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Clinical Chemistry Made Easy®



DM Vasudevan
Sreekumari S
Kannan Vaidyanathan
Geetha Damodaran K

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CHAPTER 4

Clinical Enzymology

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There are a few non-functional enzymes in plasma, which are coming out from cells of various tissues due to normal wear and tear. Their normal levels in blood are very low; but are drastically increased during cell death (necrosis) or disease. Therefore assays of these enzymes are very useful in diagnosis of diseases.

Markers for Cardiac Diseases

Serial testing of the following cardiac enzymes is usually done to guide the prognosis (Box 4.1). No single marker can successfully identify or exclude acute MI within the first 6 hr.

i. Creatine kinase (CK)
ii. Lactate dehydrogenase (LDH)
iii. Aspartate amino transferase (AST)
iv. Cardiac troponin I (CTI) and Cardiac troponin T (CTT). These are not true enzymes.
v. Brain Natriuretic Peptide (BNP). It is a reliable marker of congestive cardiac failure.

CREATINE KINASE (CK)

It is an enzyme normally present in all muscles. It is used for the reaction to produce creatine phosphate, as the energy

Box 4.1: Cardiac markers to be used in

- Any chest pain
- Unstable angina
- Suspicious ECG changes
- History suggestive of myocardial infarction
- Following surgical coronary revascularisation
- Patients with hypotension and dyspnea

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source for muscle contraction. **Normal serum** value for CK is 50–150 U/L for males and 30–130 U/L for females (values will be slightly variable when using different kits).

CK and Heart Attack

CK value in serum is increased in **myocardial infarction.** The time course is shown in Figure 4.1 and Table 4.2. The CK level starts to rise **within 3-6 hours** of infarction. Therefore CK estimation is very useful to **detect early cases**, where ECG changes may be ambiguous. The CK level is not increased in hemolysis or in congestive cardiac failure. The area under the peak and slope of initial rise are proportional to the size of infarct. CK levels are not raised during an attack of angina pectoris or pericarditis. Successful reperfusion following an

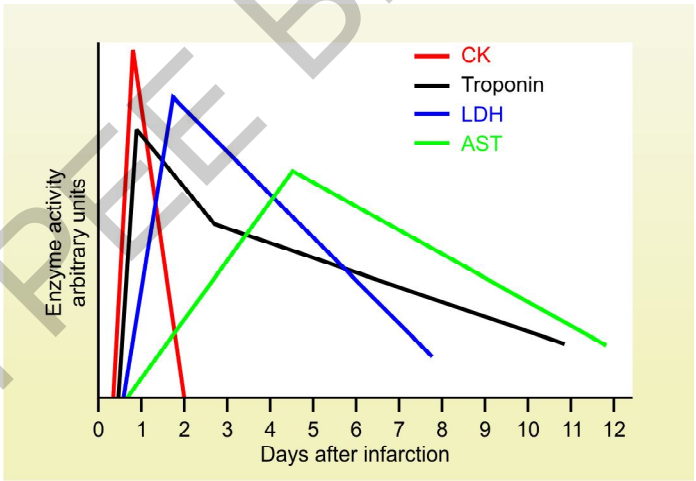

Fig. 4.1: Time course of elevation of markers in blood of myocardial infarction patients

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angioplasty or thrombolysis leads to an increased early peak and a shorter duration.

CK and Muscle Diseases

CK level is highly elevated (>10,000 U/L) in crush injury, fracture polymyositis and dermatomyositis. It is moderately increased (1000 - 10,000 U/L) in acute myositis and in adverse effect of statin. It is mildly increased (500 - 1000 U/L) in **muscular dystrophies.** In female carriers of X-linked muscular dystrophy (heterozygous), also CK is raised. **Estimation of total CK is employed in muscular dystrophies and MB iso-enzyme is estimated in myocardial infarction.**

Iso-enzymes of CK

CK is a dimer; each subunit has a molecular weight of 40 kD. The subunits are called B for brain and M for muscle. Therefore three iso-enzymes are seen in circulation. See Table 4.1.

Normally CK2 (heart iso-enzyme) is only 5% of the total activity. Even doubling the value in CK2 (MB) iso-enzyme may not be detected, if total value of CK alone is estimated. Hence the detection of **MB-isoenzyme** is important in myocardial infarction. Ratio of CK-MB to total CK

Table 4.1: Characteristics of iso-enzymes of CK

Iso-enzyme	*Electrophoretic mobility*	*Tissue of origin*	*Mean percentage in blood*
MM(CK3)	Least	Skeletal muscle	80%
MB(CK2)	Intermediate	Heart	5%
BB(CK1)	Maximum	Brain	1%

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is estimated; a ratio more than 2.5 suggests cardiac involvement.

CARDIAC TROPONINS (TnI AND TnT)

They are not enzymes; but are structural proteins present within cardiac myocytes. However, Troponins are important markers for **myocardial infarction**, and hence discussed here. The troponin complex consists of 3 components; **troponin C** (calcium binding), troponin I (binds to actin and inhibits binding of actin to myosin), and troponin T (tropomyosin binding element).

Troponin I (TnI; mol.wt. 21kD) is released into the blood **within 4 hours** after the onset of symptoms of myocardial ischemia; **peaks at 18-24 hours** and remains elevated for 8-10 days post-infarction (Fig. 4.1 and Table 4.2). Therefore, TnI is very useful as a marker at any time interval after the heart attack. TnI levels more than 1.5 ng/ml suggest myocardial infarction. A negative cardiac troponin test at 12 hr excludes an acute MI in a patient with chest pain. Troponins are not released during ischemic episodes, with no cardiac muscle damage. It is not increased in muscle injury; whereas CK2 may be elevated in some muscle injury. Troponin I has 3 isoforms; the "slow" and "fast" moving

Table 4.2: Markers of myocardial infarction

Marker	*Onset*	*Peak*	*Duration*
CK-MB	3-6 hr	18-24hr	36-48hr
Troponins	4-10 hr	18-24hr	8-14 days
LDH	6-12 hr	24-48 hr	6-8 days
AST	24-36hr	4-5 d	10-12 days
Myoglobin	1-4 hr	6-7 hr	24 hr

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forms are skeletal variety. Cardiac isoform is specific for cardiac muscle.

Troponin-T (TnT, 37kD) has two isoforms, called TnT1 and TnT2. Troponin I is specific to cardiac myocytes, but troponin T is seen in skeletal muscles in small quantities. Serum levels of TnT2 increases within 4 hours of myocardial infarction, and remains high for up to 14 days. TnT2 is 100% sensitive index for myocardial infarction. Normal level of TnT is <0.01 ug/ml and in MI, the value raise to >0.03 ug/ml. The level of Troponin will generally reflect the infarct size. Troponins are more sensitive than CK-MB to detect micro infarction.

LACTATE DEHYDROGENASE (LDH) (LD)

LDH will convert pyruvate to lactate. **Normal value** of LDH in serum is 100-200 U/L. Values in the upper range are generally seen in children. LDH level is 100 times more inside the RBC than in plasma, and therefore minor amount of hemolysis will result in a false positive test.

LDH and Heart Attack

In myocardial infarction, total LDH activity is increased, while **H4 iso-enzyme** is increased 5–10 times more. The time course of LDH level after a heart attack is given in Figure 4.1 and Table 4.2. The magnitude of the peak value as well as the area under the graph will be roughly proportional to the size of the myocardial infarct.

Increase in total LDH level is seen in hemolytic anemias, hepatocellular damage, muscular dystrophy, carcinomas, leukemias, and any condition which causes necrosis of body cells. Since total LDH is increased in many conditions, the study of iso-enzymes of LDH is of great importance.

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Iso-enzymes of LDH

LDH enzyme has 4 subunits. The subunit may be either H (heart) or M (muscle) polypeptide chains. So 5 combinations of H and M chains are possible (Table 4.3). All these 5 iso-enzymes are seen in all persons. The iso-enzymes are usually separated by **electrophoresis** (Table 4.3). M4 form is seen in skeletal muscles while **H4 form is seen in heart**. Normally LDH-2 (H3M1) concentration in blood is greater than LDH-1 (H4); but this pattern is reversed in myocardial infarction; this is called **flipped pattern.**

ASPARTATE AMINO TRANSFERASE (AST)

Normal serum level of AST is 8–20 U / L (See Table 6.5). The level is significantly elevated in **myocardial infarction.** The level is started to raise by 24 hr, reaches a peak on 4-5 days (Fig. 4.1 and Table 4.2). But the level is moderately elevated in **liver diseases** and a marked increase is seen in primary hepatoma.

Table 4.3: Features of LDH iso-enzymes

Name of iso-enzyme	*Subunit* make up of iso-*enzyme*	*Electro* phoretic mobility at *pH 8.6*	*Tissue from* which iso-enzyme has *originated*
LDH-1	H4	Fastest	Heart muscle
LDH-2	H3M1	Faster	RBC
LDH-3	H2M2	Fast	Brain
LDH-4	H1M3	Slow	Liver
LDH-5	M4	Slowest	Skeletal muscle

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Brain Natriuretic Peptide (BNP)

The natriuretic peptide family consists of three peptides: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). ANP is produced primarily in the cardiac atria. BNP is present in human brain, but more in the cardiac ventricles. These natriuretic peptides defend against excess salt and water retention.

Patients with congestive heart failure have high plasma concentrations of ANP and BNP. The concentrations are correlated with the extent of ventricular dysfunction. High concentrations of BNP predict poor long-term survival. In breathlessness, BNP test helps in the differentiation of the cause as heart failure or obstructive lung disease. Patients with COPD and worsening of their cor pulmonale with signs of RV volume overload (including edema and ascites) manifested increased BNP levels, and, indeed, these patients had increased mortality.

Enzyme Profile in Liver Diseases

Enzymes commonly studied for diagnosis of liver diseases are:

1. Alanine amino transferase (ALT)
2. Alkaline phosphatase (ALP)
3. Nucleotide phosphatase (NTP)
4. Gamma glutamyl transferase (GGT)

These are described in detail in Chapter 6.

ACID PHOSPHATASE (ACP)

It hydrolyses phosphoric acid ester at pH between 4 and 6.
Normal serum value for ACP is 2.5–12 U/L.

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ACP is secreted by prostate cells, RBC, platelets and WBC.

The prostate iso-enzyme is inactivated by **tartaric acid**. Normal level of tartrate labile fraction of ACP is 1 U/L.

ACP total value is increased in **prostate cancer** and highly elevated in bone metastasis of prostate cancer. In these conditions, the tartrate labile iso-enzyme is elevated. This assay is very helpful in follow up of treatment of prostate cancers. So, ACP is an important **tumour marker**.

Since blood cells contain excess quantity of ACP, care must be taken to prevent hemolysis while taking blood from the patient. Prostate massage may also increase the value. So blood may be collected for ACP estimation before rectal examination of patient.

PROSTATE SPECIFIC ANTIGEN (PSA)

It is produced from the secretory epithelium of prostate gland. It is normally secreted into seminal fluid, where it is necessary for the liquefaction of seminal coagulum. Normal value is 1-5 microgram/L. It is very specific for prostate activity. Values above 10 µg/L is indicative of **prostate cancer.**

Glucose-6-phosphate dehydrogenase (GPD)

It is an important enzyme in the hexose mono-phosphate shunt pathway of glucose. It is mainly used for production of NADPH. **Hydrogen peroxide i**s continuously formed inside the RBC. Peroxide will destroy RBC cell membrane. NADPH will prevent this process. Therefore, NADPH is very essential for preserving the RBC integrity.

Drug induced hemolytic anemia is characteristic of GPD deficiency. Normal value of GPD in RBC is 6-12 U/g of Hb. In

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the GPD deficient individuals, RBC life-span may be reduced, but there will be no disease manifestations. However, when certain drugs (**aspirin,** mepacrine, pamaquine, **sulpha)** are taken by such individuals, there will be sudden damage to RBCs.

Met-hemoglobinemia: NADPH is also necessary for reduction of met-hemoglobin (oxidised form) to hemoglobin. Hence met-hemoglobinemia is also manifested in GPD deficient individuals.

Table 4.4: Therapeutic use of enzymes

	Enzyme	*Therapeutic application*
1.	Asparaginase	Acute lymphoblastic leukemia
2.	Streptokinase	To lyse intravascular clot
3.	Urokinase	do
4.	Streptodornase	DNAse; applied locally
5.	Pancreatin (trypsin and lipase)	Pancreatic insufficiency; oral administration
6.	Papain	Anti-inflammatory
7.	Alpha1-antitrypsin	AAT deficiency; emphysema

Table 4.5: Enzymes in other body fluids

Enzyme	*Clinical significance*
Adenosine deaminase in pleural fluid	Elevated in tuberculous pleural effusion, but not in malignant effusion
Lactate dehydrogenase in CSF, pleural fluid, ascetic fluid	Elevated levels indicate the presence of a malignant tumour. But not diagnostic, as the enzyme is not tissue specific.

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Box 4.2: Enzyme patterns (Enzyme profiles) in diseases

I. Hepatic diseases
   1. Alanine amino transferase (ALT)
      Marked increase in parenchymal liver diseases
   2. Nucleotide phosphatase (NTP)
      Elevated in liver dysfunction with cholestasis
   3. Alkaline phosphatase (ALP)
      Marked increase in obstructive liver disease
   4. Gamma glutamyl transferase (GGT)
      Increase in obstructive and alcoholic liver

II.Myocardial infarction

1. Creatine kinase (CK-MB)
   First enzyme to rise following infarction
   CK-MB isoenzyme is specific
2. Aspartate amino transferase (AST)
   Rises after the rise in CK and returns to normal in 4–5 days
3. Lactate dehydrogenase (LDH)
   LDH-1 becomes more than 2 (flipped pattern)

III. Muscle diseases
   1. Creatine kinase (CK-MM)
      Marked increase in muscle diseases.
      CK-MM fraction is elevated
   2. Aspartate amino transferase (AST)
      Increase in muscle disease; not specific

IV. Bone diseases
   1. Alkaline phosphatase (ALP)
      Marked elevation in rickets, and Paget's disease

V. Prostate cancer
   1. Prostate specific antigen (PSA)
      Marker for prostate cancer.
      Mild increase in benign prostate enlargement
   2. Acid phosphatase (ACP)
      Marker for prostate cancer.

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Amylase and Lipase

It splits starch to maltose. 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. The peak values are seen between 5–12 hours after the onset of disease and returns to normal levels within 2–4 days after the acute phase has subsided. Moderate increase in serum levels are seen in chronic pancreatitis, mumps (parotitis) and obstruction of pancreatic duct.

Normal value of **amylase in urine** is less than 375 U/L. It is increased in acute pancreatitis. It is increased on the 1st day and remains to be elevated for 7-10 days. Amylase creatinine ratio is calculated. Creatinine mg/L divided by serum amylase x urine creatinine mg/L x 100. Normal value is 2-5%. In acute pancreatitis, it is increased to 8 %, but below 2 % in macroamylasemia.

Lipase is present in pancreatic secretion. The level in blood is highly elevated in **acute pancreatitis** and persists for 7-14 days. Thus lipase remains elevated longer than amylase. Moreover, lipase is not increased in mumps.

Enolase

It is a glycolytic enzyme. Neuron-specific enolase (NSE) is an iso-enzyme seen in neural tissues and Apudomas. NSE is a **tumour marker** for cancers associated with neuro-endocrine origin, small cell lung cancer, neuroblastoma, pheochromocytoma, medullary carcinoma of thyroid, etc.

Enzyme patterns in some important diseases are given in Box 4.2. A list of therapeutically useful enzymes is given in Table 4.4. Enzymes in other body fluids are listed in Table 4.5.

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