Biochemical Diagnosis of Myocardial Infarction

Structure of Myocardium

Myocardium (Cardiac muscle) is a special form of striated muscle, which is in permanent action. Cardiac muscle cells - cardiomyocytes contain one or two centrally placed oval shaped nuclei. Close to nuclei there are many mitochondria and glycogen granules. In the cytoplasm of cardiomyocytes (sarcoplasm) there is also myoglobin. Sarcoplasmic reticulum, which is composed of a system of vesicles and cisternae, is a reservoir for Ca^{2+} ions.

A part of cardiomyocytes are muscle fibers, that consists of two main types of myofilaments:

1. *thick (myosin) myofilaments consist of molecules of myosine - fibrilar part and globular part, in which the ATPase is located;*
2. *thin (actin) myofilaments. Their structure is based on fibers twisted in a double helix, resulting from polymerization of globular actin monomers.*

Troponin-tropomyosin complex

Actin is closely related to troponin-tropomyosin complex, which regulates the contraction of the muscle. **Tropomyosin** is constructed from two α-helical chains that wrap around one another to form a coiled-coil rod, that lies in the grooves of the actin helix in thin filaments.

Troponin is a complex of three globular proteins:

- **troponin T (TnT)**;
- **troponin C (TnC)** (not specific for heart; we can find indentical one in skeletal muscle);
- **troponin I (TnI)**.

TnT, which has the biggest molecular weight helps with connecting troponin complex to tropomyosin. TnI, when in a resting state, prevents the formation of a bridge between myosin and actin. TnC binds Calcium ions, which are released into the sarcoplasm from the sarcoplasmic reticulum. When Ca^{2+} is bound to TnC, it induces changes in configuration of troponin complexes and that leads to TnI being displaced further away from the binding site of myosin. That means that the globular parts of myosin are able to bind with actin myofilaments. The muscle contracts after the ATPase in the globular parts of myosin molecules is activated.

Biochemical Markers in Acute Myocardial Infarction

Acute coronary syndromes (ACS; Acute myocardial infarction, AIM and Unstable angina pectoris) are caused by blockage of coronary artery, usually due to thrombotic complications. When the blood flow is decreased, *myocardial ischemia* occurs. At first it's *reversible*, but if the blood flow can't be restored quick enough, after rougly an hour the changes are *irreversible.* Those changes are accompanied with death of cells and necrosis (definite myocardial infarction).

Laboratory diagnostic methods are a significant factor in diagnosis of acute coronary syndrome. Biochemically important parts of cardyomyocyte are located in *cytoplasm or mictochondria*, other are a part of *contractile apparatus*. They are being released into the circulation during the myocardial infarction. The course of their serum levels depends on many factors:

- localisation in the cell During a *brief ischemia*, **cytoplasmic proteins** are being released into the blood circulation due to functional and structural changes. During a *long-term ischemia* tissue necrotizes. That means **structural proteins** are being relesed into the blood circulation along with cytoplasmic proteins (cytoplasmic proteins are released quicker)
- relative molecular mass - the smaller proteins are quicker to be released into the circulation.
- rate of excretion - smaller molecules are quicker to be eliminated by kidneys.
- blood flow in the affected area.

Basic characteristics of biochemical parameters of myocardial infarction

Components	Mr [Da]	Biological Half-life	Localization in the Cell
Creatine Kinase (CK)	86 000	17 h	cytoplasm
• isoenzyme MB (CK-MB)	86 000	13 h	
Lactate Dehydrogenase (LD) (mostly isoenzyme LD_1)	135 000	110 h	
Myoglobin	17 800	15 min	
Cardiac troponin T (cTnT) (cytoplasmic fraction)	37 000	2–4 h	
Cardiac troponin I (cTnI) (cytoplasmic fraction)	22 500	2–4 h	
Cardiac troponin T (cTnT)	37 000	2–4 h	fibrillar contractile complex
Cardiac troponin I (cTnI)	22 500	2–4 h	
Aspartate Aminotransferase (AST) (mitochondrial isoenzyme)	93 000	34 h	mitochondria

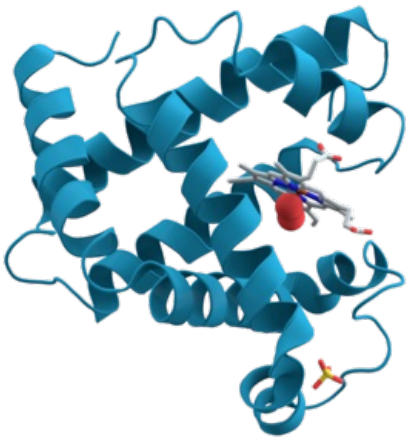
The course of levels of biochemical markers in acute myocardial infarction

Parameter	Start of rising levels of markes [h]	Peak of levels of markers [h]	Normalisation [days]	Maximal increase of levels of markers [multiple of the upper limit of the normal value]	Normal Values
Myoglobin	0,5–2	4–10	0,5–1	20×	M 19–92 µg/l F 2–76 µg/l
CK mass	2–6	12–24	2–3		0,0–5,0 µg/l
CK-MB	3–6	16–36	3–5	25×	M 0,2–3,6 µkat/l[tab2 1] F 0,2–3,1 µkat/l
cTnT	3–8	12–18 (1st peak) 72–96 (2nd peak)	7–14	300×	0,00–0,05 µg/l
cTnI	3–12	12–24	5–10		0,0–0,1 µg/l
AST	4-8	16–48	3–6	25×	0,05–0,72 µkat/l
LD	6–12	24–60	7–15	8×	3,5–7,7 µkat/l

1. Horní hranice závisí na věku – uvedené hodnoty jso pro věk 40–50 let.

Cytoplasmic Proteins

Myoglobin



Conformation of myoglobin molecule

Myoglobin is globular protein made of only 1 amino acid chain, which contains hem as a prosthetic group. It reversibly binds and transfers oxygen in skeletal muscle cells. Myoglobin in **skeletal muscle and myocardium is identical.** It's filtrated in kidneys through the glomerular membrane and excreted into the urine. Myoglobin has a very short biological half-time - 10-20 minutes.

Unlike the hemoglobin, myoglobin only contains one hem group and one globin chain. That's why it can only transfer one molecule of O_2.The affinity to oxygen of myoglobin is higher compared to hemoglobin.

Since myoglobin is a cytoplasmic protein with low molecular mass, it's quickly released from the affected tissue. The rise of myoglobin serum levels in acute myocardial infarction begins quickly (0,5-2 hours) after the onset of chest pain. Levels of myoglobin that might reach 20 times the physiological levels culminate in 6-12 hours and decrease to normal levels in 12-24 hours. Myoglobin is considered the most sensitive biochemical marker of acute myocardial infarction suitable for **the early diagnosis**. However, the disadvantage of using myoglobin as a marker in acute myocardial infarction is **not enough cardiospecifity**. The rising of myoglobin levels can be seen in:

- any damage of skeletal muscle (including e.g. intramuscular injections or small contusion after the fall)
- excessive muscular exertion (including e.g. use of abdominal press during prolonged vomiting)
- renal insufficiency

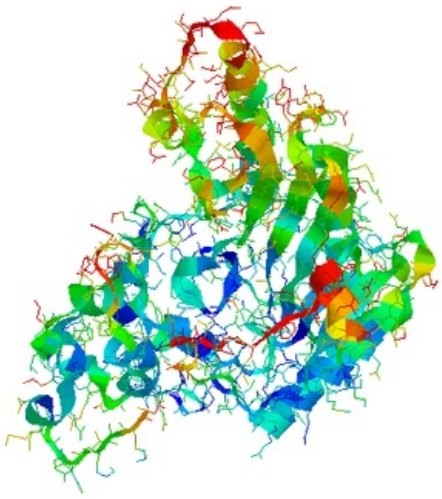
*Determination of myoglobin can **exclude** acute myocardial infarction : if we're certain that blood sample collection was not performed in window period - not sooner than 2 hours after the onset of chest pain or other signs. In this case, if levels of myoglobin are not increased, diagnosis of acute myocardial infarction is excluded. If myoglobin levels are increased, it is necessary to differentialy consider causes of the increase (myocardium, skeletal muscle, renal insufficiency) and it is necessary to determine more specific cardiomarkers - troponins or CK-MB mass.*

Myoglobin levels are determined by a variety of immunochemical methods (immunoturbidimetry, immunonephelometry, enzyme immunoassay, quick immunochemical tests.)

Creatine Kinase

Creatine Kinase (**CK**, EC 2.7.3.2) is mostly cytoplasmic enzyme, which calatyses phosporylation of creatine to creatine phosphate with the help of ATP. When there is lack of ATP, the reaction runs backwards. CK is contained mostly in skeletal muscle, myocardion and brain tissue. It consists of *two subunits* and there are two types of them - **M** (muscle) and **B** (brain), each with relative molecular mass of 40 000. There are 3 *isoenzymes of creatine kinase*, that vary in combination of those 2 subunits:

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



Molecule of creatine kinase

In skeletal muscle, CK-MM is prevalent, but isoenzyme CK-MB is also present. In the brain, there is isoenzyme CK-BB, which cannot be found in the blood if the hematoencephalic barier is intact. CK-MB is typical isoenzyme of myocard, but the heart muscle also contains CK-MM.

Catalytic concentration of **total CK** rises in 3-6 hours since the onset of myocardial ischemia. Its determination in acute myocardial infarction is limited due to insufficient cardiospecifity. Levels of total CK are affected by various factors (age, gender, muscle mass and physical activity)

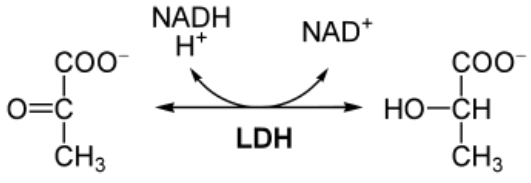
Isoenzyme CK-MB has higher diagnostic value, but not even CK-MB is fully cardiospecific. Increase of levels can be caused by damage to skeletal muscle (trauma, muscular dystrophy, intramuscular injections, resuscitation, defibrilation), extreme exercise or chronic renal insufficiency.

It is possible to determine *enzyme activity* of CK-MB, which means that only active molecules of enzyme are recognized. Another option is to to immunochemicaly determine its *mass concentration* as a protein. It's called **CK-MB mass** and it's definitely more preferred method nowadays. Determination of CK-MB mass is *more specific and more sensitive* because it also recognizes partially degraded molecules that already lost their enzymatic function.

Determination of CK-MB mass is, according to current recommendations, acceptable only if the determination of cardiospecific troponins isn't available. CK-MB mass is also used as a proof of reinfarction at time, when levels of cTn are still high.

Lactate Dehydrogenase

Lactate Dehydrogenase (**LD** nebo **LDH**, EC 1.1.1.27) is a oxidoreductase enzyme that catalyses reversible conversion of lactate to pyruvate. This molecule consists of 4 subunits with relative molecular mass of 34 000. All of those subunits might be either M (muscle) or H (heart). That means that there are 5 isoenzymes : LD_1 (4 H subunits) - LD_5 (4 M subunits). LD is found in cytoplasm of cells in many tissues. Mild tissue damage is enough for it to be released into the circulation.



Pyruvate is converted into Lactate using NADH

Isoenzymes of Lactate Dehydrogenase

isoenzyme	subunits	presence
LD_1	H_4	myocardium + erythrocytes
LD_2	H_3M	myokardium + erythrocytes
LD_3	H_2M_2	skeletal msucle
LD_4	HM_3	liver + skeletal muscle
LD_5	M_4	liver + skeletal muscle

Examination

Increased catalytic concentration of total LD in serum is found in many illnesses. Currently, determination of LD activity is used as a nonspecific marker of cell breakdown e.g. in cancer (leukemia, testicular tumors). Late increase of total LD that might last up to 15 days is characteristic for myocardial infarction. Since erythrocytes contain a high ammount of LD, hemolysis can falsely increase levels of LD in serum. Use of LD and its isoenzymes for diagnosing acute myocardial infarction is now considered obsolete.

Physiological upper limit of LD for adult men and women is 4,10 μkat/l.

LD is determined by optical test, isoenyzyme constitution is determined by electrophoresis.

Mitochondrial Proteins

Aspartate Aminotransferase

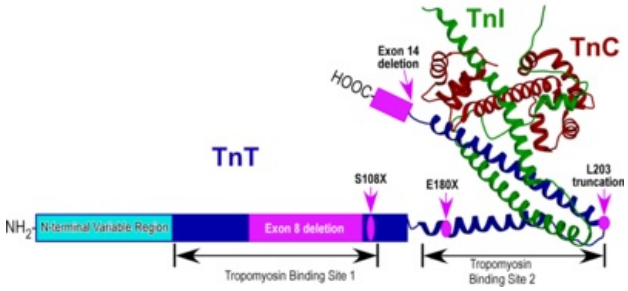
Aspartate Aminotranferase (**AST**) has a relatively high concentration in myocardium. Historically, It's one of the first biochemical markers of acute myocardial infarction, but nowadays its use is not recommended.

Structural Proteins

Troponins

Troponin T (TnT) and **troponin I** (TnI) are considered cardiac markers. TnT and TnI are found in skeletal muscle and myocardium. **Cardial isoforms** (cTnT and cTnI) have unique amino acid structure that makes them specific for **myocardium**. Cardial isoformes TnT and TnI are usually contained in the contractile apparatus and they are released due to proteolytic degradation. Only 6-8% od cTnT and 2,8-8,3% of cTnI are unbound cytosolic fraction.

Troponin cTnT normally cannot be found in blood. The process of cTnT relasing is *biphasic.* Troponin levels start to rise **in 3-8 hours** since the onset of acute myocardial infarction and the; first peak is seen in 12-18 hours after the damage of the heart muscle. This peak is caused by quick release of unbound cytosolic fraction of cTnT. The first peak is followed by another one in 3-4 days. It is caused by slower relasing of cTnT bound in *troponin-tropomyosin complex* located in the necrotic area. In 7-10 days cTnT decreases to undetectable levels.



Structure of troponin

If the blood flow in coronary artery is restored quickly, maximum levels of cTnT are detectable after circa 14 hours. Later, a second, significantly lower peak follows. The duration of increased levels depends on the size of infarction. In **extensive myocardial infarctions**, cTnT can be detected for up to 21 days. However, there is a slight disadvantage - cTnT levels are non-specifically increased in patients with renal insufficiency.

Levels of **cTnI** (which is characterized by high specifity) start to rise **in circa 3 hours** since the onset of ischemia. Increased levels are present for 5-10 days. In comparison with cTnT, levels of cTnI usually don't reach a second maximum (the cytosolic fraction is smaller).

Troponin C (TnC) is not suitable for diagnostics of acute coronary lesion, because it is identical in heart and skeletal muscle.

Troponins are determined by sensitive immunochemical methods.

Quick cTnT determinating test

Quick diagnostics is necessary in acute myocardial infarction. Biochemical tests used to determine myoglobin and troponins that can be done by the patient's bed might take a part in the diagnostics.

This is a description of quick test used to determine cardiac troponin T based on GLORIA (**G**old **L**abelled **O**ptically **R**ead **I**mmuno **A**ssay) technology produced by Roche. It uses two different monoclonal antibodies against cTnT - one of them is marked with biotin, the other one with colloidal gold.

Performing of the Test

Patient's blood is applied into the *application zone*, which contains marked antibodies. If molecules of troponin are present in the sample, sandwich complex is formed in the *reaction zone*. Before reaching the *detection zone,* glass fibers separate erythrocytes from plasma which contains immunocomplexes. The detection zone consists of two stripes: signal strip, which contains streptavidin (protein with high affinity to biotin) and control strip with immobilized troponin. Abundant antibodies marked with gold are bound to control strip. Coloration of the control strip confirms that the test is working and that the examination is valid. Sandwich immunocomplex with troponin is bound to streptavidin, which results in second colored strip. If the test is positive, two stripes appear - both in control and in signal zone. If the test is negative, only one colored strip shows - the control strip.

Recommended Procedure in suspected acute myocardial infarction

In diagnostics of acute myocardial infarction it is recommended to repeatedly determine two lab markers - *quick and definitive.*

Myoglobin has the highest diagnostic sensitivity in the early phase of acute myocardial infarction - *it is the quick marker.*

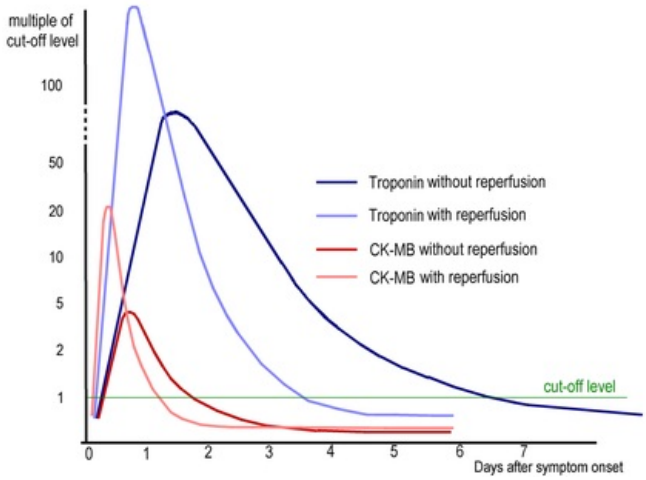
- - Early but not specific marker, that helps to distinguish if the patient with chest pains and missing or unclear ECG diagnosis has acute myocardial infarction or not.
  - Negative (not risen) level of myoglobin negates acute myocardial infarction.
  - Final diagnosis of acute myocardial infarction must me confirmed with second marker.

Troponins are definitive marker of myocardial damage.

- They are very sensitive and specific proof of myocardial necrosis - *even microscopic*.

There are quite a few benefits of troponins in diagnostics of acute myocardial infarction:

- high specifity for myocardium
- levels of troponins are almost undetectable in healthy individuals
- multiple increase of concentrations in acute myocardial infarction
- high sensitivity allows to detect even minimal myocardial damage
- increased levels staying increased for a longer time also allow late diagnostics of myocardial infarction



Changes of levels of cardiac troponins and CK-MB in acute myocardial infarction

Increased levels of cardiac troponins reflect myocardial damage. However it doesn't have to be myocardial infarction. Myocardial damage can be caused by other causes such as inflammation (myocarditis), pulmonary embolism or cardiac surgery.

Determination of CK-MB mass is acceptable only in situations when determination of troponins is not available.

- Moreover, CK, CK-MB and/or CK-MB mass are used for determination of size of the ischemic area.

Recommended schedule of blood sample collection in determining cardiac markers in suspected acute myocardial infarction.

Marker	Collection of blood sample			
	in the time of admittion	4 hours after the admittion	8 hours after the admittion	12 hours after the admittion
Early (myoglobin)	yes	yes	(yes)	–
Definitive (cardiac troponin T or I)	yes	yes	yes	yes

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Reference