BIOMARKERS OF CARDIOVASCULAR DISEASES

Dr. Ata Firouzi

MD, professor of interventional cardiology

Dr. Ermia Tabande

MD, assistant professor of cardiology

Dr. Amin Elahifar

MD, assistant professor of cardiology

1- A 70-year-old man came to the emergency room complaining of chest pain since last night, nausea and sweating. Ischemic changes can be seen in the ECG. Which biomarker is more specific for diagnosing myocardial infarction?

A) CK-MB
B) Myoglobin
C) D-dimer
D) Troponin

2- A young lady with a history of taking oral contraception pills has complained of lower limb swelling and subsequently shortness of breath and heart palpitations. Which biomarker is more sensitive to check the presence or absence of blood clots?

A) Pro-BNP
B) D-Dimer
C) CRP
D) Troponin

3- A middle-aged man with a history of heart attack and angioplasty went to the emergency room last week complaining of the same chest pain. Which test is more helpful in diagnosing myocardial infarction again?

A) Troponin
B) Pro-BNP
C) CK-MB
D) Myoglobin

4- An elderly smoker has come to the clinic complaining of shortness of breath and orthopnea. Which biomarker is more useful for investigating cardiac causes than non-cardiac ones?

A) Troponin
B) Homocyctein
C) Pro-BNP
D) D-dimer

5. The patient complained of shortness of breath, weakness and evidence of a recent myocardial infarction in the electrocardiogram, went to the emergency room. The patient's chest pain was a week ago. The high level of which biomarker is useful for diagnosing a recent heart attack?

A) Troponin
B) D-dimer
C) CRP
D) Pro-BNP

Cardiac Biomarker

- Cardiac biomarkers are protein molecules released into the blood stream from damaged heart muscle.

three criteria for biomarkers :

1. Accurate repeated measurements at reasonable cost.
2. Must provide additional information
3. Should aid treatment.

Diagnosis of Acute MI

- Recommended by the European Society of Cardiology and American College of Cardiology;

Requires presence of at least two of the following characteristics:

1. Ischemic symptoms
2. Typical rise and gradual fall of cardiac troponins or More rapid rise and fall of CK- MB
3. Typical ECG pattern(changes)

Characteristics of an ideal cardiac marker

- The ideal marker in myocardial injury would persist in circulation for several days to provide a late diagnostic time window for patient who arrived late after the event.

- Marker should play a designed role in the treatment and management of clinical subject.

- High concentration in the myocardium.

- Absence from non-myocardial tissue (high specificity).

- Rapid release into plasma following myocardial injury.

- Correlation between blood level and extent of myocardial injury for prognosis.

- Can be measured by rapid, simple and automated methods.

History of cardiac biomarkers

1954 - SGOT (AST)

1955 - LDH

1960 - CPK

1972 - CPK isoforms by Electrophoresis

1975 - CK - MB by immunoinhibition

1975 - Myoglobin

1985 - CK - MB Mass immunoassay

1989 - Troponin T

1992 - Troponin I

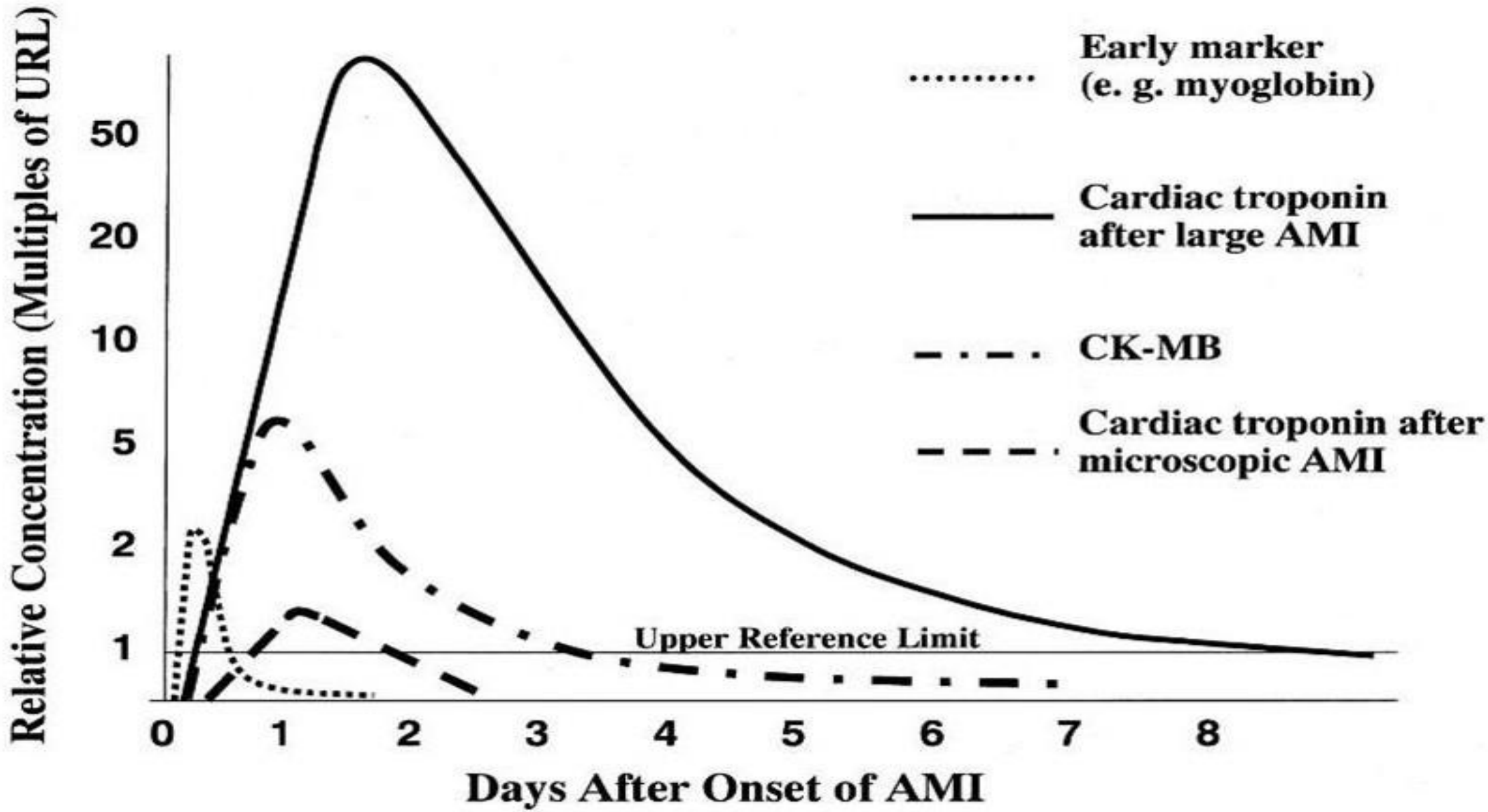
Classification of cardiac biomarkers

- **Biomarkers of myocardial injury:**

- Creatine kinase - MB
- Myoglobin
- Cardiac troponins

- **Biomarkers of hemodynamic stress and inflammation:**

- Natriuretic peptides
- CRP


Relative Concentration (Multiples of URL)
50
20
10
5
2
1
Upper Reference Limit
0
1
2
3
4
5
6
7
8
Days After Onset of AMI
Early marker
(e. g. myoglobin)
Cardiac troponin
after large AMI
CK-MB
Cardiac troponin after
microscopic AMI

MI marker changes in plasma

Enzyme / Protein	Detectable (hours)	Peak value (hours)	Duration (days)
CK-MB	3-10	12-24	1.5-3
Total CK	5-12	18-30	2-5
Cardiac troponins	3-4	12-24	upto 10

Creatine kinase

- Creatine kinase is an enzyme expressed in a number of tissues.
- CK-MB is more sensitive and specific for MI than total CK.
- It rises and falls transiently after MI.
- Appears in blood within 4-6 hours of heart attack.
- Returns to normal within 2-3 days.
- it catalyses the conversion of creatine to phosphocreatine degrading ATP to ADP.
- The CK enzyme consists of two subunits, B (Brain type) or M (Muscle type), Making three different isoenzymes: CK-MM,CK-BB and CK-MB.

CK-MB

CK-MB Advantages:

Useful for early diagnosis of MI.

Useful for diagnosis of re-infarction.

Disadvantages:

Not significant if measured after 2 days of MI (delayed admission)

Not highly specific (elevated in skeletal muscle damage)

- **False positive** (for MI) CK-MB elevation can be seen in:

- Significant skeletal muscle injury.
- Cardiac injury for reason other than MI.
- Defibrillation.
- Blunt chest trauma.

Myoglobin

- Small-size heme protein found in all tissues mainly assists in oxygen transport.
- It is released from all damaged tissues.
- Increases often occur more rapidly than Troponin and CK.
- Released from damaged tissue within 1 hour.
- Normal value:

17.4-105.7 ng/ml

- Timing:

Earliest Rise: 1-3 hrs

Return to normal :6-9 hrs

Conditions for myoglobin increase;

- Acute myocardial infarction
- Skeletal muscle damage
- muscular dystrophy and inflammatory myopathies
- Renal failure
- severe uremia
- Shock
- Trauma

CLINICAL USEFULNESS OF MYOGLOBIN

- Rapid monitor of success of thrombolytic therapy
- Negative predictor of MI

Due to poor specificity, myoglobin levels do not always predict myocardial injury and

Not utilized often for AMI/cardiac damage, because of its very rapid metabolism.

Cardiac troponins;

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

- Troponin is attached to the tropomyosin sitting in the groove between actin filaments in muscle tissue.

- CTns are structurally different from muscle troponins.

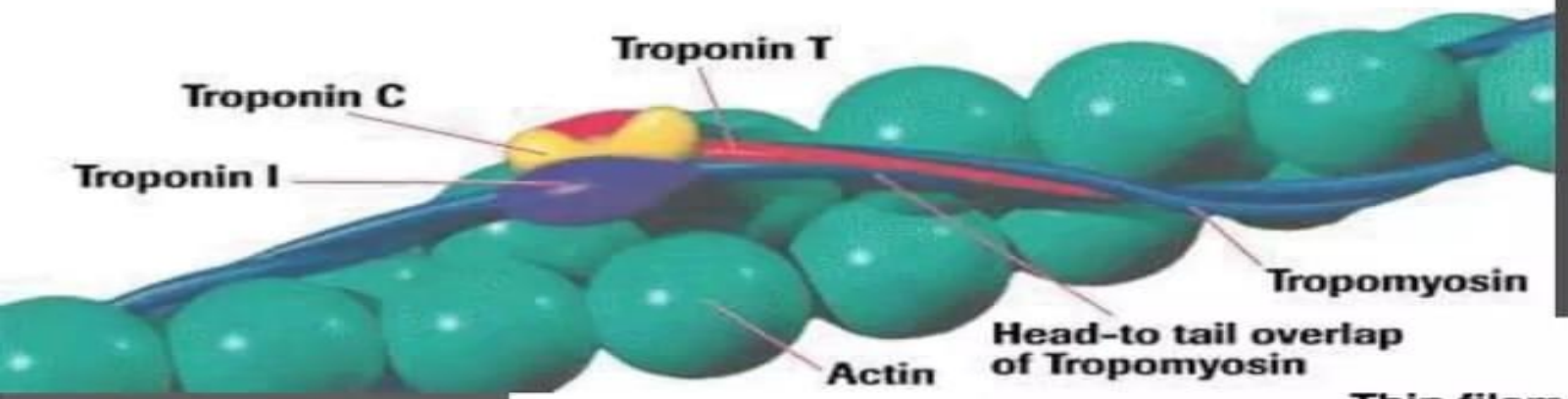
- Highly specific markers for detecting MI.

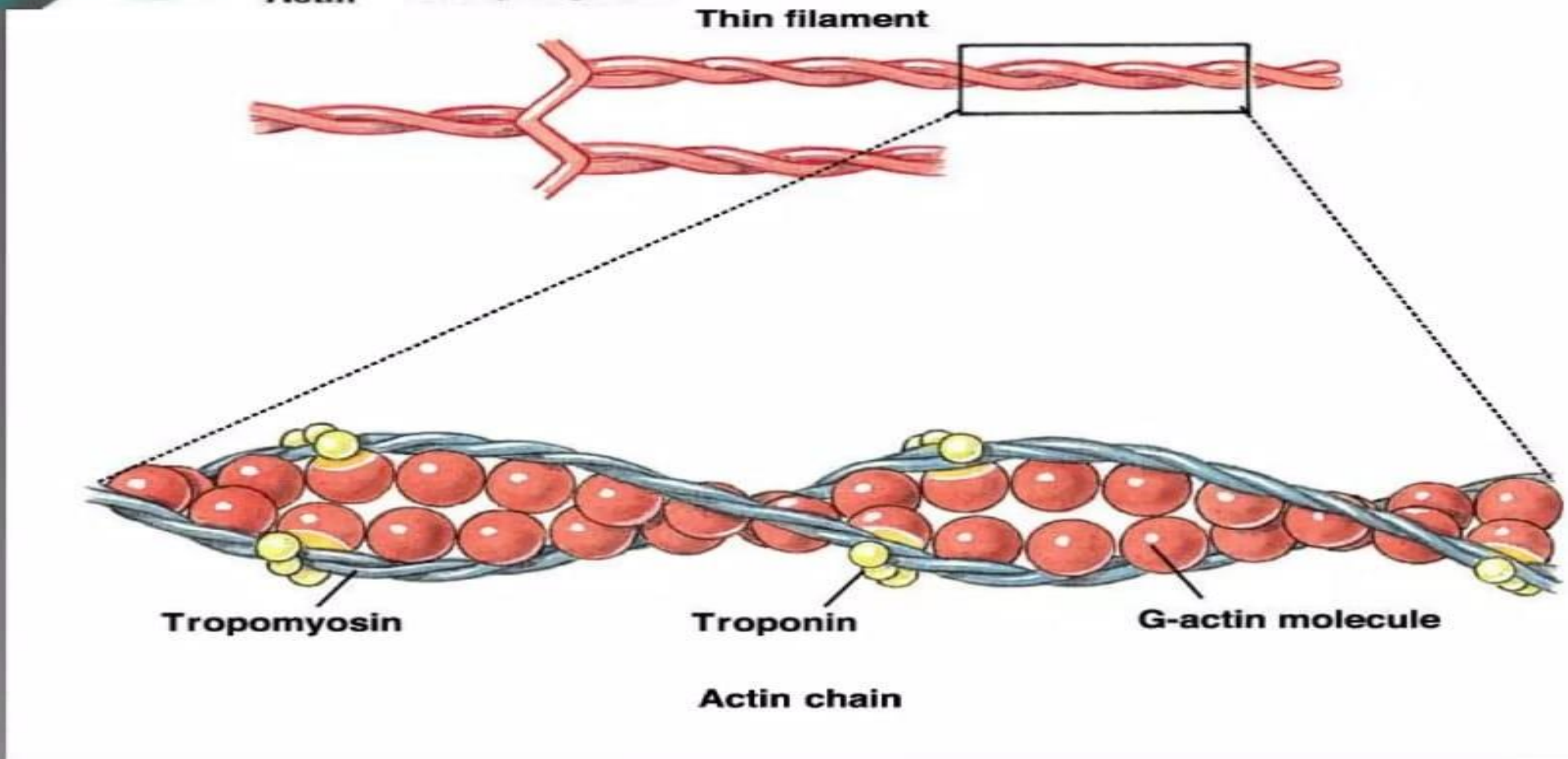
- Appear in plasma in 3-4 h after MI

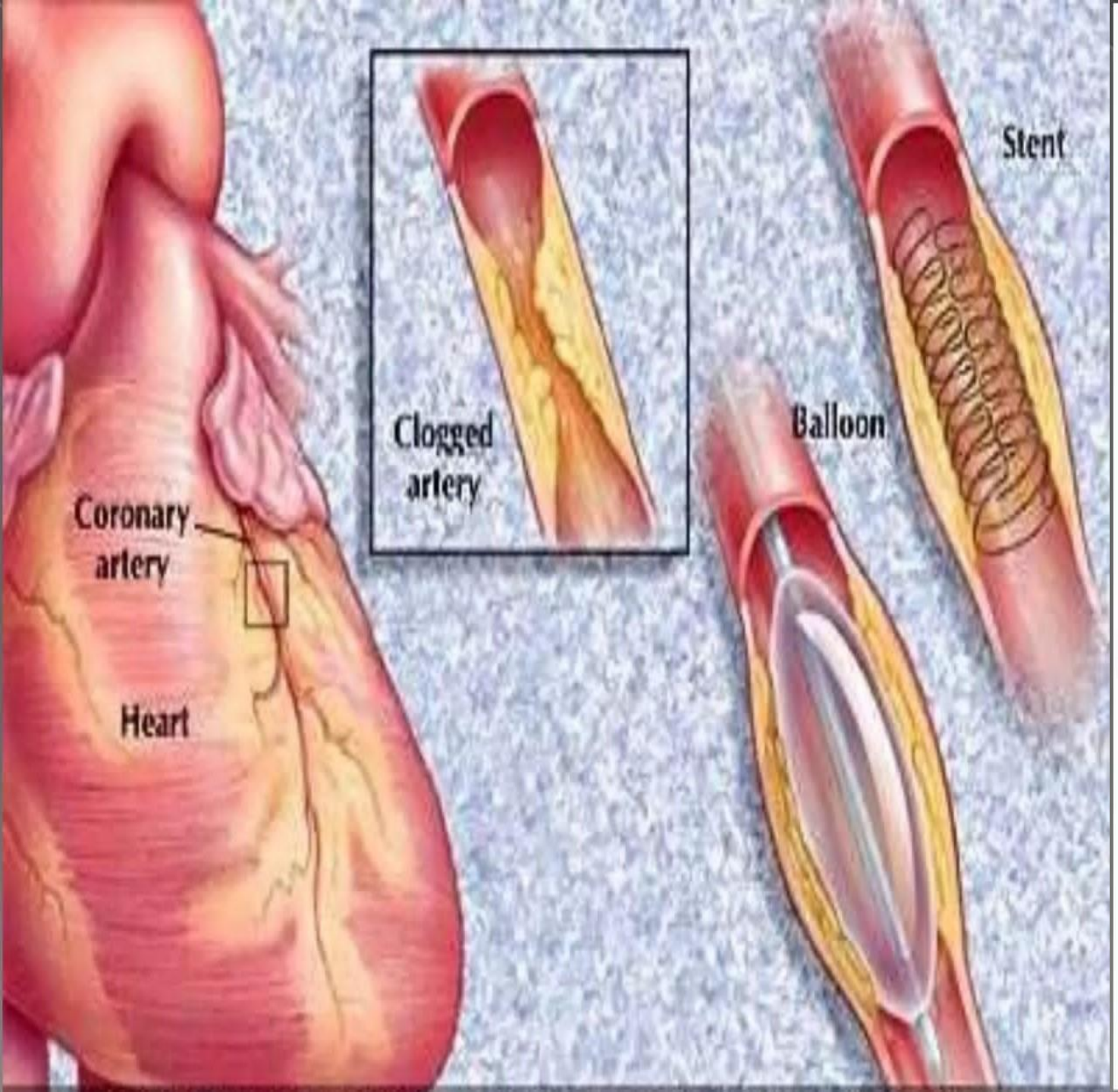
- Remain elevated for up to 10 days

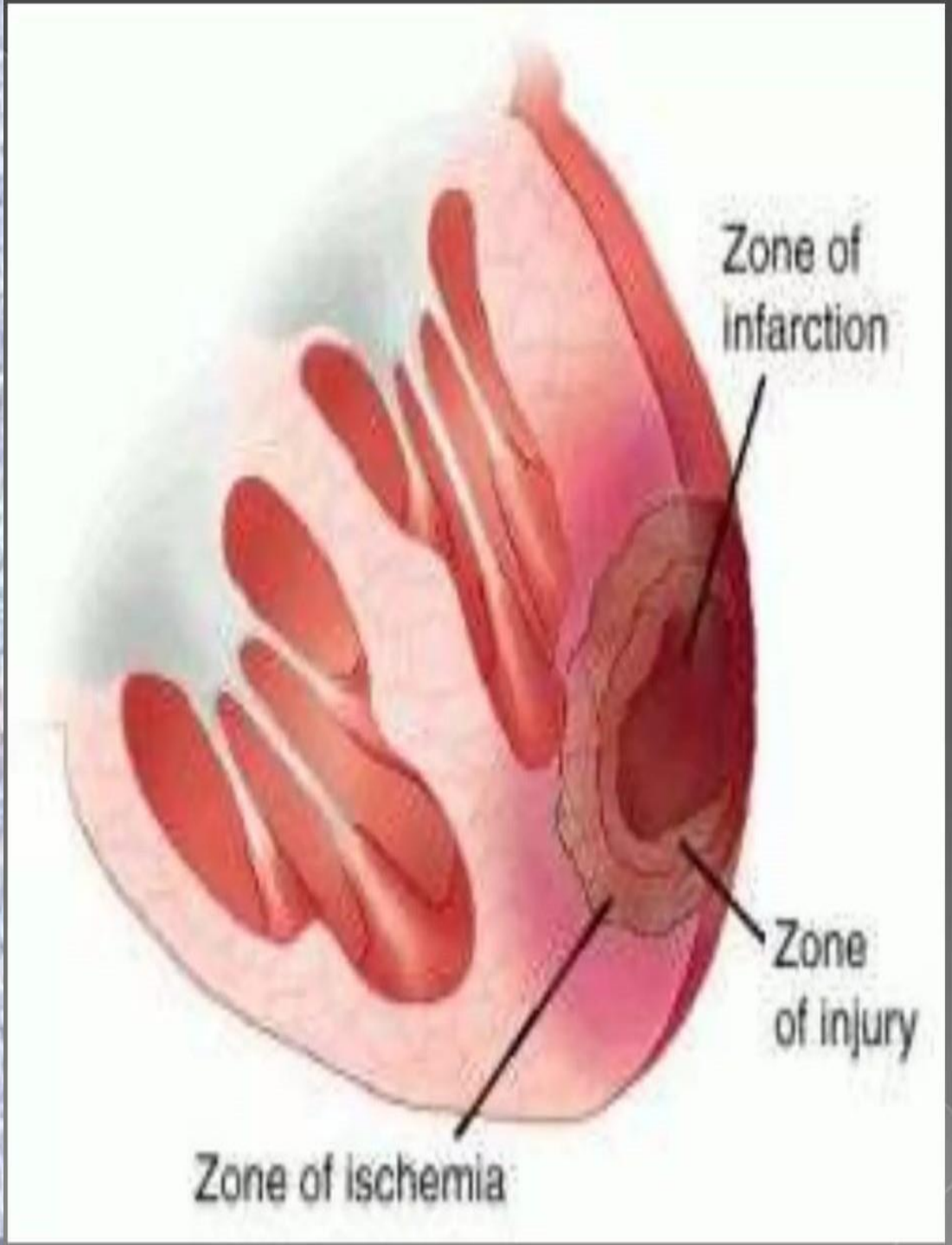
TROPONIN T

A regulatory protein released when cardiac cell necrosis occurs.






Clogged artery
Stent
Balloon
Coronary artery
Heart


Zone of infarction
Zone of injury
Zone of ischemia

Cardiac troponins;

- cTns measurements are useful in excluding AMI after 12 h following chest pain or other symptoms.

- (soluble)are released rapidly into the blood (first few hours) and Structurally bound troponins(insoluble) are released later for several days which account for the prolonged plateau of troponin release.

- Under normal circumstances there is no cardiac troponin T or I detectable in blood.

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 and Troponin-I is an inhibitory protein.

Troponin I

- Cardiac Troponin I (cTnl) is a cardiac muscle protein with a molecular weight of 24 kilo-Daltons and has a additional amino acid residues on its N-terminal that are not exist on the skeletal form.

The half life = 2~4 hours.

Serum increase = 2-8 hours

Troponin T

- Cardiac Troponin T (cTnT) is present in fetal skeletal muscle and In healthy adult skeletal muscle c TnT is absent.

- Biological half life and early serum increases of c TnT are similar to that of cTnI.

Troponin levels

- Less than 5% in cytosol.

- Troponin levels begin to rise 2-3 hours after onset of myocardial injury, Elevations in Troponin-I and Troponin-T can persist for up to 10 days after MI.

- studies have failed to find a source of Troponin-I outside the heart, but have found some Troponin-T in skeletal muscle.

Conditions commonly associated with cardiac troponin elevations;

- Arrhythmias/ Congestive heart failure/ Coronary artery disease/

Coronary vasospasm/ Critically ill patient/ Hypertension crisis/ Myocarditis/

Pericarditis/ Pulmonary embolism/ Pulmonary hypertension/ Renal failure/ Sepsis-

related myocardial dysfunction / inflammatory diseases/ Trauma …

Diagnosis based on sensitive troponin I assay

- They used the concentration of 0.04 ng per mL as the upper reference limit and established the diagnosis of myocardial infarction if one value of more than 0.04 ng per mL was documented, combined with a rise or fall in the value of 30% or more within 6 hours after admission.

High-sensitivity Troponin;

- this test detects the same protein that the standard test does, just at much lower levels.

- Because this version of the test is more sensitive, it becomes positive sooner and may help detect ACS earlier than the standard test.

- The hs-troponin test may also be positive in people with stable angina and even in people with no symptoms. When it is elevated in these individuals, it indicates an increased risk of future heart events such as heart attacks.

- This test may not be available in all labs.

- The introduction of a high-sensitivity troponin (hs-Trop) assay has been very useful in patients with non–ST-elevation myocardial infarction (NSTEMI), which allows diagnosis by a single blood test, thus permitting early treatment than otherwise might be advised.

- Some studies have concluded that a single hsTnT level ≤ 6 ng/L indicated a very low risk of AMI, whereas serial levels exceeding 19 ng/L identified patients with < 1% risk of adverse cardiac events.

- In highly suspected cases of AMI, high sensitive troponin assay can be used effectively to “rule out” in about 60% cases when the value remains low at 0 hour with no change after 1 hour.

- When it is elevated at 0 hour with a large increase at 1 hour, it is a “rule-in” and is diagnostic of an AMI.

- **False negative results** happen when troponin is not elevated after a heart attack occurs;

- It can take a few hours for troponin levels to rise after a heart attack, so initial testing may not show detectable amounts of troponin, In order to rule out a false negative result, troponin levels are typically tested again over a 3-6 hr later to look for rising values.

- **False positive results** occur when troponin is elevated but no heart attack actually occurred, These misleading results can occur because of other medical conditions that can increase troponin levels.

Biomarkers of hemodynamic stress;

Natriuretic peptides

- The natriuretic peptides are a group of structurally similar but genetically distinct peptides.

- NPs are identified as regulatory diuretic-natriuretic substances responsible for salt and water homeostasis and Lowers blood pressure.

The NP family includes

- ANP : atrial natriuretic peptide
- N-terminal proANP
- BNP : brain natriuretic peptide
- N-terminal proBNP
- CNP : C-type natriuretic peptide

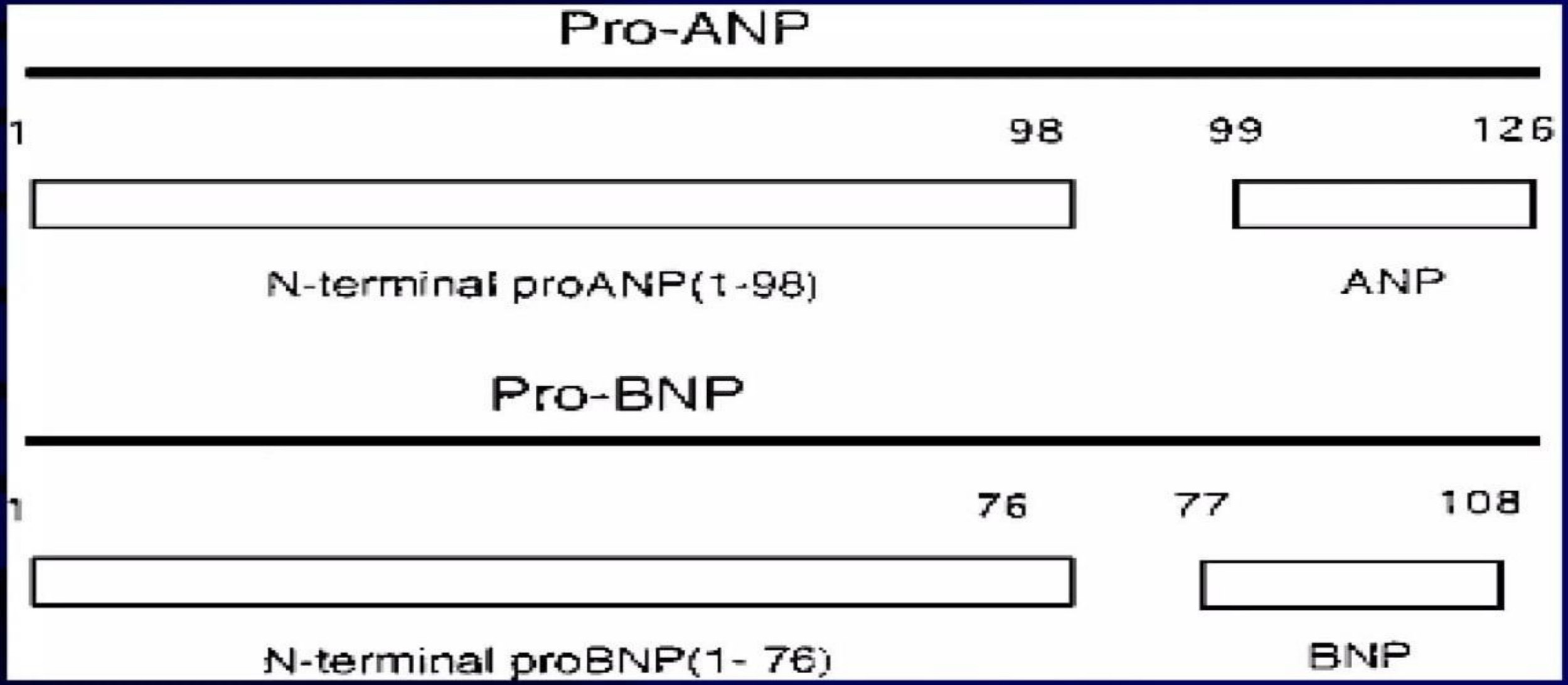
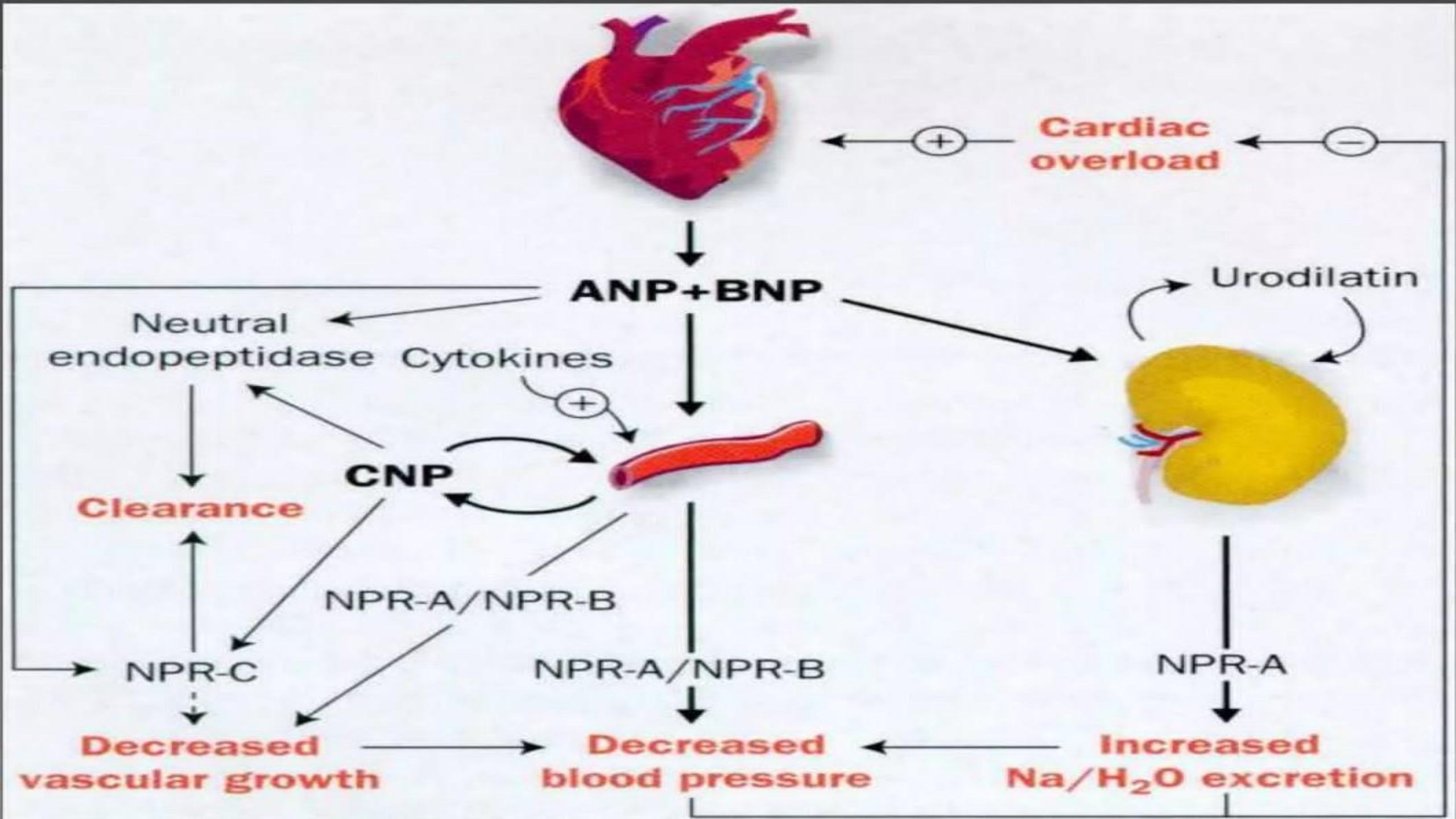


Fig. Schematic representation of the ANP and BNP precursors with sequence numbering defining low-molecular-mass forms, N-terminal forms and high-molecular-mass precursors


Cardiac overload
ANP+BNP
Urodilatin
Neutral endopeptidase
Cytokines
CNP
Clearance
NPR-A/NPR-B
NPR-C
NPR-A/NPR-B
NPR-A
Decreased vascular growth
Decreased blood pressure
Increased Na/H2O excretion

- **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.

Natriuretic peptide;

- originally isolated from porcine brain and Subsequently also isolated from human heart.

- Circulating levels of BNP are raised in patients with cardiovascular or renal disease.

- More important than ANP in heart failure Greatest proportion of circulating BNP is thought to come from the ventricles (left).

- ANP and BNP concentrations increase in response to volume expansion and pressure overload of the heart.

• ANP and BNP have been shown to be physiological antagonists of the effects of;

(1) angiotensin II on vascular tone

(2) aldosterone secretion

(3) renal-tubule sodium reabsorption

(4) vascular-cell growth

Conditions or factors commonly associated with BNP or NT-Pro-BNP elevations;

- Age
- Arrhythmias
- Cardiomyopathy: hypertrophic, ischemic, or dilated
- Congestive heart failure
- Coronary artery disease
- Gender
- Hypertension
- Left ventricular diastolic dysfunction
- Pulmonary embolism
- Renal failure
- Right heart failure
- Right ventricular overloading: fluid, or pressure overloading
- Sepsis-related myocardial dysfunction

Biomarkers of inflammation, prognostic markers and markers of risk stratification;

1. **C-reactive protein**

- CRP is an acute-phase protein produced by the liver.

- Pentameric structure consisting of five 23-kDa identical subunits.

- Plasma levels can increase rapidly to 10000x levels.

- CRP previously known to be a marker of high risk in cardiovascular disease.

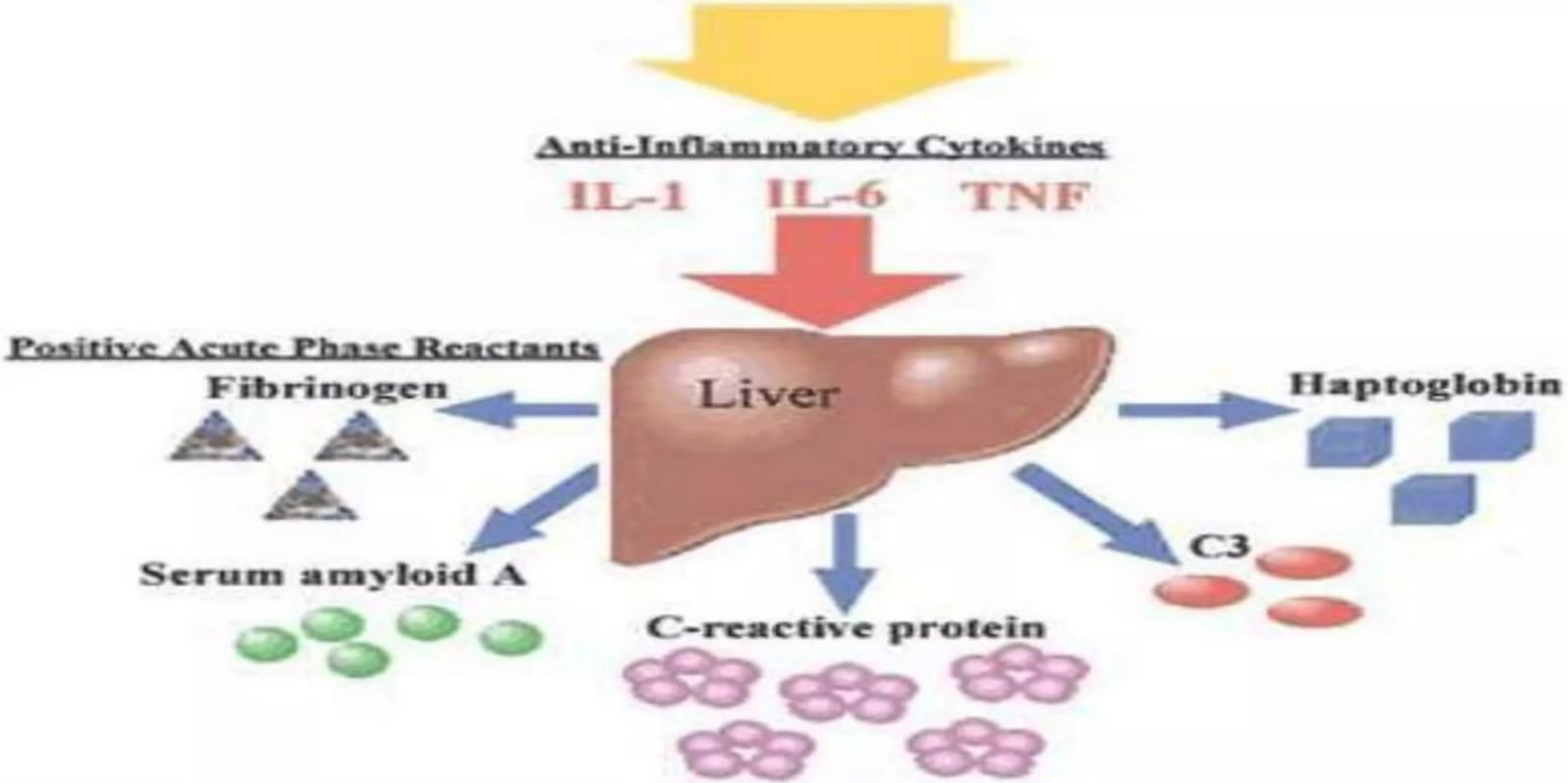
- More recent data may implicate CRP as an **actual mediator of atherogenesis**.

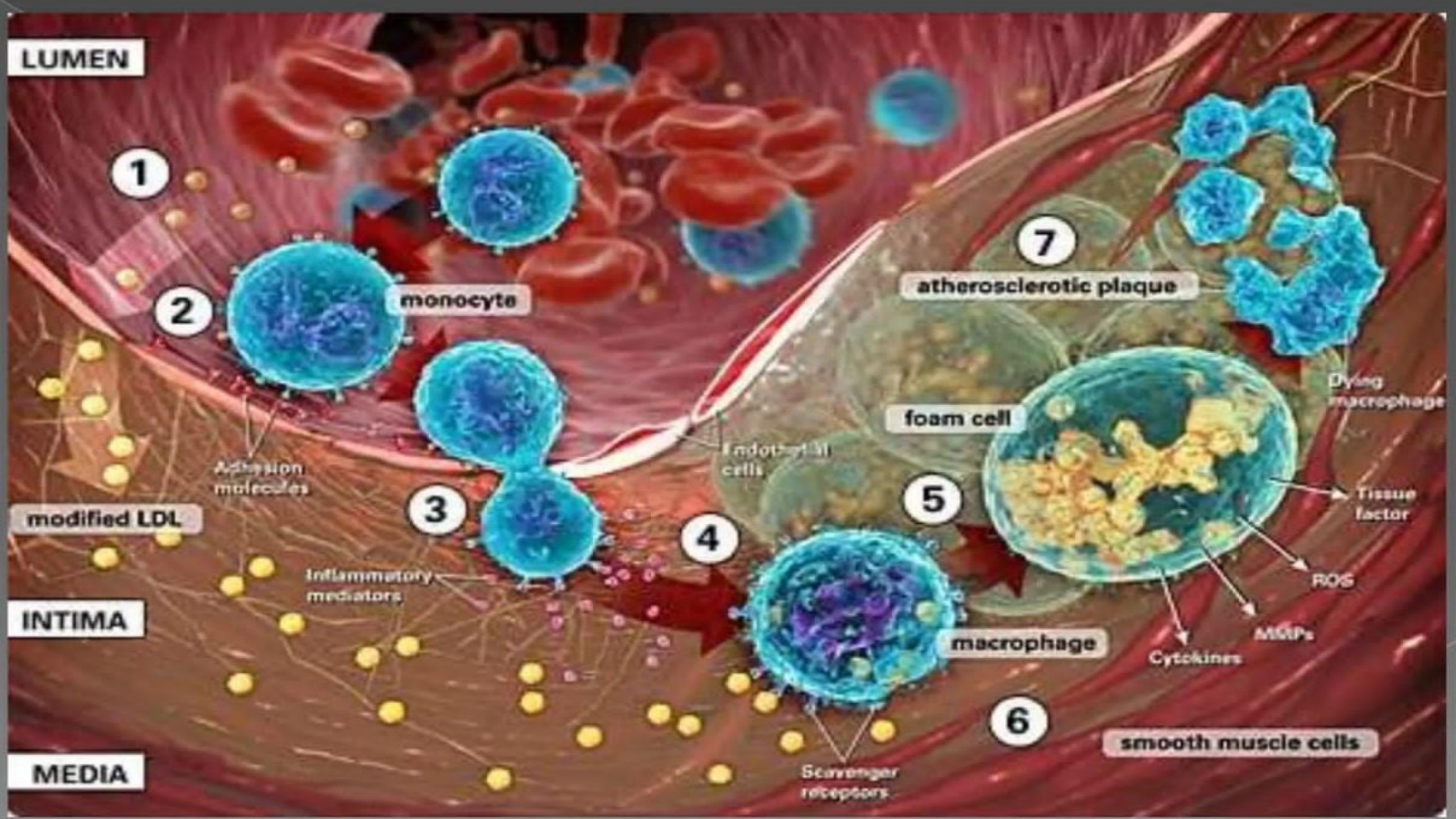
<u>CRP can:</u>

- Activate the classical compliment pathway/ Stimulate phagocytosis/ Bind to immunoglobulin receptors/ Endothelial dysfunction via † NO synthesis

- **LDL deposition in plaque by CRP-stimulated macrophages.**

INFLAMMATION




LUMEN
1
2
monocyte
Adhesion molecules
modified LDL
3
Inflammatory mediators
INTIMA
MEDIA
4
Scavenger receptors
macrophage
5
foam cell
Endothelial cells
6
smooth muscle cells
7
atherosclerotic plaque
Dying macrophage
Tissue factor
ROS
MMPs
Cytokines

2. Homocystein

- Moderate hyperhomocysteinemia occurs in 5-7% of the population.

- Recognized as an independent risk factor for the development of atherosclerotic vascular disease and venous thrombosis Can result from genetic defects, drugs, vitamin deficiencies.

Homocysteine implicated directly in vascular injury including:

- Intimal thickening/ Disruption of elastic lamina/ Smooth muscle hypertrophy/ Platelet aggregation.

- Vascular injury induced by leukocyte recruitment, foam cell formation, and inhibition of NO synthesis.

- Elevated levels appear to be an independent risk factor, though less important than the classic CV risk factors.

- Treatment includes supplementation with folate, B6 and B12.

D Dimer test;

- D-dimer is a fibrin degradation product, a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis.

If there is suspicion that a dangerous blood clot may be present a D-dimer test orderd.

- The test helps doctors rule out two conditions that can be fatal: deep vein thrombosis, a blood clot in a vein, and pulmonary embolism, a blood clot in the lung.

- As many as 100,000 deaths occur each year in the US due to DVT and PE.

- The symptoms you may have include:

Swelling or redness, usually in the lower leg but sometimes in the thigh, pelvis, or an arm/ Pain in the leg, thigh, pelvis, or arm/ Difficulty breathing/ Fast heartbeat/ Chest pain/ Sweating a lot.

- A negative test result means you probably don't have a blood clot and Usually, you won't need any further tests. However, if your results come back high, that doesn't necessarily mean you have a clot.

- **D-dimer levels** are used as a predictive biomarker for the blood disorder; disseminated intravascular coagulation and in the coagulation disorders associated with COVID-19 infection.

- A four-fold increase in the protein is an indicator of poor prognosis in people hospitalized with COVID-19.

- D-dimer normal value is anything less than 0.50 (or <500 ng/mL FEU).

- Other conditions that can cause high D-dimer levels include; pregnancy, heart disease, and recent surgery.

- If your D-dimer results were not normal, your provider will probably order more tests to make a diagnosis.

Thank You for Your Attention