

BIOCHEMICAL EXAMINATION OF ACUTE MYOCARDIAL INFARCTION

Written by Lenka Fialová, translated by Jan Pláteník

1 Structure of heart muscle

Heart muscle (myocardium) is a particular form of striated muscle, which is permanently active. The heart muscle fiber cells – cardiomyocytes – contain one or two ovoid nuclei, surrounded by numerous mitochondria and granules of glycogen. The cardiomyocyte cytoplasm (sarcoplasm) also contains myoglobin. Sarcoplasmic reticulum formed by a system of sacs and cisternae functions as a store for Ca^{2+} ions.

The contractile apparatus of cardiomyocytes consists of two main types of myofilaments:

- a) **thick (myosin) filaments** formed by myosin molecules with fibrous and globular parts, the latter possessing ATPase activity.
- b) **thin (actin) filaments** resulting from assembly of globular actin monomers into double helical filaments.

The heart muscle contraction is regulated by **troponin-tropomyosin complex** that associates with actin. **Tropomyosin** is a filamentous protein placed in the groove of double helix of the actin filament. Troponin is a complex of three globular proteins:

- **troponin T (TnT)**
- **troponin C (TnC)**
- **troponin I (TnI)**

Troponin T is the largest protein of the three; and provides a link between the troponin complex and the tropomyosin. Troponin I under resting condition inhibits interaction between actin and myosin filaments. Troponin C binds calcium ions, following their release from sarcoplasmic reticulum to the sarcoplasm. Binding of Ca^{2+} to TnC induces a conformation change of the troponin complex resulting in movement of TnI away from the myosin binding sites on the actin filament. As a consequence, globular parts of myosin filaments attach to actin filaments; and activation of myosin ATPase activity by interaction with actin starts the muscle contraction.

2 Biochemical markers of acute myocardial infarction (AMI)

The acute coronary syndromes, such as myocardial infarction, and unstable angina pectoris, occur due to coronary arterial occlusion, caused usually by a thrombotic complication of atherosclerosis. Restriction of blood flow results in heart **ischemia**, which is initially reversible. Unless the blood flow is restored, the condition in about one hour progresses to irreversible alterations ending in heart muscle cell death and myocardial **necrosis**.

The laboratory examinations significantly contribute to the diagnosis of acute coronary syndromes.

Biochemical components of heart muscle differ in their subcellular localisation: some are found in the **cytosol**, others in the **mitochondria**, yet others constitute the **contractile apparatus** of the cardiomyocyte.

In general, course of serum levels of proteins released from the heart muscle due to its infarction depends on:

- cellular localisation

Short-time ischemia alters functional and later also structural features of cytoplasmic membranes resulting in release of **cytosolic proteins**. On the other hand, **long-term ischemia** includes tissue necrosis and also **structural proteins** are released. Hence, cytosolic proteins in general appear in the circulation earlier than the structural ones.

- relative molecular mass – small proteins reach the circulation more readily

- rate of excretion – small proteins are efficiently removed from the circulation by kidney
- blood flow through the affected area.

Basic features of all used cardiometers summarises table 1.

Table 1: Basic features of biochemical markers of myocardial infarction:

<i>Component</i>	<i>MW (Da)</i>	<i>Biologic half-life</i>	<i>Cellular localisation</i>	<i>Cause of elevated levels in circulation</i>
Creatine kinase (CK)	86 000	17 hours	cytosol	cellular ischemia
CK isoenzyme MB (CK-MB)	86 000	13 hours		
Lactate dehydrogenase (mostly isoenzyme LD1)	135 000	110 hours		
Myoglobin (Myo)	17 800	15 minutes		
Heart troponin T (cTnT), cytosolic fraction	37 000	2-4 hours	cytosol	
Heart troponin I (cTnI), cytosolic fraction	22 500	2-4 hours		
Heart troponin T (cTnT)	37 000	2-4 hours	contractile complex	cellular necrosis
Heart troponin I (cTnI)	22 500	2-4 hours		
Aspartate aminotransferase (AST), mitochondrial isoenzyme	93 000	34 hours	mitochondria	

2.1 Cytoplasmic proteins

2.1.1 Myoglobin

Myoglobin is a globular protein consisting of a single amino acid chain, and heme as a prosthetic group. It reversibly binds and transfers oxygen inside muscle cells. The *myoglobin in skeletal and heart muscles is identical*. It is filtered through the glomerular membrane and passes into urine; therefore, its biologic half-life is short: 10-20 minutes.

As a *cytosolic protein with small molecular mass* it is rapidly released from the damaged heart tissue. Elevation of serum myoglobin in AMI occurs as early as 0.5-2 hours after start of the chest pain. The myoglobin levels can reach 20-fold of normal values; they peak at 6-12 hours, and within 12-24 hours return to baseline level. Myoglobin is nowadays considered as the most sensitive biochemical marker of the acute myocardial infarction especially suitable for an *early detection*. Its disadvantage is *lack of specificity for myocardium*: increase of myoglobin can also be observed in damage of skeletal muscles.

2.1.2 Creatine kinase (CK)

Creatine kinase is largely cytoplasmic enzyme that catalyses phosphorylation of creatine to creatine phosphate using ATP. If, on the other hand, ATP is in short supply, the reverse reaction occurs. CK is found chiefly in skeletal muscle, myocardium and brain. It consists of *two subunits* that can be of two types: *M (from muscle)*, and *B (from brain)*; each having MW about 40000. Various combinations of the subunits give rise to *the three isoenzymes of creatine kinase*:

- *CK-BB (CK-1, brain isoenzyme)*
- *CK-MB (CK-2, myocardial isoenzyme)*
- *CK-MM (CK-3, skeletal muscle isoenzyme)*

In skeletal muscle the isoenzyme CK-MM prevails, but CK-MB is also present. The brain contains isoenzyme CK-BB that does not appear in the blood unless permeability of the hematoencephalic barrier is compromised. The CK-MB is typical for heart muscle, but the heart contains also CK-MM.

Catalytic concentration of total CK in blood increases within 3-6 hours after start of myocardial ischemia. Due to insufficient specificity for myocardium, however, its estimation in AMI is of rather limited value. The level of total serum CK is influenced by various factors, such as age, gender, proportion of muscle tissue, and physical activity.

Examination targeted at CK-MB isoenzyme is much more valuable in AMI compared to the total CK, although the CK-MB is not exclusively myocardium-specific either. Its increase may result also from affections of skeletal muscle (trauma, muscular dystrophy, intramuscular injections, resuscitation and defibrillation), extreme physical exercise, and chronic renal insufficiency.

CK-MB can be estimated either as the *enzyme activity*, which detects only active enzyme molecules, or immunochemically as the *mass concentration of the CK-MB protein*. The latter is usually called '*CK-MB mass*'; and is unequivocally preferred nowadays. The estimation of CK-MB mass is both *more specific and more sensitive*, as it detects also partially degraded and inactive enzyme molecules.

2.1.3 Lactate dehydrogenase (LD)

Lactate dehydrogenase is an oxidoreductase catalysing reversible conversion of lactate to pyruvate, and vice versa. The enzyme molecule consists of 4 subunits, each having MW about 34000. LD is present in cytoplasm of many tissues; and is released into circulation even after mild tissue damage. As a result, increase of catalytic concentration of LD accompanies various disease states; and its estimation in laboratory diagnosis of acute coronary syndromes is considered as subordinate. In addition, due to relatively high content of LD in erythrocytes, hemolysis can readily make this test false positive. In the course of AMI, a *delayed increase* of LD is typically found, which can last as long as 15 days after the myocardial necrosis.

Total serum activity of LD encompasses 5 isoenzymes LD1-LD5. The myocardium contains mostly the *LD1, and less LD2*.

2.2 Mitochondrial proteins

2.2.1 Aspartate aminotransferase (AST)

In the heart muscle it is present in relatively high concentration. Historically, the AST was one of the first biochemical markers of AMI; nowadays, however, it has become obsolete in this indication. More about AST can be found in the lesson on biochemical examination of the liver.

2.3 Structural proteins

2.3.1 Troponins

As cardiomarkers the *troponins T (TnT)* and *I (TnI)* are utilised. TnT and TnI occur both in the skeletal and heart muscles. The *cardiac isoforms* (cTnT, cTnI) have unique amino acid composition, and therefore are *specific* for myocardium. Most of cTnT and cTnI are incorporated in the contractile apparatus whose proteolytic degradation is required for their release. Only about 6-8% of cTnT and 2.8-8.3% of cTnI are present unbound in the cytosol.

Troponin cTnT normally does not occur in the blood. The course of its release during AMI is typically *biphasic*. The increase of troponin comes within 3-12 hours after the onset of AMI; and it reaches its first peak 12-18 hours following myocardial damage. This increase is due to quick release of the free *cytosolic fraction of cTnT*. The second peak follows 3-4 days later; corresponding to the slower release of *cTnT bound to the troponin-tropomyosin complex* in the necrotic area. Within 7-10 days it falls back to the original, undetectable levels. If an early reperfusion of the coronary artery is achieved, the maximal increase of cTnT is observed in about 14 hours; then the second, substantially lower peak follows. In general, the larger the myocardial infarction, the longer time the cTnT elevation lasts. In very large infarctions it can be detectable for up to 21 days. Certain disadvantage of cTnT examination is its unspecific increase in patients with renal insufficiency.

Elevation of cTnI, which is distinctly specific, commences similarly to cTnT as early as 3 hours after beginning of ischemia. The increased levels last 5-10 days. In comparison to cTnT, the course of cardiac troponin I elevation usually does not display the second maximum (due to smaller cytosolic fraction).

Troponin C is not suitable for diagnosis of acute myocardial infarction, because this protein is identical both in heart and skeletal muscle.

Table 2: Dynamics of serum levels of biochemical markers of acute myocardial infarction

<i>Parameter</i>	<i>Elevation starts (hours)</i>	<i>Peak levels (hours)</i>	<i>Return to normal levels (days)</i>	<i>Maximal increase (compared to upper reference value)</i>	<i>Reference values</i>
Myoglobin	0.5-2	6-12	0.5-1	20-fold	M 19-92 µg/L F 12-76 µg/L
CK-MB mass	2-6	12-24	2-3		0.0-5.0 µg/L
CK	3-6	16-36	3-5	25-fold	M 0.2-3.6 µkat/L* F 0.2-3.1 µkat/L
cTnT	3-12	12-18 (first) 72-96 (second)	7-20	300-fold	0.0-0.05 µg/L
cTnI	3-12	12-24	5-10		0.0-0.1 µg/L
AST	4-8	16-48	3-6	25-fold	0.05-0.72 µkat/L
LD	6-12	24-60	7-15	8-fold	3.5-7.7 µkat/L

* The upper values depend on age; the included ones are for the age 40-50 years.

3 Recommended procedure for examination of suspect myocardial infarction

For diagnosis of acute coronary syndrome a consecutive determination of two laboratory markers – *rapid one and definitive one* – is nowadays recommended.

In an *early stage*, shortly after onset of an acute coronary syndrome, estimation of *myoglobin* has the highest diagnostic sensitivity - the '*rapid marker*'. It is an early, albeit unspecific marker, which in cases of unclear chest pain without positive ECG can distinguish patients surely without myocardial infarction from those with suspect coronary syndrome. In other words, in particular

negative (not elevated) level of myoglobin is informative, as it excludes acute myocardial infarction. On the other hand, diagnosis of AMI must be confirmed by a second marker.

Troponins are the best *definitive indicators* of myocardial damage. They enable very sensitive and specific detection of heart muscle necrosis – even a microscopic one. Usage of troponins in diagnosis of AMI has following advantages:

- highly specific for myocardium
- almost undetectable in healthy persons
- high sensitivity enabling even detection of minimal myocardial damage
- relatively long-lasting elevation allows also a late diagnosis of AMI
- the only parameter measurable even in a hemolytic sample

Increased levels of cardiac troponins reflect myocardial damage, which, however, can result not only from AMI, but also from other causes, such as inflammation (myocarditis), pulmonary embolism, or heart surgery.

Estimation of CK-MB mass is acceptable only if the examination of troponins is unavailable.

Recommended time schedule for taking blood samples in suspect AMI is shown in table 3.

Table 3: Recommended time schedule for examination of cardiomarkers in suspect AMI

<i>Indicator</i>	<i>Taking the blood sample:</i>		
	<i>time of admission</i>	<i>to repeat the test</i>	<i>12 hours after admission</i>
Early (myoglobin)	yes	2 to 6 hours since the onset of symptoms	-
Definitive (cardiac troponin T or I)	yes	6 to 9 hours after admission	yes

4 Rapid test for cTnT

In suspect AMI the rapid diagnosis is very important; and so even tests for myoglobin and troponins that can be used directly at patient's bed have been developed.

In this lesson, a rapid bed-side test for cTnT, based on technology GLORIA (Gold Labelled Optically Read Immune Assay) will be demonstrated. It utilises *two different monoclonal antibodies* against cTnT, one *labelled with biotin*, while the other one *with colloidal gold*.

Procedure:

The patient's blood is applied onto the *application zone* that contains both the labelled antibodies forming with troponin in the sample (if present) a sandwich complex. Glass fibers separate red blood cells from the sample so that only the blood plasma with immune complexes is allowed to diffuse towards *detection zone*. In the detection zone there is a *signal band* with immobilised streptavidin, and a *control band* with immobilised troponin. The sandwich immune complexes with troponin in the sample bind to the signal band; while an excess of gold-labelled anti-troponin antibodies stain the control band, which serves just as a proof that the test and reagents it includes are functional. Hence, if only the control band is stained the test result is negative; while in case of positive result two bands are seen – both control and signal ones.