INTRODUCTION

Pathophysiology of Coronary Artery Disease

Coronary artery disease (CAD), is a condition in which plaque builds up inside the coronary arteries. These arteries supply oxygen-rich blood to heart muscle. [1,2] It is the most common form of cardiovascular disease, whose underlying pathological feature is atherosclerosis.[3]

Atherosclerosis is a slowly progressing chronic disease of large and medium-sized arteries. It is characterised by the formation of atherosclerotic plaques consisting of necrotic cores, calcified regions, accumulated modified lipids, migrated smooth muscle cells (SMCs), foam cells, endothelial cells (ECs), and leukocytes. [3]

Other Names for CAD:[1,2]

- Atherosclerosis
- Coronary heart disease
- Hardening of the arteries
- Heart disease
- Ischemic heart disease
- Narrowing of the arteries

Epidemiology:

Cardiovascular disease (CVD) is the leading cause of mortality in developed countries and is likely to attain this status worldwide. It accounts for 16.7 million deaths each year. [4,5]

Pathophysiology:

1- Lesion Formation: (Endothelial activation)

Previously considered a cholesterol storage disease, currently atherogenesis is a complex interaction of risk factors including cells of the artery wall and the blood and molecular messages that they exchange. Inflammation play a major role in all stages of atherogenesis. Inflammation also participates in the local, myocardial, and systemic complications of atherosclerosis. [6]

The healthy endothelial monolayer in arteries resists prolonged contact with blood leukocytes, produces endogenous vasodilator molecules, combats thrombosis, favors fibrinolysis, and expresses enzymes, such as superoxide dismutase, that can degrade reactive oxygen species. Laminar shear stress, as prevails in normal arteries, fosters these homeostatic endothelial functions. [7,8]

When the arterial endothelium encounters disturbed flow instead of laminar sheer stress, risk factors as diverse as dyslipidemia, vasoconstrictor hormones inculpated in hypertension, the products of glycoxidation associated with hyperglycemia, or proinflammatory cytokines derived from excess adipose tissue or certain bacterial

products, these cells augment the expression of adhesion molecules (vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule 1 (ICAM-1), P- and E-selectin) that promote the sticking of blood leukocytes to the inner surface of the arterial wall. [9-13] **(Figure 1)**

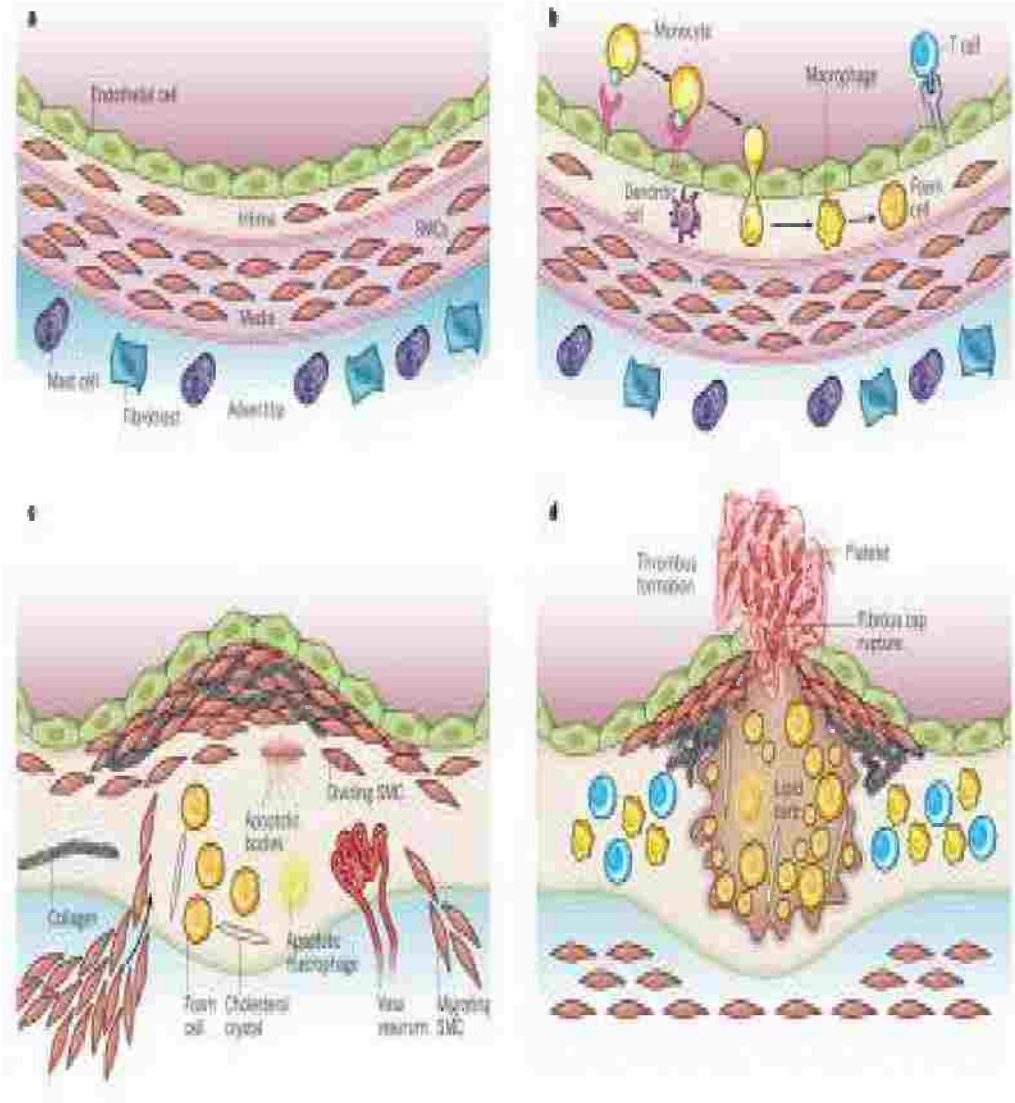


Figure (1): Stages in the development of atherosclerotic lesions. [14]

The normal muscular artery and the cell changes that occur during disease progression to thrombosis are shown. **a**, The normal artery contains three layers. **b**, The initial steps of atherosclerosis include adhesion of blood leukocytes to the activated endothelial monolayer, migration into the intima, maturation of monocytes into macrophages, and their uptake of lipid, yielding foam cells. **c**, Lesion progression involves the migration of smooth muscle cells (SMCs) and the heightened synthesis of extracellular matrix macromolecules. Plaque macrophages and SMCs can die in advancing lesions and extracellular lipid derived from dead and dying cells can accumulate in the central region

of a plaque, often denoted the lipid or necrotic core. **d**, Thrombosis often complicates a physical disruption of the atherosclerotic plaque.

2- Monocyte diapedesis:

Once activated, endothelial cells secrete chemokines, e.g., monocyte chemo attractant protein 1 (MCP-1), which together with adhesion molecules expression, attract monocytes, dendritic cells, and T-cells in the sub endothelial space. This process is characterized by monocytes tethering and rolling on endothelial cells. After firm adhesion to the dysfunctional endothelium, monocytes enter to the sub endothelial space (diapedesis). [15] T-cells gain access to the intima with similar mechanisms. [16]

3- Monocyte differentiation into macrophages:

Once in the intima monocytes are stimulated by macrophage colony-stimulating factor (M-CSF) produced by activated endothelial cells to differentiate into macrophage and/or dendritic cell-like features[17].These cells up regulate the scavenger receptors and acquire a proinflammatory pattern of functions characteristic of macrophages, producing high levels of cytokines like interleukin-(IL)1ß and tumor necrosis factor (TNF).[18]

4- Foam cells formation:

Low-density lipoproteins (LDLs) have a shell of phospholipids, free cholesterol and apolipoprotein B100 (ApoB100) with a core comprising esterified cholesterol and triglycerides. These particles can accumulate in the subendothelial space, where the ApoB100 binds to proteoglycans of the extracellular matrix, trapping the LDLs.[19]

Once LDLs accumulate in the subendothelial extracellular space they promote recruitment of monocytes and their differentiation into macrophages under the influence of proinflammatory cytokines, these cells produce reactive nitrogen and oxygen species (ROS) that oxidize LDLs, which are deemed to play a major role in the initiation and progression of the atherosclerotic process. With small increase in cellular cholesterol content and no formation of foam cells even when exposed to very high LDL concentrations. Whereas, oxidative modified LDLs bind with high affinity to macrophages, are actively internalized, and lead to intracellular cholesterol accumulation. [20]

Oxidation of LDL causes a loss of their affinity for the LDL receptor, which is downregulated by cholesterol intracellular overload, and a gain of affinity for the scavenger receptors (SR) which are up-regulated by cholesterol excess in macrophages. Thus, upon binding of oxidized LDL (oxLDL) these receptors are internalized with ensuing foam cells formation. **(Figure 2)**

Presumably the rate of production of oxLDL in the arterial intimais a function of the concetration of native LDL present and is proportional to the plasma LDL. Oxidation of LDL was originally thought to be involved in pathogenesis because this could account for the loading of macrophages with cholesterol, but it quickly became apparent that oxLDL had many other properties that were potentially proatherogenic. For example, oxLDL is itself directly chemotactic for monocytes and T cells. Among other biological effects, oxLDL (and its various oxidized lipid components) are cytotoxic for endothelial cells, for macrophages and smooth muscle cells (SMC) and stimulate the release of MCP-1 and of M-CSF from endothelial cells. [21,22]

Once internalized by macrophages cholesteryl esters of lipoproteins are hydrolyzed to free cholesterol and fatty acids. [23]

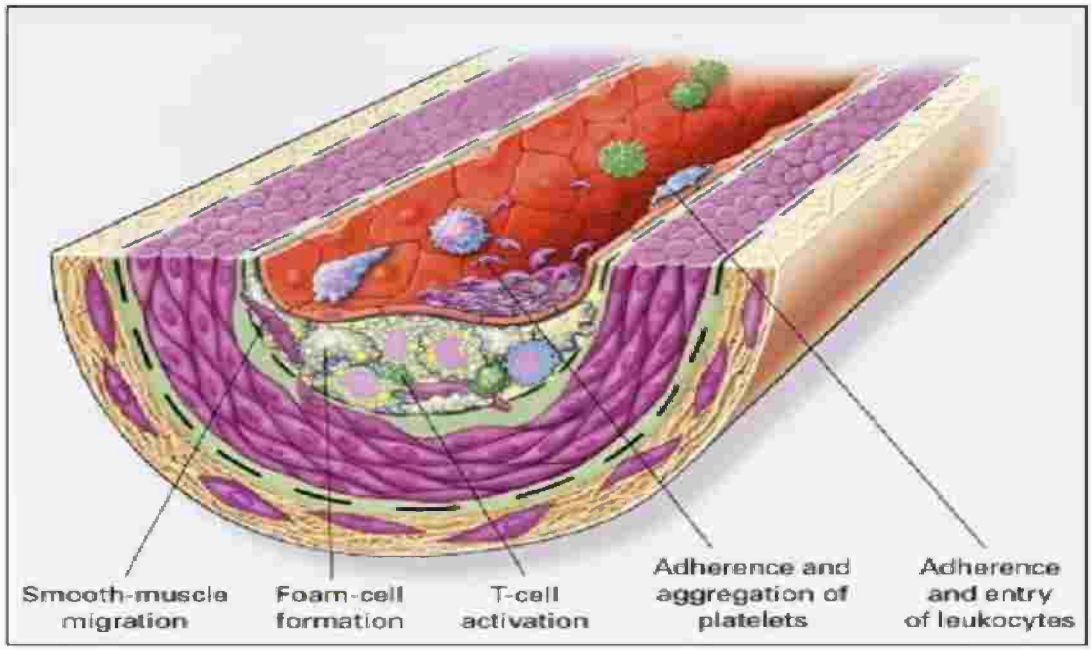


Figure (2): Fatty streaks initially consist of lipid-laden monocytes and macrophages (foam cells) together with T lymphocytes. [1]

Other atheroma cell components:

Atheroma formation includes migration of smooth muscle cells (SMC) from the tunica media into the intima. Here they proliferate under the stimulus of platelet derived growth factor (PDGF) and produce extracellular matrix molecules, like collagen and elastin, forming the fibrous cap that overlies the atherosclerotic plaque. [14]

With time, the activated leukocytes in concert with the arterial cells produce fibrogenic mediators and growth factors that enhance SMC division and subsequent formation of a dense extracellular matrix, a fibrous cap. [24]

5- Atherosclerotic plaque evolution and destabilization:

Evolution of the atherosclerotic plaque is characterized by enlargement over timedue to the accumulation of foam cells. Macrophages are crucial in plaque morphology modification, which could lend to vulnerable plaques through necrotic core formation and thinning of the fibrous cap. In fact, most lesions responsible of acute events recognized at pathology are ruptured sites at plaque shoulders, in close proximity to areas of plaque necrosis, with a thinned overlying fibrous cap.[25]

The fibrous cap thinning is secondary to processes that either decrease the collagen deposition by SMCs or enhances its degradation. Macrophages are crucial in this mechanism, reducing their local release of transforming growth factor ß (TGF ß). [26] which decreases collagen deposition by SMCs, and triggering SMCs apoptosis. [27] Moreover, macrophages might be involved in collagen degradation through the production of matrix metalloproteinases and serine proteases. [28,29]

Important Receptors:

1- Toll-Like Receptors:

Are receptors and transporters for cholesterol loading and efflux in the macrophages . In the innate immune system. toll-like receptors (TLRs) are the primary receptors that recognise highly conserved structural motifs of pathogens [30] Under hyperlipidemic conditions, TLRs likely participate in the regulation of atherosclerosis. The activation of TLRs induces the production of proinflammatory cytokines and nitric oxide in macrophages and the induction of DC maturation, TLRs activated by oxidised (ox) LDL. [31]

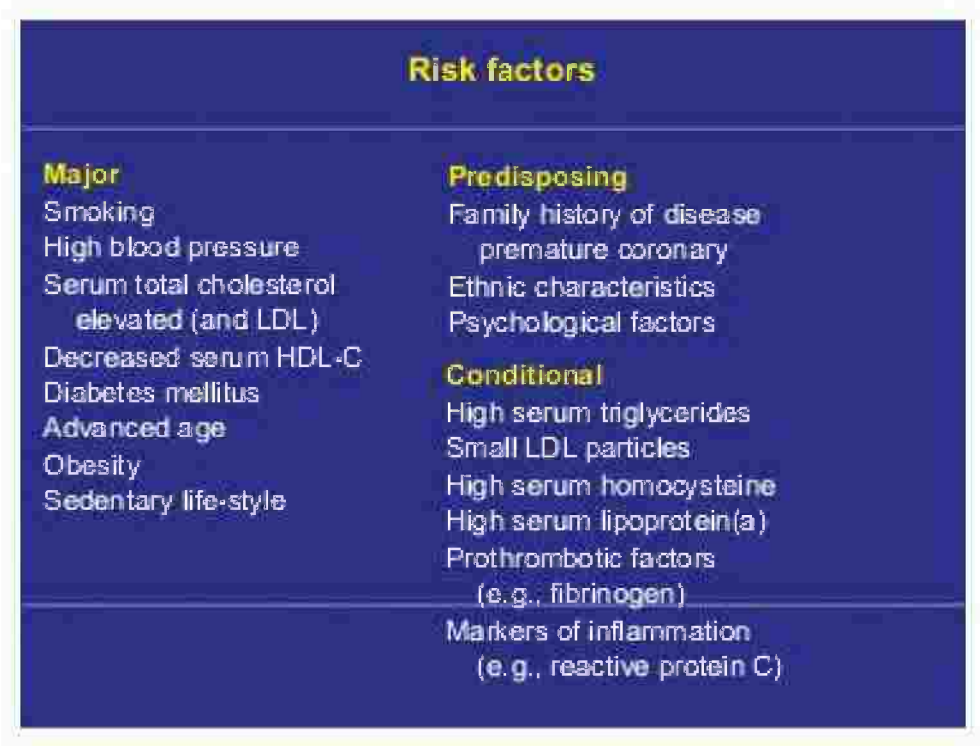
2- Scavenger Receptors:

Macrophage scavenger receptors (SRs) are found to bind and internalise modified forms of LDL through mechanisms that are not inhibited by cellular cholesterol content, and they are likely responsible for macrophage cholesterol accumulation. [32]

Risk factors for Coronary Artery Disease

Risk factors for CAD were not formally established until the initial findings of the Framingham Heart Study in the early 1960s. The understanding of such factors is critical for a clinician to prevent cardiovascular morbidities and mortality. [33,34] **(Table I)**

Table (I): Classification of risk factors. [35]



The treatment of factors included as conditional risk factors should be considered only if associated with other risk factors (major or predisposing).

I- Non modifiable Risk Factors:

1- Age and sex:

The risk of developing CAD increases with age, and includes age greater than 45 years in men and greater than 55 years in women; due to estrogen protection. [36,37]

2- Family history:

A family history of early heart disease is also a risk factor, including heart disease in the father or a brother diagnosed before age 55 years and in the mother or a sister diagnosed before age 65 years. [38,39]

3- Race:

The rate of cardiovascular death was significantly higher in blacks, and cardiovascular death rates were significantly lower in the Asian groups. [40]

II- Modifiable Risk Factors:

Increasing number of protective lifestyle factors are associated with a marked decrease in risk of coronary heart disease, cerebrovascular disease, and overall CVD mortality in men and women. Protective lifestyle factors include dietary pattern, physical activity, alcohol intake, sleep, smoking cessation, and body mass index.[41] **(Figure 3)**

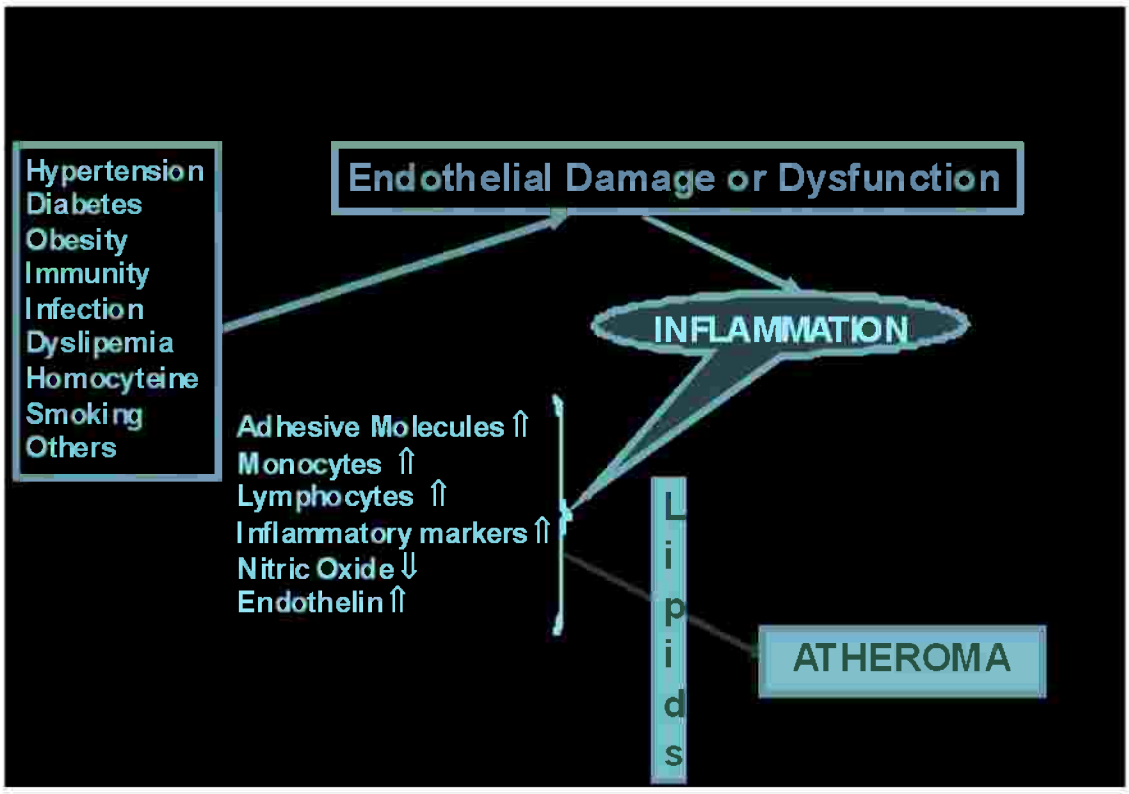


Figure (3): Risk factors for CAD. [42]

1- High blood cholesterol levels (Hyperchosterolemia):

A high level of LDL cholesterol in the blood is the principal cause of injury to the artery and vascular SMCs. [1,6]

The Framingham Heart Study results demonstrated that the higher the cholesterol level, the greater the risk of CAD; alternatively, CAD was uncommon in people with cholesterol levels below 150 mg/dL. More recent series of clinical trials using statin drugs have provided conclusive evidence that lowering LDL cholesterol reduces the rate of myocardial infarction (MI), the need for percutaneous coronary intervention and the mortality associated with CAD-related causes.[43]

2- High blood pressure (hypertension):

Angiotensin II (AII) is considered as a powerful vasoconstrictor and plays a role in atherosclerosis by enhancing growth of SMCs [44]. AII speeds up inflammation by facilitaing smooth muscle lipooxygenase activity, an activity that yields oxLDL [1] .Free radicals (superoxide anion) generated in the plasma due to hypertensive reactions decrease nitrogen oxide synthesis and increase leukocyte adhesion [45]. AII produced during

hypertension can orchestrate inflammation of the endothelial intima causing the endothelium and SMC of the artery to produce superoxide anion. [24]

In the Framingham Heart Study, even high-normal blood pressure (defined as a systolic blood pressure of 130-139 mm Hg, diastolic blood pressure of 85-89 mm Hg, or both) increased the risk of cardiovascular disease 2-fold, as compared with healthy individuals.[46]

Hypertension, along with other factors such as obesity, have been said to contribute to the development of left ventricular hypertrophy (LVH). LVH has been found to be an independent risk factor to cardiovascular disease morbidity and mortality. It roughly doubles the risk of cardiovascular death in both men and women. [47]

3- Cigarette smoking:

Nicotine and carbon monoxide contents of cigarette have damaging effects on arteries by causing them to lose their compliance and to set up a stage for plaque development. Cigarette smoking results in high levels of circulating non esterified fatty acids which can be injurious to the cell by eliciting inflammatory response. The free radicals generated from smoking result in oxidative stress and increase oxidation of LDL which triggers the recruitment of monocytes and T cells; these lead to formation of macrophages and other processes that promote atherosclerosis. [48]

Cessation of cigarette smoking constitutes the single most important preventive measure for CAD. Persons who consume more than 20 cigarettes daily have a 2- to 3-fold increase in total heart disease. Continued smoking is a major risk factor for recurrent heart attacks.[49]

4- Diabetes mellitus:

Lipoproteins can undergo glycation in conditions of chronic hyperglycaemia; Glycated lipoproteins support the action of proinflammatory cytokines in the arterial endothelium. [24]

Patients with diabetes are 2-8 times more likely to experience future cardiovascular events than age-matched and ethnically matched individuals without diabetes.[34]

5- Obesity:

When visceral fat is high, more free fatty acids are generated and this leads to elevated levels of VLDL that can decrease HDL by the action of cholesteryl ester transfer protein which converts HDL into VLDL; high levels of VLDL can instigate atherosclerosis. TNF-α and IL-6 synthesis by adipose tissue participate in inflammation response, these inflammatory processes and high levels of VLDL can bring about atherosclerosis.[24,50]

Obesity is associated with elevated vascular risk in population studies. In addition, this condition has been associated with glucose intolerance, insulin resistance, hypertension, physical inactivity, and dyslipidemia. Patients who are extremely obese (body mass index [BMI] >40) present at a younger age with STEMI, their in-hospital mortality following STEMI is increased.[51,52]

6- Lack of physical activity:

The cardioprotective benefits of exercise include reducing adipose tissue, which decreases obesity; lowering blood pressure, lipids, and vascular inflammation; improving endothelial dysfunction, improving insulin sensitivity, and improving endogenous fibrinolysis.[53] In addition, regular exercise reduces myocardial oxygen demand and increases exercise capacity, translating into reduced coronary risk.[54] Adherence to a healthy lifestyle is associated with a low risk of sudden cardiac death among women. [55]

7- Metabolic syndrome:

Metabolic syndrome is characterized by a group of medical conditions that places people at risk for both heart disease and type 2 diabetes mellitus, Patients with metabolic syndrome had significantly higher rates of coronary, cardiovascular mortality.[56]

People with metabolic syndrome have 3 of the following 5 traits and medical conditions:[57]

1- Elevated waist circumference - Waist measurement of 40 inches or more in men, 35 inches or more in women.
2- Elevated levels of triglycerides - 150 mg/dL or higher or taking medication for elevated triglyceride levels.
3- Low levels of HDL (high-density lipoprotein) or good cholesterol - Below 40 mg/dL in men, below 50 mg/dL in women, or taking medication for low HDL cholesterol level.
4- Elevated blood pressure levels - For systolic blood pressure, 130 mm Hg or higher; 85 mm Hg or higher for diastolic blood pressure; or taking medication for elevated blood pressure levels.
5- Elevated fasting blood glucose levels - 100 mg/dL or higher or taking medication for elevated blood glucose levels.

8- Mental stress, depression:

Depression has been strongly implicated in predicting CAD[58]. Adrenergic stimulation during stress can increase myocardial oxygen requirements, can cause vasoconstriction, and has been linked to platelet and endothelial dysfunction[59] and metabolic syndrome.[60]

III- Nontraditional or Novel Risk Factors (Systemic Indicators of inflammation):

1- C-reactive protein:

C-reactive protein (CRP) is an acute phase protein in the blood that demonstrates the presence of inflammation, which is the body's response to injury or infection; CRP levels rise if inflammation is present. The inflammation process appears to contribute to the growth of arterial plaque, and in fact, inflammation characterizes all phases of atherothrombosis and is actively involved in plaque formation and rupture. [61,62]

According to some research results, high blood levels of CRP may be associated with an increased risk of developing CAD and having a heart attack.[63]

2- Lipoprotein associated phospholipase A_2:

Lipoprotein associated phospholipase A_2 ($LpPLA_2$) has proinflammatory and proatherogenic functions by generating lysophosphatidylcholine (lysoPC) and non-esterified fatty acid moieties [64]. LysoPC promotes monocyte chemotaxis and increases expression of mononuclear leukocyte adhesion molecules in endothelial cells; LysoPC is suspected to facilitate initiation of atherosclerotic lesion through such activities. lysoPC binds to peptides and become antigenic and in turn, stimulate T cells. [65]

3- Lipoprotein (a):

An elevated lipoprotein (a) [Lp(a)] level is an independent risk factor of premature CAD and is particularly a significant risk factor for premature atherothrombosis and cardiovascular events. Measurement of Lp(a) is more useful for young individuals with a personal or family history of premature vascular disease and repeat coronary interventions. [66]

4- Homocysteine:

Homocysteine has prothrombotic properties; it inhibits nitrogen oxide activity, it is lethal to the endothelium and increases collagen synthesis. [1]

Studies have demonstrated that homocysteine may induce vascular damage by promoting platelet activation, oxidative stress, endothelial dysfunction, hypercoagulability, vascular smooth muscle cell proliferation, and endoplasmic reticulum stress. [67]

5- Tissue plasminogen activator:

An imbalance of the clot dissolving enzymes (eg, tissue plasminogen activator [tPA]) and their respective inhibitors (plasminogen activator inhibitor-1 [PAI-1]) may predispose individuals to myocardial infarctions. [68,69]

6- Small, Dense LDL:

Individuals with a predominance of small, dense LDL particles are at increased risk for CAD. Thus, core lipid composition and lipoprotein particle size and concentration may provide a better measure of cardiovascular risk prediction.[70]

One study suggests that the risk of coronary heart disease contributed by LDL appeared to result to a large extent from LDL that contains apolipoprotein C-III.[71]

7- Fibrinogen:

Levels of fibrinogen, an acute-phase reactant, increase during an inflammatory response. This soluble protein is involved in platelet aggregation and blood viscosity, and it mediates the final step in clot formation. Significant associations were found between fibrinogen level and risk of cardiovascular events in the Framingham heart studies.[72]

8- Other factors:

Medical conditions such as end-stage renal disease (ESRD),[73] chronic inflammatory diseases affecting connective tissues (eg, lupus, rheumatoid arthritis),[74,75] human

immunodeficiency virus (HIV) infection (acquired immunodeficiency syndrome [AIDS], highly active antiretroviral therapy [HAART]),[76]and other markers of inflammation have all been widely reported to contribute to the development of CAD.

ESRD is associated with anemia, hyperhomocysteinemia, increased calcium phosphate product, calcium deposits, hypoalbuminemia, increased troponin, increased markers of inflammation, increased oxidant stress, and decreased nitric oxide activity factors, all of which may contribute to increased CAD risk.[73]

Low serum testosterone levels have a significant negative impact on patients with CAD.[77]

A population-based study found that insomnia is associated with a moderately increased risk of acute myocardial infarction.[78]

Vitamin D deficiency was associated with increased mortality and myocardial infarction. PTH excess was associated with a 30% increased risk of heart failure.[79]

Myocardial infarction

Definition:

Myocardial infarction (MI) reflects cell death of cardiac myocytes caused by ischaemia, which is the result of a perfusion imbalance between supply and demand. [80-82]

Coronary atherosclerosis is a chronic disease with stable and unstable periods. During unstable periods with activated inflammation in the vascular wall, patients may develop a myocardial infarction. MI may be a minor event in a lifelong chronic disease, it may even go undetected, but it may also be a major catastrophic event leading to sudden death or severe haemodynamic deterioration. MI may be the first manifestation of CAD.[1]

Complete necrosis of myocardial cells at risk requires at least 2-4 h, or longer, depending on the presence of collateral circulation to the ischemic zone, persistent or intermittent coronary arterial occlusion, the sensitivity of the myocytes to ischemia and individual demand for oxygen and nutrients. [81]

The incidence of MI increases with age; however, the actual incidence is dependent on predisposing risk factors for atherosclerosis, approximately 50% of all MIs in the united states occur in people younger than 65 years. However, in the future, as demographics shift and the mean age of the population increase, a larger percentage of patients presenting with MI will be older than 65 years. [83]

Risk factors of myocardial infarction:

Six primary risk factors have been identified with the development of atherosclerotic CAD and MI: hyperlipidemia, diabetes mellitus, hypertension, tobacco use, male gender, and family history of atherosclerotic arterial disease. [84]

Pathophysiology of myocardial infarction:

Atherothrombotic coronary occulusion; as the fibrous cap grows weaker, it becomes highly susceptible to hemodynamic stresses. Such stress results in disruption of the atherosclerotic plaque which can trigger thrombosis and possibly lead to acute myocardial infarction (AMI). [85]

Diagnosis:

I- Clinical presentation:

The ischemia is usually the cause of the symptoms of an MI, although other mechanisms such as hypotension or spasm are possible. [86]

The most common symptom of MI is acute severe chest pain. The chest pain may also be characterized by pressure, burning, or aching. The pain can radiate toward the jaw, neck, arms, or back and usually lasts more than 15 minutes. [87-89]

Symptoms other than chest pain that may occur in those suffering an MI include weakness and fatigue[90,91] shortness of breath ,nausea, cold sweat, and dizziness.[92,93]

II- Electrocardiogram:

MI can also be described in terms of what an electrocardiogram (ECG) shows: [94]

1- ST-elevation myocardial infarction (STEMI).
2- Non-ST-elevation myocardial infarctin (NSTEMI).

A STEMI is a more severe type of MI where the coronary artery is completely blocked off, as opposed to an NSTEMI, where the artery is only partly occluded.

III- Cardiac markers:

Markers of Myocardial Necrosis:

1. Cardiac troponins T and I (cTnT & cTnI).
2. Total creatine kinase (CK), its isoenzymes and isoforms.
3. Myoglobin.

Markers of Inflammation:

1. High Sensitivity C Reactive Protein (hs-CRP).
2. Lipoprotein Associated Phospholipase A_2 (Lp-PLA_2).
3. Fibrinogen.
4. Interleukin-6 (IL-6).
5. Tumor Necrosis Factor α (TNFα).
6. Myeloperoxidase (MPO).

Markers of Plaque Destabilization:

1. Matrix Metalloproteinase-9 (MMP-9).
2. Iintercellular adhesion molecule (ICAM).
3. Vascular Cell Adhesion Molecule (VCAM).

Markers of Plaque Rupture:

1. Pregnancy-associated plasma protein A (PAPP-A).
2. Soluble CD40L (sCD40L).
3. Placental Growth Factor (PLGF).

Markers of Ischemia:

1. Ischemia modified albumin (IMA).
2. Free fatty acid (FFA).
3. Choline.

Markers of Myocardial Dysfunction:

1. B-type natriuretic peptide (BNP).
2. N-terminal fragment of pro-BNP.

A biomarker is defined as a substance used as an indicator of a biological state. Through various techniques it is objectively measured and used to assess normal or

pathogenic biological processes, or of pharmacologic responses to a therapeutic intervention. [95]

Criteria of ideal biomarke:

Table (II): Characteristics of an ideal cardiac marker. [96]

High sensitivity:	Abundant in cardiac tissue
High specificity:	Absent from non-myocardial tissue Not detectable in blood from non-diseased subjects
Release:	Rapid release for early diagnosis Long half-life in blood for late diagnosis
Analytical:	Cost effective Short turnaround time Precise Accurate
Clinical:	Ability to infuence therapy and so improve patient outcome Validated by clinical studies

Criteria for acute, evolving, or recent MI: [97]

Either one of the following criteria satisfies the diagnosis for an acute, evolving or recent MI:

1- Typical rise and gradual fall (troponin) or more rapid rise and fall (CK-MB) of biochemical markers of myocardial necrosis with at least one of the following.

   i) Ischaemic symptom.
   ii) Development of pathologic Q waves on the ECG.
   iii) ECG changes indicative of ischaemia (ST segment elevation or depression); or
   iv) Coronary artery intervention (e.g. coronary angioplasty

2- Pathologic findings of an acute MI.

Cardiac biomarkers

I- Markers of Myocardial Necrosis:

1- Troponin:

Troponin is a regulatory complex of 3 protein subunits located on the thin filament of the myocardial contractile apparatus. The 3 subunits are designated troponin C (the calcium-binding component), TnT (the tropomyosin-binding component), and TnI (the inhibitory component). Both cTnT and cTnI are stored in the myocyte (bound to the myofibrils), including a small cytosolic pool (4%-6%), with the majority of the remaining troponin found in the sarcomere. Thus, TnT and TnI have similar release kinetics from damaged myocardium. Both troponins increase in serum within 4 to 6 hours after AMI peak at 12 to 24 hours, and remain elevated for up to 14days. [98-100] cTnT and cTnI have equal myocardial tissue specificity, as well as high sensitivity. [101]

Troponins have a number of advantages over other cardiac markers, which has led to their adoption as the new gold standard for myonecrosis. [102-104]

* It is important to note that cTnT and cTnI are not detected normally in the blood of healthy persons. Consequently, significant elevations of cTnT or cTnI are thought to most likely reflect myocardial necrosis. Patients with detectable troponin but no CK-MB in the blood may exhibit microscopic zones of myocardial necrosis (microinfarction).

** Another important factor leading to the adoption of serum troponin testing as the marker of choice for the evaluation of patients with chest pain relates to the ability of the marker to predict outcomes following acute coronary syndrome (ACS). There is a quantitative relationship between the quantity of cardiac troponin measured in blood and the risk of death in patients with ACSs.

Due to its high sensitivity and nearly absolute myocardial tissue specificity, cTn assays have become the corner stone in the diagnosis and risk stratification of ACS.[105] **(Figure 4)**

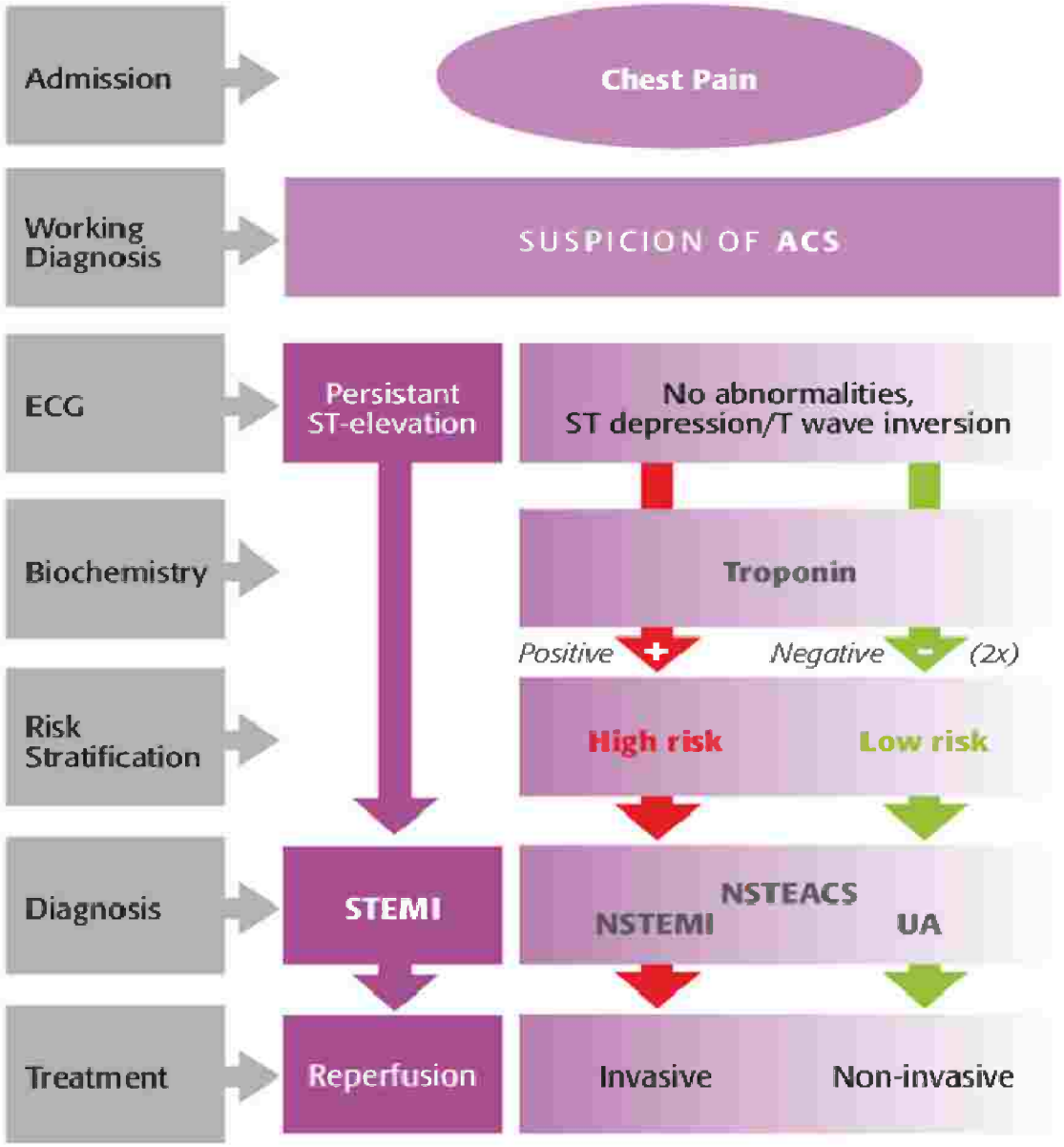


Figure (4): Approach to diagnosis and risk stratification (ACC/AHA 2OO7 Guidelines).[106]

2- CK total and CK-MB:

The enzyme creatinine kinase exists as three isoenzyme forms: CK-MM,CK-MB, and CK-BB. These isoenzymes are found in the cytosol. [107-109]

CK-BB: Increased in neurological diseases ; prostatectomy; digestive cancers
CK-MB: Increased with AMI
CK-MM: Increased in myopathy, hypothyroidism, polymyositis, rhabdomyolysis, muscle trauma, intensive exercise, AMI

CK is widely distributed in tissues, elevations in total serum CK lack specificity for cardiac damage, which improves with measurement of the MB fraction. Elevated CK-MB is relatively specific for myocardial injury, particularly in patients with ischemic symptoms when skeletal muscle damage is not present

CK-MB starts rising in the blood 4-6 hours after the onset of chest pain. It peaks at 10-24 hours and then returns to normal after 48-72 hours. Since CK-MB levels return to baseline 48 to 72 hours after infarction, it can be used to detect reinfarction. [108,110] **(Figure 5)**

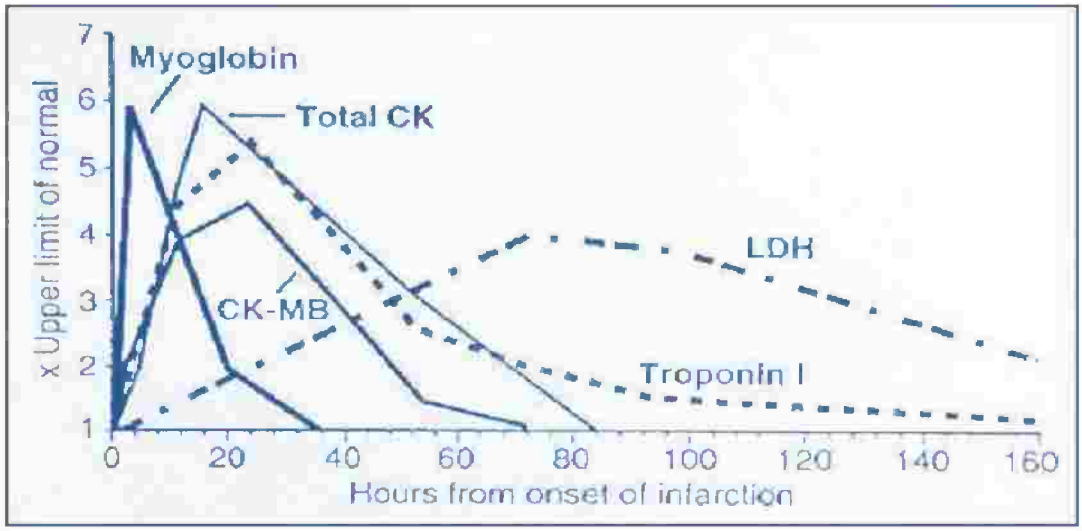


Figure (5): Temporal profile of cardiac necrosis markers after acute myocardial infarction. [111]

3- Myoglobin:

Myoglobin is a heme protein found in skeletal and cardiac muscle that has attracted considerable interest as an early marker of MI. Its low molecular weight and cytoplasmic location account for its early release profile: myoglobin typically rises 1-4 hours after onset of infarction, peaks at 6-12 hours, and returns to normal within 24-36 hours. The major advantage of myoglobin as a cardiac marker is that it is released earlier from damaged cells than other cardiac markers, permitting earlier detection of AMI. The main reason that myoglobin has not been used for the evaluation of chest pain is its poor clinical specificity (60%-90%) owing to the presence of large quantities of myoglobin in skeletal muscle. Myoglobin, therefore is potentially useful for ruling out but not for confirming the diagnosis of AMI. [112-114]

II- Markers of Inflammation:

1- C-reactive protein and High sensitivity C-reactive protein:

CRP is an acute phase reactant synthesized in liver and is elevated in inflammatory conditions, High-sensitivity only means that the concentration of CRP was determined using an assay designed to measure very low levels of CRP.[115-117]

CRP present in the early stages of plaque development and also involved in facilitating processes ranging from the initial recruitment of leukocytes to the arterial wall to the eventual rupture of the plaque itself. [118,119]

CRP levels measured at admission also may be useful as an independent predictor of new coronary events, including AMI and death in patients with ischemic heart disease. [120]

CRP is a strong independent predictor of future cardiovascular events, including MI, ischemic stroke, peripheral vascular disease and sudden cardiac death. [121,122]

CRP concentration might reflect the vulnerability of the atheromatous lesion and the likelihood of a plaque to rupture. [123]

2- Lipoprotein Associated Phospholipase A_2:

It is the main topic of our study that will be discussed later in details.

3- Myeloperoxidase (MPO):

Myeloperoxidase is a haem containing enzyme secreted by inflammatory cells (activated neutrophils and monocytes) and is found in atherosclerotic plaques[118], MPO can generate several reactive nitrogen intermediates concomitantly inducing oxidative damage of cells and tissues[124],Such reactive intermediates have been found at increased levels in LDL isolated from arthero-sclerotic lesions[125]. Moreover, high MPO expression levels have been reported in patients with sudden cardiac death, at sites of plaque rupture, superficial erosions and in the lipid core whereas less harmful fatty streaks were observed to exhibit little MPO expression[126]

III- Markers of Plaque Rupture:

1- Pregnancy-associated plasma protein A (PAPP-A):

PAPP-A is a high-molecular-weight, zinc binding metallo-proteinase, originally identified in the plasma of pregnant women[127]. Elevated levels of PAPP-A were found in patients presenting with unstable plaques, aggravated unstable angina and acute MI.[128]

2- Soluble CD40L (sCD40L):

sCD40 ligand is a signaling protein that reflects both inflammatory and platelet interaction[129]. It was reported that elevations of sCD40L correlated with an increased risk of cardiac events during six months of follow up[130,131]. However, a rise in sCD40L was associated with many other inflammatory conditions as rheumatoid arthritis.[132]

IV- Markers of Ischemia:

1- Ischemia modified albumin (IMA):

In 2003, IMA became the first FDA-approved ischemic biomarker and was recommended for use in ruling out ACS, especially in patients presenting with typical acute chest pain but normal or non-diagnostic ECGs.[133]

2- Choline:

Choline is an organic compound involved in several processes such as cell signaling, nerve impulse transmission and the transport and metabolism of lipids. It was found that phospholipase D enzyme activation, followed by the release of choline into the bloodstream, are related to the cascade mechanisms of coronary plaque destabilization.[134]

V- Markers of Myocardial Dysfunction:

B- Type natriuretic peptide (BNP) and N-terminal fragment of pro-BNP:

The natriuretic peptide family consists of 3 peptides: atrial natriuretic peptide (ANP), brain (or B-type) natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). These neurohormones are released in response to hemodynamic stress and are involved in the regulation of intravascular volume homeostasis BNP is secreted by the ventricles, and to a lesser extent by the atria, and appears in blood after cleavage of the precursor molecule proBNP. This cleavage also results in the release of NT-proBNP, the N-terminal counterpart Therefore, the blood levels of both molecules are increased in HF. [135,136]

NT-proBNP also elevated in absence of HF in cases of: heart muscle disease, heart valvular disease, arrhythmia (atrial fibrillation), acute coronary syndrome, stroke and Pulmonary embolism. [137]

Other markers:

Lactate dehydrogenase: [138]

LDH enzyme composed of two subunits M (muscle) and H(heart) and consist of five isoenzymes LD-1 to 5.LD-1 contains four H subunits and is the predominant form in the heart ,but also occurs in pancreas, brain, kidney, stomach and erythrocytes. After AMI start to rise 12-18 hours after onset of symptoms, peak at 48-72 hours and return to normal by 6-10 days.

Isoenzymes LD1 and LD2 appear primarily in the heart, red blood cells and kidneys. LD3 is primarily in the lungs. LD4 and LD5 are located in the liver, skin, and the skeletal muscles. LD 1is astrong indicative of AMI.

Because LDH is present in almost all body tissues, cellular damage increases total serum LDH, limiting the diagnostic usefulness of this test.

The LDH-1 isoenzyme level is more sensitive and specific than the total LDH. Normally, the level of LDH-2 is higher than the level of LDH-1. An LDH-1 level higher than that of LDH-2, a phenomenon known as "flipped LDH," is strongly indicative of a heart attack.

Table (III): Biochemical markers of acute coronary syndrome. [96]

Marker	Main use of marker	MW (kDa)	Initial elevation	Time to peak	Return to normal
Creatine kinase MB subform (CK-MB)	Re-infarction diagnosis	85	4–6 h	12–24 h	3–4 days
Myoglobin	Re-infarction diagnosis	18	1–3 h	6–12 h	24–48 h
Troponin I (cTnI)	Benchmark for MI diagnosis	23.5	4–6 h	12–24 h	6–8 days
Troponin T (cTnT)	Benchmark for MI diagnosis	37	4–6 h	12–24 h	7–10 days
Ischemia-modified albumin (IMA)	Myocardial ischemia	65	ND	ND	ND
B-type natriuretic peptide (BNP)	Ventricular overload	3.4	ND	ND	ND
N-terminal pro-B-type natriuretic peptide (NT-proBNP)	Ventricular overload	8.5	ND	ND	ND
C-reactive protein (CRP)	Inflammation	125	ND	ND	ND
Myeloperoxidase (MPO)	Inflammation	150	ND	ND	ND
Choline	Plaque instability/disruption	ND	ND	ND	ND
Soluble fragment CD40 ligand (sCD40L)	Inflammation	18	ND	ND	ND
Heart fatty acid binding protein (H-FABP)	Myocardial necrosis	15	2–3 h	8–10 h	18–30 h
Free fatty acids unbound to albumin (FFAu)	Myocardial ischemia	ND	ND	ND	ND
Pregnancy-associated plasma protein A (PAPP-A)	Plaque instability/disruption	200	ND	ND	ND

ND = not defined.

Lipoprotein Associated Phospholipase A_2

Phospholipases A_2 (PLA_2s) belong to a superfamily of enzymes that catalyze the hydrolysis of glycerophospholipids at the sn-2 position, producing nonesterified fatty acids such as arachidonic acid, and lysophospholipids. [139,140]

The PLA_2 lipid products lead to the generation of a variety of downstream signaling molecules, including prostaglandins, leukotrienes, lysophospholipids, platelet-activating factor (PAF), and oxidized lipids, that induce a multitude of biological actions in virtually all tissues, including the cardiovascular system. [141-146]

PLA_2s comprise distinct sets of enzymes with different localizations: [147]

(1) Cytosolic enzymes:

i) Ca+2 dependent ($cPLA_2$).
ii) Ca+2 independent ($iPLA_2$).
iii) Specific for PAF (intracellular PAF-acetylhydrolase).

(2) Extracellular enzymes:

i) Associated with lipoproteins (Lp-PLA_2, also known as the secreted PAF acetylhydrolase).
ii) Typically secreted ($sPLA_2$) and present in the extracellular space.

Lp-PLA_2 exhibits unique substrate specificity toward PAF and oxidized phospholipids (oxPLs). [148]

The $sPLA_2$ family consists of 10 isozymes with low molecular mass that are involved in a number of biological processes, including inflammation, and host defense against bacterial infection. [149]

Arterial inflammation plays a central role in the pathogenesis of atherosclerosis and adverse cardiovascular events Although Ca+2 dependent ($cPLA_2$) and Ca+2 independent ($iPLA_2$) are likely to contribute to vascular inflammation and atherosclerosis via the production of various lipid mediators in different cell types. [150-153]

A wealth of evidence supports a major role for Lp-PLA_2 and a subset of sPLA isoforms (notably sPLA IIA, -III, -V, and -X) in the pathophysiology of atherosclerosis, from initiation and progression to cardiovascular complications. The molecular basis for their unique roles in vascular inflammation and atherosclerosis likely stems from the fact that Lp-PLA_2 and $sPLA_2$s have the unique capacity to bind and hydrolyze lipoproteins in distinct and specific manners, thereby producing various lipid mediators and modifying the lipid particles Notably, Lp-PLA_2 and the $sPLA_2$ members are molecularly and biochemically distinct, and thus their roles and underlying mechanisms of action in the blood and within the vascular wall are likely to different. Low-density lipoprotein (LDL) modified by Lp-PLA_2 or $sPLA_2$s acquires potent proatherogenic activities. [154,155]

Structural and Biochemical Features of Lp-PLA_2

Lipoprotein-Associated PLA_2:

Lp-PLA_2 is a Ca+2 independent, 45-kDa (EC 3.1.1.47) secreted protein that circulates in plasma in a constitutively active form.[148]

The enzyme is actively secreted by monocyte-derived macrophages, T lymphocytes, and mast cells, and these cells likely contribute the major source of Lp-PLA_2 in plasma.[156]

The vast majority of Lp-PLA_2 in normolipidemic subjects is closely associated with LDL (85%), in particular small, dense LDL particles, which explains why it is referred to as lipoprotein-associated PLA_2. [157]

A small proportion of the circulating enzyme activity is also associated with high-density lipoprotein. It has been estimated that only approximately 0.1% of lipoprotein particles are laden with the enzyme. This latter is particularly enriched into lipoprotein (a), an atherogenic lipoprotein particle that appears to be a preferential carrier of oxPLs in human plasma. [157-160]

The enzyme is more closely related to neutral lipases and serine esterases than other PLA_2s. As a result, the enzyme also exhibits PLA1 and lipase activities [161] ,Unlike other PLA_2s that must bind to the water-lipid interface for optimal enzymatic activity, Lp-PLA_2 catalyzes the hydrolysis of its various substrates essentially from the aqueous phase. [162]

Lp-PLA_2 was discovered because of its ability to catalyze the hydrolysis of PAF and thus was originally named PAF-acetylhydrolase. Besides PAF, the enzyme has a broad specificity for polar phospholipids and hydrolyzes several types of short-chain and oxPLs. oxPLs appear as major physiological substrate that can be hydrolyzed both in vitro and in vivo. [148,163,164]

This suggests a major specific role of Lp-PLA_2 in the depletion of oxPLs from lipoproteins. Alternatively, Lp-PLA_2 is involved in the production of Lyso-PC and oxidized nonest-erified fatty acids, which are proinflammatory and proapoptotic lipid mediators.[165] **(Figure 6)**

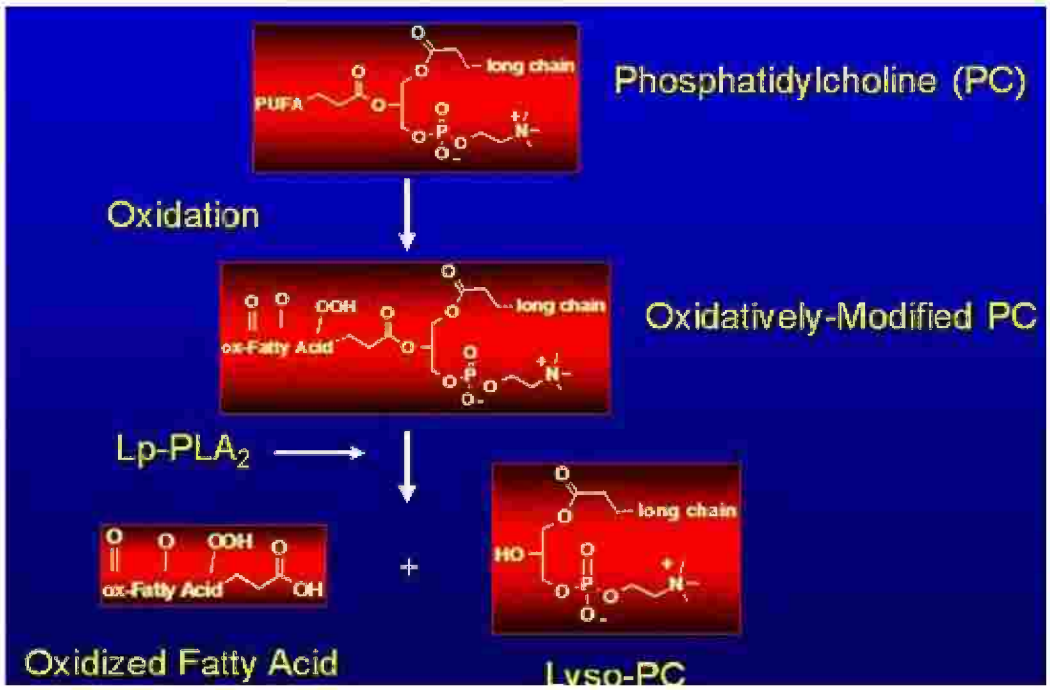


Figure (6): Lp-PLA$_2$ hydrolyzes oxidized LDL to release proinflammatory lipids. [166]

Oxidative enzymes can oxidize phospholipids in LDL particles. Oxidized phosphatidylcholine is hydrolyzed by Lp-PLA$_2$ to release oxidized fatty acid and Lyso-PC.

Proatherogenic Biological Activities:

The biological role of Lp-PLA$_2$ has been controversial, with contradictory antiathero genic and proatherogenic functions. The anti-atherogenic properties of Lp-PLA$_2$ were first suggested because of the enzymatic catabolism of biologically active oxPLs in LDL and degradation of PAF[167]. Interestingly mildly oxidized LDL and other apolipoprotein B (apoB) containing lipoproteins depleted of Lp-PLA$_2$ activity exhibit increased stimulation of monocyte chemotaxis and adhesion compared with Lp-PLA$_2$ non depleted lipoproteins. [168]

As more recent studies have ascribed anti-inflammatory properties to oxPLs, It has been suggested that hydrolysis of oxPLs by Lp-PLA$_2$ might promote inflammation. [169]

The most compelling basic evidence in favor of an atherogenic role of Lp-PLA$_2$ comes from the observation that hydrolysis of oxPLs by the enzyme generates Lyso-PC and oxidized free fatty acids, which both exhibit a number of proatherogenic effects[170] including recruitment of inflammatory cells to lesion-prone areas, local increases of inflammatory cytokines and also cytotoxic to macrophages, and may facilitate the formation of a necrotic lipid core in advanced atherosclerotic lesions.[64,171,172]

Lyso-PC is capable of modulating macrophage and T-cell migration, neutrophil and macrophage activation[173] and phagocytic clearance of apoptotic cells; These Lyso-PC effects may contribute to the initiation and progression of atherosclerosis.[174] **(Figure 7)**

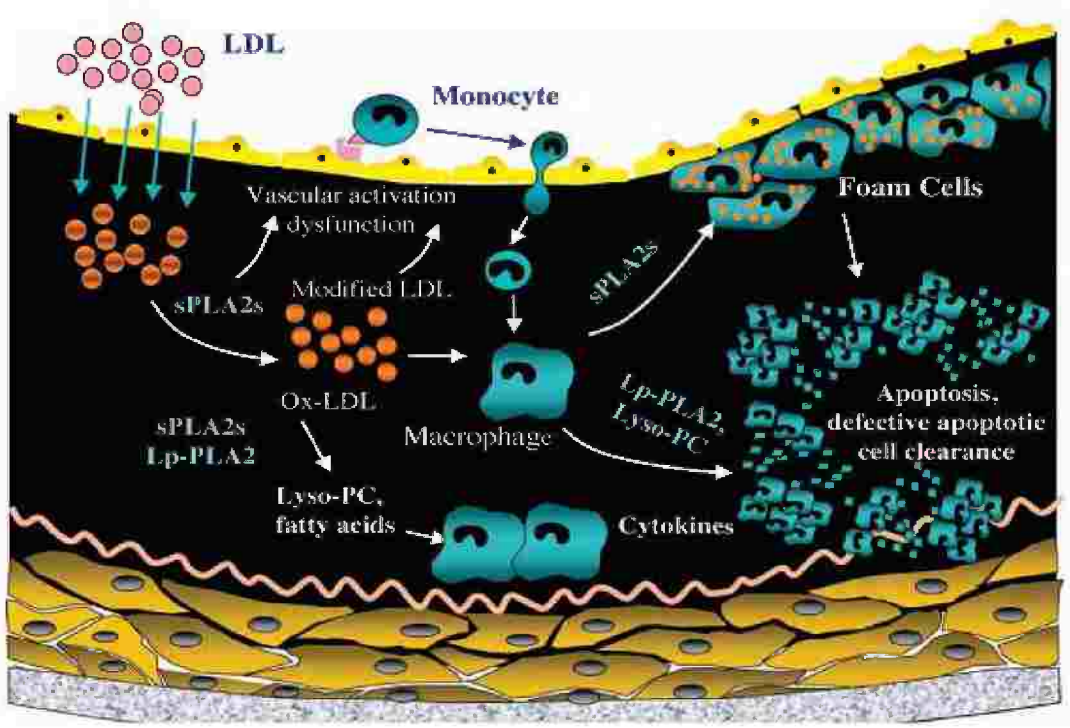


Figure (7): Schematic representation of proatherogenic pathways induced by $sPLA_2s$ or Lp-PLA_2. [175]

Several $sPLA_2$ enzymes have been shown to promote the modification of LDLs, enhancing their binding to matrix proteoglycans and facilitating their aggregation and oxida-tion. Both sPLA and Lp-PLA_2activities lead to the generation of bioactive fatty acids and Lyso-PC thereby promoting cell activation and production of inflammatory cytokines $sPLA_2s$, but not Lp-PLA_2, also promote macrophage foam cell formation by modifying lipoprotein particles. Lp-PLA_2 is strongly expressed within the necrotic core and surrounding macrophages of vulnerable and ruptured plaques in humans and is thought to promote apo-ptotic cell death. Excess production of Lyso-PC inresponse to PLA_2 activation may inhibit apoptotic cell clearance, thereby perpetuating vascular inflammation and pro-moting necrotic core formation. Ox-LDL indicates oxidized LDL.

Expression in Atherosclerotic Lesions:

Extensive Lp-PLA_2 expression was found in human advanced lesions and rupture-prone lesion the so-called thin-cap fibroatheromas [176] , Lp-$PLA_2$was found within apoptotic macrophages, which suggests that its products either represent a marker of apoptosis or might play a causal role in the induction of cell death. Lyso-PC a major product of Lp-PLA_2 activity in oxPLs, potently inhibits apoptotic cell clearance [174] which potentially may explain the preferential localization of Lp-PLA_2 in necrotic cores. Only minimal reactivity to Lp-PLA_2 was detected in pathological intimal thickening or fibroatheromas, and when present, it was mostly localized to the lipid pool or necrotic core. Minimal expression was found in smooth muscle cells. [176]

Anti-inflamatory effects of Lp-PLA$_2$:

Lp-PLA$_2$ appears to be particularly represented in small dense LDL particles, which are believed to be more proatherogenic and to promote Lp-PLA$_2$ activity. Bound to these lipoproteins Lp-PLA$_2$ hydrolyses potentially dangerous oxidized phospholipids reducing their ability to promote monocyte chemotaxis and adhesion, and it decreases the bioavailability of the prothrombotic platelet-activating factor. Thus, in a physiological state, Lp-PLA2 might actually have a protective, anti-inflammatory and antiplatelet function. [148] **(Figure 8)**

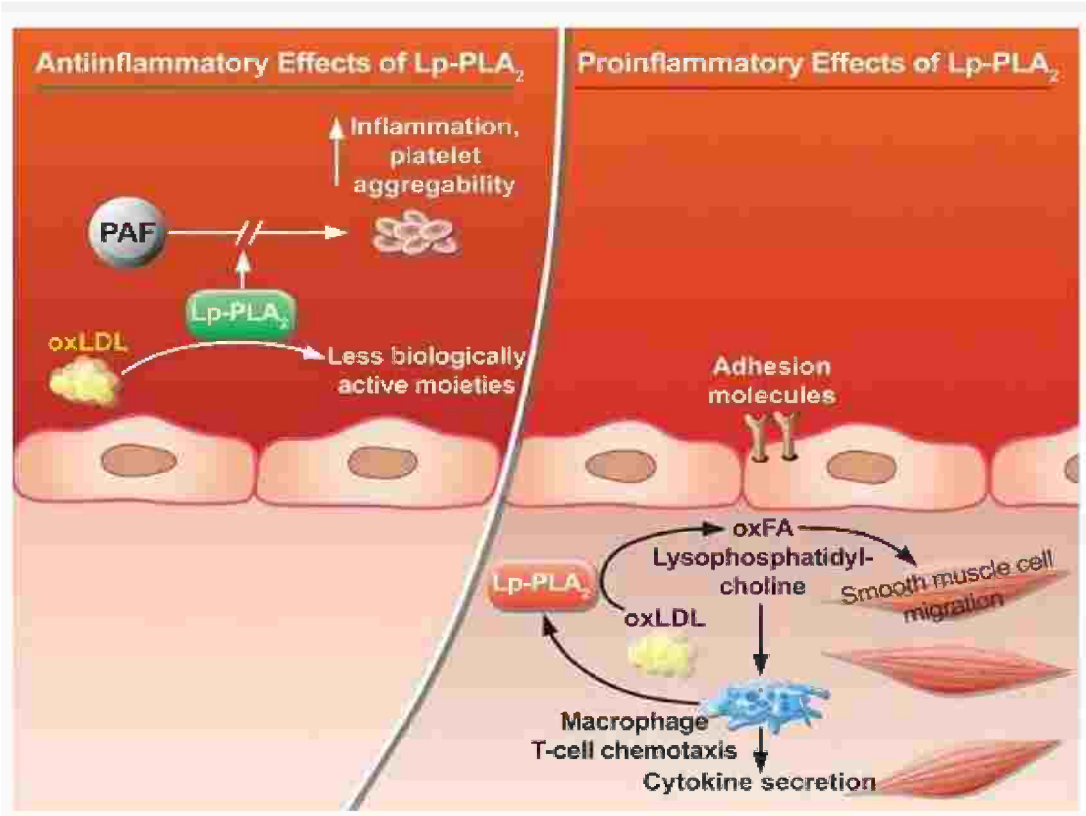


Figure (8): The controversial role of Lp-PLA$_2$. [177]

The enzyme hydrolyses a number of mediators potentially involved in atherogenesis, such as oxidized low-density lipoproteins (oxLDL) and platelet-activating factor (PAF), thus reducing their negative impact. At the same time, products of Lp-PLA$_2$ mediated degradation of these molecules may also have proinflammatory, proliferative, and ultimately proatherogenic roles Notably, Lp-PLA$_2$ is itself hyperexpressed in the setting of inflammation, and it is inhibited by peroxynitrite (ONOO-) What causes this equilibrium to tip from a beneficial role for Lp-PLA$_2$ to a proatherogenic role of the enzyme remains unknown.

Lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) is a novel biomarker of vascular-specific inflammation that provides information about atherosclerotic plaque inflammation and stability. Elevated levels of serum Lp-PLA$_2$ are indicative of rupture prone plaque and a strong independent predictor of cardiovascular risk, including CAD, MI, and stroke.[178]