped pattern of LDH
- Elevated LDH are observed in hemolytic anemias, leukemias, germ cell tumors.

- LDH 4 is increased in pulmonary embolism
- LDH 5 in muscular dystrophies
- LDH levels are elevated in CSF in bacterial meningitis

Alkaline Phosphatase (ALP)

- ALP is nonspecif ic enzyme.
- It hydrolyses aliphatic, aromatic or heterocyclic compounds containing phosphate group.
- Optimum pH-9 &10 & it is activated by Mg2+ &Mn.

- Zn is a constituent of ALP.

- It is produced by osteoblasts of bone, and is associated with the calcif ication process.
- It is localised in cell membranes : ecto- enzyme.
- It is associated with transport mechanisms in liver, kidney & intestinal mucosa.

ALKALINE phosphatase

- Normal range; 40-125 U/L
- 6 isoenzymes are known: liver, bones, kidney, intestine, placenta, WBC.
- Vary in the content of sialic acid. Hence not true isoenzymes
- Placental isoenzyme is most heat stable
- Bone isoenzyme is heat labile

Clinical significance

- Increased ALP occurs in bone diseases and hepatobiliary diseases
- Very high levels: 10-25 times upper normal limit is found in Paget's disease, rickets ,osteomalacia, osteogenic cancers and hyperparathyroidism
- Transient elevation in fractures(during healing)
- Not increased in osteoporosis

- 10-12 times upper limit in obstructive jaundice
- ALP levels are elevated in normal pregnancy
- An isoenzyme identical to placental ALP is found in patients with lung cancer: REGAN isoenzyme

- The leukocyte alkaline phosphatase (LAP) is signif cantly decreased in chronic myeloid leukemia & it is increased in lymphomas.

Methods of separation of isoenzymes

- **Electrophoresis:** agarose gel electrophoresis is used to separate LDH isoenzymes.
- LDH 1 is the fastest moving LDH5 is slowest moving

- **Heating:** placental isoenzyme of ALP is heat stable while bone isoenzyme is heat labile.

- **Enzyme inhibition:** placental isoenzyme is inhibited by phenylalanine.

- **Immunoinhibition:** this is done for separation of creatine kinase isoenzymes. Antibodies against B is used to precipitate CKBB and CKMB.

CARDIAC BIOMARKERS

- A biomarker is a clinical laboratory test which is useful in detecting dysfunction of an organ

Uses of cardiac biomarkers:

- 1 Diagnosis:
- A. Acute coronary syndromes
- B. congestive cardiac failure
- 2 Monitoring the success of therapy
- 3 For prognosis.

When to test for cardiac biomarkers

- Cardiac biomarkers are tested when the patient presents with:
- Acute chest pain
- Unstable angina
- Suspicious ECG
- History suggestive of AMI
- Following Cardiac bypass surgery or angioplasty

CLASSIFICATION OF CARDIAC BIOMARKERS

- **Biomarkers of myocardial injury**
  - markers of myocardial necrosis
  - markers of myocardial ischemia e.g: enzymes like CKMB, LDH,AST proteins like troponin, myoglobin

- **Biomarkers of haemodynamic stress:** BNP, pro BNP
- **Inflammatory markers:** hs CRP, Homocysteine

Cardiac markers for myocardial ischemia

- **Enzymes:**
- CKMB
- Lactate dehydrogenase
- Aspartate transaminase

- **Proteins:**
- Myoglobin
- Troponins(TnT and TnI)
- Ischemia modified albumin

Creatine kinase

- It catalyses the conversion of creatine to creatine phosphate.
- CK has 3 isoenzymes of which CKMB is specific for heart muscles.

- CKMB values are increased in acute myocardial infarction.

- It starts rising within 3-6 hours of onset of MI.
- Peak values are seen at 18-24 hours
- Returns to baseline at 72 hours.

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

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

*A relative index exceeding 3 is indicative of AMI

Advantages

- **It can detect very early cases where ECG changes are ambiguous.**

- **Not increased in hemolysis (unlike LDH and AST) or in CCF.**
- **Rise of CKMB proportional to size of infarct**

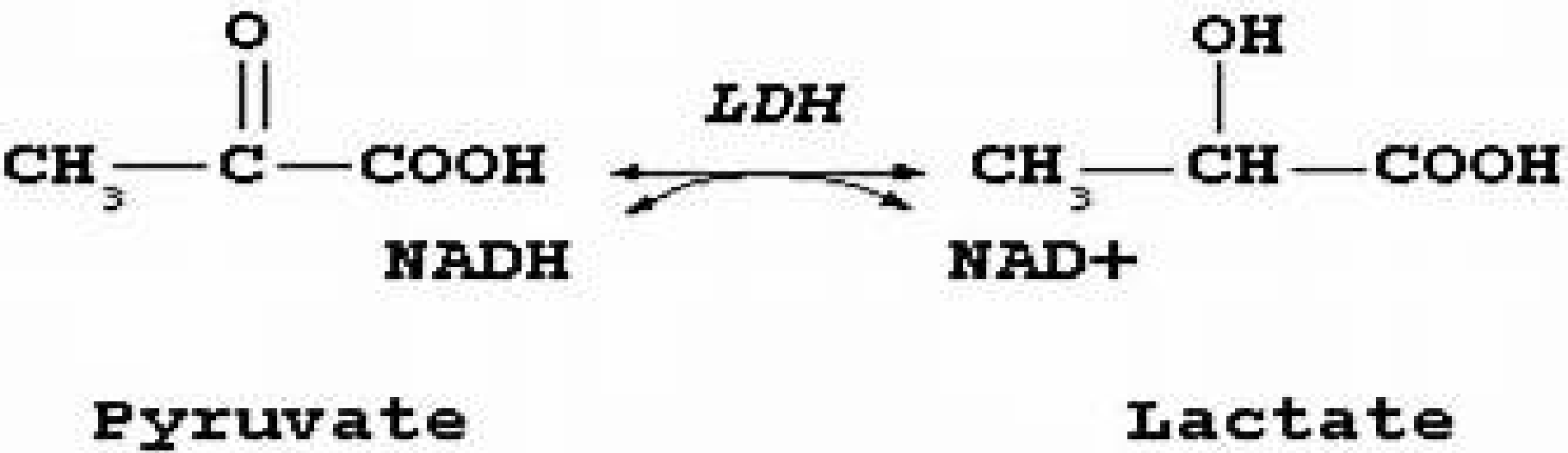
- **Useful in early diagnosis of MI**
- **Useful in reinfarction cases** if level normalizes and then increases again

Disadvantage

- Not useful in delayed admissions (after 48 hours)
- Values rise after cardio pulmonary resuscitation
- High values obtained after:
- Defibrillation
- Cocaine abuse
- Blunt chest trauma

Lactate dehydrogenase

- This enzyme catalyses the conversion of lactate to pyruvate and vice versa



- LDH 1 isoenzyme is elevated 10 -12 hours after AMI , peak values are seen at 48 hours and returns normal at 7-10 days

- Usually LDH 2 is more than LDH 1
- But the ratio is reversed after AMI.

- This is called flipped pattern of LDH

- LDH 1: LDH 2 > 0.75 is diagnostic of AMI

ADVANTAGES

- LDH1: 2 ratio is specific for AMI

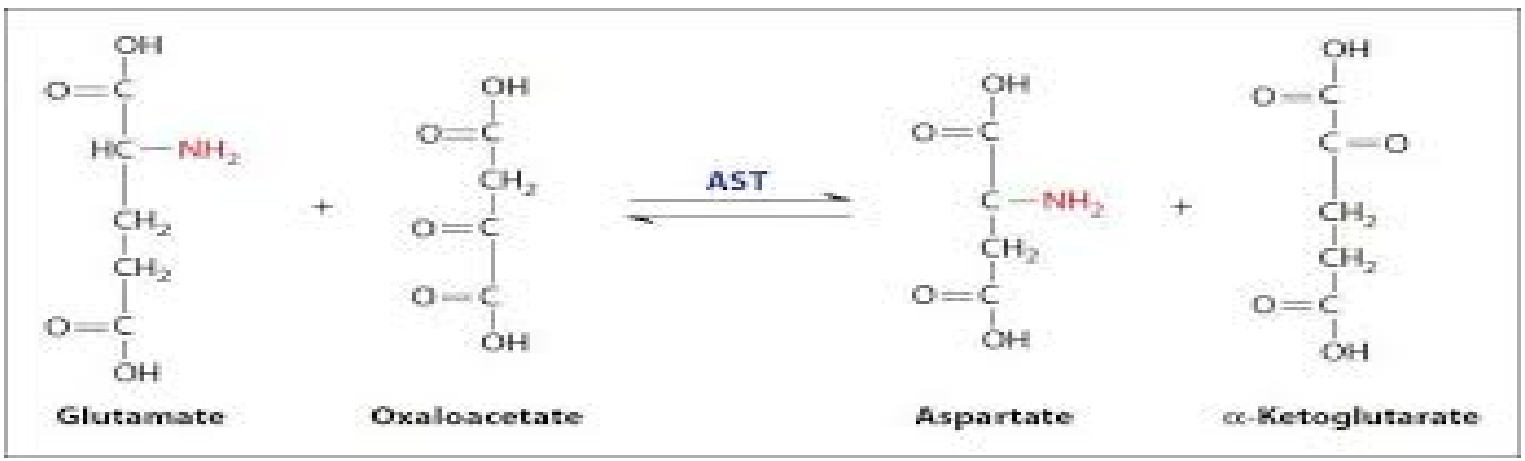
- LDH levels remain elevated upto 2 weeks.
- So AMI with late admissions can be diagnosed

DISADVANTAGES

- LDH is present in almost all the tissues
- So it is not specific for cardiac muscle
- Also LDH rises only after 10 hours. For the treatment of AMI first 6 hours is vital to save the cardiac muscle.
- Thrombolysis is not successful if done after 6 hours
- Reinfarction cannot be diagnosed within 2 weeks

Aspartate amino transferase

- AST catalyses the following reaction



Detected in blood: 6-12 hours after onset of MI attacks (not for early cases)

Reaches a maximum peak level: in 30 hours

Returns to normal : after 2 - 6 days after MI

- AST was the earliest marker enzyme used for diagnosis of AMI
- However it is non specific so it is not done nowadays

MYOGLOBIN

- Small-size heme protein found in all tissues mainly assists in oxygen transport

- It is released from all damaged tissues like cardiac tissue, renal tissues, skeletal muscles
- Therefore it is not specific to heart muscles.
- Increases often occur more rapidly than Trop and CK
- It is a negative predictor of MI, i.e normal levels rules out MI

- Timing:
  - Earliest Rise: 1-3 hrs
  - Peak 6-9 hrs
  - Return to normal: 12 hrs

CARDIAC TROPONINS

- Troponin is a complex of three regulatory proteins that is integral to muscle contraction in skeletal as well as cardiac muscle

- Troponin is attached to the tropomyosin sitting in the groove between actin f laments in muscle tissue

TROPONINS

- Troponin has three subunits, TnC, TnT, and TnI
  - Troponin-C has calcium binding ability and has no diagnostic value
  - Troponin-T binds the troponin tropomyosin complex,
  - Troponin-I is an inhibitory protein
- Cardiac Troponin I and T are specific for heart muscle.

Cardiac TROPONIN I

1. Cardiac Troponin I (cTnI) is a cardiac muscle protein

2. cTnI has an additional aminoacid residues on its N-terminal that are not exist on the skeletal form.

3. The half life = 2~4 hours.

4. Serum level increases within 2-8 hours

- cTrop I is released in blood within 2-8 hours of onset of MI.
- PEAK AT 14-24 HOURS, REMAIN ELEVATED FOR 3-7 DAYS

Cardiac TROPONIN T

1. Cardiac Troponin T (cTnT) is present in fetal skeletal muscle.

2. In healthy adult skeletal muscle cTnT is absent.

3. Biological half life and early serum increases of cTnT are similar to that of cTnI.

- Normally there is no circulating troponin
- So any rise is diagnostic of myocardial injury

Timing Summary

TEST	ONSET	PEAK	DURATION
CK/CK-MB	3-6 hours	18-24 hours	36-48 hours
Troponins	2-8 hours	18-24 hours	Up to 10 days
Myoglobin	1-3 hours	6-7 hours	24 hours
LDH	6-12 hours	24-48 hours	6-8 days

High sensitivity troponin

- Elevated troponin is also found in stroke, acute coronary syndromes, pulmonary embolism,heart failure etc
- To eliminate such causes high sensitivity troponin has been developed
- It rises within 3 hours of AMI
- Two measurements are required for diagnosis , first at onset and second at 6 hours.
- If needed 3rd sample after 12 hours to be checked
- conc > 4 ng/L is diagnostic

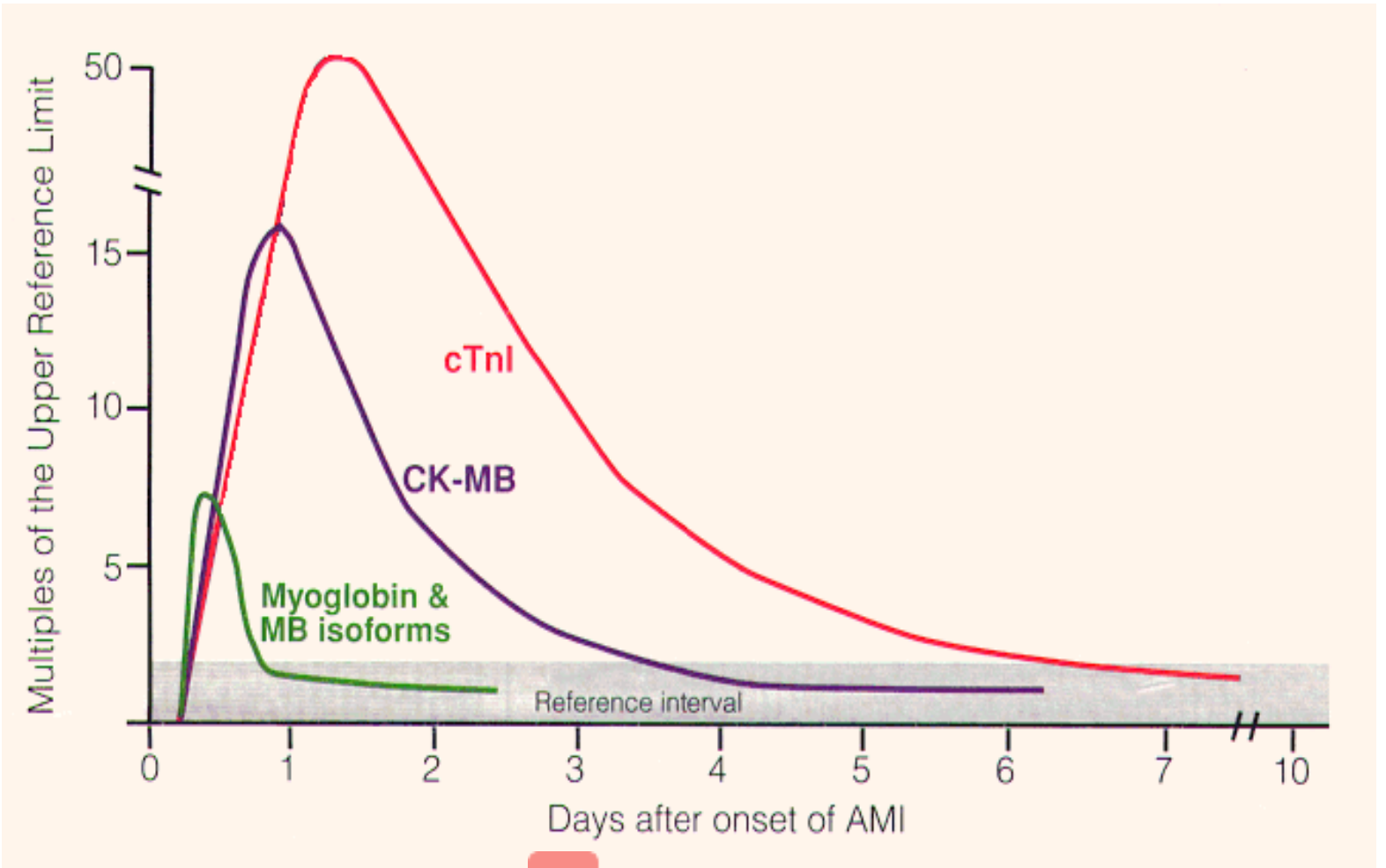
Ischemia Modified Albumin (IMA)

- A novel marker of ischemia, is produced when circulating serum albumin contacts ischemic heart tissues

- Mechanism- due to structural change in the amino terminal end of albumin

- IMA levels rise within 6 hours

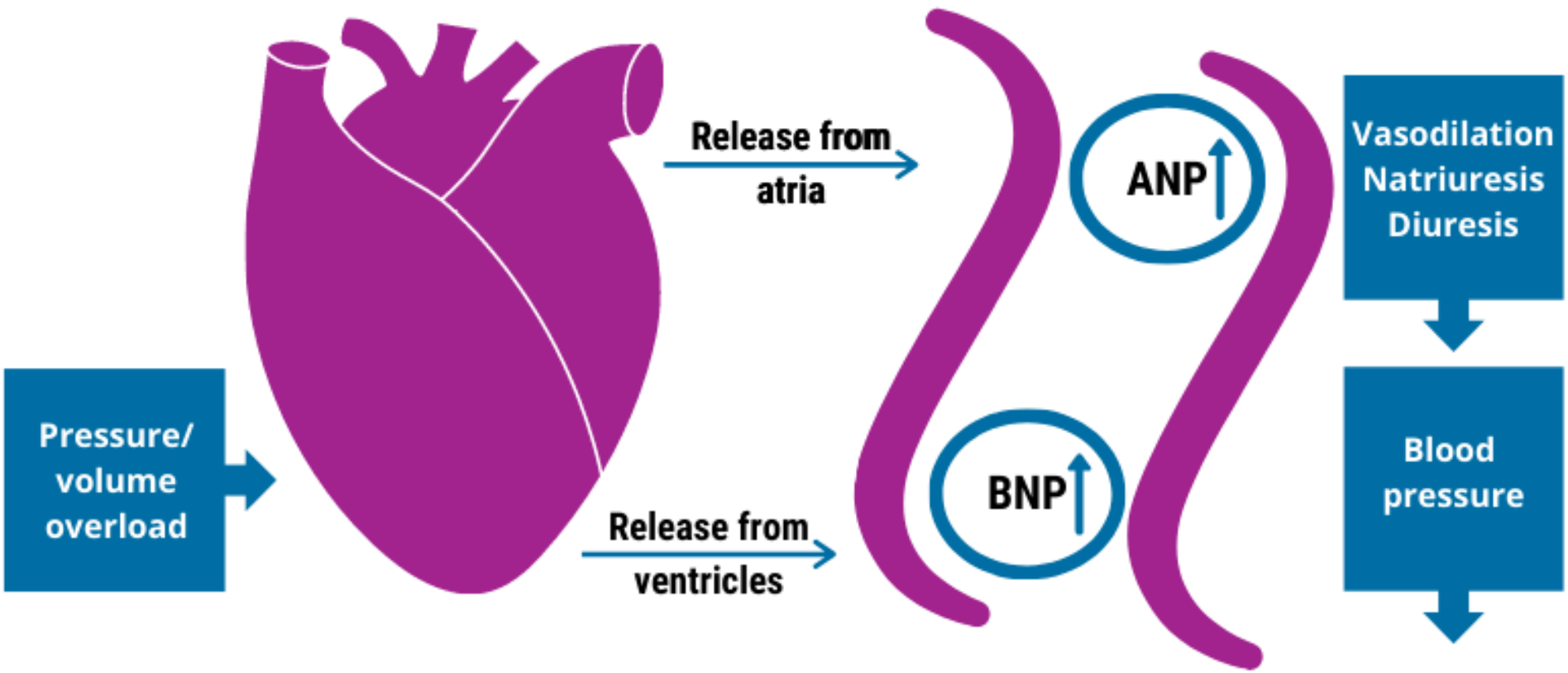
- Remains elevated for 12 hours



Markers of hemodynamic stress

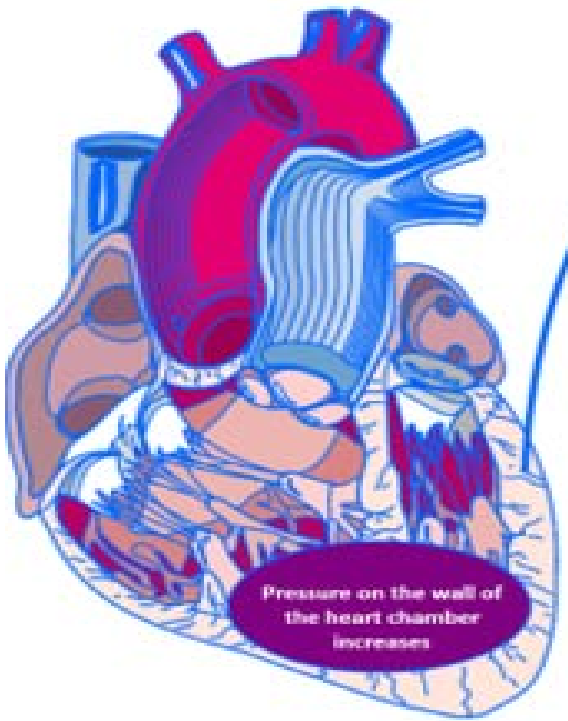
NATRIURETIC PEPTIDES

- ANP is released primarily in response to atrial wall stretching and intravascular volume expansion.
- BNP is mainly secreted by the ventricles
- CNP is found predominantly in the brain and also synthesized by vascular endothelial cells



BNP

- MARKER OF VENTRCULAR DYSFUNCTION
- INCREASED IN HEART FAILURE
- Pro BNP is the best marker for ventricular dysfunction
- Used to differentiate symptoms of lung obstruction from ventricular dysfunction



Pre-pro-BNP$_{1-134}$

26-aa signal sequence

pro-BNP$_{1-108}$

Myocardial cells

Blood

NT-proBNP$_{1-76}$

BNP$_{77-108}$

$t_{1/2}$ = 60-120 min

$t_{1/2}$ = 18 min

INFLAMMATORY MARKERS

- C-Reactive Protein is a pentameric protein.

- Although considered to be a general nonspecif ic marker of inf lammation, elevated baseline levels of high sensitivity CRP are correlated with higher risk of future cardiovascular morbidity and mortality.

Clinical Uses of crp

- Screeningfor cardiovascular risk in otherwise “healthy” individuals

Elevated levels are predictive of

- Long-term risk of first MI
- Ischemic stroke

Clinical applications of enzymes

Plasma Enzymes

Sources of plasma enzymes: Two sources:

Plasma derived

Cell derived

Plasma derived enzymes: **Functional plasma enzymes**
These enzymes act on substrates in plasma & their activity is higher in plasma than cells.

E.g. coagulation enzymes.

Functional plasma enzymes

- Certain enzymes are present at all times in circulation in normal individuals and perform physiological functions.
- Examples: pseudocholinesterase, blood clotting enzymes, lipoprotein lipase.
- They become clinically significant when the plasma levels become lower than normal.

Non functional plasma enzymes
(cell derived enzymes)

- Most of the enzymes detected in plasma have no function in plasma
- They are present in high concentration inside the cells
- They are released into plasma due to breakdown of cells during various disease processes
- Increase in serum levels of such enzymes indicate organ dysfunction/ diseases
- E.g: ALT,AST, ALP, CK etc

Diagnostic significance of enzymes

1) Enzymes can act as diagnostic markers of underlying diseases .

2) Enzymes can also act as reagents for various biochemical estimations and detections

- Enzymes as diagnostic markers

NAME OF THE ENZYME	Conditions in which level of activity in serum is elevated
Aspartate Amino transferase (AST) Serum glutamate-oxaloacetate transaminase (SGOT)	Myocardial infarction, Liver disease especially with liver cell damage
Alanine Amino transferase (ALT) Serum glutamate-pyruvate transaminase (SGPT)	Liver disease especially with liver cell damage
Alkaline Phosphatase (ALP)	Liver disease- biliary obstruction Osteoblastic bone disease-rickets
Acid Phosphatase (ACP)	Prostatic carcinoma
γ glutamyl Transferase (γ GT)	Liver disorder like liver cirrhosis and alcoholism
Creatine kinase (CK)	Myocardial infarction and skeletal muscle disease(muscular dystrophy
Lactate Dehydrogenase (LDH)	Myocardial infarction, hemolysis, liver diseases

Enzymes as Tumour Markers

Enzyme	Disease
Serum acid phosphatase	Cancer prostate
Serum Alkaline phosphatase	Metastasis in liver, jaundice due to carcinoma head of pancreas, osteoblastic metastasis in bones
Serum LDH	Advanced malignancies and Leukemias

Enzymes as diagnostic reagents

Enzyme	Used for testing
Urease	Urea
Uricase	Uric acid
Glucose oxidase	Glucose
Cholesterol oxidase	Cholesterol
Lipase	Triglycerides
Alkaline phosphatase	ELISA
Horse radish Peroxidase	ELISA
Restriction endonuclease	Recombinant DNA technology
Reverse transcriptase	Polymerase chain reaction

Enzymes as therapeutic agents

Enzyme	Therapeutic Application
Streptokinase/Urokinase	Acute MI, Pulmonary embolism, DVT(Deep vein thrombosis)
Trypsin, lipase and amylase	Pancreatic insufficiency
Asparaginase/Glutaminase	Acute lymphoblastic leukemias
Hyaluronidase	Enhanced local anesthesia and for easy diffusion of fluids
Papain	Anti inflammatory
Chymotrypsin	Pain killer and Anti inflammatory
Alpha- 1 Antitrypsin	Deficiency and Emphysema
Serratopeptidase	Pain killer and Anti inflammatory

Enzyme profiles in liver diseases

Enzymes commonly studied for diagnosis of liver diseases are:

- Alanine transaminase (ALT)
- Aspartate transaminase(AST)
- Alkaline phosphatase (ALP)

- Nucleotide phosphatase (NTP)

- Gamma glutamyl transferase (GGT)

Aspartate aminotransferase (AST)

- It was also called as serum glutamate oxaloacetate transaminase (SGOT).
- Normal serum level: 8 to 20 U/L.
- It is a marker of liver injury - increases in liver diseases like hepatitis and malignancies of liver.
- AST was used as a marker of myocardial ischemia.
- The level is signif cantly elevated in myocardial infarction.
- But troponins have replaced AST as a diagnostic marker in IHD

Alanine aminotransferase (ALT)

- It was called as serum glutamate pyruvate transaminase (SGPT)
- Normal serum level: 13-35 U/L
- Very high values (300 to 1000 U/L) are seen in acute hepatitis, either toxic or viral in origin.
- Both ALT and AST levels are increased in liver disease, but
- ALT > AST.

- Rise in ALT levels may be noticed several days before clinical signs such as jaundice are manifested.
- Moderate increase (50 to 100 U/L) of ALT may be seen in chronic liver diseases such as cirrhosis, hepatitis C and non-alcoholic steatohepatitis (NASH).

Alkaline Phosphatase (ALP)

It is localised in cell membranes -ecto-enzyme

- It is produced by osteoblasts of bone and is associated with the calcif ɨcation process.

Normal range-40-125 U/L.

In children, higher levels are seen, due to increased osteoblastic activity.

Moderate (2-3times) increase in ALP level is seen in hepatic diseases such as infective hepatitis, alcoholic hepatitis or hepatocellular carcinoma.

Very high levels of ALP (10-12 times of upper limit) may be noticed in extrahepatic obstruction (obstructive jaundice) caused by gallstones or by pressure on bile duct by carcinoma of head of pancreas

Drastically high levels of ALP (10-25 times of upper limit) are seen in bone diseases where osteoblastic activity is enhanced such as

- Paget's disease (osteitis deformans),
- Rickets and osteomalacia,
- osteoblastoma,
- metastatic carcinoma of bone
- hyperparathyroidism.

Nucleotide Phosphatase (NTP)

- It is also known as 5' nucleotidase.
- This enzyme hydrolyses 5' nucleotides to corresponding nucleosides
- It is a marker enzyme for plasma membranes

- It is moderately increased in hepatitis and highly elevated in biliary obstruction.
- Unaffected by bone diseases.

Gamma Glutamyl Transferase (GGT)

- It is used in the synthesis of glutathione

- It is seen in liver, kidney, pancreas, intestinal cells & prostate gland.

GGT is clinically important because of its sensitivity to detect alcohol abuse.

GGT is increased in alcoholics.

Increase in GGT level is generally proportional to the amount of alcohol intake.

Enzyme profiles in muscle diseases

Enzymes commonly studied for diagnosis of muscle diseases are:

- AST
- CPK
- Aldolase

CPK is most reliable indicator of muscular diseases.

Aldolase

- It is a glycolytic enzyme.
- It is drastically elevated in muscle damages such as progressive muscular dystrophy, poliomyelitis, myasthenia gravis and multiple sclerosis.
- It is a very sensitive early index in muscle wasting diseases.

Enzyme profiles in bone diseases

- Serum alkaline phosphatase remains the only useful enzyme
- ALP is most valuable index of osteoblastic activity.
- Increased ALP activity is seen in rickets, osteomalacia, hyperparathyroidism & in Paget's disease.

Enzyme profile in pancreatic diseases

Amylase

- The enzyme splits starch to maltose & activated by Ca^{2+} & Cl^{-} ions.
- It is produced by pancreas and salivary glands.
- Normal serum value is 50-120 IU/L.

- The value is increased about 1000 times in acute pancreatitis which is a life- threatening condition.

- Moderate increase is seen in chronic pancreatitis, mumps (parotitis) and obstruction of pancreatic duct.

lipase

The level in blood is highly elevated in acute pancreatitis

Lipase is not increased in mumps.

Enzyme Markers of Prostatic diseases

- **Acid Phosphatase:**
- **It is an enzyme secretd by prostate, RBC and WBC**
- **High levels are found in prostate cancer with metastasis**
- **Prostate Specific Antigen(PSA):**
- **It is an enzyme secreted by epithelial cells of prostate.**
- **It increases in BHP and prostate cancer**