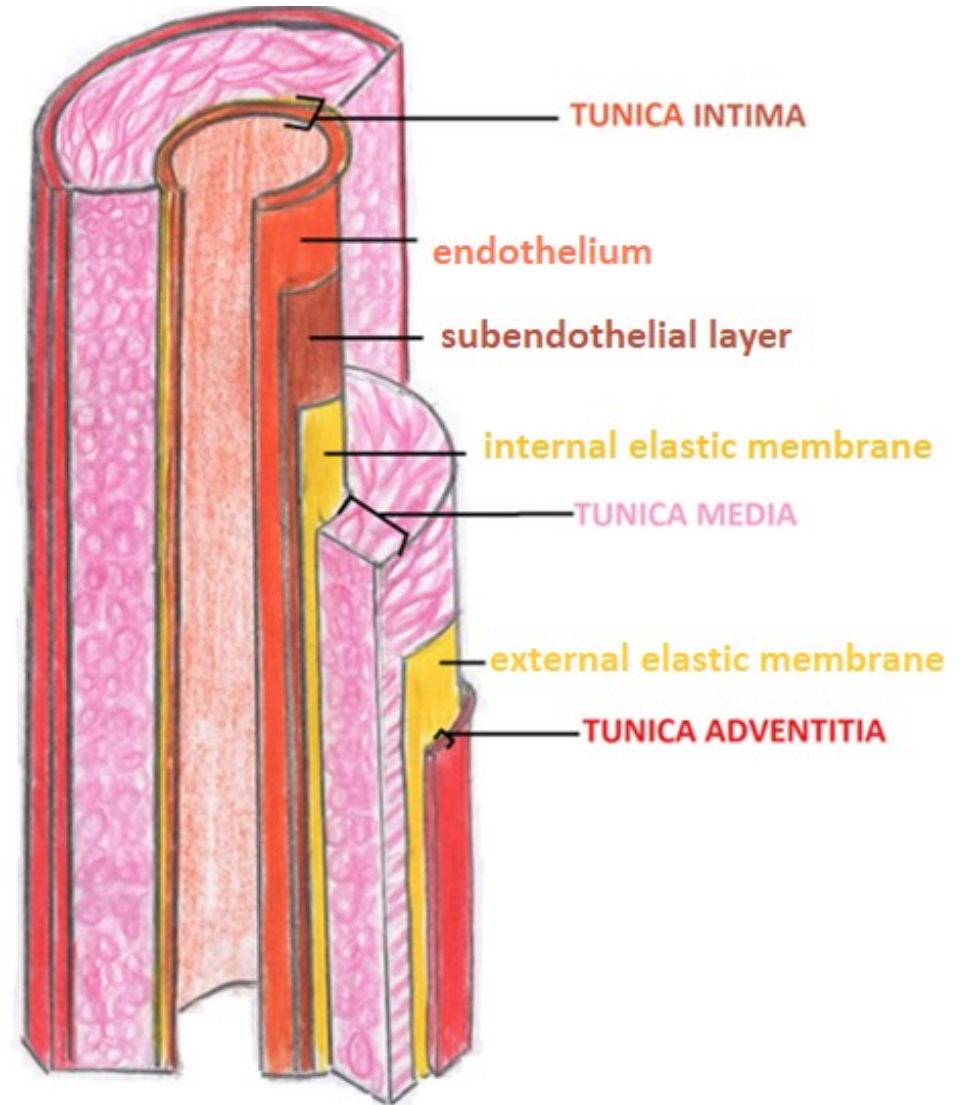# Pathobiochemistry of atherosclerosis, ischemic heart disease, cardiac markers

Vascular structure

- Blood vessels consist of 3 layers:
    1. **Tunica intima** (formed by endothelial cells).
    2. **Tunica media** (formed by smooth muscle cells and elastic fibers).
    3. **Tunica adventitia** (formed by collagen fibers).

Atherosclerosis

= chronic progressive disease of a vascular wall.

- It is characterized by the accumulation of lipids and other components of blood and fibrous tissue in the intima of the arteries, accompanied by changes in a media of vascular wall.

- Atherosclerosis develops as a chronic inflammation with an excessive proliferative response of the intima and the media to various stimuli, mainly modified LDL (low density lipoproteins).

- The development of atherosclerosis is the result of interactions between lipoproteins and cells of the immune system (mainly macrophages and T-lymphocytes) and other cell types (endothelial cells, smooth muscle cells and platelets).

Development of atherosclerosis

- The development of atherosclerotic lesion (damage) is a long-term process.

- The first step is probably endothelial cell damage.

- Monocytes and T lymphocytes are adhered to the damaged endothelial cells. These then penetrate into the space of intima.

- Conversion of monocytes and T-lymphocytes into macrophages in the intima space. Macrophages = major cells involved in atherogenesis.

- Increased permeability of endothelial lining → increased penetration of low-density lipoprotein particles into the subendothelial space.

- Lipoperoxidation in lipoprotein particles by reactive forms of oxygen and nitrogen. Absorption of lipoprotein particles by macrophages. Lipoproteins are taken up by macrophages by scavenger receptors → accumulation of lipoprotein particles in macrophages and their transformation into foam cells = basis for the formation of atheroma plaques.

- Scavenger receptors recognize oxidized LDL particles due to structural modification in Apo B100. LDL particles that have undergone lipoperoxidation cannot be removed by LDL receptors (do not recognize modified Apo B100).
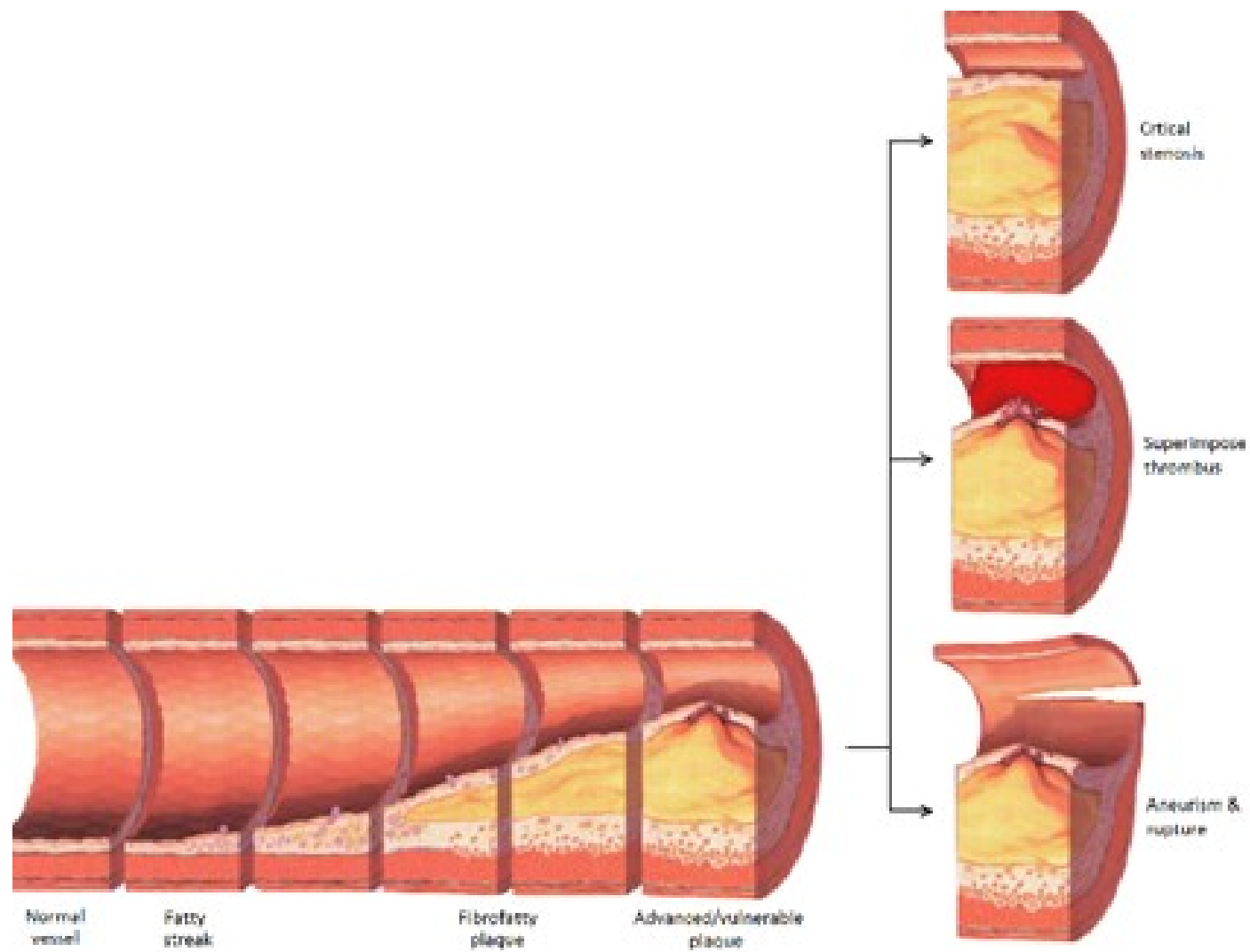
Development of atherosclerosis

- Oxidized LDL stimulates cells of smooth muscle, endothelial cells and monocytes to produce chemotactic and growth factors such as: PDFG (platelet growth factor), FGF (fibroblast growth factor), IL 1β, TNFα, and heparin-binding epidermal growth factor. → increased smooth muscle cell replication → accelerated atherogenesis process.

- Endothelial cells, due to oxidized LDL, induce formation of tissue factor and reduce formation of plasminogen activator inhibitor → conditions to accelerate platelet aggregation and thrombus formation, especially where the atheroma plaque is calcified and thus more susceptible to rupture.

Atheroma

= atherosclerotic plaque, atheroma plaque.

- Composed of a central lipid core (mainly cholesterol) covered with a fibrin cap (smooth muscle of vascular cells, collagen and elastic fibers).

- The composition of atheroma affects its stability, we distinguish: Stable atheroma: characterized by a strong and intact cap. Unstable atheroma: characterized by a higher content of lipids, foam cells, T-lymphocytes in the nucleus. The fibrous cap is thin and contains less collagen.

- Unstable plaques are more susceptible to rupture due to ongoing inflammation. Macrophages produce proteolytic enzymes (metalloproteinases such as collagenases, elastases, stromelysin) that weaken the fibrous cover. T-lymphocytes produce interferon gamma suppressing collagen production and CD40 promoting metalloproteinase synthesis.

- Plaque rupture → plaque hemorrhage + thrombus formation → acute coronary syndrom.

Normal vessel

Fatty streak

Fibrofatty plaque

Advanced/vulnerable plaque

Critical stenosis

Superimpose thrombus

Aneurism & rupture

Areas affected by atherosclerosis + its clinical manifestations

- Atherosclerosis occurs most frequently in the artery branching areas - turbulent flow (hemodynamic stress, inflammatory response).

- Clinical manifestation:
  in coronary arteries → ischemic heart disease (IHD).
  In cerebral arteries → stroke.
  In lower extremity arteries → ischemic disease of the lower extremities (leg ischemia).

Risk factors for the development of atherosclerosis:

Uncontrollable risk factors:

- Older age

- Male gender

- Positive family history

- Genetic predisposition (DM, hypertension, but also hereditary disorders of lipid metabolism such as familial hypercholesterolaemia).

Controllable risk factors:

 - Obesity

- Wrong lifestyle (physical inactivity, high intake of sugars, cholesterol and trans unsaturated fatty acids).

- Hypertension (untreated hypertension contributes to vascular endothelial damage).

- Diabetes mellitus

- Smoking (contributes to vascular endothelial damage).

- Dyslipidemia

Other risk factors:

- Estrogen deficiency in postmenopausal women.
- Infections (contributing to epithelial dysfunction, e.g. *Chlamydia pneumoniae*, Cytomegalovirus, *Haemophilus influenzae*, *Helicobacetr pylori*, Epstein-Barr virus).

Biochemical risk factors for the development of atherosclerosis:

- ↑ total cholesterol
- ↑ LDL-cholesterol
- ↓ HDL-cholesterol
- ↑ TAG (triacylglycerols)
- Hyperglycemia
- Hyperinsulinemia
- ↑ level of homocysteine

Gender and estrogen deficiency in postmenopausal women

 - Women in reproductive age are protected from atherosclerosis and its consequences against men of the same age.

- This protection in women decreases during menopause, when estrogen decreases and as a result LDL cholesterol and triglyceride levels increase and protective HDL cholesterol levels are stagnating or decreasing.

↑ total cholesterol, ↑ LDL-cholesterol, ↓ HDL-cholesterol

- Cholesterol is always present in the atherosclerotic plaque.

- Atherogenicity of cholesterol is due multiplication lipoproteins containing apolipoprotein B100 that transport cholesterol to peripheral tissues = atherogenic lipoproteins.

- HDL is a lipoprotein that transports cholesterol into other lipoproteins and to the liver = anti-atherogenic lipoprotein.

- ↑ pro-atherogenic lipoproteins (VLDL, IDL, LDL, Lp (a)) + ↓ HDL = higher risk development of atherosclerosis.

↑ level of homocysteine

- Homocysteine is a non-essential amino acid that is produced by the metabolism of methionine.

- ↑ level of homocysteine support the process of atherosclerosis by directly damaging the vascular endothelium, stimulating oxidative stress and vascular myocyte proliferation, and reducing local nitric oxide production.

- Vitamins B6, B13 and folic acid play an important role in the metabolism of homocysteine → a diet with a higher content of these vitamins or their supplementation can help reduce homocysteine levels.

Diabetes mellitus, hyperglycemia, hyperinsulinemia

- Long-term hyperglycaemia contributes to oxidative stress. Hyperglycaemia affects the formation of free radicals, which can lead to lower levels of antioxidants and increase lipid peroxidase.

- In hyperglycemia, a non-enzymatic protein glycation process occurs, resulting in advanced glycation end products (AGEs). AGEs react with specific receptors (RAGE). Binding of AGE to RAGE leads to activation of NF-κB and oxidative stress → damage of the vascular endothelium.

- Hyperinsulinemia accelerates atherosclerosis by several mechanisms: it stimulates lipogenesis leading to higher VLDL synthesis/secretion, affects the growth and proliferation of vascular smooth muscle cells, activates genes involved in the anti-inflammatory response, and stimulates collagen synthesis. Furthermore, hyperinsulinemia causes sodium retention, arterial hypertension and weight gain.

Ischemic heart disease (IHD)

- IHD is defined as myocardial ischemia due to a pathological process in the coronary arteries.

- Causes of disorder of myocardial perfusion:
  A) Causes of organic origin (atherosclerosis, thrombus, embolism, arteriitis, dissection of the coronary artery).
  B) Causes of functional origin (spasm of the coronary artery).

- Very common is combination of causes of organic origin with cause of functional origin.

- The most common cause of IHD is unstable atherosclerotic plaque in coronary artery → thrombus formation above plaque → coronary artery occlusion → acute MI

- Basic classification of IHD:
  - Acute forms of IHD (unstable angina pectoris, acute myocardial infarction (MI), sudden death)
  - Chronic forms of IHD (angina pectoris, vasospastic angina pectoris, silent ischemia, syndrome X, IHD with arrhythmias, IHD with heart failure).

IHD

- Ischemia occurs when oxygen supply demands outweigh the possibilities of perfusion.

- Increased oxygen demand for the myocardium can be induced by physical exertion, increase in systolic pressure or tachycardia. Myocardium responds to chronic ischemia by developing a collateral vascular bed that helps blood to circumvent stenosis, allowing the supply of myocardium.

- Risk factors contributing to IHD: hypertension, lipid metabolism disorders, smoking, diabetes mellitus, obesity, lack of physical activity, stress, positive family history, male gender, etc.

Symptoms of IHD

- The main symptom is chest pain (astringent, burning) = stenocardia, chest pressure, feeling of shortness of breath. The pain response to nitroglycerin administration is important. The patient may also be pain free (silent ischemia, e.g. in diabetics). Patients with DM may have damaged nerve fibers transmitting painful stimuli → they do not feel stenocardia, but myocardium is not supplied with oxygen).

- Other symptoms of IHD include dyspnea (after exertion, at rest, nighttime), swelling of the lower limbs, syncope, palpitations.

- The basic examination method is ECG + determination of cardiac markers.

Chronic forms of IHD

- Angina pectoris (AP): is characterized by seizure stenocardia due to transient myocardial ischemia due to physical stress, emotional stress ... Due to atherosclerotic coronary artery damage, there is a disproportion between the supply and consumption of oxygen in the myocardium. Stenocardia disappears after finishing of physical stress, when demand of myocardium for oxygen decreases.

- Vasospastic AP: arises due to spasm of coronary artery, stenocardia not manifesting in physical activity. Factors causing vasospastic AP include smoking, emotional stress, rapid cooling...

- Syndrome X: The cause of the syndrome is damage to small arterioles (microvascular AP). Stenocardia after exercise and normal coronarography is present.

Acute forms of IHD

- Unstable AP: is defined as a newly formed AP or an existing AP that has deteriorated in the last 4 weeks. The deterioration may relate to a higher frequency of complaints, increased pain intensity or prolonged pain.

- Sudden death: is defined as death within 1 hour of the onset of symptoms. 70% is caused by IHD.

- Acute MI = acute necrosis of the area of cardiomyocyties due to prolonged ischemia. The cause of ischemia is a sudden coronary artery occlusion or extreme progressive stenosis. Stenocardia occurs regardless of physical stress. Pain persists after nitrate administration. Coronary artery occlusion is most often caused by a thrombus formed after the rupture of an unstable atherosclerotic plaque. Coronary artery occlusion may also be due to the following causes: arteriitis, trauma, aortic dissection or embolism in the coronary artery. Necrotic tissue is replaced by connective tissue (infarct scar).

Cardiac markers

- Cardiac markers = biochemical indicators of ischemia, necrosis and hemodynamic changes affecting the heart. It is used to detect and evaluate the severity of acute forms of IHD (acute MI, unstable AP) and to determine the prognosis of people who have had MI. Appropriate treatment may be initiated according to cardiac marker levels.

- cardiac markers = enzymes, proteins and hormones found in cardiomyocytes. Normally, their blood concentration is very low. When the heart musce cells are damaged (mainly when they die), these enzymes, proteins and hormones begin to release → ↑ their blood concentration.

- Cardiac markers are examined from a blood sample.

- Different cardiac markers have different times of their rise, peak and decrease → we can determine the progression of MI, the time of onset of IM or predict its possible recurrence.

- Specific cardiac markers (their levels are increased only when the heart is damaged) x non-specific cardiac markers (their levels may also be increased when other tissues, such as skeletal muscle, are damaged).

Major cardiac markers - troponin

- Troponins are proteins, which in the form of the so-called troponin complex, together with actin and tropomyosin, are part of the thin muscle fibers.

- Troponin exists in several molecular isoforms.

- Troponin T (TnT) and troponin I (TnI) are used as cardiac markers. TnT and TnI occur in skeletal muscle and myocardium. Cardiac isoforms (cTnT and cTnI) have a unique amino acid composition and are therefore specific for the myocardium.

- Troponin levels remain elevated for up to 2 weeks → the possibility of revealing myocardial damage.

- Examination: immunochemical methods.

- Troponin = cardiac marker of the first choice.

Major cardiac markers – creatinine kinase

- Creatine kinase (CK) catalyzes the reversible phosphorylation of creatine to creatine phosphate by ATP

- CK consists of 2 subunits: B (brain), M (muscle).

- According to the representation of individual subunits we can distinguish 3 isoforms of CK: CK-BB (brain isoenzyme), CK-MM (muscle isoenzyme) and CK-MB (myocardial isoenzyme).

- CK-MB is not completely cardiospecific (an increase may also be caused by skeletal muscle damage).

- CK-MB can be determined as an enzyme activity or preferably immunochemically as a protein in the form of a mass concentration (CK-MB mass).

- Determination of CK-MB mass is more sensitive and specific → we also show partially degraded molecules that have lost enzymatic activity.

Major cardiac markers – lactate dehydrogenase

- Lactate dehydrogenase (LDH, LD) = oxidative-reducing enzyme catalysing the conversion of lactate to pyruvate.

- LDH consists of 4 subunits. Each of these subunits can be either M (muscle) or H (heart) → we recognize 5 isoenzymes:

Isoenzyme	Subunits	Occurrence
LD_1	H_4	Myocardium + erytrocytes
LD_2	H_3M_1	Myocardium + erytrocytes
LD_3	H_2M_2	Skeletal muscle
LD_4	H_1M_3	Liver + skeletal muscles
LD_5	M_4	Liver + skeletal muscles

- Increased catalytic LDH concentration in serum accompanies many diseases. It is currently used as a non-specific marker of cell breakdown, e.g. in cancer. It is characterized by an increase in total LDH after MI, which may persist for up to 15 days. Hemolysis (high LDH content in the erytrocytes) may falsely increase serum LDH concentrations. The use of LDH and its isoenzymes for the diagnosis of acute coronary syndrome is currently obsolete.

- LDH determination: optical tests, electrophoretic determination of LDH isoforms.

Major cardiac markers – aspartate aminotransferase

- Aspartate aminotransferase (AST) catalyzes the reversible transfer of the amino group from aspartate to 2-oxoglutarate.

- AST is highly present in the myocardium, but it can also be found in the liver, skeletal muscle, erythrocytes, kidneys, pancreas...

- AST is currently not recommended for the diagnosis of acute coronary syndrome.

- The determination of AST is used in liver assays to determine hepatocyte damage.

Major cardiac markers - myoglobin

- Myoglobin = hemoprotein, consisting of a single polypeptide chain and one heme group.

- Reversibly binds and carries oxygen in muscle cells.

- Skeletal muscle and myocardial myoglobin is identical.

- After myocardial damage, it is rapidly released into the blood → suitable for early diagnosis of acute MI.

- Disadvantage: insufficient cardiospecificity. It may also be increased due to skeletal muscle damage, after muscle load, in renal insufficiency.

Enzyme	Mr [Da]	Biological half-life	Localization in the cell
CK	86 000	17 h	cytoplasm
CK-MB	86 000	13 h	
LD (mainly LD$_1$)	135 000	110 h	
Myoglobin	17 800	15 min.	
cTnT (cytoplasmic fraction)	37 000	2 – 4 h	
cTnI (cytoplasmic fraction)	22 500	2 – 4 h	
cTnT	37 000	2 – 4 h	fibrillar contractile complex
cTnI	22 500	2 – 4 h	
AST (mitochondrial isoenzyme)	93 000	34 h	mitochondria

Basic characterization of biochemical markers of myocardial infarction

Markers	Increase	Peak level	Normalization	Physiological value
Myoglobin	0,5 – 2 h	4 – 10 h	0,5 – 1 day	M 19 – 92 µg/l F 12 – 76 µg/l
CK-mass	2 – 6 h	12 – 24 h	2 – 3 days	0,0 – 5,0 µg/l
CK-MB	3 – 6 h	16 – 36 h	3 – 5 days	M 0,2 – 3,6 µkat/l F 0,2 – 3,1 µkat/l
cTnT	3 – 8 h	12 – 18 h (1. peak) 72 – 96 h (2. peak)	7 – 14 days	0,00 – 0,05 µg/l
cTnI	3 – 12 h	12 – 24 h	5 – 10 days	0,0 – 0,1 µg/l
AST	4 – 8 h	16 – 48 h	3 – 6 days	0,05 – 0,72 µkat/l
LD	6 – 12 h	24 – 60 h	7 – 15 days	3,5 – 7,7 µkat/l

Levels of biochemical markers in acute myocardial infarction

Major cardiac markers – natriuretic peptides

- Natriuretic peptides = tissue hormones whose function is to stimulate the secretion of Na^+ (and water) in response to an increase in blood pressure or cardiac muscle tension, thereby reducing circulating volume.

- There are several natriuretic peptides:
  1. Natriuretic peptide A (ANP, atrial): is secreted by cardiomyocytes in the form of prohormone, which is cleaved into 2 fragments → self ANP (biologically active) and N-terminal fragment (N-BNP, biologically inactive). Its secretion is increased by tension in the wall of the atrium.
  2. Natriuretic peptide B (BNP, brain): it was first described in pig brain (hence its name). In humans, it is secreted by ventricular cardiomyocytes in response to increased tension in wall of ventricle or ventricular dilatation. It is secreted in the form of prohormone, which is cleaved into 2 fragments → self BNP (biologically active) and N-terminal fragment (NT-proBNP biologically inactive).
  3. Natriuretic peptide C (CNP): is secreted by the vessel endothelium in response to endothelial stress.

- BNP or its N-terminal propeptide NT-proBNP is most suitable for diagnosis of cardiac insufficiency.

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