Biochemical Changes in Disease

Biochemical Changes in Disease

Edited by

Inês Lopes Cardoso and Fernanda Leal

Cambridge Scholars Publishing

Biochemical Changes in Disease

Edited by Inês Lopes Cardoso and Fernanda Leal

This book first published 2022

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN (10): 1-5275-8874-2
ISBN (13): 978-1-5275-8874-5

TABLE OF CONTENTS

Preface viii

Chapter 1 1
Biochemical Changes in Pancreatic Diseases
Ayse Ceylan Hamamcioglu and Kubra Gultekin
Introduction 3
1.1. Pancreatic disorders 3
1.1.1. Pancreatitis 3
1.1.2. Cystic fibrosis 6
1.1.3. Diabetes mellitus 11
Conclusion 19

Chapter 2 36
Biochemical Changes in Cardiovascular Diseases
Carla Moutinho, Carla Matos and Carla Sousa
Introduction 38
2.1. Pathogenesis of atherosclerosis 39
2.2. Oxidative stress in cardiovascular diseases 40
2.3. Haemostatic process activation 42
2.4. Endothelial layer disfunction 44
2.5. Role of perivascular adipose tissue 45
2.6. Autophagy 46
2.7. Inflammatory factors 47
2.8. Biochemical markers of atherosclerosis 49
2.8.1. Biomarkers of the inflammatory process 51
2.8.2. Biomarkers of atherosclerotic plaque destabilization 55
2.8.3. Biomarkers of shear stress in the vascular endothelium 59
2.8.4. Biomarkers of blood vessel microcalcification 59
2.8.5. Biomarkers of thrombocyte activation 60
2.8.6. Biomarkers of neurohormonal activation 60
2.9. Treatment of atherosclerosis 61
Conclusion 63

Chapter 3 73
Biochemical Changes in Musculoskeletal Diseases
Inês Lopes Cardoso, Fernanda Leal and Ana Cláudia Fonseca
Introduction 74
3.1. Bone diseases 75
3.1.1. Bone metabolism 75
3.1.2. Biochemical markers of bone remodelling 76
3.1.3. Rickets 79
3.1.4. Osteoporosis 82
3.1.5. Osteomalacia 85
3.1.6. Paget's disease 88
3.2. Joint diseases 89
3.2.1. Rheumatoid arthritis 91
3.2.2. Gout 94
3.2.3. Osteoarthritis 98
3.3. Muscular diseases 100
3.3.1. Muscular dystrophies 102
3.3.2. Metabolic myopathies 105
Conclusion 108

Chapter 4 118
Biochemical Changes in Common Psychiatric Diseases
Joana Queiroz-Machado and Sandra Rebelo
Introduction 119
4.1. Major depressive disorder 120
4.1.1. Pathophysiology and treatment – present and future 120
4.2. Anxiety disorders 126
4.2.1. Pathophysiology and treatment – present and future 126
4.3. Schizophrenia and other psychotic disorders 131
4.3.1. Pathophysiology and treatment – present and future 132
Conclusion 138

Chapter 5 140
Biochemical Changes in Nutritional Diseases
Fernanda Leal and Inês Lopes Cardoso
Introduction 141
5.1. Malnutrition 142
5.1.1. Malnutrition in children 143
5.1.2. Malnutrition in the elderly 145
5.1.3. Metabolic changes 148
5.1.4. Symptoms and diagnosis 150

5.1.5. Biochemical parameters 151
5.2. Obesity 154
5.2.1. Obesity in children and adolescents 154
5.2.2. Obesity in the elderly 156
5.3. Eating disorders 159
5.3.1. Anorexia nervosa 160
5.3.2. Bulimia nervosa 162
5.3.3. Binge eating disorder 164
5.3.4. Orthorexia nervosa and bigorexia 165
Conclusion 167

Chapter 6 172
Biochemical Changes in Infectious Diseases
Inês Lopes Cardoso, Fernanda Leal and Léa Borderie
Introduction 173
6.1. Infectious diseases of viral origin 174
6.1.1. COVID-19 175
6.1.2. AIDS 182
6.2. Infectious diseases of bacterial origin 185
6.2.1. Tuberculosis 185
6.2.2. *Helicobacter pylori* infection 187
6.3. Infectious diseases of fungal origin 191
6.3.1. *Tinea* infection 191
6.3.2. *Candida* infection 192
6.4. Infectious diseases of parasitic origin 194
6.4.1. Toxoplasmosis 195
6.4.2. Malaria 197
Conclusion 200

CHAPTER 2

BIOCHEMICAL CHANGES IN CARDIOVASCULAR DISEASES

CARLA MOUTINHO[1], CARLA MATOS[1] AND CARLA SOUSA[1]

[1]FP-I3ID - INSTITUTE OF INVESTIGATION, INNOVATION AND DEVELOPMENT FERNANDO PESSOA, FACULTY OF HEALTH SCIENCES, UNIVERSITY FERNANDO PESSOA, PORTO, PORTUGAL

List of abbreviations

AA: Arachidonic acid
ADM: Adrenomedullin
ADMA: Asymmetric dimethylarginine
ADP: Adenosine diphosphate
AMPK: Adenosine monophosphate-activated protein kinase
Apo: Apolipoprotein
ATG6: Autophagy protein 6
Bcl: Beclin
CETP: Cholesteryl ester transfer protein
CRP: C-reactive protein
CVD: Cardiovascular diseases
COX-1: Cyclooxygenase-1
DAG: Diacylglycerol
DHA: Docosahexaenoic acid
DM: Diabetes mellitus
DNA: Deoxyribonucleic acid
ECM: Extracellular matrix
eNOS: Endothelial nitric oxide synthase

EPA: Eicosapentaenoic acid
GDF15: Growth/differentiation factor 15
GPVI: Glycoprotein VI
GPx: Glutathione peroxidase
HDL: High-density lipoproteins
HDL-c: High-density lipoproteins cholesterol
HMG-CoA: 3-Hydroxy-3-methyl-glutaryl-coenzyme A
hs-CRP: High sensitivity assays to quantify the low C-reactive protein
IBD: Inflammatory bowel disease
ICAM-1: Intercellular adhesion molecule 1
Ig: Immunoglobulin
IL: Interleukin
IP3: 1,4,5-Trisphosphate
LDL: Low-density lipoproteins
LDL-c: Low-density lipoproteins cholesterol
LDL-r: Low-density lipoproteins receptors
LFA-1: Lymphocyte function-associated antigen 1
LOX-1: Lectin-like oxidized low-density lipoprotein receptor 1
Lp(a): Lipoprotein(a)
Lp-PLA2: Lipoprotein-associated phospholipase A2
MCP-1: Monocyte chemoattractant protein 1
miRNA or miR: Micro ribonucleic acid
MMP-9: Matrix metalloproteinase 9
MPO: Myeloperoxidase
MR-proADM: Midregional pro-adrenomedullin
mTOR: Mechanistic target of rapamycin
NADPH: Nicotinamide adenine dinucleotide phosphate
NOX: Nicotinamide adenine dinucleotide phosphate oxidases
OPN: Osteopontin
OPG: Osteoprotegerin
oxLDL: Oxidized low-density lipoproteins
P2Y: Peptide 2Y
P53: Tumour protein 53
P62: Tumour protein 62
PA: Plasminogen activator
PAI: Plasminogen activator inhibitors
PAPP-A: Pregnancy-associated plasma protein-A
PCSK-9: Proprotein convertase subtilisin/kexin type 9
PDGF: Platelet-derived growth factor
PECAM-1: Platelet endothelial cell adhesion molecule 1
PIGF: Placental growth factor

PN-1: Protease nexin 1
PPAR-α: Peroxisome proliferator-activated receptor-α
PTP: Protein tyrosine-phosphatases
RA: Rheumatoid arthritis
RNA: Ribonucleic acid
RNS: Reactive nitrogen species
ROS: Reactive oxygen species
SAA: Serum amyloid-A protein
sCD40L: Soluble CD40 ligand
SLE: Systemic lupus erythematosus
sPLA2: Secretory phospholipase A2
SOD: Superoxide dismutase
TXA2: Thromboxane A2
TG: Triglycerides
TNF-α: Tumour necrosis factor-α
tPA: Tissue plasminogen activator
TRAIL: Tumour necrosis factor-related apoptosis-inducing ligand
uPA: Urokinase plasminogen activator
VCAM-1: Vascular adhesion molecule 1
VLA-4: Very late antigen 4
VSMC: Vascular smooth muscle cells
XO: Xanthine oxidase

Introduction

Cardiovascular diseases (CVD), mainly ischemic heart disease and stroke, with atherosclerosis as the key underlying factor, are one of the most common causes of death in both developing and developed countries worldwide, with an ever-increasing prevalence. Total CVD cases nearly doubled from 271 million in 1990 to 523 million in 2019, and the number of CVD deaths increased from 12.1 million in 1990 to 18.6 million in 2019. Nevertheless, CVD mortality rates have continued to decline worldwide. In the last three decades, more than half of the reduction in CVD mortality has been attributed to changes in risk factor levels in the population, primarily the reduction in cholesterol and blood pressure levels and smoking. Other risk factors, as unhealthy diet, physical inactivity, harmful use of alcohol, obesity and type 2 diabetes mellitus (DM), together with non-modifiable factors, as sex and age, partly offset this favourable trend.

Atherosclerosis results from low-grade chronic inflammation that arises from an interaction between immunological mechanisms and metabolic

abnormalities within the vessel wall. In addition to inflammation, risk factors for this condition include high cholesterol and low-density lipoproteins (LDL), low level of high-density lipoproteins (HDL) in the blood, hypertension, tobacco smoke, diabetes mellitus, obesity, inactive lifestyle, unhealthy diet, body mass index, waist circumference, older age, family history of early earth disease, high blood levels of triglycerides (TG), inflammation, sleep apnoea, stress, alcohol consumption.

This chapter concentrates on the pathogenesis and biochemical changes of atherosclerosis, that is the major cause of CVD, and it is driven by oxidative stress and enhanced inflammation in the artery wall.

2.1. Pathogenesis of atherosclerosis

Blood vessel thickening due to the formation of plaques in the subendothelial intimal space is the main feature of atherosclerosis. Cholesterol, TG and lipoproteins are directly related to the pathogenesis of this disease. Atherosclerosis begins with the accumulation of LDL, which are sequestered in the subendothelial space by adhesion to extracellular matrix proteins rich in proteoglycans. This vessel thickening is more frequent in coronary artery, carotid artery, abdominal aorta, descending aorta, and iliac artery.

When LDL reaches the sub-intimate space, it can be aggregated and/or oxidized, becoming strong chemo attractants. The aggregation of LDL gives rise to complexes, which can undergo pinocytosis or phagocytosis by macrophages, that become foam cells. Macrophages are originated from circulating monocytes, that adhere to the endothelial cells that express adhesion molecules, such as vascular adhesion molecule 1 (VCAM-1), monocyte chemoattractant protein 1 (MCP-1) and P and E-selectins and migrate via diapedesis in the subendothelial space. Monocytes differentiate into macrophages and engulf oxidized LDL, becoming foam cells and contributing to plaque/atheroma development by secreting multiple mediators of the inflammatory process.

The increased inflammation triggers the migration of vascular smooth muscle cells (VSMC) from the tunica media into the subendothelial space where they abnormally proliferate and secrete extracellular matrix (ECM) proteins, contributing to atheroma growth. VSMC present in the intimal layer form a fibrous cap that contains the plaque. The atheroma plaque is mainly composed of a mixture of macrophages, lymphocytes, VSMC, cholesterol, necrotic debris, and foam cells. In the advanced stages, there is

intra-plaque neovascularization and haemorrhages. Platelet aggregation and clotting activation have a role in the development of thrombotic complications by adhering to the exposed sub endothelium at the site of plaque rupture and erosion. The rupture of the fibrous cap leads to thrombus formation causing blockage of the blood flow, or embolism in areas of the vascular bed far from the atherosclerotic area.

Thus, according to Bergheanu et al. (2017), the vascular modifications observable in atherosclerosis are progressively:

1- Intimal thickening, with deposition of VSMC and ECM proteins;
2- Fatty streak, where macrophage foam cells develop, mixed with VSMC;
3- Pathologic intimal thickening: VSMC and ECM proteins aggregate near the lumen, over an acellular area rich in hyaluronan and proteoglycans with lipid infiltrates;
4- Fibroatheromas, with an acellular necrotic core covered by a thick fibrous cap of VSMC in proteoglycan-collagen matrix;
5- Vulnerable plaque, with a thin type I collagen fibrous cap;
6- Ruptured plaque: the rupture of the fibrous cap leads to the presence of luminal thrombus and the increased macrophage infiltration.

The initial phase of atherosclerosis, which can last decades, is asymptomatic, as the plaque forms in the vessel wall of multiple arterial beds in proximity to bifurcations. The progressive narrowing of the arterial lumen is initially counterbalanced by vasodilation of arteries and collateral vascularization. The reduction of blood flow caused by a stenosis, which overcomes the reserve dilation capacities of the arteries, leads to the clinical features of atherosclerosis, which are angina pectoris, intermittent claudication or a transient ischemic attack. If an acute thrombotic obstruction, triggered by plaque disruption or, more frequently erosion, took place, an acute coronary syndrome or a stroke can occur.

2.2. Oxidative stress in cardiovascular diseases

The heart needs adequate oxygen supply to maintain its contractile function. At the cellular level, oxygen undergoes a reduction to superoxide anion (O_2^-) through the action of different types of oxidases (*e.g.,* uncoupled endothelial nitric oxide synthase (eNOS), mitochondria and xanthine oxidase (XO), NADPH oxidases (NOX)). Reactive oxygen species (ROS) are subcellular messengers in signal transduction pathways with beneficial and harmful functions. Under physiological conditions, ROS, as reactive nitrogen

species (RNS), play important roles in different signalling pathways, through the oxidation of specific targets. However, after increasing the activation of ROS/RNS-producing enzymes and/or in the face of a deficiency in endogenous antioxidant capacity, oxidative stress may occur. This can lead to reversible changes that can transiently alter the activity of the protein involved in the physiological adaptations, or irreversible oxidations, which originate pathophysiological processes. ROS are by-products of mitochondrial respiration or metabolism, being produced by specific enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, peroxiredoxins and myeloperoxidase. In the heart and skeletal muscle, ROS are essentially originated by NOX, eNOS and XO, producing superoxide anion and/or hydrogen peroxide (H_2O_2). O_2^- reacts with nitric oxide (NO) and forms peroxynitrite ($ONOO^-$), that is a very RNS. H_2O_2 and O_2^- are the predominant redox signalling agents originated under the control of growth factors and cytokines by more than 40 enzymes, prominently including NOX and the mitochondrial electron transport chain. However, several other reactive species are involved in redox signalling, such as nitric oxide, hydrogen sulphide and oxidized lipids. ROS/RNS, that are produced in cardiomyocytes in response to specific stimuli (acute, transient or sustained), originate lipid peroxidation, interact with DNA repair enzymes and transcription factors or cause DNA damage, lead to the oxidation/nitration of key proteins involved in contractility, calcium manipulation, metabolism, antioxidant defence mechanisms, among others. ROS/RNS also stimulate the inflammatory process, signs of stress inducing cardiac hypertrophy, fibrosis or cell death via apoptosis/necrosis and deregulate autophagy. ROS involved in signalling cardiac redox may have several origins, but NADPH oxidases, as dedicated sources of signalling of reactive oxygen species, seem to be of great importance. In fact, NADPH oxidases are a family of enzymes whose primary function is to produce ROS, mediating adaptive and maladaptive changes in the heart. The activity of NADPH oxidases is increased in the diabetic heart, characterized by an increase in oxidative stress.

There are 7 different isoforms of NOX (NOX1-NOX5 and DUOX1 and 2), being NOX1, 2, 4 and 5 identified and characterized in the cardiovascular system. Since cardiomyocytes contain a high concentration of mitochondria, which provide the main source of endogenous ROS, these organelles suffer oxidative damage, which often leads to the death of apoptotic cells and initiates cardiac pathology.

NOX4 has its highest levels of expression in proximal tubular cells of the kidneys, but is also expressed in other types of cells, including cardiomyocytes.

In the cardiovascular system, an increase in NOX4 expression can be triggered by various situations, such as pressure overload, hypoxia, and inflammation, which significantly affects cell function. This isoform essentially produces H_2O_2. Although NOX4 may play a protective role in cases of cardiac hypertrophy, fibrosis, contractile function, harmful effects are observed on the overloaded heart due to increased ROS production and consequent mitochondrial damage.

2.3. Haemostatic process activation

The haemostatic process can be divided in three steps: primary haemostasis, in which an interaction between platelets and the damaged endothelium occurs; secondary haemostasis, in which the activation of the clotting system happens, consists of activation of the protease cascade resulting in the production of fibrin clots; and the last step is fibrinolysis, a counterbalance system that degrades fibrin and dissolves the clot.

Primary haemostasis involves the adhesion, activation, and recruitment of platelets. The activated platelets release proinflammatory and pro aggregating factors from their granules, as adenosine diphosphate (ADP) and thromboxane A2 (TXA2), inducing the production of secondary messengers, such as diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) which, in turn, lead to an increased concentration of cytosolic calcium. Studies showed that ROS are able to mimic the roles of ADP and IP3 by influencing primary haemostasis through platelet activation. Platelets could be a target of ROS but also a source of free radicals. Indeed, an imbalance between ROS production and the antioxidant system dysregulates and amplifies platelet activation due to isoprostane formation, the modulation of platelet receptors, and the oxidation of LDLs. Platelet activation, in turn, leads to further ROS production through NOX activation, triggering mitochondrial dysfunction (Fig. 2-1).

Secondary haemostasis can also be impaired by ROS, especially those generated by NOX, promoting clotting activation, as they are able to upregulate the tissue factor, causing prothrombotic effects within blood vessels. ROS overproduction provokes the reaction of NO with O_2^-, generating $ONOO^-$, which, in turn, leads to serine-protease and fibrinogen nitration resulting in a pro-coagulant environment. Besides the increase and dysregulation of coagulation cascade factors, thrombosis occurs following a decrease in anticoagulant factors, such as protein S. Fibrinolysis consists of plasminogen activator (PA) stimulation to produce plasmin, which is able to promote the lysis of fibrin, accelerating clotting degradation. However, a

relative increase in the plasmin concentration is avoided through the actions of plasminogen activator inhibitors (PAI-1 and PAI-2). ROS are able to upregulate PAI-1, which is also implicated in the development of the atherothrombotic process. There is experimental evidence showing that PAI-1 expression is strongly increased during the inflammatory process and during atherothrombogenesis, and studies have shown that the oxidation of LDL, with consequent formation of oxLDL, induces the overproduction and release of PAI-1.

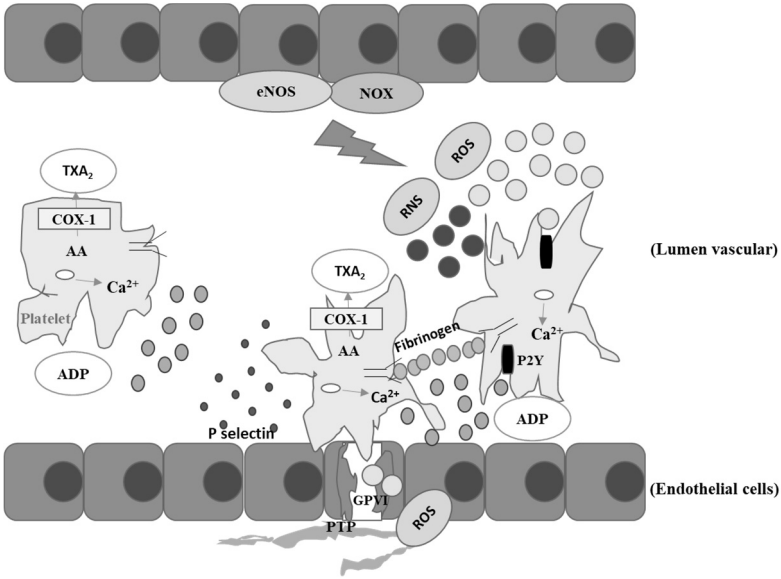

Fig. 2-1. Platelet activation after exposure to collagen and the production of free radicals.

Protein tyrosine-phosphatases (PTP) are the primary targets of ROS which, in turn, determine the upregulation of glycoprotein VI (GPVI) signalling. This event causes the overstimulation of platelets, inducing cytosolic calcium ions mobilization and the release of pro aggregating factors from granules, as ADP and TXA2. Overdue to endothelial dysfunction, eNOS and NOX stimulation lead to the overproduction of ROS and RNS which are able to interact with platelets through the ADP receptor, triggering the same intracellular signalling pathway.

(Platelet TXA2 production from arachidonic acid (AA) is dependent on COX-1 (cyclooxygenase-1); P2Y is a peptide receptor for ADP)

The role of proteases as thrombin, uPA/tPA (urokinase PA/tissue PA) or plasmin, in the pathophysiology of atherosclerosis is being put in evidence, with an unbalanced ratio between proteases and their inhibitors favouring

the chronic evolution of the plaque. Protease nexin 1 (PN-1), a serpin closed to plasminogen activator inhibitor type-1 (PAI-1), has emerged as a key regulator in vascular biology, even though its mechanism of action is hitherto unknown. Protease Nexin 1 is present in platelets and monocytes and is released from platelet α-granules during their activation. Its physiological role in coagulation, fibrinolysis and tissue remodelling and inflammation can be explained by the inhibition of a broad range of serine proteases. It displays anti-thrombotic properties via its ability to block thrombin generation and activity, and anti-fibrinolytic properties thanks to its ability to block plasmin generation and activity. PN-1 is involved in the different stages of atherosclerotic plaque progression. In the early stage, PN-1 may be involved in endothelial dysfunction and may represent a cell defence reaction against proteases present in the atherosclerotic plaque. In the advanced plaque, it is able to form covalent complexes with plasmin. Overexpression of PN-1 by VSMC has been shown to significantly reduce their adhesion, spreading and migration on vitronectin, an adhesive protein found in atherosclerotic plaques. At the most complicated stage of atherosclerosis, rupture of the plaque, platelet PN-1 is assumed to contribute to thrombus stabilization.

2.4. Endothelial layer disfunction

The arterial endothelial cells present a diversity of homeostatic functions: i) the enzymatic remodelling of extracellular matrix components; ii) the biosynthesis of vasoactive mediators, various growth factors, cytokines and hormone-like substances; iii) the enzymatic buffering of ROS, iv) the transport and metabolism of lipoproteins; and v) the synthesis of prostaglandins. When confronted with certain proinflammatory cytokines endothelial cells undergo a coordinated program of gene activation, which alters many of these vital functional properties. Endothelial layer cell dysfunction results in the earliest detectable changes in the life history of an atherosclerotic lesion, initially involving the selective recruitment of circulating monocytes from the blood to become foam cells.

An imbalance between the activity of antioxidant enzymes, such as GPx, catalase, and SOD, and the pro-oxidant system produces an uncontrolled increase in oxidative stress, leading to endothelial dysfunction. The production of oxidized LDL promotes the production of proinflammatory factors, such as interleukin-1 (IL-1), IL-6, tumour necrosis factor-α (TNF-α) and C-reactive protein (CRP) that generate the endothelial proinflammatory phenotype characterized by an increase in E-selectin,

VCAM-1 and intercellular adhesion molecule 1 (ICAM-1) expression.

Animal studies have provided compelling evidence demonstrating the roles of vascular oxidative stress and NO in atherosclerosis. ROS are also considered crucial mediators of vascular homeostasis and the atherosclerosis pathogenesis. ROS and RNS overproduction are responsible for endothelial dysregulation. Endothelial cells as well as fibroblasts and VSMC express several NOX (1, 2, 4 and 5). In particular, NOX-1 and NOX-2 are involved in the development of hypertension, inflammation, and endothelial dysfunction. The production of free radicals induced by the different isoforms of NOX influences the activity of other enzymes such as eNOS, producing NO. The endogenous production of NO in endothelial cells, at nanomolar concentrations, through NOS activation represents a vasoprotective mechanism of the vascular endothelium, while exaggerated release of NO as a consequence of a cytokine-inducible NOS activation leads, as described above, to the rapid reaction of NO with O_2^-, generating $ONOO^-$, the main compound responsible for the onset of endothelial dysfunction and, in the late stages, the development of atherothrombosis. The subsequent imbalance between vasoconstriction and vasodilatation increases the endothelial permeability and triggers a local inflammatory response. All established cardiovascular risk factors such as hypercholesterolaemia, hypertension, diabetes mellitus, and smoking enhance ROS generation and decrease endothelial NO production. Key molecular events in atherogenesis such as oxidative modification of lipoproteins and phospholipids, endothelial cell activation, and macrophage infiltration/activation are facilitated by vascular oxidative stress and inhibited by endothelial NO. Atherosclerosis develops preferentially in vascular regions with disturbed blood flow (arches, branches, and bifurcations).

2.5. Role of perivascular adipose tissue

The perivascular adipose tissue surrounds almost all the vessels with the exception of cerebral arteries, and is composed of brown or white adipocytes, or a mixture of both types, and of a stromal vascular fraction with fibroblasts, endothelial cells and immune cells. The perivascular adipose tissue has also been described as having a role in the pathophysiology of CVD, namely for modulating the vascular tone, since it presents an anticontractile function, in response to several agonists such as phenylephrine, serotonin, angiotensin II, and TXA2. In the presence of cardiovascular risk factors, such as obesity, atherosclerosis, diabetes,

hypertension and chronic alcohol consumption, oxidative stress emerges, predisposing to the vascular damage and affecting the anticontractile function of the perivascular adipose tissue. ROS are formed in the adipose tissue by different mechanisms.

In obesity, the perivascular adipose tissue inflammation and oxidative stress is caused by NOX activation, eNOS uncoupling, reduced expression of antioxidant molecules, mitochondria-derived ROS and proinflammatory cytokines. In aging and chronic alcohol consumption, ROS generation by NOX is a common feature. In response to a proatherogenic lipid profile, increased adiponectin and eNOS-derived NO is characteristic.

2.6. Autophagy

Apoptosis and autophagy are two forms of programmed cell death associated with the development of CVD. Autophagy is an intracellular catabolic mechanism for the degradation of dysfunctional proteins and organelles, essential for the maintenance of cellular homeostasis, and associated with increased longevity and health. While autophagy is characterized by the early degradation of organelles, with preservation of the cytoskeleton until the last stage, apoptosis involves the early degradation of the cytoskeleton, with preservation of the organelles until the final stage.

At the cardiovascular level, autophagy is a fundamental process for homeostasis in most cells of cardiovascular origin and for the function of the heart and vessels. Apoptosis and autophagy interact through the Beclin complex pathways (Bcl1-Bcl2/Bcl-xL), mTOR (mechanistic target of rapamycin), TRAIL (tumour necrosis factor-related apoptosis-inducing ligand), TNF-α, endoplasmic reticulum stress and p53 pathways of the nucleus. It is essential to promote cardiac and vascular health during aging, and there are some therapeutic approaches, such as pharmaceutical compounds involving mTOR inhibitor and AMPK activator to regulate apoptosis and autophagy, genetic interventions and caloric restriction, which show cardioprotective activity. Normal levels of autophagy can protect cells, however defective or excessive autophagic activity appears to contribute to cardiovascular disorders, such as heart failure or atherosclerosis.

As autophagy decreases with age, it triggers harmful cellular processes that lead to stiffening and functional decline of the arterial system. It is known that the autophagic process becomes dysfunctional as atherosclerosis progresses, even though there are, in the atherosclerotic plaque, factors that stimulate the autophagy, such as ROS. Autophagy can occur in the main

cell types of atherosclerotic plaques, that are macrophages, vascular smooth muscle cells and endothelial cells, promoting these cells survival.

Oxidized lipids, present in atherosclerotic plaques, can stimulate autophagy in macrophages. Autophagy in vascular smooth muscle cells can be initiated by atherosclerosis-related stimuli such as oxLDL, 7-ketocholesterol, TNF-α, and platelet-derived growth factor (PDGF). On the other hand, autophagy caused by factors such as osteopontin (OPN), angiotensin II and nicotine can accelerate the death of VSMC, further enhancing atherosclerotic lesions. In endothelial cells autophagic process can be stimulated by oxLDL, advanced glycation end products and saturated fatty acids. At the level of atherosclerosis, miRNA-30-mediated translational control of ATG6 regulates endothelial cell autophagy initiated by oxLDL.

However, defective autophagy can provide an accumulation of damaged proteins and organelles, such as mitochondria, resulting in increased oxidative stress and apoptosis, but accelerates stress-induced premature senescence and atherogenesis in VSMC, and in endothelial cells promotes apoptosis and also senescence. The reason for these differences is still unclear, but it may be related to increased levels of the linker molecule p62 in atherosclerotic plaques, or it may depend on the cell's origin and/or its proliferative capacity.

2.7. Inflammatory factors

Atherosclerosis results from low-grade chronic inflammation, related to unhealthy lifestyles, and also to the interaction between the immunological system and metabolic abnormalities. In a similar manner, chronic inflammatory diseases confer a significantly increase in the risk of accelerated atherosclerosis leading to enlarged morbidity and mortality and reduced life expectancy compared to the general population. In fact, there are common molecular pathways shared by atherosclerosis and inflammatory diseases. This is the case with inflammatory bowel disease, psoriasis and psoriatic arthritis, chronic obstructive pulmonary disease, rheumatoid arthritis, between others.

Patients with inflammatory bowel disease (IBD) have a higher risk of endothelial dysfunction and, consequently, of subclinical atherosclerosis than healthy controls, but the cause of the increased cardiovascular risk is not fully known. Structural changes in the arterial vessel wall occur because of long-term exposure to inflammation or cardiovascular risk factors. The pulse wave velocity levels were higher, while flow-mediated dilatation

levels were significantly decreased, in patients with Crohn's disease or ulcerative colitis relatively to healthy persons.

Psoriasis is associated with an increased risk of multiple comorbidities, including CVD at younger ages, unidentifiable by traditional risk factors. Insulin resistance appears to play a major role in the development of atherosclerosis in these cases. Patients with psoriasis show abnormalities in the innate and adaptive immune system that lead to high serum levels of proinflammatory cytokines, capable of increasing cell-mediated immunity, promoting the migration of inflammatory cells through the vascular endothelium, resulting in endothelial dysfunction and, consequently, in the formation of plaques. It seems that chronic systemic inflammation, characteristic of psoriasis and psoriatic arthritis, leads to insulin resistance, that originates endothelial dysfunction and atherosclerosis.

Chronic obstructive pulmonary disease is associated with subclinical atherosclerosis. Patients with chronic obstructive pulmonary disease seem to have a more pronounced atherosclerotic process, which is evidenced by elevated intima-media thickness, increased prevalence of carotid plaques, enlarged pulse wave velocity and reduced flow-mediated dilation. In addition, this pathology has been associated with increased levels of vascular biomarkers, regardless of physiological confounding factors, smoking or other cardiovascular risk factors. Thus, chronic obstructive pulmonary disease appears to be an independent risk factor for CVD.

Atherosclerosis, as an inflammatory process, shares mediators and activation mechanisms with rheumatoid arthritis (RA). For example, the mechanism of atherosclerotic plaque rupture has similarities with rheumatoid synovitis and destruction of joint structures. RA is associated with an increased risk of morbidity and mortality, mainly due to augmented atherosclerotic disease. RA patients evidence a higher intima-media thickness and an increased prevalence of carotid plaques.

Patients with systemic lupus erythematosus (SLE) generally have increased levels of total cholesterol, LDL, TG and apolipoprotein B (Apo B), and decreased levels of HDL, and a prevalence of metabolic syndrome of about 10%, with SLE being associated with endothelial damage and coronary atherosclerosis.

2.8. Biochemical markers of atherosclerosis

Biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”. Biomarkers are characterized by elevated sensitivity, high repeatability of results and the possibility of usage in clinical procedures. They have applications in several areas, such as screening, diagnosis, prognostication, prediction of recurrences, and monitoring of therapy.

There are presently several clinical biomarkers that are related with cardiovascular outcomes. These biomarkers comprise: cardiac troponins I and T, B-type natriuretic peptides, haptoglobin, and D-dimer. Even though these biomarkers are routinely used in clinical laboratory and have helped clinicians save lives, they are late-stage biomarkers. The challenge is to find biomarkers that detect early-stage CVD to considerably reduce morbidity and mortality associated with cardiovascular effects and improve prognosis.

Recognition of classical biomarkers of atherosclerosis, such as LDL, HDL, and TG may not be effective in patients with moderate or atypical cardiovascular risk. For more precise management, non-classical atherosclerosis biomarkers may be helpful in these patients.

Biomarkers of atherosclerosis are characteristic of its phases. These comprise biomarkers of i) the inflammatory process, ii) destabilization of atherosclerotic plaque, iii) shear stress in the vascular endothelium, iv) blood vessel microcalcification, v) thrombocyte activation and vi) neurohormonal activation (Table 2-1). In the following divisions, the roles of validated biomarkers for atherosclerosis are summarized, referring on the promising candidates, the microRNAs.

Table 2-1. Main biomarkers of atherosclerosis.

Inflammatory process	Destabilization of atherosclerotic plaque	Shear stress in the vascular endothelium	Blood vessel microcalcification	Thrombocyte activation	Neurohormonal activation
Cathepsins SAA ICAM-1 VCAM-1 IL-6 TNF-α CRP MPO MMP-9 GDF15 Lp(a) ADMA	sCD40L oxLDL anti oxLDL antibody Selectins MicroRNAs PIGF PAPP-A MPO MMP-9 CRP	MicroRNAs	MicroRNAs OPN OPG	Lp-PLA2 sPLA2 sCD40L	Copeptin MR-proADM

ADMA - Asymmetric dimethylarginine; CRP - C-reactive protein; GDF15 - Growth/differentiation factor 15; ICAM-1 - Intercellular adhesion molecule 1; IL-6 - Interleukin 6; Lp(a) - Lipoprotein(a); Lp-PLA2 - Lipoprotein-associated phospholipase A2; MMP-9 - Matrix metalloproteinase 9; MPO - Myeloperoxidase; MR-proADM - Midregional pro-adrenomedullin; OPN - osteopontin; OPG – Osteoprotegerin; oxLDL - Oxidized low-density lipoprotein; PAPP-A - Pregnancy-associated plasma protein-A; PIGF - Placental growth factor; SAA - Serum amyloid-A protein; sCD40L - soluble CD40 ligand; sPLA2 - secretory phospholipase A2; TNF-α - tumour necrosis factor-alfa; VCAM-1 - vascular cell adhesion protein 1.

2.8.1. Biomarkers of the inflammatory process

Cathepsins

Cathepsins, enzymes typically concentrated in the lysosomes and endosomes of macrophages, are proteases that degrade undesirable endocytosed or intracellular proteins. Emerging research has demonstrated that cathepsins, specifically cathepsins B and X, cysteine proteases, and cathepsin D, an aspartic protease, are upregulated in atherosclerotic lesion. Another study has showed the potential of cathepsins as a diagnostic tool, having revealed that the circulating levels of cathepsin S, K and L and their endogenous inhibitor, cystatin C, could be biomarkers in the diagnosis of some pathologies, such as coronary artery disease, aneurysm, peripheral arterial disease, and coronary artery calcification. An imbalance in expression between cathepsins and its inhibitor can trigger the proteolysis of extracellular matrix in the pathogenesis of CVD. In the development phase of the disease, inflammatory cytokines, growth factors, oxidative stress, hypertensive stimuli, among others, regulate the expression and activities of cathepsins.

Serum amyloid-A protein (SAA)

SAA is an acute phase apolipoprotein that increases the expression of prothrombotic and proinflammatory molecules. Concentrations of greater than 10 mg/L were suggestively related with a greater number of new cerebral lesions detected on diffusion weighted magnetic resonance imaging during carotid artery stenting and significantly related with progressive atherosclerosis measured by ultrasound examination. Higher levels can identify patients with ischemic stroke caused by atherothrombosis *versus* cardioembolic stroke.

Intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion protein 1 (VCAM-1)

Increased expression of ICAM-1 and VCAM-1 has been demonstrated in the first stage of leukocyte penetration into the vascular endothelium in vessels predisposed to the development of atherosclerosis and within existing atherosclerotic lesions. VCAM-1 and ICAM-1 are endothelial cell surface glycoproteins that allow/aid, respectively, endothelial cell-leukocyte adhesion in inflammation. The receptor for ICAM-1 is LFA-1 (CD11a/CD18, Lymphocyte function-associated antigen 1, alphaL beta2 integrin), which occurs on all types of leukocytes. The receptor for VCAM-1 is VLA-4 (CD49d/CD29, very late antigen 4, alpha4 beta1 integrin),

located on monocytes and lymphocytes. The interaction between ICAM-1/LFA-1 and VCAM-1/VLA-4 is supported by platelet endothelial cell adhesion molecule 1 (PECAM-1). This molecule transduces mechanical signals in endothelial cells and regulates migration of leukocytes under the vascular endothelium where inflammation occurs.

VCAM-1 levels have been reported to be positively linked with cardiovascular mortality, the presence of carotid atherosclerotic lesions, and magnetic resonance markers of plaque instability. There is support for a predictive function of circulating concentrations of ICAM-1 in initially healthy people and as a significant correlation was detected with cardiovascular mortality. ICAM-1 was found elevated in more than 300 patients who undertook carotid endarterectomy contrasted with healthy controls.

Interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α)

Cytokines are key regulatory glycoproteins related to inflammatory/immunological processes which modulate all aspects of vascular inflammation. IL-6 is one of the most important and most multi-functional interleukins, mainly produced by monocytes and macrophages. IL-6 enhances cell adhesion molecule expression and the production of acute phase reactants such as CRP and TNF-α. Therefore, it is associated with the development of atherosclerotic plaques. Quantifications of IL-6 may be convenient to reclassify intermediate risk patients into higher risk categories. According to the Atherosclerotic Cardiovascular Disease risk score, serum IL-6 > 1 pg/mL in patients with chest pain and intermediate risk examined for coronary angiography was predictive of major coronary artery disease. Findings on IL-6 receptors indicate that IL-6 inhibition could provide an innovative therapeutic approach to coronary heart disease prevention, but strong clinical trials and genetic testing in large populations are needed to validate and select new therapeutic targets.

On the other hand, TNF-α is a proinflammatory cytokine implicated in atherosclerotic progression from the initial phases of intimal thickening to the subsequent vessel occlusion. TNF-α is an inhibitor of endothelial nitric oxide synthase, improves the production of reactive oxygen species and reduces the effect of endothelium-derived hyperpolarizing factor. It is involved in cell differentiation and proliferation, platelet activation, and apoptosis. Senior patients who had elevated levels of TNF-α in the blood more often had clinically diagnosed atherosclerosis. TNF-α is linked with a larger plaque size and is increased in patients with plaque instability.

C-reactive protein (CRP)

CRP is a pattern recognition molecule that is elevated in inflammatory conditions, such as atherosclerosis, being an acute phase biomarker of inflammation and destabilization of atherosclerotic plaque. It belongs to the pentraxin family and is produced mainly in hepatocytes because of IL-6 stimulation. Investigations have reliably described that concentration in blood above 10 mg/L indicates an inflammatory process and higher CRP levels have a prognostic value for cardiovascular events and mortality.

CRP has been suggested to increase LDL oxidation and to induce a prothrombotic state through induction of tissue factor expression in human monocytes. It can activate or inhibit the complement cascade, driving the inflammation in atherosclerotic lesions. CRP has additionally been exhibited to reduce the expression and bioactivity of endothelial nitric oxide synthase with a subsequent effect on vasodilatation. CRP downregulates angiogenesis stimulated by vascular endothelial growth factor, while it promotes endothelial apoptosis in a nitrous oxide-dependent approach. CRP has also been found to synergistically augment angiotensin II-induced proinflammatory outcomes, involving cellular migration and proliferation as well as lesion collagen and elastin content. Ultimately, it induces the release of monocyte chemoattractant protein-1 and endothelin-1 upregulating adhesion molecules and chemoattractant chemokines in endothelial and vascular smooth muscle cells.

The high sensitivity assays (hs-CRP) quantify the low CRP concentration which cannot be assessed by the routine biochemistry analysis. A hs-CRP level of >3 mg/L should be considered as risk factor for perioperative adverse cardiovascular event in patients with asymptomatic atherosclerosis or stable ischemic heart disease and levels >10 mg/L have a greater prognostic value in those undergoing from acute coronary syndrome.

Myeloperoxidase (MPO)

MPO is a haemoprotein produced by monocytes and that activates neutrophils and catalyses the formation of hyperchlorite from chloride and H_2O_2. It promotes oxidation of LDL and oxidative modification of Apo A. MPO and metalloproteases disrupt the collagen layer in an atherosclerotic plaque, therefore leading to its erosion and rupture. Clinical trials have found that superior MPO levels are early indicators of coronary artery disease prior to detection by angiography or high cardiac troponin values.

Matrix metalloproteinase 9 (MMP-9)

Matrix metalloproteinases are zinc dependent endopeptidases produced by several cell types. Metalloproteinases are responsible for degradation of collagen and other extracellular matrix components. Matrix metalloproteinases, particularly MMP-9, are involved in all stages of atherosclerosis process. MMP-9 increases the infiltration of monocytes under the vascular endothelium. In addition, MMP-9 also influences intraplaque angiogenesis through interaction between integrins and proteinases. The aneurysm formation during atherosclerosis process is equally due to the arterial remodelling and increased extracellular matrix components breakdown by metalloproteinases.

Growth/differentiation factor 15 (GDF15)

GDF15 is produced by macrophages, cardiomyocytes, and endothelial cells in response to acute inflammatory process. The rise in GDF-15 concentrations is associated, among other conditions, to atherosclerosis, atrial fibrillation, heart failure, pulmonary embolism, and renal failure.

Lipoprotein(a) (Lp(a))

Lp(a) is a modified LDL lipoprotein by attaching a specific apolipoprotein(a) to Apo B100. The physiological functions of Lp(a) include proatherosclerotic, prothrombotic and proinflammatory roles. Lp(a) concentrations are transiently increased by inflammatory processes and by tissue damage caused by acute phase proteins, being elevated Lp(a) plasmatic level a genetically determined, independent, causative risk factor for CVD.

Alike other lipoproteins, Lp(a) is also susceptible to oxidative changes, leading to extensive formation of oxidized phospholipids, oxysterols, oxidized lipid-protein adducts in Lp(a) molecules that consolidate the progression of atherosclerotic lesions and intimal thickening by induction of M1 macrophages, inflammation, autoimmunity, and apoptosis.

Asymmetric dimethylarginine (ADMA)

ADMA is derived from the methylation of arginine residues within proteins by the activity of protein arginine N-methyltransferases. Enhanced oxidative stress upregulates this protein expression and ADMA consequently synthesis. On the other hand, pro-oxidant and proinflammatory stimuli inhibit dimethylarginine dimethylaminohydrolase activity, the enzymes responsible for ADMA degradation in cell culture research.

ADMA has been of attention to vascular researchers because this biomarker was demonstrated to be endogenous inhibitors of eNOS via competition with L-arginine, the requisite eNOS substrate and structural analogue of ADMA. Clinical data proposes that ADMA plasma concentrations are related with endothelial function, mainly in patients with coronary atherosclerosis or atherosclerosis risk factors, whereas this association is significant but weak in healthy individuals. Additionally, in prospective studies, plasma ADMA has been independently linked with clinical outcome and mortality in diabetic subjects and patients with atherosclerosis.

2.8.2. Biomarkers of atherosclerotic plaque destabilization

Circulating soluble CD40 ligand (sCD40L)

sCD40L, largely derived from activated platelets, stimulates an inflammatory reaction in vascular endothelial cells by the secretion of cytokines and chemokines. Membrane-bound CD40L and sCD40L types interact with the CD40 receptor molecule, leading to the release of MMPs and subsequent destabilization of the atherosclerotic plaque. High plasma concentrations of sCD40L were demonstrated in patients with myocardial infarction and unstable ischemic heart disease.

Oxidized low density lipoprotein (oxLDL) and anti oxLDL antibody

oxLDL can trigger the expression of adhesion molecules on the cell surface and thus promote the activation of endothelial cells. These adhesion molecules mediate the rolling and adhesion of macrophages, that adhere to the endothelium and then, in response to chemokines, migrate into the intima. As the consequence of the leukocyte activation, proinflammatory cytokines are released, ROS are synthesized, and MMPs are also produced, contributing to the matrix degradation. OxLDL also induces apoptosis of smooth muscle cells. Furthermore, oxLDL impairs nitric oxide production in endothelial cells. This leads to atherosclerotic plaque destabilization and rupture.

Apart from the scavenger receptors, oxLDL also binds to lectin-like oxidized low density lipoprotein receptor 1 (LOX-1). Later, the same receptor was shown on the surface of smooth muscle cells and macrophages.

The oxLDL molecule acts as an antigen leading to the production of anti oxLDL antibodies. LDL oxidation can affect different parts of its molecule,

which is the reason why different anti-oxLDL antibodies can be produced. IgM anti-oxLDL antibodies have been shown to reduce the risk of severe coronary artery disease. In the case of IgG anti-oxLDL class, this compound is more complex and needs additional investigation.

Selectins

Selectins are a group of cell-surface glycoproteins involved in the rolling and anchoring of leukocytes on the vascular wall. For instance, L-selectin is expressed on all granulocytes and monocytes and on most lymphocytes. It has been associated to larger plaque size estimated by ultrasound imaging in patients with carotid atherosclerotic plaques. Also, the important function of P-selectin in both leukocyte recruitment and atherosclerosis progression has been confirmed in various animal models and several authors have confirmed that a deficiency of this adhesion molecule has a protective result against atherosclerosis and an increase level is associated with progression of atherosclerosis, coronary artery disease, and atrial fibrillation.

MicroRNAs (miRNAs or miRs)

MicroRNAs are short, non-coding segments of RNA containing 18-26 nucleotides that function to silence mRNAs and thus prevent the translation of messenger RNAs (mRNAs) to proteins. They relocate from one cell to another in a process of intercellular communication to silence specific mRNAs in the target cell. miRNAs distribute in the body in membrane-derived vesicles, such as micro vesicles, exosomes, and apoptotic bodies, as well as bound to RNA-binding proteins, or by HDL cholesterol. The function of miRNAs has been recently proposed as next generation biomarkers due to their integral role in mediating cellular and molecular roles. miRNAs are involved in the pathogenesis of many diseases. Selected miRNAs implicated in the development of the atherosclerotic formation/rupture are shown in the Table 2-2.

Table 2-2. miRNAs involved in atherosclerotic process - most important examples.

miRNAs	Function
Plaque inflammation	
miRNA-126	Inhibition of VCAM-1
miRNA-155, -222, -424, -503, -9, -17, -20a, -106a	Regulation of monocyte differentiation into macrophages within the plaque
miRNA-147, -155, and -342-5p	Stimulation of plaque macrophages in M1 phenotype; Up-regulation of TNF-α and IL-6 cytokines
miRNA-125a, -146a, -33, and -155	Inhibition of lipid agglomeration
miRNA-15a, -16 s	Modulation of macrophage apoptosis
miRNA-21, miRNA-34a	Synthesis of MMP-9; VSMC proliferation
miRNA-210	Linked to intraplaque angiogenesis and, possibly, to the formation of unstable plaques; regulates endothelial apoptosis
miRNA-146a	Formation of the T helper type-1 mononuclear phenotype
miRNA-29	Inhibition of elastin expression
miRNA-221/222	Stimulation of cell proliferation or apoptosis
miRNA-365	Stimulation of endothelial cells apoptosis
miRNA-100, -127, -145, -133a, -133b	High-level expression in symptomatic carotid plaque
Endothelial shear stress	
miRNA-143 and 145	Development of VSMC transfer into the atheroprotective contractile phenotype
miRNA-126-5p	Limitation of endothelial cell proliferation at sites of low endothelial shear stress
miRNA-92a	Related with low endothelial shear stress, expansion of inflammation
Microcalcification	
miRNA-29a and miRNA-29b	Suppression of the disintegrin and metalloproteinase expression.
miR-125b	Differentiation of VSMC into an osteoblast-like phenotype

The most important advantage of employing miRNAs as biomarkers is the opportunity for assessment of selected miRNAs by using the standard technology for the detection and/or comparison of RNA concentrations (the quantitative reverse transcriptase-polymerase chain reaction method), with great sensitivity and specificity. However, prospective large-scale human studies are required to authenticate the real potential of circulating miRNAs and changes in miRNA expression; hence, circulating miRNAs can serve as independent biomarkers of atherosclerotic diseases, and, moreover, whether other more readily accessible body fluids, such as urine or saliva, may be suitable for diagnosis.

Placental growth factor (PIGF)

PIGF is a growth factor that belongs to the family of endothelial growth factor. It plays an important role in the pathogenesis of atherosclerosis by stimulating angiogenesis and increasing the migration of monocytes and macrophages into the vascular endothelium, which subsequently produces inflammatory and vascular mediators, resulting in an increased risk of plaque rupture. It has been shown that overweight children and with the metabolic syndrome have higher PIGF levels in the blood compared to healthy children. In addition, a positive correlation was found between PIGF and troponin plasmatic concentrations.

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

PAPP-A, a metalloproteinase produced by the placenta, can enhance local insulin-like growth factor (IGF) bioavailability through proteolytic cleavage of three IGF binding proteins events. This enzyme exerts a proatherogenic effect by altering a variety of pathological processes implicated in atherosclerosis, including lipid accumulation, vascular inflammation, endothelial dysfunction, vascular smooth muscle cell proliferation and migration, plaque stability, and thrombus formation. In patients with coronary atherosclerosis disease, increased PAPP-A levels are significantly associated with a higher risk of CVDs.

MPO, MMP-9 and CRP

Biomarkers of atherosclerotic plaque destabilization similarly include MPO, MMP-9 and CRP described above.

2.8.3. Biomarkers of shear stress in the vascular endothelium

A variety of miRNAs are included as shear stress biomarkers. For instance, and as showed in Table 3.2, microRNA-143 and microRNA-145 switch the phenotype of VSMC to contractile ones. microRNA-126-5p restricts the proliferation of vascular endothelial cells, while microRNA-92a improves the development of inflammatory processes in the vascular wall.

2.8.4. Biomarkers of blood vessel microcalcification

microRNAs

Vascular calcification is a prominent aspect of atherosclerosis, and some miRNAs are involved. microRNA-29a and microRNA-29b inhibited calcification of VSMC by suppressing the expression of a disintegrin and metalloproteinase with thrombospondin motifs 7. Moreover, miR-125b downregulation can promote calcification of vascular smooth muscle cells by targeting Ets1, a transcription factor protein. Furthermore, VSMC trans-differentiation into osteoblast-like cells can be promoted by the inhibition of endogenous miR-125b with the osteoblast transcription factor SP7, as its target, which can regulate osteoblast differentiation.

Osteopontin (OPN) and osteoprotegerin (OPG)

A growing number of stimulatory and inhibitory molecules imply that vascular calcification is an actively controlled process. Among these molecules OPN, an acidic phosphoprotein, and OPG, a member of the TNF-α receptor super family, have been proved to inhibit mineral deposition as well as osteoclast genesis and they are constitutively expressed by an extensive range of cell types in the vasculature

These bone-matrix proteins, which attenuate vascular microcalcification, are biomarkers of atherosclerotic plaque composition and CVD prognosis. Data derived from clinical investigations support the notion that increased serum levels of this markers are positively associated with acute cardiovascular events, coronary disease severity and poor long-term cardiovascular results. Circulating OPN-OPG amounts were higher in patients bearing carotid stenosis with unstable atherosclerotic and in symptomatic *versus* asymptomatic patients, who presented superior calcification.

2.8.5. Biomarkers of thrombocyte activation

Lipoprotein-associated phospholipase A2 (Lp-PLA2) and secretory phospholipase A2 (sPLA2)

Lp-PLA2 is a proinflammatory protein produced by monocytes, lymphocytes, and mast cells; 80% is bound to LDL cholesterol, and 20% to HDL cholesterol. Numerous studies have demonstrated that Lp-PLA2 plays a causal role in atherosclerosis and inhibiting Lp-PLA2 improves vascular inflammation and decelerates the progression of atherosclerosis. Additionally, several meta-analyses and epidemiological studies and have also reliably proven that increased plasma levels of Lp-PLA2 are associated with an increased risk of cardiovascular events. sPLA2 is the phospholipase A2 isozyme and hydrolyses the sn-2 ester bond in glyceroacyl phospholipids of lipoproteins and cell membranes, producing non-esterified fatty acids and bioactive lysophospholipids implicated in acute and chronic inflammatory processes. Expression of sPLA2 is up-regulated in response to cytokines such as interferon-γ, TNF-α, IL-1β, and oxLDL. The relationship between sPLA2 concentration and CVD prospect was properly demonstrated. For example, in coronary artery disease patients, an increase in circulating sPLA2 levels is a significant risk factor of clinical coronary events during follow-up.

sCD40L

The biomarkers of thrombocyte activation also include the previously described sCD40L.

2.8.6. Biomarkers of neurohormonal activation

Copeptin

Copeptin is secreted from the posterior pituitary gland into the circulation in stoichiometric amounts along with vasopressin. Both neuropeptides are primarily released in response to hemodynamic or osmotic changes. In contrast with vasopressin, copeptin exhibits higher plasma and serum stability, allowing its use in laboratory diagnostics. After release, it remains stable for several days and copeptin levels increase rapidly in conditions, such as CVD, stroke, sepsis, and shock. Some researchers have been demonstrated that the increased concentration of copeptin in blood positively correlates with the risk of developing coronary heart disease and the risk of death due to CVD.

Midregional pro-adrenomedullin (MR-proADM)

MR-proADM is a stable and surrogate measure for mature adrenomedullin (ADM), and provides useful information, particularly in the short-term. ADM is a peptide hormone produced by the adrenal medulla, heart, and vascular endothelial cells that acts as a vasodilator and plays important roles in the microcirculation and in endothelial dysfunction. ADM has some cardiovascular actions, including those promoting vasorelaxation, natriuresis and increasing cardiac output. A potential role of ADM in calcification processes in the heart and aorta has also been shown in animal and cell culture studies. Immunoluminometric assays for the measurement of the MR-proADM precursor fragment in human plasma have been reported, thus making it a promising biomarker of the risk of developing coronary heart disease and heart failure. Additionally, MR-proADM may be a prognostic biomarker after ST-elevation myocardial infarction.

2.9. Treatment of atherosclerosis

Dyslipidaemia, and particularly hypercholesterolaemia, is considered the main cause of atherosclerosis, with LDL-c, TG, and HDL-c as strong independent predictors of atherosclerotic disease after the analysis of the data from the Framingham study. The high level of HDL-c is considered a protective parameter, while low HDL-c has been shown to be a strong independent predictor of premature atherosclerosis.

Lp(a) is a specialised form of LDL, consisting of an LDL-like particle and the specific ApoA. Its elevation in plasma is an additional independent risk marker and can be related to the pathophysiology of atherosclerotic vascular disease and aortic stenosis.

The control of dyslipidaemia is the cornerstone of prevention and treatment of atherosclerosis, and can be achieved by lifestyle modifications, eventually with the use of specific lipid lowering drugs.

Non-pharmacological measures

Measures such as dietary changes, weight control, stimulation of physical activity and smoking and alcohol eviction, preferably to be implemented since infancy, are effective for the prevention and control of dyslipidaemia.

Pharmacological measures

Medication to adequately control lipoprotein levels needs to be initiated when the plasma lipid values are altered, after a variable period of lifestyle modifications and depending on the global cardiovascular risk of the patient. In secondary prevention, medical therapy is almost invariably needed in addition to lifestyle optimisation.

Statins (3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors) induce an increased expression of LDL receptors (LDL-r) on the surface of the hepatocytes, decreasing plasma concentration of LDL and other Apo B-containing lipoproteins, including TG-rich particles (although benefits of lowering elevated TG levels are modest for reducing cardiovascular risk). Statins can also elevate HDL-C levels between 5-10%.

The most used cholesterol absorption inhibitor is ezetimibe. It is usually used in combination with statins, or in monotherapy when statins are not tolerated. In monotherapy, ezetimibe can reduce LDL by 15-22% and when combined with a statin it induced an incremental reduction in LDL levels of 15-20%.

Bile acid sequestrants (cholestyramine, colestipol) can produce a reduction in LDL of 18-25%, but their use is limited by gastrointestinal adverse effects and major drug interactions with other frequently prescribed medications.

Proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK-9 inhibitors), evolocumab and alirocumab, two monoclonal antibodies that inhibit PCSK-9, offer the prospect of achieving even lower LDL levels than statins in combination with ezetimibe. PCSK-9 stimulates the absorption and degradation of the receptors for LDL in the hepatocytes. Through inhibition of PCSK-9, the degradation of LDL-r is prevented, improving the absorption of LDL particles and lowering LDL plasma concentrations. In clinical trials, the PCSK-9 therapy lowered LDL by 50% and demonstrated a significant percentage atheroma volume decrease. The PCSK-9 therapy is suitable in a wide range of patients provided that they express the LDL receptor, including those with heterozygous and homozygous familial hypercholesterolaemia.

Fibrates are agonists of peroxisome proliferator-activated receptor-α (PPAR-α); they are effective in lowering fasting TG, post-prandial TGs and TG-rich lipoprotein remnant particles, lowering TG levels up to more than 50%. Fibrates increase HDL-c in a similar proportion with statins, namely between 5% and 15%.

n-3 fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) can lower TG up to 45%, possibly through interaction with PPAR.

Cholesteryl ester transfer protein (CETP) inhibitors, a new class of pharmacologically active molecules, seems to be effective in increasing HDL-c levels, with an effect of ≥ 100% increase in HDL-c and frequently a reduction of LDL-c levels as well.

Conclusion

Atherosclerosis is a pathologic process by the accumulation of lipids in the plasma and its deposition on the cell wall triggers a cascade of events leading to vascular wall thickening, luminal stenosis, calcification, and thrombosis. This process leads to coronary artery disease and myocardial infarction, carotid artery stenosis and stroke, abdominal aortic aneurysms, peripheral vascular disease with lower-extremity claudication and, in some cases, death.

ROS are significant contributors to atherosclerosis, causing oxidative modification of LDL. ROS can also promote endothelial dysfunction and a vascular inflammatory response. Excessive oxLDL and the upregulation of LOX-1 expression can lead to defective autophagic mechanisms and can trigger inflammatory and oxidative stress responses. Proinflammatory factors, such as IL-1, IL-6, TNF-α and CRP generate the endothelial proinflammatory phenotype and endothelial cells dysfunction. Moreover, there is a prothrombotic effect of vascular-derived and platelet-derived ROS and an outcome over the perivascular adipose tissue, affecting its anticontractile function, predisposing to vascular damage.

Classical biomarkers of atherosclerosis, such as LDL, HDL, and TG are suitable for the average patient, but may not be effective in patients with moderate or atypical cardiovascular risk. Other biomarkers are under study, comprising biomarkers of the inflammatory process, destabilization of atherosclerotic plaque, shear stress, microcalcifications, thrombocyte activation and neurohormonal activation, and the promising candidates, the microRNAs.

Treatment of atherosclerotic is based on lowering LDL by statin therapy. In high-risk patients with statin intolerance or who do not obtain the target level of LDL, association with ezetimibe, or other drugs should be considered. PCSK-9 inhibitors are used in hypercholesterolaemia patients that do not respond satisfactorily to other therapies.

Bibliography

Abdellatif, M., Sedej, S., Carmona-Gutierrez, D., Madeo, F., & Kroemer, G. (2018). Autophagy in cardiovascular aging. *Circulation Research*, 123 (7), 803-824.

Abdolmaleki, F., Gheibi-Hayat, S. M., Bianconi, V., Johnston, T. P., & Sahebkar, A. (2019). Atherosclerosis and immunity: A perspective. *Trends in Cardiovascular Medicine*, 6, 363-371.

Alipov, V. I., Sukhorukov, V. N., Karagodin, V. P., Grechko, A. V., & Orekhov, A. N. (2017). Chemical composition of circulating native and desialylated low density lipoprotein: what is the difference? *Vessel Plus*, 1, 107-115.

Ambrosino, P., Lupoli, R., Di Minno, A., Tasso, M., Peluso, R., & Di Minno, M. N. (2015). Subclinical atherosclerosis in patients with rheumatoid arthritis. A meta-analysis of literature studies. *Thrombosis and Haemostasis*, 113 (5), 916-930.

Bakhtiyar, S., Barari, A., & Abdi, A. (2021). The effect of combination training program on the expression of placental growth factor and vascular endothelial growth factor receptor 1 indicators among coronary artery bypass graft patients. *Journal of Nursing and Midwifery Sciences*, 8, 20-26.

Benjamin, E. J., Muntner, P., Alonso, A., Bittencourt, M. S., Callaway, C. W., Carson, A. P., Chamberlain, A. M., Chang, A. R., Cheng, S., Das, S. R., Delling, F. N., Djousse, L., Elkind, V., Ferguson, J. F., Fornage, M., Jordan, L. C., Khan, S. S., Kissela, B. M., Knutson, K. L., Kwan, T. W., Lackland, D. T., Lewis, T. T., Lichtman, J. H., Longenecker, C. T., Loop, M. S., Lutsey, P. L., Martin, S. S., Matsushita, K., Moran, A. E., Mussolino, M. E., O'Flaherty, M., Pandey, A., Perak, A. M., Rosamond, W. D., Roth, G. A., Sampson, A., Satou, G. M., Schroeder, E. B., Shah, S. H., Spartano, N. L., Stokes, A., Tirschwell, D. L., Tsao, C. W., Turakhia, M. P., VanWagner, L. B., Wilkins, J. T., Wong, S. S., Virani, S. S. & American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee (2019). Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. *Circulation Research*, 139 (10), e56-e528.

Bergheanu, S. C., Bodde, M. C., & Jukema, J. W. (2017). Pathophysiology and treatment of atherosclerosis Current view and future perspective on lipoprotein modification treatment. *Netherlands Heart Journal*, 25, 231-242.

Bonaca, M., Hamburg, N., & Creager, M. (2021). Contemporary Medical Management of Peripheral Artery Disease. *Circulation Research,* 128 (12), 1868-1884.

Bosmans, L. A., Bosch, L., Kusters, P. J. Lutgens, E., & Seijkens, T. P. (2021). The CD40-CD40L dyad as immunotherapeutic target in cardiovascular disease. *Journal of Cardiovascular Translational Research*, 14, 13-22.

Bravo-San Pedro, J. M., Kroemer, G., & Galluzzi, L. (2017). Autophagy and mitophagy in cardiovascular disease. *Circulation Research*, 120 (11), 1812-1824.

Bruen, R., Fitzsimons, S., & Belton, O. (2019). MiR-155 in the resolution of atherosclerosis. *Frontiers in Pharmacology*, 10, 1-14.

Buja, L. M. (2020). Innovators in atherosclerosis research: A historical review. *International Journal of Cardiology*, 307, 8-14.

Burgoyne, J. R., Mongue-Din, H., Eaton, P., & Shah, A M. (2012). Redox signaling in cardiac physiology and pathology. *Circulation Research*, 111 (8), 1091-1106.

Cai, H., & Harrison, D. G. (2000). Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circulation Research*, 87, 840-844.

Cao, H., Jia, Q., Yan, L., Chen, C., Xing, S., & Shen, D. (2019). Quercetin suppresses the progression of atherosclerosis by regulating MST1-mediated autophagy in ox-LDL-induced RAW264.7 macrophage foam cells. *International Journal of Molecular Sciences*, 20 (23), 6093.

Carresi, C., Mollace, R., Macrì, R., Scicchitano, M., Bosco, F., Scarano, F., Coppoletta, A. R., Guarnieri, L,, Ruga, S., Zito, M. C., Nucera, S., Gliozzi, M., Musolino, V., Maiuolo, J., Palma, E., & Mollace, V. (2021). Oxidative stress triggers defective autophagy in endothelial cells: Role in atherothrombosis development. *Antioxidants*, 10, 387.

Clemmons, D. R. (2018). Role of IGF-binding proteins in regulating IGF responses to changes in metabolism. *Journal of Molecular Endocrinology*, 61, T139-T169.

Choudhury, M., & Devagourou, V. (2017). Biomarkers of Atherosclerosis: Is the evaluation essential preoperatively? *Atherosclerosis*, 2 (3), 1000116.

Churov, A., Summerhill, V., Grechko, A., Orekhova, V., & Orekhov, A. (2019). MicroRNAs as potential biomarkers in Atherosclerosis. *International Journal of Molecular Sciences*, 20 (22), 5547.

Dégano, I. R., Camps-Vilaró, A., Subirana, I., García-Mateo, N., Cidad, P., MuñozAguayo, D., Puigdecanet, E., Nonell, L., Vila, J., Crepaldi, F. M., de Gonzalo-Calvo, D., Llorente-Cortés, V., Pérez-García, M. T., Elosua, R., Fit,ó M., & Marrugat, J. (2020). Association of circulating microRNAs with coronary artery disease and usefulness for reclassification of healthy individuals: the REGICOR study. *Journal of Clinical Medicine*, 9 (5), 1402.

Dong, Y., Chen, H., Gao, J., Liu, Y., Li, J., & Wang, J. (2019). Molecular machinery and interplay of apoptosis and autophagy in coronary heart disease. *Journal of Molecular and Cellular Cardiology*, 136, 27-41.

Dong, Y., Xu, W., Liu, C., Liu, P., Li, P., & Wang, K. (2019). Reactive oxygen species related noncoding RNAs as regulators of cardiovascular diseases. *International Journal of Biological Sciences*, 15 (3), 680-687.

Dowsett, L., Higgins, E., Alanazi, S., Alshuwayer, N. A., Leiper, F. C., & Leiper, J. (2020). ADMA: A Key player in the relationship between vascular dysfunction and inflammation in atherosclerosis. *Journal of Clinical Medicine*, 9 (9), 3026.

Dubois-Deruy, E., Peugnet, V., Turkieh, A., & Pinet, F. (2020). Oxidative stress in cardiovascular diseases. *Antioxidants (Basel)*, 9 (9), 864.

Eder, L., & Gladman, D. D. (2015). Atherosclerosis in psoriatic disease: latest evidence and clinical implications. *Therapeutic Advances in Musculoskeletal Disease*, 7 (5), 187-195.

El Dayem, S. M. A., Battah, A. A., El Bohy, A. M., Yousef, R. N., & Talaat, A. (2020). Copeptin as a biomarker of atherosclerosis in type 1 diabetic patients. *International Journal of Medical Sciences*, 7 (23), 3975-3978.

Falkentoft, A. C., Rørth, R., Iversen, K., Høfsten, D. E., Kelbæk, H., Holmvang, L., Frydland, M., Schoos, M. M., Helqvist, S., Axelsson, A., Clemmensen. P., Jørgensen, E., Saunamäki, K., Tilsted, H. H., Pedersen, F., Torp-Pedersen, C., Kofoed, K. F., Goetze, J. P., Engstrøm, T., & Køber, L. (2018). MR-proADM as a prognostic marker in patients with ST-segment-elevation myocardial infarction-DANAMI-3 (a Danish Study of Optima Acute Treatment of Patients With STEMI) Substudy. *Journal of American Heart Association*, 7 (11), e008123.

Fisk, M., McEniery, C. M., Gale, N., Mäki-Petäjä, K., Forman, J. R., Munnery, M., Woodcock-Smith, J., Cheriyan, J., Mohan, D., Fuld, J., Tal-Singer, R., Polkey, M. I., Cockcroft, J. R., & Wilkinson, I. B. (2018). Surrogate markers of cardiovascular risk and chronic obstructive pulmonary disease: A large case-controlled study. *Hypertension*, 71 (3), 499-506.

Förstermann, U., Xia, N., & Li, H. (2017). Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. *Circulation Journal*, 120 (4), 713-735.

Full, L. E., Ruisanchez, C., & Monaco, C. (2009). The inextricable link between atherosclerosis and prototypical inflammatory diseases rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Research Therapy*, 11 (2), 217.

Ge, P., Chen, Z., Pan, R., Ding, X., Liu, J., Jia, Q., Liu, Z., He, S., An, F., Li, L., Li, Z., Gu, Y., Zhu, T., Li, C., Wang, L., Ma, W., Yang, Z., & Jia, E. (2016). Synergistic effect of lipoprotein-associated phospholipase A2 with classical risk factors on Coronary Heart Disease: A multi-ethnic study in China. *Cellular Physiology and Biochemistry*, 40, 953-968.

Ghantous, C. M., Kamareddine, L., Farhat, R., Zouein, F. A., Mondello, S., Kobeissy, F., & Zeidan, A. (2020). Advances in cardiovascular biomarker discovery. *Biomedicines*, 8 (12), 552.

Golembiewska, E., Qureshi, A.R., Dai, L., Lindholm, B., Heimbürger, O., Söderberg, M, Brismar, T. B., Ripsweden, J., Barany, P., Johnson, R., & Stenvinkel, P. (2020). Copeptin is independently associated with vascular calcification in chronic kidney disease stage 5. *BMC Nephrology*, 21 (43), 1-8.

Gray, S. P., Shah, A. M., & Smyrnias, I. (2019). NADPH oxidase 4 and its role in the cardiovascular system. *Vascular Biology*, 1 (1), H59-H66.

Grootaert, M. O. J., Moulis, M., Roth, L., Martinet, W., Vindis, C., Bennett, M. R., & De Meyer, G. R. Y. (2018). Vascular smooth muscle cell death, autophagy and senescence in atherosclerosis. *Cardiovascular Research*, 114 (4), 622-634.

Grootaert, M. O. J., Roth, L., Schrijvers, D. M., De Meyer, G. R. Y., & Martinet, W. (2018). Defective Autophagy in Atherosclerosis: To die or to senesce? *Oxidative Medicine and Cellular Longevity*, 7687083.

Hansen, S. S., Aasum, E., & Hafstad, A. D. (2018). The role of NADPH oxidases in diabetic cardiomyopathy. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 1864 (5 Pt B), 1908-1913.

Hartley, A., Haskard, D., & Khamis, R. (2019). Oxidized LDL and anti-oxidized LDL antibodies in atherosclerosis - Novel insights and future directions in diagnosis and therapy. *Trends in Cardiovascular Medicine*, 29 (1), 22-26.

Hasni, S. A., & Kaplan, M. J. (2021). Chapter 36 - Mechanisms of Vascular Damage. in Systemic Lupus Erythematosus In: *Systemic Lupus Erythematosus - Basic, Applied and Clinical Aspects,* 325-331, Academic Press.

Held, C., White, H. D., Stewart, R. H., Budaj, A., Cannon, C. P., Hochman, J. S., Koenig, W., Siegbahn, A., Steg, P. G., Soffer, J., Weaver, W. D., Östlund, O., & Wallentin, L. (2017). Inflammatory biomarkers interleukin-6 and C-reactive protein and outcomes in stable coronary heart disease: experiences from the STABILITY (stabilization of atherosclerotic plaque by initiation of darapladib therapy) trial. *Journal of the American Heart Association*, 6, e005077.

Heriansyah, T., Chomsy, I. N., Kumboyono, K., Pratiwi, P. A., & Wihastuti, T. A. (2020). Expression of hypoxia-inducible factor-1α (HIF1A) and Lp-PLA2 in low, intermediate, and high cardiovascular disease risk population. *Vascular Health and Risk Management*, 6, 507-513.

Ivanova, E. A., Myasoedova, V. A., Melnichenko, A. A., Grechko, A. V., & Orekhov, A. N. (2017). Small dense low-density lipoprotein as biomarker for atherosclerotic diseases. *Oxidative Medicine and Cellular Longevity*, 2017, 1273042.

Jang, A.Y., Han, S. H., Sohn, I. S., Oh, P. C., & Koh, K. K. (2020). Lipoprotein(a) and Cardiovascular Diseases - Revisited. *Circulation Journal*, 84 (6), 867-874.

Kattoor, A. J., Goel, A., & Mehta, J. L. (2019). LOX-1: regulation, signaling and its role in atherosclerosis. *Antioxidants*, 8 (7), 1-15.

Kuroda, J., Ago, T., Matsushima, S., Zhai, P., Schneider, M. D., & Sadoshima, J. (2010). NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart. *Proceedings of the National Academy of Sciences,* 107, 15565-15570.

Laffont, B., & Rayner, K. J. (2017). MicroRNAs in the pathobiology and therapy of atherosclerosis. *Canadian Journal of Cardiology*, 33 (3), 313-324.

Lara-Guzmán, O. J., Gil-Izquierdo, Á., Medina, S., Osorio, E., ÁlvarezQuintero, R., Zuluaga, N., Oger, C., Galano, J. M., Durand, T., & Muñoz-Durango, K. O. (2018). Oxidized LDL triggers changes in oxidative stress and inflammatory biomarkers in human macrophages. *Redox Biology*, 15, 1-11.

Likozar, A., Zavrtanik, M. & Šebeštjen, M. (2020). Lipoprotein(a) in atherosclerosis: from pathophysiology to clinical relevance and treatment options, *Annals of Medicine*, 52 (5), 162-177.

Lind, L., Gigante, B., Borné, Y., Feldreich, T., Leppert, J., Hedberg, P., Östgren, C. J., Nyström, F. H., Sundström, J., Ärnlöv, J., Baldassarre, D., Tremoli, E., Veglia, F., Hamsten, A., O'Donnell, C. J., Franceschini, N., Orho-Melander, M., Nilsson, J., Melander, O., Engström, G., & Mälarstig, A. (2021). Plasma protein profile of Carotid Artery Atherosclerosis and atherosclerotic outcomes: Meta-analyses and Mendelian randomization analyses. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 41 (5), 1777-1788.

Lubitz, M., & Mukerji, V. (2018). Lipoprotein(a) and Atherosclerosis: A case report and literature review. *International Archives of Cardiovascular Diseases*, 2 (1), 1-5.

Madjene, C., Boutigny, A., Bouton, M-C., Arocas, V., & Richard, B. (2021). Protease nexin-1 in the cardiovascular system: Wherefore art thou?.

Cardiovascular Medicine, 8, 652852.
Marcovina, S. M., & Albers, J. J. (2016). Lipoprotein (a) measurements for clinical application. *Journal of Lipid Research*, 57 (4), 526-537.
Marsche, G., Holzer, M., & Curcic, S. (2017). Secretory phospholipase A2 modified high-density lipoprotein potently suppresses platelet activation. *Atherosclerosis*, 263, e94.
Martinez, E., Martorell, J., & Riambau, V. (2020). Review of serum biomarkers in carotid atherosclerosis. *Journal of Vascular Surgery*, 71 (1), 329-341.
Masuyama, A., Mita, T., Azuma, K., Osonoi, Y., Nakajima, K., Goto, H., Nishida, Y., Miyatsuka, T., Mitsumata, M., & Watada, H. (2018). Defective autophagy in vascular smooth muscle cells enhances atherosclerotic plaque instability. *Biochemical and Biophysical Research Communications*, 505 (4), 1141-1147.
Matsui, M., Uemura, S., Takeda, Y., Samejima, K., Matsumoto, T., Hasegawa, A., Tsushima, H., Hoshino, E., Ueda, T., Morimoto, K., Okamoto, K., Okada, S., Onoue, K., Okayama, S., Kawata, H., Kawakami, R., Maruyama, N., Akai, Y., Iwano, M., Shiiki, H., Saito, H., & NARA-CKD Investigators (2015). Placental growth factor as a predictor of cardiovascular events in patients with CKD from the NARA-CKD study. *Journal of the American Society of Nephrology*, 26 (11), 2871-2881.
Mialet-Perez, J., & Vindis, C. (2017). Autophagy in health and disease: focus on the cardiovascular system. *Essays Biochemistry*, 61 (6), 721-732.
Monget, P., & Oxvig, C. (2016). PAPP-A and the IGF system. *Annales d'Endocrinologie*, 77, 90-96.
Mongue-Din, H., Patel, A. S., Looi, Y. H., Grieve, D. J., Anilkumar, N., Sirker, A., Dong, X., Brewer, A. C., Zhang, M., Smith, A., & Shah, A. M. (2017). NADPH Oxidase-4 Driven Cardiac Macrophage Polarization Protects Against Myocardial Infarction-Induced Remodeling. *JACC: Basic to Translational Science*, 2 (6), 688-698.
Murakami, M., Sato, H., Miki, Y., Yamamoto, K., & Taketomi, Y. (2015). A new era of secreted phospholipase A. *Journal of Lipid Research*, 56 (7), 1248-1261.
Nilsson, J., & Hansson, G. K. (2020). Vaccination strategies and immune modulation of atherosclerosis. *Circulation Research*, 126 (9), 1281-1296.
Obermayer, G., Afonyushkin, T., & Binder, C. J. (2018). Oxidized low-density lipoprotein in inflammation-driven thrombosis. *Journal of Thrombosis and Haemostasis*, 16 (3), 418-428.
Orekhov, A. N. (2018). LDL and foam cell formation as the basis of atherogenesis. *Current Opinion in Lipidology*, 29 (4), 279-284.
Orekhov, A. N., & Sobenin, I. A. (2019). Modified and dysfunctional lipoproteins in atherosclerosis: effectors or biomarkers? *Current Medicinal Chemistry*, 26 (9), 1512-1524.
Orsó, E., & Schmitz, G. (2017). Lipoprotein(a) and its role in inflammation, atherosclerosis and malignancies. *Clinical Research in Cardiology Supplements*,12 (1), 31-37.
Ozturk, K., Guler, A. K., Cakir, M., Ozen, A., Demirci, H., Turker, T., Demirbas, S., Uygun, A., Gulsen, M., Bagci, S. (2015). Pulse wave velocity, intima media thickness, and flow-mediated dilatation in patients with normotensive

normoglycemic inflammatory bowel disease. *Inflammatory Bowel Diseases*, 21 (6), 1314-1320.

Patanè, S. (2018). Growth differentiation factor-15 in chronic heart failure. *JACC: Heart Failure,* 6 (2), 537-547.

Pereira-da-Silva, T., Cruz, M.C., Carrusca, C., Ferreira, R.C., Napoleão, P., & Carmo, M. M. (2018). Circulating microRNA profiles in different arterial territories of stable atherosclerotic disease: A systematic review. *American Journal of Cardiovascular Disease,* 8, 1-13.

Pereira-da-Silva, T., Ferreira, V., Castelo, A., Caldeira, D., Napoleão, P., Pinheiro, T., Ferreira, R. C., & Carmo, M. M. (2021). Soluble CD40 ligand expression in stable atherosclerosis: A systematic review and meta-analysis. *Atherosclerosis,* 319, 6-100.

Piepoli, M. F., Hoes, A, W., Agewall, S., Albus, C., Brotons, C., Catapano, A. L., Cooney, M.-T., Corrà, U., Cosyns, B., Deaton, C., Graham, I., Hall, M. S., Hobbs, F. D. R., Lochen, M.-L., Lollgen, H., Marques-Vidal, P., Perk, J., Prescott, E., Redon, J., Richter, D. J., Sattar, N., Smulders, Y., Tiberi, M., van der Worp, H. B., van Dis, I., Verschuren, W. M. M., Binno, S. (2016). 2016 European Guidelines on cardiovascular disease prevention in clinical practice. *Kardiologia Polska*, 74 (9), 821-936.

Poznyak, V., Nikiforov, G., Markin, M., Kashirskikh, A., Myasoedova, A., Gerasimova, V., & Orekhov N. (2021). Overview of OxLDL and Its Impact on Cardiovascular Health: Focus on Atherosclerosis. *Frontiers in Pharmacology*, 11, 2248-2259.

Principi, M., Mastrolonardo, M., Scicchitano, P., Gesualdo, M., Sassara, M., Guida, P., Bucci, A., Zito, A., Caputo, P., Albano, F., Ierardi, E., Leo, A. D., Ciccone, M. M. (2013). Endothelial function and cardiovascular risk in active inflammatory bowel diseases. *Journal of Crohn's and Colitis*, 7 (10), e427-433.

Rafieian-Kopaei, M., Setorki, M., Doudi, M., Baradaran, A., & Nasri, H. (2014). Atherosclerosis: process, indicators, risk factors and new hopes. *International Journal of Preventive Medicine*, 5 (8), 927-946.

Raggi, P., Genest, J., Giles, J. T., Rayner, K. J., Dwivedi, G., Beanlands, R. S., & Gupta, M. (2018). Role of inflammation in the pathogenesis of atherosclerosis and therapeutic interventions. *Atherosclerosis*, 276, 98-108.

Reinstadler, S. J., Klug, G., Feistritzer, H. J., Metzler, B., & Mair, J. (2015). Copeptin testing in acute myocardial infarction: ready for routine use? *Disease Markers*, 2015, 614145.

Reiss, A. B., Siegart, M. N., & DeLeon, J. (2017). Interleukin-6 in atherosclerosis: atherogenic or atheroprotective? *Clinical Lipidology*, 12 (1), 14-23.

Reyes-Soffer, G. (2021). The impact of race and ethnicity on lipoprotein(a) levels and cardiovascular risk. *Current Opinion in Lipidology*, 32 (3), 163-166.

Roth, G.A., Mensah, G.A., Johnson, C.O., Addolorato, G., Ammirati, E., Baddour, L.M., Barengo, N.C., Beaton, A.Z., Benjamin, E.J., Benziger, C.P., Bonny, A., Brauer, M., Brodmann, M., Cahill, T.J., Carapetis, J., Catapano, A.L., Chugh, S.S., Cooper, L.T., Coresh, J., Criqui, M., DeCleene, N., Eagle, K.A., Emmons-Bell, S., Feigin, V.L., Fernández-Solà, J., Fowkes, G., Gakidou, E., Grundy, S.M., He, F.J., Howard, G., Hu, F., Inker, L., Karthikeyan, G., Kassebaum, N.,

Koroshetz, W., Lavie, C., Lloyd-Jones, D., Lu, H.S., Mirijello, A., Temesgen, A.M., Mokdad, A., Moran, A.E., Muntner, P., Narula, J., Neal, B., Ntsekhe, M., Moraes de Oliveira, G., Otto, C., Owolabi, M., Pratt, M., Rajagopalan, S., Reitsma, M., Ribeiro, A.L.P., Rigotti, N., Rodgers, A., Sable, C., Shakil, S., Sliwa-Hahnle, K., Stark, B., Sundström, J., Timpel, P., Tleyjeh, I.M., Valgimigli, M., Vos, T., Whelton, P.K., Yacoub, M., Zuhlke, L., Murray, C., & Fuster, V. (2020). Global burden of Cardiovascular Diseases and risk factors, 1990-2019: Update from the GBD 2019 study. GBD-NHLBI-JACC Global Burden of Cardiovascular Diseases Writing Group. *Journal of the American College of Cardiology*, 76 (25), 2982-3021.

Ruscitti, P., Cipriani, P., Liakouli, V., Iacono, D., Pantano, I., Margiotta, D. P. E., Navarini, L., Destro-Castaniti, G. M., Maruotti, N., Di Scala, G., Picciariello, L., Caso, F., Bongiovanni, S., Grembiale, R. D., Atzeni, F., Scarpa, R., Perosa, F., Emmi, G., Cantatore, F. P., Guggino, G., Afeltra, A., Ciccia, F., & Giacomelli, R. (2019). Subclinical and clinical atherosclerosis in rheumatoid arthritis: results from the 3-year, multicentre, prospective, observational GIRRCS (Gruppo Italiano di Ricerca in Reumatologia Clinica e Sperimentale) study. *Arthritis Research Therapy*, 21 (1), 204.

Sárközy, M., Kovács, Z.Z.A., Kovács, M.G., Gáspár, R., Szűcs, G., & Dux, L. (2018). Mechanisms and modulation of oxidative/nitrative stress in type 4 Cardio-Renal syndrome and renal sarcopenia. *Frontiers in Physiology*, 9, 1648.

Shao, D., Lian, Z., Di, Y., Zhang, L., Rajoka, M. R., Zhang, Y., Kong, J., Jiang, C., & Shi, J. (2018). Dietary compounds have potential in controlling atherosclerosis by modulating macrophage cholesterol metabolism and inflammation via miRNA. *NPJ Science of Food Journal*, 2, 13.

Shrivastava A, Singh H, Raizada A, & Singh, S. K. (2015). C-reactive protein, inflammation and coronary heart disease. *The Egyptian Heart Journal*, 67 (2), 89-97.

Shlyankevich, J., Mehta, N. N., Krueger, J. G., Strober, B., Gudjonsson, J. E., Qureshi, A. A., Tebbey, P. W., & Kimball, A. B. (2014). Accumulating evidence for the association and shared pathogenic mechanisms between psoriasis and cardiovascular-related comorbidities. *The American Journal of Medicine,* 127 (12), 1148-1153.

Sies, H., Jones, & D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature Reviews Molecular Cell Biology*, 21 (7), 363-383.

Soufi-Zomorrod, M., Hajifathali, A., Kouhkan, F., Mehdizadeh, M., Rad, S. H., & Soleimani, M. (2016). MicroRNAs modulating angiogenesis: miR-129-1 and miR-133 act as angio-miR in HUVECs. *Tumor Biology*, 37, 9527-9534.

Sun, C. Q., Zhong, C. Y., Sun, W. W., Xiao, H., Zhu, P., Lin, Y. Z., Zhang, C. L., Gao, H., & Song, Z. Y. (2016). Elevated type II secretory phospholipase A2 increases the risk of early atherosclerosis in patients with newly diagnosed metabolic syndrome. *Scientific reports*, 6, 34929.

Surma, S., Czober, T., Lepich, T., Sierka, O., & Bajor, G. (2020). Selected biomarkers of atherosclerosis: clinical aspects. *Acta Angiologica*, 26 (1), 28-39.

Szabó, M. Z., Szodoray, P., & Kiss, E. (2017). Dyslipidemia in systemic lupus erythematosus. *Immunologic Research*, 65 (2), 543-550.

Takac, I., Schröder, K., Zhang, L., Lardy, B., Anilkumar, N., Lambeth, J. D., Shah, A. M., Morel, F., & Brandes, R. P. (2011). The E-loop is involved in hydrogen peroxide formation by the NADPH oxidase Nox4. *Journal of Biological Chemistry*, 286 (15), 13304-13313.

Toutouzas, K., Benetos, G., Karanasos, A., Chatzizisis, Y. S., Giannopoulos, A. A., & Tousoulis, D. (2015). Vulnerable plaque imaging: updates on new pathobiological mechanisms. *European Heart Journal*, 36 (45), 3147-3154.

Ueshima, H., Kadowaki, T., Hisamatsu, T., Fujiyoshi, A., Miura, K., Ohkubo, T., Sekikawa, A., Kadota, A., Kadowaki, S., Nakamura, Y., Miyagawa, N., Okamura, T., Kita, Y., Takashima, N., Kashiwagi, A., Maegawa, H., Horie, M., Yamamoto, T., Kimura, T., & Kita, T. (2016). Lipoprotein-associated phospholipase A2 is related to risk of subclinical atherosclerosis but is not supported by Mendelian randomization analysis in a general Japanese population. *Atherosclerosis*, 246, 141-147.

Varona, J. F., Ortiz-Regalón, R., Sánchez-Vera, I., López-Melgar, B., García-Durango, C., Castellano-Vázquez, J. M., Solís, J., Fernández-Friera, L., & Vidal-Vanaclocha, F. (2019). Soluble ICAM 1 and VCAM 1 blood levels alert on subclinical atherosclerosis in non smokers with asymptomatic metabolic syndrome. *Archives of Medical Research*, 50 (2), 20-28.

Wainstein, M. V., Mossmann, M., Araujo, G. N., Gonçalves, S. C., Gravina, G. L., Sangalli, M., Veadrigo, F., Matte, R., Reich, R., Costa, F. G., Andrades, M., da Silva, A. .V., & Bertoluci, M. C. (2017). Elevated serum interleukin-6 is predictive of coronary artery disease in intermediate risk overweight patients referred for coronary angiography. *Diabetology & Metabolic Syndrome*, 9 (67), 1-7.

Wang, J., Tan, G. J., Han, L. N., Bai, Y. Y., He, M., & Liu, H. B. (2017). Novel biomarkers for cardiovascular risk prediction. *Journal of Geriatric Cardiology*, 14, 135-150.

Wang, L., Wang, J., Cretoiu, D., Li, G., & Xiao, J. (2020). Exercise-mediated regulation of autophagy in the cardiovascular system. *Journal of Sport and Health Science*, 9 (3), 203-210.

Wang, L. Y., Zhu, Y. N., Cui, J. J., Yin, K. Q., Liu, S. X., & Gao, Y. H. (2017). Subclinical atherosclerosis risk markers in patients with chronic obstructive pulmonary disease: A systematic review and meta-analysis. *Respiratory Medicine*, 123, 18-27.

Weber, C., & Noels, H. (2011). Atherosclerosis: current pathogenesis and therapeutic options. *Nature Medicine*, 17 (11), 1410-1422.

Wojciechowska, A., Braniewska, A., & Kozar-Kaminska, K. (2017). MicroRNA in cardiovascular biology and disease. *Advances in Clinical and Experimental Medicine*, 26, 868-874.

Wu, H., Du, Q., Dai, Q., Ge, J., & Cheng, X. J. (2018). Cysteine protease cathepsins in atherosclerotic cardiovascular diseases. *Atherosclerosis & Thrombosis Journal*, 25 (2), 111-123.

Wu, M. Y., Li, C. J., Hou, M. F., & Chu, P. Y. (2017). New insights into the role of inflammation in the pathogenesis of atherosclerosis. *International Journal of Molecular Sciences*, 18 (10), 2034.

Yadati, T., Houben, T., Bitorina, A., & Shiri-Sverdlov, R. (2020). The ins and outs of cathepsins: Physiological function and role in disease management. *Cells*, 9 (7), 1679.

Yilmaz, S. G., Isbir, S., Kunt, A. T., & Isbir, T. (2018). Circulating microRNAs as novel biomarkers for atherosclerosis. *in vivo*, 32 (3), 561-565.

Zhang, J., Du, R., Peng, K., & Wu, X. (2019). Serum lipoprotein (a) is associated with increased risk of stroke in Chinese adults: A prospective study. *Atherosclerosis*, 289, 8-13.

Zhang, M., Brewer, A. C., Schröder, K., Santos, C. X., Grieve, D. J., Wang, M., Anilkumar, N., Yu, B., Dong, X., Walker, S. J., Brandes, R. P., & Shah, A. M. (2010). NADPH oxidase-4 mediates protection against chronic load-induced stress in mouse hearts by enhancing angiogenesis. *Proceedings of the National Academy of Sciences*, 107 (42), 18121-18126.

Zhang, X., Luo, S., Wang, M., & Shi, G. P. (2020). Cysteinyl cathepsins in cardiovascular diseases. *Biochimica et Biophysica Acta - Proteins and Proteomics*, 1868 (4), 140360.

Zhang, Y., Murugesan, P., Huang, K., & Cai, H. (2020). NADPH oxidases and oxidase crosstalk in cardiovascular diseases: novel therapeutic targets. *Nature Reviews Cardiology,* 17 (3), 170-194.

Zhao, C. F., & Herrington, D. M. (2016). The function of cathepsins B, D, and X in atherosclerosis. A*merican Journal of Cardiovascular Disease*, 6 (4), 163-170.

Ziviello, F., Conte, S., Cimmino, G., Sasso, F. C., Trimarco, B. & Cirillo, P. (2017). Pregnancy-associated plasma protein-A and its role in cardiovascular disease. Biology, experimental/clinical evidences and potential therapeutic approaches. *Current Vascular Pharmacology*, 15, 197-206.