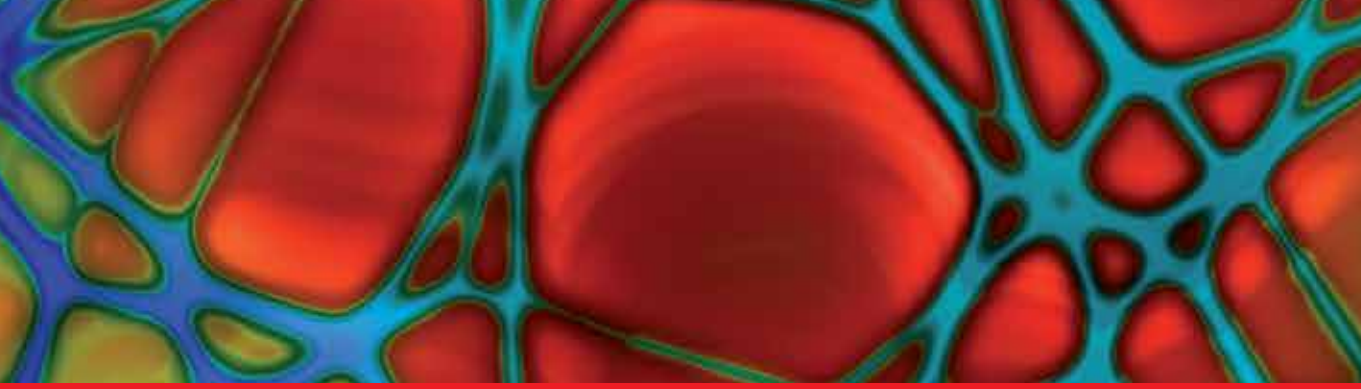

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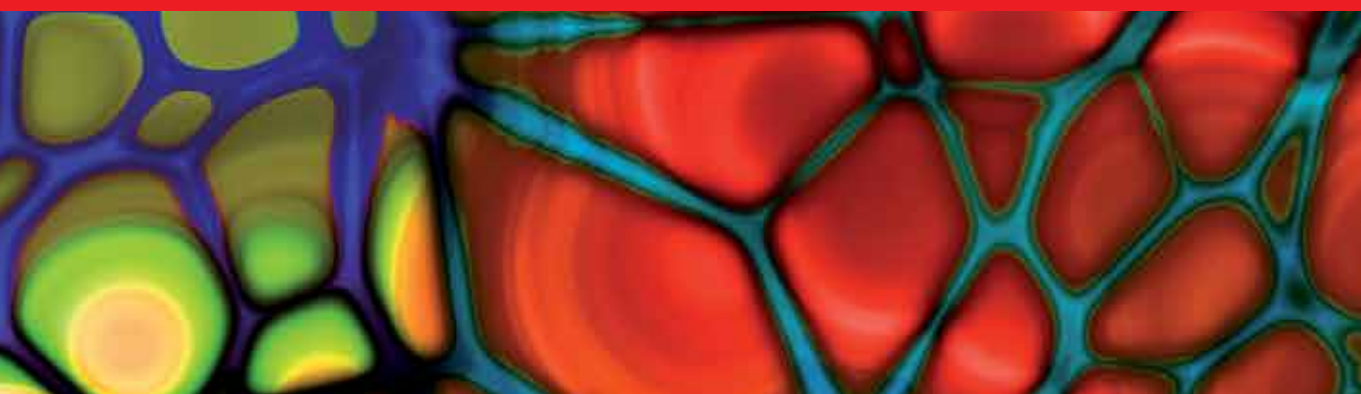
Atherosclerosis

Yesterday, Today and Tomorrow

Edited by Luigi Gianturco



ATHEROSCLEROSIS - YESTERDAY, TODAY AND TOMORROW

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Notice

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Meet the editor

Luigi Gianturco, MD, is a cardiologist at ICBM Hospital of Vigevano, Italy. From 2009 to 2017 he was a cardiologist at IRCCS, Orthopedic Institute R. Galeazzi, University of Milan, where he was also Vice Chief of the Cardiology Unit and Assistant Manager. He graduated cum laude from University "La Sapienza" of Rome in 2003 and gained a postgraduate master's degree in Clinical Echocardiography at the University of Milan in 2009. He has several communications or published works in national and international journals. Luigi is Chief of the Italian Soccer Referees' Medical Committee (since 2013), a member of the e-Cardiology Working Group of the European Society of Cardiology, and has a passion for reading and writing.

Contents

Preface

Atherosclerosis is one of the most feared enemies for individuals. In this book we focus our attention on the main characteristics of atherosclerosis. However, a comprehensive book should have many chapters, therefore this book is not conclusive but goes some way in tackling the theme of atherosclerosis.

Many studies are necessary to completely clarify the phenomenon of atherosclerosis, so we provide a concise window of "yesterday" and "today" into the world of atheroma.

Modern medicine and modern cardiology cannot ignore continuing medical education and literature is the way to achieve this.

Using innovation and prevention as our main tools for the future we will attempt to overcome any difficulties or at least we try to provide a work of modern cardiovascular history.

I would like to thank all my team for collaborating in this book. In particular, my thanks go to Bruno (my expert colleague) and Aurel (my secretary) for analyzing and reading all the texts; Vincenzo, Stefano, and Andrea for English evaluation; and finally Rebecca (my future wife) for encouraging me in all my professional activities and giving me the power of daily life!

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Introduction

Chapter 1

Focus on Coronary Atherosclerosis

Hakan Saçlı, İbrahim Kara and Mehmet Kaan Kırali

Additional information is available at the end of the chapter

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Abstract

Atherosclerosis is a vascular disorder consisting of thickening of arteries and lack of elasticity. Result of atherosclerosis is that arteries become narrowed and hardened due to an excessive buildup of plaque around the artery wall. The disease disrupts the flow of blood around the body, posing serious cardiovascular complications. Arteries contain what is called an endothelium, a thin layer of cells that keeps the artery smooth and allows blood to flow easily. Endothelial damage starts the first step of atherosclerosis. After this, low-density lipoprotein (LDL) cholesterol accumulates in the artery wall. Inflammatory process starts after this accumulation, and macrophages reach the endothelium to clean up cholesterol. But some macrophages are stuck in the affected part of the artery wall in this process. Over time, this results in plaque being built up, consisting of cholesterol and macrophage white blood cells. The plaque clogs up the artery, disrupting the flow of blood. This potentially causes blood clots that can result in life-threatening conditions such as heart attack and other cardiovascular diseases. Atherosclerosis can be seen in all arteries in the body. Atherosclerosis is the most common cause of death in the western countries. Some risk factors are as follows: age, sex, familial predisposition, hyperlipidemia, hypertension, diabetes mellitus, smoking, obesity, insufficient physical activity, etc. Whatever the main reason or the risk factor is, once atherosclerosis is formed, several life-threatening cardiovascular disorders can be seen. So, it has to be revealed.

Keywords: atherosclerosis, coronary atherosclerosis, coronary artery disease, ischemic heart disease

1. Introduction

Atherosclerosis locution originates from the Greek-Latin word "atera" meaning "oat or milky mush." It describes a vascular disorder consisting of thickening of arteries and lack of elasticity. The general pathology refers to three situations:

The predominant type is often seen with intimal fatty plaque formation including central lipid-rich core. We want to mention about this type of atherosclerosis.

The second morphologic type of atherosclerosis, Mönckeberg's medial calcific sclerosis, is seen in the muscular arteries with a medial calcification. This form is not prominent as classic atherosclerosis and mostly seen after the age of 50. This can be seen radiologically and can be felt with palpation.

The third type is the disorders of the small arteries and arterioles, named arteriosclerosis. This is seen mostly with hypertensive and diabetic patients. This refers to stiffening or hardening of the artery walls.

The final result of atherosclerosis is that arteries become narrowed and hardened due to an excessive buildup of plaque around the artery wall. The disease disrupts the flow of blood around the body, posing serious cardiovascular complications.

Atherosclerosis can be seen in all arteries in the body. But we will instruct about the coronary atherosclerosis. Atherosclerosis is the most common cause of death in the western countries [1].

Arteries contain what is called an endothelium, a thin layer of cells that keeps the artery smooth and allows blood to flow easily. Endothelial damage starts the first step of atherosclerosis. After this LDL cholesterol accumulates in the artery wall. Inflammatory process starts after this accumulation, and macrophages reach the endothelium to clean up cholesterol. But some macrophages stuck in the affected part of the artery wall in this process. Over time this results in plaque being built up, consisting of cholesterol and macrophage white blood cells.

The plaque clogs up the artery, disrupting the flow of blood. This potentially causes blood clots that can result in life-threatening conditions such as *heart attack* and other cardiovascular diseases.

Some risk factors are follows: age, sex, familial predisposition, hyperlipidemia, hypertension, diabetes mellitus, smoking, obesity, insufficient physical activity, etc. Whatever the main reason or the risk factor is, once atherosclerosis is formed, several life-threatening cardiovascular disorders can be seen. So, it has to be revealed.

2. Background

The role of the circulation is to service the needs of the tissues. It includes transporting nutrients to thecells of the body and waste products away from the cells of the body. And finally transporting hormones from one part of the body to another. Naturally, circulation maintains an appropriate environment in all the tissue fluids of the body for optimal survival and function of the cells.

Systemic circulation, pulmonary circulation, peripheral circulation, etc. have some details to maintain the blood flow. The arteries are the large conductive vessels that transport blood

under high pressure to the tissues. The arterioles are the last small branches of the arterial system. And, the capillaries are where the exchange of fluid, electrolytes, nutrients, hormones, and other substances occurs. And, of course veins, venules, collect blood from the capillaries. Our main subject is atherosclerosis of arterial vessels.

We have to learn the normal state before discussing the pathological status. The normal artery wall is consisting of intima, media, and adventitia. Lumen is lined by a monolayer of endothelial cells that overlies smooth muscle cells. The inner layer of smooth muscle cells, known as the intima, is circumscribed by the internal elastic lamina. The media layer is between the internal elastic lamina and external elastic lamina. The media is another layer of smooth muscle cells. Outside the external elastic lamina is an adventitia part that is rarely populated by cells and microvessels of the vasa vasorum.

Atherosclerosis is a progressive disease of medium- and large-sized arteries characterized by focal intimal lesions called atheromas or atherosclerotic plaques that protrude into vessel lumen and eventually leading to various complications [2]. There are several diseases led by atherosclerosis: coronary artery disease, peripheral artery disease, and carotid artery disease. These are real threats for mortality and morbidity in the developed countries.

3. Pathophysiology

Atherosclerosis is a chronic, inflammatory, fibroproliferative disease of medium- and large-sized arteries [3]. There are different stages to form the atherosclerotic plaque. The initiation phase is the beginning and the progression of the plaque and the final complication stage.

Chronic or recurrent endothelial damage is the cornerstone of the "response to damage" hypothesis. Hyperlipidemia, hypertension, smoking, immunoreactions, hemodynamic factors, toxins, and viruses can cause this chronic endothelial damage. Hemodynamic deformities such as endothelial shear stress, turbulent flow, or unfavorable effects of hypercholesterolemia have a role in the initiation phase. Due to endothelial damage and turbulent flow, endothelial permeability, cell regeneration, and receptor-mediated LDL endocytosis and leukocyte adhesion to endothelium increase.

Hyperlipidemia has an important role in the atherogenesis [4]. Chronic hyperlipidemia especially hypercholesterolemia can start the endothelial damage. After all, lipoproteins accumulate in these damaged endothelial sites. The cellular response after endothelial damage continues with increased permeability, leukocyte adhesion, monocyte migration, and increased adhesion. This is no longer the initiation phase after this stage; progression has started.

Some cellular events take an important part in this phase. Smooth muscles migrate from the media layer to intima and macrophages activated. Monocytes turn to macrophages. Activated macrophages, and smooth muscle cells absorb lipids. Modified lipid molecules due to the oxidative mechanisms of modified LDL arise. Oxide LDL makes some additive affects, in order; (a) with the help of altered receptors LDL absorbed easily by macrophages (b) they

are chemotactic to circulating monocytes (c) they enhance adhesion of monocytes (d) they prevent the mobility of macrophages because of this macrophages remain their position and hold on to there (e) they are cytotoxic to endothelium and smooth muscle cells (f) they are immunogenic.

The endothelial damage is like as peeling of the endothelium, because of this damage platelets hold on to the endothelium. Smooth muscle cells derived from media layer migrate to here and starts to duplicate and some of them absorb lipids inside and turns to the foam like cells. And this is shown as fatty streaks.

After this stage macrophages take a leading role in atherosclerosis. Macrophages secrete interleukin-1 (IL-1) and tumor necrosis factor (TNF), and they are increasing leukocyte adhesion. Again, monocyte chemoattractant protein-1 (MCP-1) produced by macrophages collects leukocytes in the plaque. They have a role to oxidate the LDL. And finally, they secrete stimulators to affecting the smooth muscle cell growth [5].

Fatty streaks are seen in the childhood phase. This lesion starts as a small 1-mm-diameter intimal color change. With the organization of atherosclerosis, this lesion varies 1–3 mm in diameter and 1–2 cm long. Some of them are raised and some of them not.

Atheromatous plaque is the definitive lesion, and it is rich in lipids, but more often it is a lipid and fibrotic lesion. Sometimes, this solid and fibrotic characterized plaque can be rich with cells. Plaques' diameter can reach to a few cm. Its color changes according to the amount of the lipid. It is changed to a round shape and has an irregular shape.

Atheromatous plaques can be seen in the different parts of the body. The prevelence of involvement is in order; Abdominal aorta, coronary arteries, popliteal arteries, descending thoracic aorta, internal carotid arteries and the circle of Willis.

Finally, microscopically atherosclerotic plaque has got the main components. These are lipids, vascular smooth cells, monocytes/macrophages, rarely lymphocytes, connective tissue matrix, and fibrils (**Figure 1**).

But more importantly, atherosclerotic plaque changes to four different types. These are complicated plaques:

a. Calcification of the arteries. They can be seen as a consecutive island, and some of them can be in the whole artery.

b. Ulceration of the surface of the atherosclerotic plaque. This can cause embolization.

c. Platelet aggregation can occur on the ulcerated plaque. This can lead to total occlusion of the artery. The most devastating effect of atherosclerosis such as heart attack and stroke is caused by the superimposed thrombosis.

d. Atherosclerotic aneurysm can occur due to atherosclerosis.

e. This ulceration can break endothelial integrity, and this can causes rupture of the plaque and can cause bleeding.

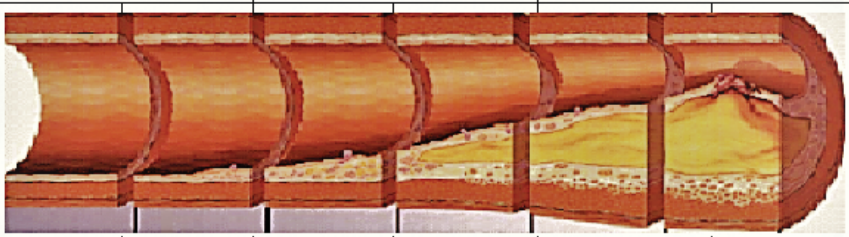
Clinical findings	Asymptomatic			Asymptomatic or Symptomatic		
Growth mechanism	Growth is mainly with lipid deposition				Proliferation of smooth muscle cells and increase of collagen	Thrombosis and /or hematoma
Onset of time	From first decade		From Third decade		From fourth decade	
Phases of progression of Atherosclerosis						
Main Histology of the progression	***First lesion***: -Normal Histology - Macrophage migration -Isolated foam cells	***Fatty Streak:*** -Mainly intracellular lipid deposition	***Intermediate phases:*** -New fatty streaks -Intracellular Lipid deposition and lipid pools	***Atheroma:*** -New fatty streaks -Intracellular and Extracellular lipid accumulation	***Fibroatheroma:*** -New fatty streaks -Single or multiple lipid cores -Fibrotic and calcific layers	***Complicated Lesions:*** -Disrupted surface (ulcerated plaque) -Thrombosis -Hematoma and hemorrhage

Figure 1. The stages of the progression of atherosclerosis.

4. Epidemiology and etiology

Due to the asymptomatic phase of atherosclerosis, it is impossible to say the frequency of atherosclerosis, because the process of atherosclerosis starts with fatty streak in the first decade of lifetime. More advanced lesions begin to develop when individuals are in their second and third decade. Complicated coronary atherosclerosis causes coronary artery disease (CAD) after all. CAD remains the most common pathology with which cardiologists and cardiac surgeons are facing. It is the most common cause of death in Turkey in 2013 [6]; 38.8% of the deaths were due to the ischemic cardiovascular disease. Ischemic heart disease is the most common cause of death in the world as reported by the World Health Organization (WHO) in 2012 [7].

Inactivation of genes coding for monocyte chemotactic protein-1 (MCP-1), its receptor on monocyte/macrophages (CCR2), and macrophage colony-stimulating factor has a profound impact on the development of atherosclerosis in otherwise identical mice that have been shown in the experimental studies [8]. The etiology of atherosclerosis is unknown, but in the development process of atherosclerosis, the pathophysiology is important to explain the nature. There are some important risk factors in this process. We have to classify risk factors in two. These are modifiable and non-modifiable risk factors.

5. Risk factors

5.1. Non-modifiable risk factors

a. Increased age.

b. Male gender: lack of atheroprotective properties of estrogen which raises HDL and lowers LDL.

c. Hereditary factors: history of coronary artery disease (CAD) among first-degree relatives at a young age (before 55 for males and before 65 for females). New markers of the cardiovascular risk factors:

d. Increased lipoprotein(a) level.

e. Increased homocysteine level: high levels may promote oxidative stress, vascular inflammation, and platelet adhesiveness. And, this process leads to atherosclerosis. A meta-analysis that collected a large number of prospective studies showed a significant association between the serum level of homocysteine and the incidence of cardiovascular disease [9]. Not just with it, increased blood homocysteine levels are shown in patients with acute myocardial infarction [10].

f. C-reactive protein (CRP), high-sensitivity CRP (hs-CRP), and other markers of inflammation: activate complement and contribute to a sustained inflammatory state. CRP is a biomarker of tissue damage and inflammation. It is an acute-phase reactant and increases in the inflammatory process. But nowadays, it has been used in the diagnosis of the cardiovascular diseases such as CAD. Sara et al. have showed that hs-CRP is associated with coronary endothelial dysfunction in the asymptomatic coronary artery disease [11].

5.2. Modifiable risk factors

a. Dyslipidemia: increased LDL and decreased high-density lipoprotein (HDL).

b. Tobacco smoking: enhances oxidative modification of LDL, contributes to endothelial dysfunction via oxidant stress, and increases expression of leukocyte adhesion molecules.

c. Hypertension: increases permeability of the vessel wall to lipoproteins and promotes retention of LDL in the vessel intima by accentuating production of LDL-binding proteoglycans by smooth muscle cells.

d. Diabetes mellitus: enhances glycation of LDL and is associated with endothelial dysfunction.

e. Obesity and lack of physical activity: can cause dyslipidemia, hypertension, and insulin resistance.

f. Stressful lifestyle: better known as Type A personality.

5.3. Atheroprotective factors

a. Exercise

b. High-density lipoprotein (HDL) and its major apolipoprotein (ApoA1)

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Coronary atherosclerosis is an important site of atherosclerosis. There are various types of results due to coronary atherosclerosis. Especially, the size of the plaque and the type of the complicated plaque are important for this. Whatever the beginning of the atheromatous plaque, the result can be a fatal heart attack. In developed countries atherosclerosis causes more than half of total mortality. Coronary artery disease (CAD) is responsible for a major proportion of these deaths [12].

6. Signs and symptoms

Onset of the atherosclerotic plaque and speed of the growth and complications, there are several signs and symptoms. Atherosclerosis can be seen in every artery, but for the coronary atherosclerosis, the result of the disease is coronary artery disease, and the symptoms and the signs are due to this. Because of the impaired blood flow, there is a sort of symptoms. Some of them are in the side of the chest, and some of them are systemic because of the impaired circulation.

- Chest pain
- Shortness of breath
- Weakness, tiredness, reduced exertional capacity
- Dizziness, palpitations
- Leg swelling
- Weight gain
- Diaphoresis
- Tachycardia: common in persons with acute coronary syndrome (ACS) and acute myocardial infarction (AMI)
- High or low blood pressure
- S_4 gallop: a common early finding
- S_3 gallop: an indication of reduced left ventricular function
- Heart murmurs
- Tachypnea
- Xanthelasmas
- Livedo reticularis
- Syncope
- Leg edema
- Rales

Coronary atherosclerosis causes coronary artery disease. Complicated atherosclerotic plaque disrupts the blood flow in the coronary circulation. Impaired blood flow causes a corrupted supply and demand of the oxygen and the metabolites in the heart. This results in a decrease in coronary arterial blood flow and a decrease in oxygen supply. There are several symptoms such as chest pain (angina pectoris), dyspnea, syncope, and sometimes pulmonary edema. Increased demand of blood supply and oxygen starts the angina pectoris. Because of the decreased blood flow in coronary artery, sufficient blood cannot be supplied in the increased effort capacity. The spectrum of presentation includes symptoms and signs consistent with the following conditions:

- Asymptomatic state (subclinical phase)
- Stable angina pectoris
- Unstable angina (i.e., acute coronary syndrome)
- Acute myocardial infarction (AMI)
- Chronic ischemic cardiomyopathy
- Congestive heart failure
- Sudden cardiac arrest

7. Diagnosis and treatment

Atherosclerosis can be seen in all the arterial sites in the whole body as mentioned before. So, the physical examination can give us very important findings. A well-taken medical history and physical examination can be helpful for the diagnosis. Suspicious findings can lead us to make a decision for the advanced examination.

Medical history is the cornerstone of diagnosis. A positive history of typical chest pain, shortness of breath, impaired physical capacity, and the other signs and symptoms are very useful to diagnosis.

Atherosclerosis can cause both coronary artery and peripheral artery diseases. Concomitant coronary and peripheral artery disease prevalence is varied 28–94% in published reports [13]. So, on the calcified peripheral artery, palpation or lack of pulse in the peripheral arteries or signs of the peripheral artery disease are important parts of the physical examination.

What are the parts of the advanced examination?

Electrocardiography (ECG): impaired blood flow in acute events such as acute myocardial infarction and acute coronary syndromes are the changes we can see in the ECG.

Echocardiography (ECO): atherosclerotic calcification or plaque and thickness of aortic wall can be seen in ECO. Ventricular low ejection fraction and impaired contraction of ventricular segments can suspect us for coronary atherosclerosis.

Stress echocardiography: this echocardiography can be performed either by exercise method or pharmacological drugs that increase cardiac contractility and rate.

Exercise echocardiography: images are taken before and after the treadmill or stationary bike effort test. If exercise echocardiography cannot be performed due to peripheral artery disease, musculoskeletal disorders, etc., drug-stimulated (dobutamine, adenosine, dipyridamole) stress echocardiography can be performed. These drugs increase the cardiac contractility and rhythm. Doses of the drugs increases step by step, and images are taken gradually.

The purpose is to assess the exercise tolerance of the heart. If there is a myocardial perfusion defect due to coronary artery disease, stress echocardiography can give information about this. The severity of the coronary artery disease can be assessed with this test. Before and after revascularization either PCI or CABG cardiac risk can be evaluated. It can be performed for cardiac risk analyses for noncardiac surgeries. Exercise echocardiography can be used for risk stratification in asymptomatic patients with severe aortic stenosis too [14]. Yao et al. have showed in their clinical study; as the result of the exercise tests, monophasic/normal wall motion was associated with a benign prognosis, but abnormal wall motion responses were associated with a worse prognosis [15].

Myocardial perfusion scintigraphy: can show us the ischemic parts of the heart due to the occlusive effect of coronary atherosclerosis leading to coronary artery disease.

Computed tomography (CT): conventional thoracoabdominal CT scan can show atherosclerotic calcification and plaques in the aortic or arterial wall. But coronary CT scan can show us the presence of coronary atherosclerosis, the degree of the coronary artery disease, and the occlusive lesions.

Intravenous ultrasound (IVUS): can be useful for the controversial lesions. This is an invasive technique that localizes plaques and quantifies plaque seriousness. Virtual histology-intravascular ultrasound (VH-IVUS) can identify plaque components. Optical coherence tomography (OCT), also known as optical frequency domain imaging (OFDI), identifies intimal hyperplasia and also detects and quantifies the key features of vulnerable plaque [16].

Coronary angiography: is the gold standard for diagnosis of coronary atherosclerosis and coronary artery disease. Moving image of each coronary artery and the atherosclerotic lesions can be seen. It is the most specific and sensitive test for the diagnosis of coronary artery disease.

Coronary angiography can come out to such results. This can be a follow-up with medical therapy, a percutaneous coronary intervention can be necessary, or a coronary artery bypass grafting (CABG) is essential to be performed to the patient. All of the interventions are selected due to the percentage of the affected coronary artery lesion, the lesion type, lesion location, the number of the affected coronary artery, and of course the general condition of the patient.

Treatment: There are several treatment modalities. These include lifestyle changes, risk factor modification, and medical therapies. But we want to mention about the clinically important occlusive coronary artery diseases' invasive treatment.

Percutaneous coronary intervention (PCI): it is also known as coronary angioplasty, and this is a nonsurgical technique to treat obstructive coronary artery disease. It can be a choice in stable angina pectoris, in acute myocardial infarction, or in multivessel coronary artery disease. The procedure is performed in angiography catheter laboratory. An x-ray fluoroscopy and opaque fluid are necessary for the procedure. Entry ways for the procedure are femoral arteries and radial arteries for the individual cases.

Some urgent cases such as acute myocardial infarction PCI can be performed emergent. Primary PCI is called in this situation. But also PCI is used for elective coronary artery disease usually. The procedure starts with a local anesthesia from the arterial puncture side; this can be even femoral or radial artery. Hydrophilic and micro-catheters and guidewires are used to reach coronary arteries. These radiopaque wires are seen easily on fluoroscopy. A balloon angioplasty can be performed to the occlusive lesion. Coronary stents can be implanted to the occluded lesion. Coronary stents vary from bare metal stents to drug eluding stent. These drug eluding stents vary to the first, second, and third generation. Nowadays, fourth-generation bioresorbable stents are mentioned in some clinical trials [17]. Whatever the kind of the stents, the main purpose is to improve blood flow of the myocardium tissue.

Coronary artery bypass grafting (CABG): so, is this the only technique that we can improve blood supply of the myocardium? Is there any other way of myocardial revascularization? The answer is yes. It is coronary artery bypass grafting (CABG). This is an open cardiac surgical procedure. This means that it is more invasive than PCI. But in some cases, PCI cannot be the concluding treatment for the coronary artery disease. Lesion type, region of the lesion, collateral and main side branches extending from the lesion, severity of the lesion, and the number of the lesions is important for the physician to make the choice.

Before explaining CABG, we have to mention the indications and guidelines (**Table 1** and **2**).

7.1. Guidelines for coronary artery bypass graft surgery

7.1.1. Asymptomatic CAD

7.1.1.1. Class I

1. LMC stenosis.[18, 20]

2. LMCE disease.

3 Three-vessel disease.

Revascularization	CABG			DES		
	No risk	DM	LVD	No risk	DM	LVD
One-vessel disease	N	N	N	Y	Y	Y
Proximal LAD	Y	Y	Y	N	N	N
Two-vessel disease without LAD	N	N	N	Y	Y	Y
Two-vessel disease with LAD	Y	Y	Y	Y	Y	Y
Two-vessel disease + proximal LAD	Y	Y	Y	N	N	N
Three-vessel disease	Y	Y	Y	C	C	C
Three-vessel + proximal LAD	Y	Y	Y	N	N	N
LMC ± other lesions	Y	Y	Y	N	N	N

CABG, coronary artery bypass grafting; DES, drug-eluting stent; DM, diabetes mellitus; LAD, left anterior descending artery; LMC, left main coronary artery disease; LVD, left ventricular dysfunction. *Y, yes; N, no; C, controversial.

Table 1. The reality of myocardial revascularization strategies in patients with isolated coronary artery disease [18].

Recommendation	CABG		PCI	
	Class	Level	Class	Level
One or two-vessel disease without LAD	IIb	C	I	C
One-vessel disease with proximal LAD	I	A	I	A
Two-vessel disease with proximal LAD	I	B	I	C
LMC with SYNTAX score < 22	I	B	I	B
LMC with SYNTAX score 23–32	I	B	IIb	B
LMC with SYNTAX score > 32	I	B	III	B
Three-vessel disease SYNTAX score > 22	I	A	I	B
Three-vessel disease SYNTAX score 23–32	I	A	III	B
Three-vessel disease SYNTAX score > 32	I	A	III	B

Recommendation for the type of revascularization (CABG or PCI) in patients with stable CAD with suitable coronary anatomy for both procedures [19].

Table 2. 2014 ESC/EACTS Guidelines on myocardial revascularization guidelines.

7.1.1.2. Class IIa

(1) Proximal LAD (one- or two-vessel disease)

7.1.1.3. Class IIb

(1) One- or two-vessel disease not involving proximal LAD (if a large territory at risk on non-invasive studies or LVEF <50%, class IIa and IIb become class I indications)

7.1.2. Stable angina

7.1.2.1. Class I

1. LMC stenosis.

2. LMCE disease.

3. Three-vessel disease.

4. Two-vessel disease with proximal LAD stenosis and LVEF <50% or demonstrable ischemia.

5. One- or two-vessel disease without proximal LAD stenosis but with a large territory at risk and high-risk criteria on noninvasive testing.

6. Disabling angina refractory to medical therapy.

7.1.2.2. Class IIa

1. Proximal LAD stenosis with one-vessel disease.

2. One- or two-vessel disease without proximal LAD stenosis, but with a moderate territory at risk and demonstrable ischemia.

7.1.3. Unstable angina/non-ST-segment elevation MI (non-STEMI)

7.1.3.1. Class I

1. LMC stenosis.

2. LMCE disease.

3. Ongoing ischemia not responsive to maximal nonsurgical therapy.

7.1.3.2. Class IIa

Proximal LAD stenosis with one- or two-vessel disease.

7.1.3.3. Class IIb

One- or two-vessel disease without proximal LAD stenosis when PCI not possible (becomes class I if high-risk criteria on noninvasive testing).

7.1.4. ST-segment elevation (Q wave) MI

7.1.4.1. Class I

1. Failed PCI with persistent pain or shock and anatomically feasible.

2. Persistent or recurrent ischemia refractory to medical treatment with acceptable anatomy, which has a significant territory at risk and not a candidate for PCI.

3. Requires surgical repair of post-infarct VSD or MR.

4. Cardiogenic shock in patients <75 years of age who have ST elevation, LBBB, or a posterior MI within 18 hours onset.

5. Life-threatening ventricular arrhythmias in the presence of ≥50% LMC stenosis or three-vessel disease.

7.1.4.2. Class IIa

1. Primary reperfusion in patients who have failed fibrinolytics or PCI and are in the early stages (6–12 h) of an evolving STEMI.

2. Mortality with CABG is elevated in the first 3–7 days after STEMI/NSTEMI. After 7 days, criteria for CABG in previous section are applied.

7.1.5. Poor LV function

7.1.5.1. Class I

1. LMC.

2. LMCE.

3. Proximal LAD stenosis and two- to three-vessel disease.

7.1.5.2. Class IIa

Significant viable territory and noncontractile myocardium.

7.1.6. Life-threatening ventricular arrhythmias

7.1.6.1. Class I

1. LMC.

2. Three-vessel disease.

7.1.6.2. Class IIa

1. Bypassable one- or two-vessel disease.

2. Proximal LAD disease and one- or two-vessel disease. These become class I indications if arrhythmia is resuscitated cardiac death or sustained ventricular tachycardia.

7.1.7. Failed PCI

7.1.7.1. Class I

1. Ongoing ischemia with significant territory at risk.

2. Shock.

7.1.7.2. Class IIa

1. Foreign body in critical position.

2. Shock with coagulopathy and no previous sternotomy.

7.1.7.3. Class IIb

Shock with coagulopathy and previous sternotomy.

7.1.8. Previous CABG

7.1.8.1. Class I

1. Disabling angina refractory to medical therapy.

2. Nonpatent previous bypass grafts, but with class I indications for native CAD.

7.1.8.2. Class IIa

1. Large territory at risk.

2. Vein grafts supplying LAD or large territory are "/> 50% stenosed.

Class I: Conditions for which there is evidence and/or general agreement that a given procedure or treatment is useful and effective.

Class II: Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness or efficacy of a procedure.

Class IIa: Weight of evidence/opinion is in favor of usefulness/efficacy.

Class IIb: Usefulness/efficacy is less well established by evidence/opinion.

Class III: Conditions for which there is evidence and/or general agreement that the procedure/treatment is not useful/effective and in some cases may be harmful.

ACC, American College of Cardiology; AHA, American Heart Association; CABG, coronary artery bypass grafting; CAD, coronary artery disease; LAD, left anterior descending artery; LBBB, left bundle branch block, LMC, left main coronary artery; LMCE, left main coronary equivalent; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MR, mitral regurgitation; NSTEMI, non-ST elevation myocardial infarction; PCI, percutaneous transluminal coronary angioplasty; STEMI, ST elevation myocardial infarction; VSD, ventricular septal defect [20].

There are several grafts that are used in CABG. Arterial grafts such as left internal and right internal mammary artery (LIMA and RIMA), especially LIMA has got the longest patency rate (10-year patency is 95%). Radial artery can be used, but it is a muscular artery and has got a predisposition to vasospasm. Vena saphena magna is the most used venous graft.

This procedure can be performed with cardiopulmonary bypass (CPB) machine (on-pump), without CPB (off-pump CABG-OPCAB), or beating heart procedures. In the last decade, minimally invasive techniques are rising to individual cases. MIDCAB (minimally invasive direct coronary artery bypass) can be performed without full median sternotomy. This can serve minimal surgical trauma and avoid wound complication.

TECAB: this is a robotically assisted total endoscopic coronary artery bypass procedure. This is a complex procedure; surgeon has to steep a learning curve. This procedure can perform both on-pump and off-pump CABG.

Awake coronary artery bypass (ACAB) procedure: This avoids side effects of general anesthesia. This includes a minimal invasive procedure without intubation and mechanical ventilatory support. A somatosensory and motor block is made via the T1–T8 level of vertebra. This preserves diaphragmatic ventilation.

Early outcomes after CABG continue to improve, and the early cumulative mortality rate is below 2% and lower than 1% in lower-risk patients. The most common reasons for death are heart failure (65%), neurologic events (7.5%), hemorrhage (7%), respiratory failure (5.5%), and dysrhythmia (5.5%).

The survival rate after isolated CABG is higher than 98% for the first month and 97% for first year, 92% for 5 years, 80% for 10 years, 65% for 15 years, and 51% for 20 years. Usage of LIMA is a predictive parameter for late survival.

8. Prevention

Prevention of the coronary atherosclerosis has to be lifelong. Individuals need to be careful for risk factors. Adopting a healthy lifestyle. What is inside of this healthy lifestyle?

Healthy eating habit for the heart: eating habits are very important in the process of developing atherosclerosis. Healthy diet consists of low amounts of white bread, unsaturated fat products, fast foods, salt, and sugar. It also includes eating dairy products, fruits, vegetables, whole grain, seafood, poultry without skin, lean meats, low-fat milk, or fat-free milk.

After the start of healthy diet for the heart, weight control can be achieved, because overweight and obese people have high risk for coronary atherosclerosis.

Physical activity: stressful and sedentary lifestyles are the risk factors for coronary atherosclerosis. So, a programmed physical activity can improve the fitness level and the health of the individuals.

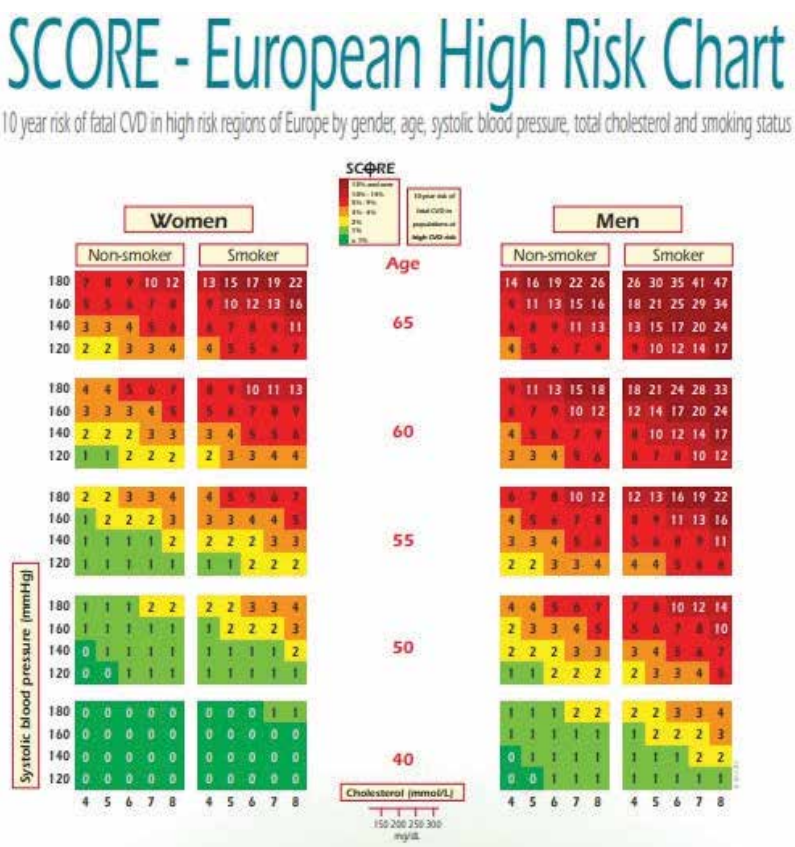

Figure 2. Ten year risk of fatal CVD in high risk regions of Europe by gender, age, systolic blood pressure, total cholesterol and smoking status.

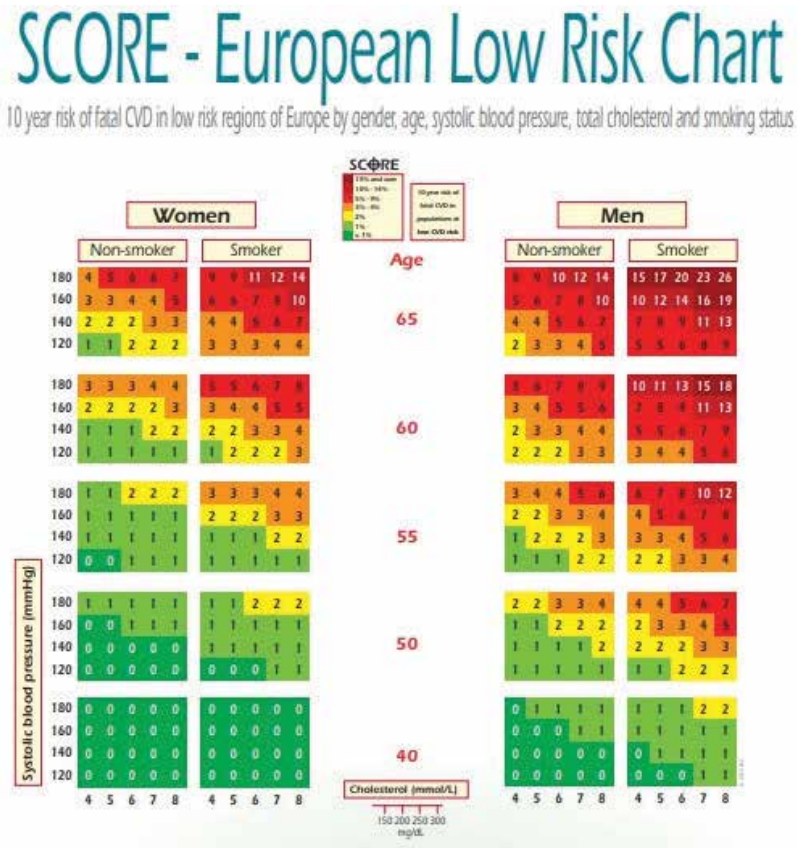

Figure 3. Ten year risk of fatal CVD in low risk regions of Europe by gender, age, systolic blood pressure, total cholesterol and smoking status.

8.1. Stopping tobacco smoking

We have to mention about risk scores and charts. Risk scores can give us several information about the cardiac risk of our body. This can lead the person to change avoidable habits.

Framingham Risk Score: age, sex, cigarette smoking, cholesterol level, high-density lipoprotein (HDL) cholesterol level, systolic blood pressure, and usage of antihypertensive drugs. Some clinics include diabetes mellitus (DM), low-density lipoprotein (LDL) cholesterol, and diastolic blood pressure to modify this risk score.

Another risk score system is SCORE risk charts. This includes SCORE—European High Risk Chart and SCORE—European Low Risk Chart. This score system is based on gender, age, total cholesterol, systolic blood pressure, and cigarette smoking (**Figures 2** and **3**).

9. Conclusion

Coronary atherosclerosis and coronary artery disease (CAD) are the most frequent causes of hospitalization in western countries. It is an important mortality and morbidity cause. The

onset of the first lesions begins in the first decade of the life period and proceeds with the lifetime. Risk factors are important and decisive for the progression of the atheromatous plague. A healthy and modified life is the key to prevent from the disease.

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Chapter 2

Early Stages of Atherosclerosis Documented in Early Embryologic Life

Bahar Uslu

Additional information is available at the end of the chapter

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Abstract

The composition of arterial bifurcations primarily changes blood flow and has a substantial role in the development of vascular disorders. Hence, it is essential to know the structural physiognomies of the common carotid artery (CCA) and its branches for the early onset of atherosclerosis in newborns. Some studies were conducted to evaluate the characteristics of CCA in newborn cadavers. Correlation between area ratios and atherosclerotic endothelial damage was determined. Investigations demonstrated that carotid bifurcation regions depicted widespread occurrence of intimal lipid accumulations, while carotid bifurcation region structure demonstrated abundant blood cells and disconnected endothelial cells. Fibrin collection on endothelial surface in low area ratios was another essential finding in the examinations of their endothelial surface erosion. The abovementioned morphological findings seemed to be matching to outflow to inflow area ratio statistics, favoring low area and degeneration. The correspondence between area ratios and the histological characteristic of cerebral vessels of newborn cadavers specifies that the early stages of atherosclerosis began in early embryologic life.

Keywords: carotid artery, cerebral blood flow, endothelium, newborn cadaver

1. Introduction

Atherosclerosis is described by a noticeable condensing and solidification of blood vessels [1–4]. Obtained risk factors such as high blood pressure, smoking, diabetes mellitus, and dyslipidemia are main factors in the onset of atherosclerosis [4–10]. Further, anatomical, histological, and hemodynamic features of the arteries and the genetic factors are other dynamics that predispose the onset of atherosclerosis [3–5, 11, 12].

2. Structural physiognomies of the common carotid artery (CCA) and its branches

Blood vessels and their luminal physiognomies have long been questioned as additional risk factors for atherosclerosis referring their stimulus on blood flow [9, 13–15]. Some arteries are more predisposed for the onset of the atherosclerotic plaques on the endothelial surface [2, 4, 10, 15, 16]. Current studies specified that the variations in the luminal diameter of the vessel have a collision on the beginning of atherosclerosis [3, 5, 6, 13].

Carotid artery is one of the two main vessels that stream blood to brain. Obstruction in the carotid artery leads to erosion and causes some brain symptoms. The anatomical elements of the common carotid artery (CCA) and its branches receive attention from researchers and clinicians, referring their scientific and clinical results. Also, another important factor is their involvement in plaque formation [7, 8, 10, 13, 17–21]. Hence, it is essential to distinguish the anatomical topographies of the CCA and its branches. CCA divides into two branches, that is, internal carotid and external carotid arteries. Atherosclerosis progresses mainly at bends and major branches of the arterial network, such as the carotid bifurcation and its subdivisions [2, 3, 15, 17]. Alike other bifurcation of large vessels, carotid bifurcation at the neck region is more prone to the initial growth of atherosclerotic plaques [3, 6, 14–16]. Flow models suggest that vessel anatomy, in particular vessel diameter and area ratios, affects plaque formation at arterial bifurcations. The carotid bifurcation is one of the most common of atherosclerotic plaques [2, 3, 9, 20, 22]. Therefore, assessing the diameters of the CCA, internal, and external carotid arteries (ICA and ECA) is important for evaluating the pathological changes [6, 22–25].

3. The correlation between area ratios and the histological characteristic of cerebral vessels of newborn cadavers

Despite the progression of atherosclerosis with aging being widely studied, there are limited studies in newborns. Many studies evaluated the diameters of peripheral vessels in adults, but only few studies were conducted on CCA and its branches in newborns [3, 6, 12, 19, 22]. Thus, an early beginning of atherosclerosis is suggested to initiate in the early period of life. Then, the purposefulness was to scrutinize the anatomical and histological characteristics of cerebral vessels in newborn cadavers. Consequently, it has been hypothesized that variations in carotid bifurcation lumen geometry would be the self-regulating prognosticators of ICA atherosclerosis, aiming to reveal the early beginning of atherosclerosis in newborn cadavers. The relation between the endothelial destruction and the outflow to inflow area ratio was also been inspected in some studies.

4. Other related predisposing risk factors

Atherosclerosis is a series of complex events that can begin in early fetal life [3, 4, 16, 26]. The onset of atherosclerosis was implied to be connected with several risk factors such as

diabetes, genetics, and so on [4, 26]. Such as aorta abdominalis, bifurcation regions have been mentioned as primary locus that is prone to atherosclerosis [6, 20]. In recent studies, it has been suggested that the vessel diameter and area ratios are hypothetically important elements of plaque improvement [9, 20, 23]. With this information in mind, it earlier-published findings have been continued [6, 17], by observing the characteristics of vessel positioned in the bifurcation regions in young cadavers [6, 11, 13, 27].

As specified in the study, a vessel diameter and area ratio (score) are approximately 1.15 [12]. Low ratios could be reflective of increased local stress and endothelial damage. As a predictable result, the endothelial reaction to the damage might be amplified permeability accompanied by monocyte adhesion and migration.

The diameters of the CCA, ECA, and ICA have again been analyzed in the recent studies. The relative vessel dimension was shown to be significant in the progress of the disease [6, 11, 13, 19, 27]. Consequently, ICA/CCA, ECA/CCA, ECA/ICA ratios as well as the outflow to inflow area ratio have been calculated. Furthermore, vessels have been histologically evaluated using histo-staining methods and scanning electron microscopy (SEM) to determine the extent to which atherosclerotic pathology exists, if any [3]. The other central aims were to calculate the mean diameters of CCA, ICA, and relationships between atherosclerosis and ECA and outflow to inflow area ratio in the newborn period group. These records can be of use in intravascular composition and also for understanding the changes in these vessels that occur in fetal life [3].

The substantial intra-individual and inter-individual alterations of the carotid artery have previously been publicized in some studies [6, 9, 11, 13, 16, 20, 27]. Recently, it has been tried to discover the answer whether these differences were present in the early period of life [3].

5. Regarding atherosclerotic plaque establishment

Atherosclerotic plaque formation has been suggested to be thoroughly related to a shrinkage in the outflow to inflow area ratio [6]. This information has been sustained in several studies [9, 18, 21, 28–30].

Fisher and Fieman, and Schultz et al., publicized that the bifurcation anatomy stimuli the blood flow that produces the endothelial destruction [9, 20]. Mortensen also declared endothelial impairment and clarified that a quantity of a pulse wave reaching a bifurcation is reflected, and the higher the quantity of reflection, the more the hemodynamic stress might progress locally. The increase in the pressure could lead to endothelial destruction and support atherosclerotic plaque improvement [19]. In terms of endothelial damage, findings presented parallel results to the literature [3].

Initial examinations of this geometric risk theory were assessed in part, owing to relatively small sample sizes. Fisher and Fieman studied the conclusions of bifurcation angle and area ratio asymmetry on the improvement of atherosclerosis [6, 9, 21]. Also, it had limited samples because of the difficulties in obtaining human cadavers [3].

It has been shown that in early lesions of the atherosclerosis, fatty streaks progress very early in fetal period [3, 4]. The creation of fatty streaks also depends on many dynamics such as the susceptibility of the arteries and genetic factors, and the maternal hypercholesterolemia. The locations of a lesion demonstrate variability, and the fatty streaks tend to occur focally in certain predisposed regions while sparing neighboring unaffected sections [3]. Abdominal aorta and common carotid are much more prone to the development of fatty streaks [4]. The intracranial arteries are less prone to laceration enlargement than extra-cranial arteries; hence, the initial lesions develop in extra-cranial arteries rather than in intracranial ones [4].

The purpose why certain arteries are more disposed to atherosclerotic changes is not well understood. The hemodynamic factors and morphologic features of the artery may play a role [9, 12, 14, 20, 21]. It has been concentrated on the carotid bifurcation [3].

Shultz et al. outcomes [16] demonstrated that variation in carotid bifurcation anatomy is not restricted to differences in absolute vessel dimension. In addition, vessel diameter and area ratios diverge between and within individuals [20].

Selected studies, which have studied the relation between bifurcation's luminal geometry and the occurrence of cerebral artery aneurysms on angiographic images, have localized atherosclerotic lesions at the bifurcations of human cerebral arteries on autopsy cases. However, in this study, there were no available data on the endothelial topography in bifurcation geometry of newborn cadavers in the CCA and its major branches. For this motivation, histologic assessment makes last studies more valuable [3, 31].

Gosling et al. analyzed the optimal area ratio of an arterial bifurcation, producing the least reflection of pressure to be 1.15. That proportion can be close to ideal in human infants; however, in the long term, the decrease in outflow to inflow area ratio can lead to atherosclerotic plaque development. Gosling et al. studied 19 cases, with ages ranging from 0 to 10 and the outflow to inflow area ratio was found to be 1.11 ± 0.02 at 0 age group. Uslu's consequences were closer to the optimum ratio [3, 12].

Sitzer attempted to deliver a mechanistic link by proposing that their angle or rotation of ICA origin may be related to the ICA angle of insertion (comparable with the ICA-CCA angle of Lee et al.), which has been linked to flow turbulences [2, 25].

There are several studies on the diameters of CCA, ICA, and ECA in adults, but few studies are on newborns. To our knowledge, there are no earlier documents available on the relationship between the diameter of newborn cadavers and the CCA, ICA, and ECA [6].

Sehirli reported the mean outflow to inflow area ratio as 1.10 ± 0.33 mm in female and 1.18 ± 0.22 mm in male newborn cadavers for the common carotid artery bifurcation [6]. The consequences of Uslu's study on intracranial bifurcations show that the means of the outflow to inflow area ratio in fetal material are close to the optimum value in fetal material for the cerebral vessels.

Consistent with the results, the luminal geometry of arterial bifurcations impacts the blood flow that produces endothelial damage [3, 9, 13, 26].

6. Potential limitations and implications for these types of diameter calculations

Numerical changes were convincing, but not perfect. Studies were retrospective, comparatively small, and focused on an inadequate number of newborn cadavers. Sample availability was insufficient for both affected groups and controls. It should also be noted that studies were not planned to be an epidemiological study, and therefore, groups did not signify the characteristics of a wide-ranging population. Recognition of blood cells and fibrin on the endothelial surface are interpreted as pathological definitions. Conversely, there might be problems with the poor fixation of specimens, that is, blood could not be washed out from the arterial lumen before fixation procedure. Lastly, scanning electron microscopy (SEM) studies can be associated with various kinds of artifacts. The authors then hope to approve their pathological findings using transmission electron microscopy in upcoming studies. Despite the above possible limitations, the current studies seem to establish a modest upper bound on the influence of local versus known or unknown systemic cardiovascular risk factors on wall setting. Thus, last results are parallel with the earlier ones to support theory that carotid bifurcation geometry (and/or local hemodynamics) is a risk factor for initial carotid wall solidifying.

7. Conclusion

In newborns, the results showed that the outflow to inflow area ratio was very close to optimum. Recent data can be very helpful for understanding the anatomical variations of the CCA, ECA, and ICA. The correlations between area ratios and the histologic assessments of cerebral vessels of newborn cadavers specify that the early stage of atherosclerosis began in early embryologic life. Last results encourage the hypothesis that carotid bifurcation anatomy is among the main risk factors for the early onset of atheroma plaques. Still, supplementary studies are needed to underline the other factors, potentials, and mechanisms.

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Section 2

Atherosclerosis Mechanisms

Chapter 3

Inflammatory Mechanisms in Atherosclerosis

Ida Gregersen and Bente Halvorsen

Additional information is available at the end of the chapter

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Abstract

Atherosclerosis is a disease of chronic inflammation, characterized by a dysfunctional interplay between the immune apparatus and lipids. Immune cells, as well as nonimmune cells, drive plaque inflammation through a complex crosstalk of inflammatory mediators. The cells are activated by risk factor–induced triggers, which are present in the circulation and in the vessel wall, such as shear stress, oxidized lipoproteins and oxidative stress. Without relief from risk factors, the activation of inflammatory processes persists, resulting in a chronic nonresolving inflammation. Inflammation is associated with severity of disease, and complex lesions, which are prone to rupture and cause acute events, are characterized by extensive inflammation. Thus, inflammation is an active driver of atherosclerotic plaque development and a risk factor for atherosclerotic events. It is therefore of utmost importance to understand the mechanisms behind these inflammatory processes and to be able to develop new diagnostics and treatment modalities for atherosclerotic disorders. This chapter provides a brief overview of the most important inflammatory players and processes during atherosclerotic plaque development and of possible therapeutic targets to combat atherosclerotic disease.

Keywords: inflammation, monocytes, macrophages, T cells, cholesterol

1. Introduction

Atherosclerosis is a complex disease of the artery wall. It is the major cause of cardiovascular disease (CVD), which is the most common cause of death in the world, killing 17.5 million people each year [1]. Although previously thought of as a disorder of age and cholesterol, it is now commonly appreciated that atherosclerosis results from a complex interplay between inflammation and lipids. As early as in the nineteenth century, Rudolf Virchow described inflammation as an active driver of plaque development; however, the importance of these findings was not appreciated until over a century later. During this time, modern immunology

evolved extensively and paved the way for in-depth understanding of how the immune system works. Identification of adhesion markers on endothelial cells and thus the ability of leukocytes to migrate into atheromas gave plausibility of inflammation as a contributor in atherogenesis [2, 3]. Furthermore, findings showing that monocytes [4], and later on that vascular cells [5, 6], secrete inflammatory mediators were important evidence supporting this. These discoveries were followed by clinical proof in the 1990s. Immune activation in atherosclerotic plaques was identified [7], and myocardial infarction was recognized as a potent trigger of CRP release [8]. Since then, an extensive number of animal as well as clinical studies have established inflammation as a major driver of atherosclerotic disease. Regardless of this acceptance, our understanding of atherogenic inflammation is far from complete.

2. Inflammatory mediators in atherosclerosis

The evidence for atherosclerosis as an inflammatory disease is solid. The importance of immune activation in atherosclerosis is demonstrated in several animal models, were removal of central inflammatory mediators or cell types have been shown to extensively reduce plaque development [9–11]. Further, both communicable and noncommunicable inflammatory conditions increase the risk of cardiovascular disease (CVD), and CRP is an independent risk factor of cardiovascular events, both in healthy individuals and in patients with established disease [12–14]. Moreover, immune cells are present within all atherosclerotic plaques, from early fatty streaks to complex atheromas. Lesional inflammation increases during the course of plaque development and is most prominent in vulnerable plaques with large necrotic cores. Immune cells, but also smooth muscle cells, platelets and endothelial cells are drivers of plaque inflammation. Further, there are numerous different inflammatory triggers contributing to the great complexity of atherosclerotic plaque inflammation.

2.1. Immune cells in atherosclerotic inflammation

2.1.1. Macrophages: linking lipid metabolism and inflammation in atherogenesis

Macrophages are involved in all stages of plaque development, and are the most important immune cell in atherogenesis. Monocytes originate from a common stem cell in the bone marrow and migrate to various tissues where they develop into tissue-specific macrophages. Although circulating monocytes are a heterogeneous population, it is not known if distinct monocyte subtypes develop into specific macrophage subtypes in humans. Monocytes from the circulation are recruited to the intimal layer of an artery by chemokines (e.g., CCL2) and neuronal guidance molecules (e.g., ephrin-B2). Inside the plaque, the monocytes differentiate to macrophages and engulf modified lipids through scavenger receptors such as SR-A1 and CD36. These lipid-filled macrophages, called foam cells, have altered phenotype and immune function. The efferocytotic capacity (ability to clear apoptotic cells) is one of the functions affected, and as extensive cholesterol accumulation is also lethal to the cells, a necrotic core consisting of cell debris and lipids forms inside the lesion. Plaques with large necrotic cores are associated with an unstable plaque phenotype and are prone to rupture. Monocyte infiltration and foam cell formation are key elements in plaque development and provide the main link between lipid metabolism and

chronic inflammation. There is, however, not only the quantity but also the phenotype of the macrophages that is important to the fate of the plaque [15]. The terms M1 and M2 describe the "classical" activated macrophage induced by T helper cell (Th) 1 cytokine interferon (IFN) γ and the "alternatively" activated macrophage induced by Th2 cytokines IL-13 and IL-4, respectively. In short, the M1 macrophages produce pro-inflammatory cytokines and chemokines, cause tissue injury and promote atherosclerotic plaque development. M2 macrophages are often divided into "wound healing" and "regulatory" macrophages, the latter induced by immune complexes and IL-10, and produce anti-inflammatory cytokines and increase plaque stability [16]. Their pro- and antiatherogenic role is supported by studies showing that plaques enriched in M2 macrophages are associated with a stable or regressive phenotype and vice versa. Growth factors, lipids and cytokines produced by vascular cells and immune cells in the plaque affect the macrophage polarization state. Due to the complexity of inflammatory stimuli present in the plaque, the terms "M1" and "M2" and "classical" and "alternative" are overly simplified, and it is more likely that there exists a range of overlapping phenotypes in the atherosclerotic lesions [15, 17–19].

2.1.2. Dendritic cells are professional antigen presenting cells in the plaque

Another cell of the innate immune system, with great importance for atherosclerotic plaque inflammation, is the dendritic cell (DC). Increased number of DCs is present in atherosclerotic plaques of both humans and mice, and also, as described later, in tertiary lymphoid organs in the adventitia. However, the circulating number of DCs has, by the majority of studies performed, been reported to be reduced in atherosclerosis, which could reflect hampered production from the bone marrow as well as increased recruitment to the plaque [20–22]. As macrophages, the DCs engulf lipids and become foam cells, thereby contributing to plaque development. On the contrary, it has also been suggested that DCs can control cholesterol homeostasis and counteract hypercholesterolemia. It is, however, their role as antigen presenting cells (APCs) that is most described in plaque inflammation [22]. Antigen presentation to T cells occurs both inside the plaque and in the lymphatic tissue, and it is shown that DCs can leave the atherosclerotic lesion upon signals from the chemokines CCL19 and CCL21 [23]. The different subgroups of DCs activate pro- and anti-inflammatory functions in T cells. Difficulties in finding DC-specific markers, as well as the broad spectrum of different DC cell subtypes, have complicated the study of DCs in atherogenesis. There is, however, without doubt that DCs are important players in atherosclerotic disease [22, 24].

2.1.3. Other innate immune cells in atherogenesis

Neutrophils, mast cells and innate lymphoid cells, such as natural killer (NK) cells, are also important contributors to inflammation in atherogenesis, and their role is increasingly appreciated. The description of these cell types is beyond the scope of this chapter, but has been reviewed elsewhere [25–28].

2.1.4. T-cell diversity in atherogenesis

CD4+ Th cells are the most abundant of the adaptive immune cells in the plaque and are therefore the most studied. In the plaque, they are activated by epitopes of native as well as oxidative

LDL presented by antigen-presenting cells (i.e., DCs). Activated T cells can affect atherosclerosis in two ways: through effector functions in the arterial wall and by activating B cells in lymphoid organs to produce circulating antibodies [29]. For CD4+ Th cells, several subsets have been identified. Most is known about the role of Th1 and Th2 in atherosclerosis; however, in recent years, it has become evident that Th17 and Tregs also are important players in atherogenesis. Polarization of Th cells is determined by the cytokine environment, and the proinflammatory Th1 cells are the most abundant T cell in the plaques. Th1 is characterized by secretion of IFN-γ, and Th2 typically secretes IL-4, IL-5 and IL-13. Th17 secretes IL-17 and IL-22, and Tregs secrete IL-10 and transforming growth factor (TGF)-β. In short, the Th1 cells are proatherogenic, while Tregs are atheroprotective. The impact of Th2, Th17 and natural killer T cells (NKTs) on atherosclerosis has shown more conflicting results, but are all present in the plaque. CD8+ cytotoxic T cells are also present in atherosclerotic lesions, although less frequent than CD4+ effector cells. Their activation and importance in atherosclerosis is not completely understood, but they can exert proatherogenic effects through IFN-γ production and macrophage activation or through their cytotoxic activity. Recently, the $CD8^+$ regulatory T cell was described, with possible atheroprotective effects, through modulatory effects on T cell–B cell interaction [30, 31].

2.1.5. B cells and atherogenic antibodies

B cells are divided into two subtypes: B1 and B2 cells, and both of these are involved in atherogenesis. B1 cells are involved in innate humoral immune response, divide upon self-renewal in the periphery and produce antibodies with low specificity. In contrast, B2 cells are conventional B cells, which differentiate to plasma cells upon antigen presentation by T cells and DCs in lymph nodes, producing antibodies with high affinity and thereby contribute in adaptive immunity [30, 32]. Several animal models with B cell depletion resulting in aggravation of atherosclerosis have suggested a protective role for B cells in atherogenesis [33, 34]. Specific depletion of B2 cells has, however, been shown to reduce the development of atherosclerosis, suggesting subset specificity with regard to B cell atherogenity [35, 36]. In contrast to B2 cells which are mainly proatherogenic, producing IgG antibodies and activating T cells, B1 cells produce IgM antibodies, which can bind and thereby block the uptake of oxLDL by macrophages, exerting atheroprotective effects [37]. Most of these studies are performed in animal models, and thus, the importance for B cells in human atherosclerosis is unclear. In contrast to macrophages and T cells, B cells are only found in some atherosclerotic plaques, and a more abundant in so-called tertiary lymph organs, in the adventitial layer of the artery.

2.2. Tertiary lymphoid organs: extended plaque inflammation

During chronic inflammatory conditions, lymph-node–like structures, termed tertiary lymphoid organs (TLOs), can develop. Immune cells in the adventitial layer of atherosclerotic arteries were discovered decades ago [38], but the importance of these TLOs for atherosclerotic plaque inflammation is still unknown. Advanced plaques are, however, associated with increased adventitial inflammation in both humans [39, 40] and $Apoe^{-/-}$ mice [41], suggesting that such extended plaque inflammation is important in the disease process. They likely evolve as a response to arterial wall inflammation in early lesion development. Medial SMCs are suggested as drivers of TLO development and are upon inflammatory stimuli shown to attract immune cells into

adventitia by production of the lymphorganogenic chemokines CXCL13 and CCL21 [42, 43]. The TLOs have a different composition of immune cells than the macrophage-rich plaques and is mostly composed of dendritic cells, T cells and a high number and diversity of B cells [43, 44]. This supports the role of TLOs as sites for T-cell training [45] and activation of local humoral immune responses [37]. A recent paper suggests that TLOs participate in atheroprotection [45], however, as the plaque itself, the TLOs can harbor both pro- and anti-inflammatory mediators, and thus, the net effect of adventitial inflammation is still elusive [46].

2.3. Inflammatory mechanisms of nonimmune cells in atherosclerosis

Plaque development does not occur randomly, but typically at curvatures and branching points in the arteries. At these sights, the shear stress activates the endothelial cells lining the arterial wall, leading to structural, molecular and functional alterations in the cells. Atheroprone flow activates the Nf-Kβ pathway and TLR2 expression in endothelial cells as well as a spectrum of other conduits leading to increased endothelial proliferation and inflammation. The activated endothelial cells adhere leukocytes and stimulate neighboring cells, e.g. vascular smooth muscle cells (VSMCs) [47–49]. Upon atherogenic stimuli, that is, from the activated endothelium, VSMCs undergo so-called phenotype switching. They progress from quiescent contractile to proliferative and migratory cells. These cells possess atheroprotective functions, as they produce extracellular matrix and proteoglycans, which protects the plaque from rupture. They do, however, also accumulate lipids and become macrophage-like foam cells, contributing to plaque development [50, 51]. Further, they express adhesion molecules such as VCAM-1 and ICAM-1 and thereby contribute to retention of monocytes and macrophages in the lesions [52, 53]. Thus, VSMCs can have both protective and destructive effects, depending on the stage of plaque development, and the stimuli present. Inflammatory monocytes can further stimulate VSMCs to secrete pro-atherogenic matrix metalloproteinases (MMPs), which increase the risk of plaque rupture through thinning of the fibrous cap [54]. Further, VSMCs produce a variety of cytokines, activating immune cells, endothelial cells and other VSMCs in the lesion [51]. The inflammatory, atheroprone contribution of VSMCs is however probably under-communicated, as lack of cell-specific markers complicates their identification. Macrophages can express "classical" smooth muscle cell markers (i.e., α-actin and SM22α), and vice versa (i.e., CD68 and Mac2), and this is determined by the presence of lipids and inflammatory stimuli in the plaque [55, 56]. The local inflammatory micro milieu will therefore decide the inflammatory contribution of smooth muscle cells to atherogenesis by regulating the transition of VSMC into inflammatory cells. Thus, there is a need for better markers to more correctly determine the role of VSMCs in atherosclerotic inflammation.

Also nonimmune cells in the circulation can contribute to the inflammatory milieu during atherogenesis. In addition to their most known roles as blood clotting cells, platelets also possess a great inflammatory potential. In a bidirectional manner, platelets interact with both leukocytes and endothelial cells to communicate inflammation. They express a variety of inflammatory mediators and receptors and contribute to atherosclerotic inflammation throughout disease development, from development of fatty streaks to thrombus formation. For an extensive review of the role of platelets in atherogenesis, see [57].

3. Inflammasome activation: a central inflammatory driver of atherosclerosis

Inflammasomes are intracellular immune sensors, which are tightly controlled. They assemble upon stimuli from tissue damage, infection or metabolic disturbances, and their activation results in production of the pro-inflammatory cytokines interleukin (IL)-1β and IL-18. There are several different inflammasomes, but the *NOD-like receptor containing a pyrin domain 3* (NLRP3) inflammasome is the most described and is an important constituent of innate immune apparatus. The NLRP3 inflammasome is a multimeric protein complex, which upon activation assembles and attracts caspase-1 molecules, which then is activated by self-cleavage. Active caspase-1 cleaves pro-IL-1β and pro-IL-18 to active cytokines ready for secretion [58, 59]. IL-1β is a prototypical proatherogenic cytokine, and NLRP3 is thus an important contributor to atherosclerotic inflammation. Cholesterol crystals, which deposit in atherosclerotic lesions, can activate the inflammasome both in vitro and in vivo [60, 61]. Further, the nonlipid danger signal ATP stimulates foam cell formation and cell migration through inflammasome activation [62]. Thus, the inflammasome promotes atherogenesis through inflammatory, as well as noninflammatory pathways, induced by lipid- as well as nonlipid stimuli. The NLRP3 inflammasome is present and activate in human atherosclerotic plaque [61]. Further, LDL receptor (LDLR)-deficient mice which received bone marrow from NLRP3-deficient mice show attenuated atherosclerosis, and silencing of NLRP3 in ApoE-deficient mice stabilizes atherosclerotic plaques, pointing to an important role in atherosclerotic disease development [60, 63].

4. Danger signals in atherosclerosis

Inflammation is a part of the body's response to harm, either from microbes, such as virus and bacteria, from burns or toxins, or from injury. The main function is to eliminate the insult, remove damaged tissue and restore tissue homeostasis. In atherosclerosis, the signals of harm, termed *triggers*, are numerous. In contrast to infectious disease, the most typical triggers in atherogenesis are however sterile. These are termed damage-associated molecular patterns (DAMPs) and are host-derived danger signals released upon tissue damage, metabolic disturbances, or environmental stress. The risk factors of CVD include hyperlipidemia, smoking, hypertension and hyperglycemia, and all these factors cause DAMPs. There is, however, also evidence supporting a role for pathogens in atherosclerosis. These are termed pathogen-associated molecular patterns (PAMPs). Bacterial and viral microbes are found in atherosclerotic plaques and are associated with disease risk [64, 65]. In addition to pathogens, gut microbiota is a potential source of PAMPs, also linked to atherogenesis [66]. The causal relationship between the endogenous DAMPs and atherosclerosis is stronger than for PAMPs. Microbes do not seem to be required for atherogenesis, as germ-free mice are not protected against disease [67]. The DAMPs comprehend the necessary evil of atherogenesis, namely lipids. As mentioned, the interaction between lipids and immune activation is the hallmark of atherosclerotic disease. Nonmodified fatty acids can activate immune responses, and while saturated fats are shown to stimulate inflammation, polyunsaturated fats are repressors [68]. It is, however, the modified lipids that are the typical triggers during atherogenesis. In hyperlipidemia, LDL undergoes oxidation, forming oxidation-specific

epitopes (OSEs), an important class of DAMPs in atherosclerosis [65, 69]. Cholesterol saturation inside the plaques leads to the formation of cholesterol crystals, which are important activators of the NLRP3 inflammasome (see Section 3) [60]. Other crystal structures can also serve as DAMPs, such as monosodium urate (MSU) crystals, which are composed of crystalized uric acid that contributes to the increased risk of atherosclerosis in patients with gout [70]. Moreover, lipids, nucleic acids and proteins can be modified in the presence of sugars, forming advanced glycation end products (AGEs), which activate immune responses through specialized receptors. These DAMPs are especially prevalent in diabetic subjects, promoting atherosclerosis through vascular dysfunction and increased inflammation [71, 72].

Necrotic cores of complex lesions are huge sources of inflammatory stimuli. In contrast to apoptosis, which is silent, necrosis and pyroptosis activate innate immune responses through the release of DAMPs such as heat shock proteins, nucleic acids, uric acid and ATP [65, 73, 74]. Further, as immune cells accumulate and the plaque develops, the demand for oxygen exceeds the availability, leading to hypoxic conditions. Hypoxia can activate the NLRP3 inflammasome and stimulate the polarization of M1 macrophages, causing increased inflammation in the plaques [75, 76]. Further, as mentioned, mechanical stress in the artery wall can also be a trigger of inflammation by stimulating endothelial activation, with subsequent activation of immune cells and VSMC in the artery wall.

The presence of risk factors provides continuous production of triggers, resulting in defective rescue mechanisms and persistent immune stimulation. Without relieve of these stimuli, a nonresolving inflammation develops, which is a hallmark of atherogenesis.

5. Defective resolution in atherosclerosis

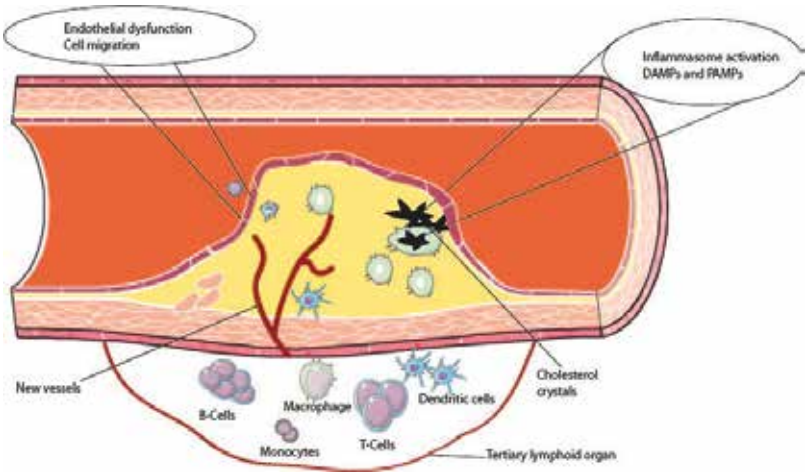
Inflammation is a beneficial process; however, it becomes detrimental if the response is too strong or too long. Cessation of inflammation was previously thought of as a passive process; however, it is now known that resolution of inflammation is a highly active process, involving a complex network of mediators. For inflammation to stay homeostatic, these mechanisms need to be intact. Atherosclerosis is characterized by a chronic nonresolving low-grade inflammation. Thus, a defective resolution of inflammation is an important contributor to atherosclerotic development and sustainability. Resolution is driven by endogenous specialized lipid-derived mediators (SPMs), which are synthesized from fatty acids, as well as proteins such as IL-10, M1 macrophages and the nucleotides inosine and adenosine. These stimulate tissue repair and regeneration and can therefore be distinguished from the classical anti-inflammatory signals, which are merely antagonists of pro-inflammatory signals. In a normal immune response, the production of SPMs is initiated by the production of the pro-inflammatory prostaglandins. Defective resolution in atherosclerosis can be summarized in three processes: (1) sustained inflammation, (2) increased infiltration/reduced egress of immune cells and (3) defective efferocytosis. In early atherosclerotic plaques, the efferocytotic capacity of macrophages (ability to clear apoptotic cells) is sufficient. Thus, inflammatory cells are cleared from the lesion, and this process elicits the release of anti-inflammatory mediators that counteract the plaque development. In advanced plaques, the efferocytotic

capacity is however, as mentioned, compromised, leading to reduced clearance of dead cells, secondary necrosis and stimulation of the pro-inflammatory environment and growth of the necrotic core. SPMs are shown to counteract these processes and stabilize plaques [73, 77]. However, in advanced atherosclerotic lesions, the ratio of SPMs to pro-inflammatory mediators is decreased, and the administration of SPMs counteracts atherosclerotic disease development. These findings provide a mechanistic explanation for the defective resolution observed in atherosclerotic disease [78, 79]. To further map the production, regulation and function of pro-resolving mediators in atherosclerosis will be of great importance to increase our understanding of how the inflammation in atherosclerosis becomes nonresolving.

6. Inflammation: a therapeutic target in atherosclerotic disease

Despite the great success of modern treatment modalities, atherosclerosis is still the leading cause of mortality and morbidity worldwide. For example, high-dose statin treatment and other standard measures only prevent a fraction of recurrent events in survivors of MI. This residual burden of events presents a pressing unmet medical need, and novel perspective on atherogenesis is needed to treat those who are not met by the current treatment regimes. An interesting new therapeutics is the enzyme proprotein convertase subtilisin-kexin type 9 (PCSK9) inhibitors. PCSK9 binds to the hepatic low-density lipoprotein (LDL) receptor, thereby inhibiting recycling of this receptor, resulting in attenuated removal of LDL cholesterol (LDL-C) from the circulation. The importance of PCSK9 for LDL-C homeostasis is illustrated in individuals with loss- and gain-of function mutations in this enzyme leading to hypo- or hypercholesterolemia, respectively, with dramatic effects on the incidence of atherosclerotic disease. Recent studies have shown that anti-PCSK9 therapies markedly reduce LDL-C levels, leading to lower incidence of adverse cardiovascular disease (CVD) outcomes in high-risk patients with hyperlipidemia [80]. In patients with LDL levels that remain above the treatment target, despite statin treatment (residual LDL risk); adding a PCSK9 inhibitor should be considered. Recent network meta-analysis demonstrates that PCSK9 inhibitors significantly reduce LDL cholesterol, on top of medium to high statin therapy [81]. Of direct relevance for inflammation, a very recent study, the CANTOS trial, suggests that those with residual inflammatory risk could benefit from interleukin-1β inhibition by Canakinumab. This anti-inflammatory treatment resulted in reduced cardiovascular risk, independent of lipid-lowering effects [82]. Another exciting approach to target inflammation in atherosclerosis is the pro-resolving mediators SPMs. In contrast to anti-inflammatory agents, these ligands will not compromise host defense, one of the most important challenges of immunosuppressive therapeutics. Many experimental studies have shown therapeutic potential for SPMs; however, more knowledge is needed to pinpoint how these mediators act, before these findings can be translated into clinical use [83].

In sum, atherosclerosis is characterized by low-grade chronic inflammation in the arteries, in tight interplay with lipids. Targeting both pathways, depending on individual risk analysis, might be the future of prevention and treatment of cardiovascular disease. However, there is still a need for tools to identify people at risk, especially for personalized treatment. There is also a need to evolve more precise targets for treatment.



Atherosclerotic plaque inflammation

Inflammation is involved in all stages of plaque development. Endothelial dysfunction allows entry of lipoproteins and migration of inflammatory cells into the intimal layer of the artery. Inside the plaque the cells are activated by PAMPs and DAMPs. Cholesterol crystals are important DAMPs which can activate the NLRP3 inflammasome and stimulate release of inflammatory cytokines. Further, accumulation of large amounts of lipids in the immune cells can lead to extensive cell death, and a necrotic core develops due to dysfunctional clearance of these cells. The necrotic core maintains the nonresolving inflammatory milieu in the lesion and is a typical feature of advanced plaques. Moreover, tertiary lymphoid organs can form in the adventitial layer of the vessel wall and feed the plaque with inflammatory cells and mediators, and can further contribute to plaque inflammation. The authors wish to acknowledge Sverre Holm for making the illustration and SERVIER Medical Art (www.servier.fr) for use of their medical art kits.

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Chapter 4

Effects of Nicotine Contained in Tobacco Mainstream Smoke on Vascular Smooth Muscle Cells

Akio Nakamura

Additional information is available at the end of the chapter

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Abstract

Cigarette smoking is a known risk factor for arteriosclerosis. In atheromatous plaques, the accumulation of vascular smooth muscle cells (VSMCs) have a phenotype differing from that of their normal contractile type. Nicotine is a major pharmacological agent in cigarette smoke. However, any direct effect of nicotine on VSMCs remains uncertain. We investigated the changes in the expression levels of differentiation markers and activity of mitogen-activated protein kinases (MAPKs) after nicotine exposure for 48 h using human aorta primary smooth muscle cells (HVSMC) differentiated with transforming growth factor-β. The results indicated that HVSMC phenotype changed to a synthetic-like phenotype after nicotine exposure. Nicotine is a factor that can change the expression of differentiation marker proteins in VSMCs. Thus, we proposed that nicotine directly affects the migration of VSMCs from the tunica media to atheromatous plaques in the vascular intima by inducing the transformation from a contractile-type to a synthetic-like type, which occurs before the development of atheromatous plaques. Nicotine is contained in nicotine patches and gums for smoking cessation. There may also promote atheromatous plaque formation. We anticipate that determining this mechanism will lead to new means of preventing and treating plaque formation and development in arteriosclerosis.

Keywords: nicotine, vascular smooth muscle, cell migration, proliferation, cigarette smoke

1. Introduction

According to the World Health Organization (WHO) World Health Statistics 2016, the world's highest cigarette smoking rates were 76.2% for men in Indonesia, and 52.0% for women in Nauru. Ranked second place was Jordan for men (70.2%), and Kiribati for women (40.9%),

third place was Kiribati for men (63.9%) and Serbia for women (39.7%). As of 2015, the gender smoking ratio was estimated as 33.7% men and 10.6% women in Japan [1]. Globally, an estimated 93.3 million people smoke, the majority of whom reside in developing countries, where smoking rates are estimated to be as high as 50% for men. It has been shown that men tend to use all tobacco products at a higher rate than women [2]. Atherosclerosis is more common in men than women [3]. It may be derived from this that men have more arteriosclerotic diseases.

Smoking, as well as second-hand smoke, induces circulatory diseases, heart attacks, strokes, cancers, and respiratory diseases [4–7]. Several studies have suggested that cigarette smoke has 7357 chemical compounds from different classes [8]. Nicotine is the most predominant alkaloid (approximately 90–95%), found in the tobacco plant, *Nicotiana tabacum* [9–10]. Plasma nicotine levels have been reported as 4–30 ng/ml after smoking a cigarette, 8–10 ng/ml after chewing nicotine gum, and 22 ng/ml after smoking a pipe [11, 12]. Nicotine is a toxic compound that should be handled with care, as it has been reported that more than 0.5 g of oral nicotine is required to kill an adult [13, 14].

Epidemiological studies show that cigarette smoking has long been known as a major risk factor for atherosclerosis [15–18]. In particular, nicotine in the cigarette smoke promotes atherogenesis [17–20]. However, little is known about the mechanism by which nicotine induces arteriosclerosis. Atherosclerosis is a specific form of arteriosclerosis in which an artery wall

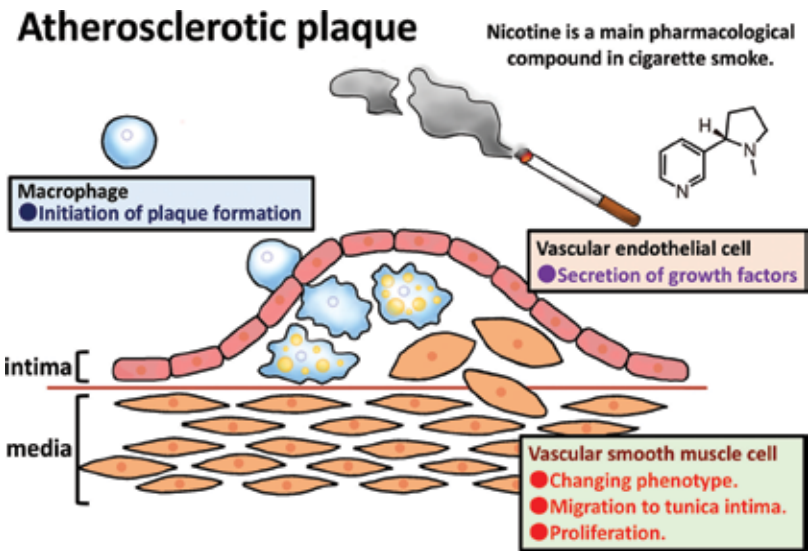

Figure 1. Atherosclerotic plaque is due to the cooperation of three types of cells. The first one is macrophages. Their invasion into tunica intima preludes the formation of plaque. The second is endothelial cells. It secretes some growth factors which affects the VSMC. The third is VSMCs. Under the stimulation, their phenotype changes before migrating to tunica intima and proliferating there. This phenotypic change is referred to as contractile-to-synthetic-type transition and it contributes to the development of plaque. This phenotypic change could be induced by different kinds of stimuli. Nicotine is one of them. Nicotine is a main pharmacological compound in cigarette smoke. It is reported to promote cell migration of rat and human VSMCs. However, little is known about whether nicotine promotes the phenotypic change of human VSMC.

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thickens as the result of invasion, accumulation of white blood cells and fatty materials such as foam cells and cholesterol [22, 23] (**Figure 1**). It has also been known that accumulation of vascular smooth muscle cells (VSMCs) can be observed in atherosclerotic lesions (**Figure 1**). The proliferation of VSMCs with the subsequent formation of intimal thickening is a major event in the development of atherosclerotic lesions [24, 25] (**Figure 1**). Normally, the differentiated VSMCs constitute the tunica media of the artery and are responsible for the vasoconstriction function. However, why the VSMCs accumulate during arteriosclerotic plaque formation is not well understood (**Figure 1**).

In this chapter, we describe that nicotine in tobacco mainstream smoke causes dedifferentiation of VSMCs to migration-proliferation types via nicotinic acetylcholine receptors (nAChRs) expressed in the VSMCs, which is a cause of arteriosclerotic plaque formation.

2. The nicotinic acetylcholine receptors (nAChRs) on VSMCs

nAChRs are transmembrane ligand-gated ion channels expressed in the cell membrane of all mammalian cells, and their endogenous ligand is acetylcholine [26]. We were the first to report that nAChRs were expressed on VSMCs [27]. Also, we found that nicotine promotes cell migration of VSMCs GbaSM-4 cells isolated from basilar arteries of guinea pigs, and this cell migration is inhibited by methyllycaconitine, an antagonist of nAChRs [27]. That was the first report on the effect of nAChRs on VSMCs [27]. In subsequent studies of other groups, it was reported that nicotine promoted the chemotaxis and migration of VSMCs isolated from rats and humans [28, 29]. Thereafter, various types of nAChRs have been discovered and reported by real-time qPCR, Western blots, etc. in several tissues [30, 31].

We exposed cell line AC01 cells derived from mouse aortic smooth muscle to 0.1 μM nicotine [32], and performed exhaustive gene expression analysis using DNA microarray for gene expression after 48 h. As a result of whole gene expression analysis, α1, α2, α6, α7, α9, β1, β2, β4, δ, ε, and γ subunits of nAChRs were detected in AC01 cells (**Figure 2A**). After AC01 cells were exposed to nicotine for 48 h, a change was observed in the ratio of the fluorescence intensity of cy3 / cy5 indicating the amount of transcription of mRNA for each subunit. As a result, the α1, α6, α7, β2, and δ subunits increased by 2.6, 2.3, 2.4, 2.0, and 3.1 times, respectively, compared to the control (**Figure 2**).

Furthermore, we measured the expression levels of nAChRs in human vascular smooth muscle cells (HVSMCs) using real-time qPCR. As a result, α2, α6, α7, and β1 subunits of the nAChRs were detected. The expression level of the α2 subunit was relatively low, and it disappeared within 72 h of nicotine exposure. The expression level of the α6 subunit increased with time, to about 20-fold after 72 h as compared with the control (0 h). The α7 subunit was the most frequently expressed in the HVSMCs. The expression level of the β1 subunit was in trace amounts, and from this result, there was no clear influence of exposure to nicotine. Thus, it was discovered that nAChRs were expressed in response to nicotine in HVSMCs [33].

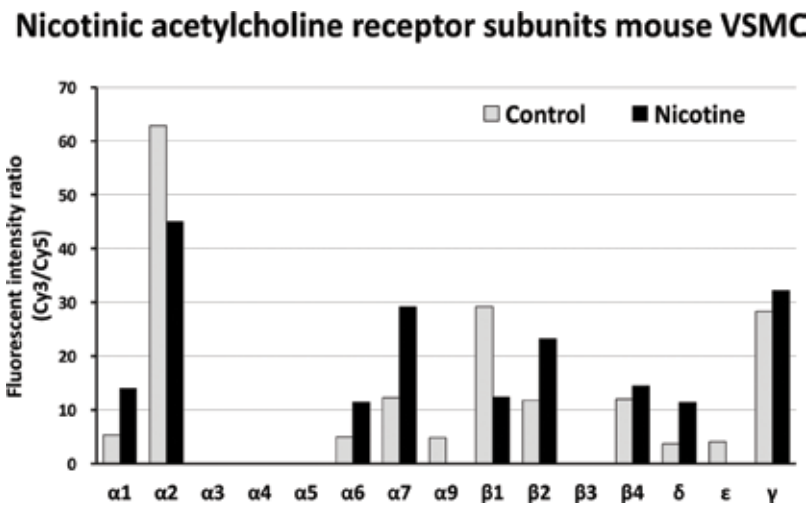

Figure 2. The mRNA levels of nAChR subunits in AC01 cells of mouse VSMC. AC01 cells were exposed to 0.1 μM of nicotine or not exposed for 48 h. Each sample was labeled with Cy3 (nicotine-treated cells) and Cy5 (non-treated cells), resulting in differently labeled samples. The labeled mixture of both samples was applied onto a 3D-gene™ mouse Oligo chip 25 K (Toray Industries, Tokyo, Japan), competitively hybridized, and washed. Scanned images were analyzed using GenePix Pro (MDS Analytical Technologies, Sunnyvale, CA, USA). All analyzed data were scaled by global normalization.

3. Remodeling of vascular smooth muscle by nicotine

Nicotine did not induce any significant changes on the relaxation of tension in isolated VSMCs, despite its effects on the cardiovascular system [34]. However, Carty et al. proposed that nicotine was a mitogenic agent for VSMCs [35]. Previous studies, including our studies, reported that nicotine promotes the chemotaxis and migration of mammalian VSMCs [28, 29]. In addition, we reported that GBaSM-4 cells were promoted in their migratory ability after chronic exposure to nicotine [36]. Normally, the differentiated VSMC have contractile function, but do not migrate or proliferate. VSMC which migrate and proliferate and differentiated VSMCs which on exposure to nicotine start migrating and proliferating in the atherosclerotic plaque of patients with arteriosclerotic disease are different in phenotype from the contractile type VSMC. Apparently, nicotine has the effect of changing VSMCs from differentiated to dedifferentiated type, that is transformation from the contractile-type to the synthetic-like (proliferative) type.

Therefore, we examined the gene and protein expression after exposing HVSMCs to nicotine using human DNA microarrays, real time qPCR, and Western blots [33]. To the best of our knowledge, our study is the first to investigate the possibility that nicotine exposure for 48 h could induce a phenotypic change in HVSMCs (**Figures 3** and **4**).

Myosin II of motor proteins plays important roles in the contraction for smooth muscles and cell migration of non-muscle cells [37–41]. Myosin II isoform 11 is expressed in the contractile type of smooth muscle cells [42]. Myosin II isoform 10 is expressed during fetal development, as a synthetic-like non-muscle isoform [42]. Thus, the expression of myosin II isoforms 11

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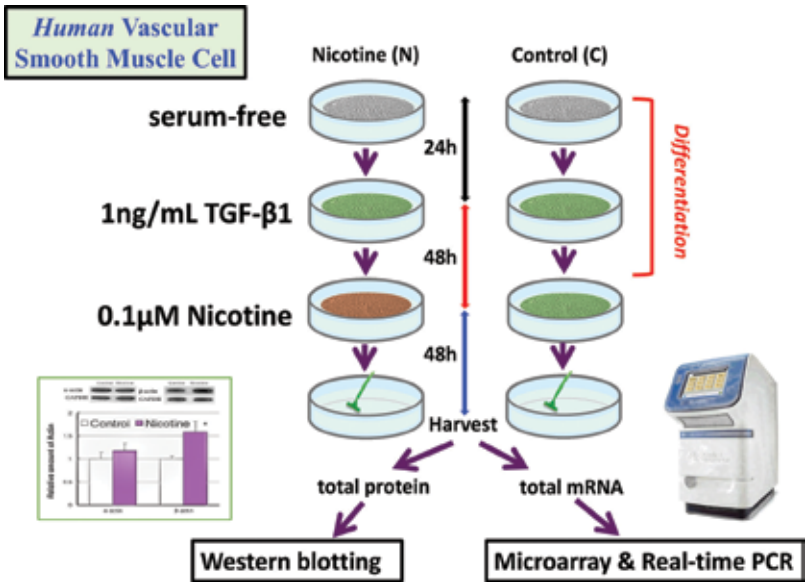

Figure 3. Primary human aorta smooth muscle cells were cultured under the differentiated condition. Upon reaching confluence, the cells were deprived of serum for 24 h. The differentiation was induced by TGF-β1 for 48 h. The cells of the nicotine group were then exposed to 0.1 μM of nicotine. In another 48 h, the total RNAs and proteins were purified for qPCR and immunoblotting, respectively.

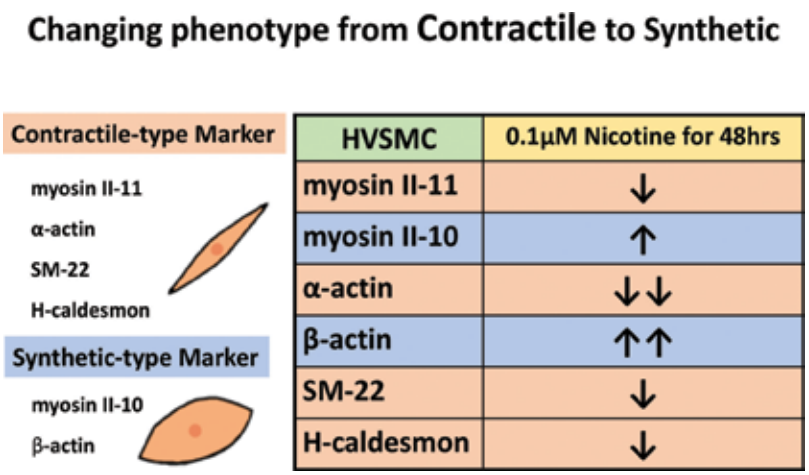

Figure 4. The change in mRNA levels upon nicotine exposure. HVSMCs induced by TGF-β were exposed to 0.1 μM of nicotine. The total RNA was extracted 48 h after exposure, and the cDNA corresponding to each time point of the cells were synthesized. Each gene was inspected using real-time PCR. The mRNA level of myosin II isoform 11, α-actin, SM-22, and H-caldesmon decreased 48 h after exposure to nicotine. On the contrary, the mRNA level of myosin II isoform 10 and β-actin increased after exposure of nicotine.

and 10 are indicative of the contractile and non-muscle (proliferative) types, respectively. In our study, myosin II isoform 11 mRNA level decreased by approximately 0.8-fold, 48 h after HVSMCs exposure to nicotine. In comparison, myosin II isoform 10 mRNA level increased in

a time-dependent manner to approximately 3-fold after 48 h [33]. During Western blot experiments using specific antibodies against each of the marker proteins, the protein expression of myosin II isoform 10 increased after 48 h exposure to nicotine. The amount of myosin II isoform 11 decreased by approximately 0.6-fold after the 48-h nicotine exposure. The myosin II isoform 10 level was increased to about 1.2-fold after exposure to nicotine [33]. These results indicated that the isoforms of myosin II had changed to the non-muscle (proliferative) type from the smooth muscle contractile type because of nicotine exposure (**Figure 4**).

Subsequently, α-actin and β-actin were used as contractile-type and synthetic-like type marker genes, respectively [43]. After exposure of HVSMCs to nicotine, the α-actin mRNA level decreased by approximately 0.4-fold, whereas, the β-actin mRNA level increased to approximately 1.7-fold after 48 h, respectively [33]. Using Western blot experiments, the protein expression of α-actin levels did not significantly change. In contrast, β-actin levels significantly increased to approximately 1.6-fold after the nicotine exposure [33]. These results indicated that the actin isoform also changed to the synthetic-like type from the contractile-type after nicotine exposure (**Figure 4**).

SM22 and high-molecular-weight caldesmon (H-caldesmon) are major smooth muscle differentiation markers [44, 45]. The SM22 mRNA level decreased by approximately 0.9-fold after the 48-h exposure of HVSMCs to nicotine. The mRNA level of the H-caldesmon, a smooth muscle contractile-type marker protein, was about 0.7-fold after 48 h [33]. Using Western blot experiments, H-caldesmon and SM22 levels, significantly decreased by approximately 0.4- and 0.7-fold, respectively after nicotine exposure [33]. The decreased H-caldesmon and SM22 expression levels also indicated the transformation to the synthetic-like type from the contractile-type after nicotine exposure (**Figure 4**).

Notch receptors are intimately involved in HVSMC differentiation. Activation of Notch receptors by cell-cell adhesion induces the expression differentiation marker proteins of contractile-type on smooth muscles [46]. However, when HVSMCs at 100% confluence were exposed to nicotine in our study, the expression of Notch receptors did not increase [33]. This indicated that nicotine had suppressed the expression and function of the Notch receptors.

Mitogen-activated protein kinases (MAPKs) play an important role in cell proliferation and migration [46, 47]. MAPKs are also intimately involved in VSMC growth and migration [48, 49]. It has been reported that nicotine induces the production of growth factors such as vascular endothelial growth factor (VEGF), Platelet-derived growth factor (PDGF-BB), and Fibroblast growth factor (FGF-2) from VSMCs, and that PDGF-BB and FGF-2 promoted the proliferation of VSMCs [29, 50–52]. Nicotine-induced VEGF production was mediated by nAChRs via activation of the VEGF and its receptor as well as the extracellular signal-regulated kinase (ERK)1/2 pathway [27]. PDGF-BB caused cytoskeletal protein remodeling, enhanced the proliferation, and migration of VSMCs [51]. In our study, the phosphorylation levels of the p38 MAPK, ERK1/2, and c-jun N-terminal kinase increased after 48 h of nicotine exposure [33]. Activation of MAPKs signaling indicated that the characteristics of VSMCs changed to migration-type cells after nicotine exposure.

Our results suggest that nicotine can decrease the expression of differentiation marker proteins in HVSMCs, and change these cells from the contractile-type to synthetic-like type,

thus, promoting cell migration [33]. Therefore, we considered that nicotine facilitated the formation of intimal lesions characteristic of atherosclerosis. Recently, it was reported that nicotine upregulated the transcription of miR-200b in VSMCs [53]. The miR-200b-mediated down-regulation of Rho-specific guanine nucleotide dissociation inhibitor A facilitated the migration and proliferation of VSMCs in a Rho GTPase-dependent manner [53].

4. New challenges on HVSMC exposure to nicotine

Regarding the influence of nicotine on HVSMCs, a new problem was found during our research. It was about how nicotine works as a signal in HVSMCs. It has been shown that nicotine binds to nAChRs, and opens the ion channels in these receptors to significantly increased intracellular Ca^{2+} levels [54, 55]. We measured the changes in intracellular Ca^{2+} level in HVSMCs upon nicotine stimulation. Our results indicated that nicotine stimulation significantly increased intracellular Ca^{2+} levels in HVSMCs. In addition, mecamylamine, a non-selective nAChR blocker, effectively blocked the nicotine effect in the nicotine-treated HVSMCs. However, mecamylamine did not exhibit complete inhibition of the nicotine stimulation. This suggests that nicotine is involved in intracellular signal transduction through receptors other than nAChRs. From the results of our comprehensive gene analysis, several receptors whose gene expression were increased by nicotine exposure have been discovered. In the future, it would be expedient to clarify the functions of these novel nicotine receptors (**Figure 5**).

Furthermore, the transformation of VSMCs by nicotine shown in our study suggested that nicotine itself promoted arteriosclerosis. In addition to cigarettes, nicotine is also contained in

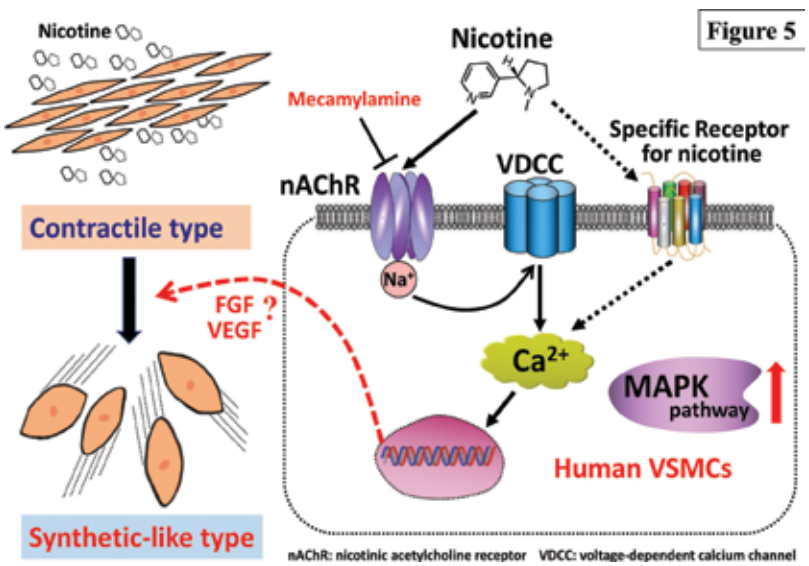

Figure 5. A schematic diagram showing the relationship between nicotine exposure and the phenotypic change in HVSMCs. The solid line arrows indicate an effect based on our results. The break line arrows indicate an effect based on our speculation.

therapeutic nicotine patches and gums used for smoking cessation. Thus, there is a possibility that these nicotine patches or gums promote atheromatous plaque formation. Moreover, smokeless tobacco contains large amounts of sodium, which enhance nicotine absorption [56]. These problems should also be considered sufficiently because nicotine used even during smoking cessation treatment and avoidance of tobacco sidestream smoke induces arteriosclerosis.

5. Conclusion

Several data have widely suggested nicotine as one of the factors responsible for the formation of atheromatous plaques in the vascular intima. Numerous studies so far, including our research, indicate that nicotine induces intracellular Ca^{2+} influx in HVSMCs via nAChRs and possibly via another nicotine-specific receptor. Consequently, HVSMCs are transformed from the contractile-type to the synthetic-like type, which occurs during the development of atheromatous plaques. Aside from cigarettes, nicotine is also contained in nicotine patches and gums used for smoking cessation. Thus, there is a possibility that these nicotine patches or gums promote atheromatous plaque formation. Therefore, we hypothesize that elucidating the mechanism of action of nicotine will lead to new means of preventing and treating atherosclerotic plaque formation and development of arteriosclerosis.

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Chapter 5

Food Restriction and Atherosclerotic Plaque Stabilization

Dorien G. De Munck, Guido R.Y. De Meyer and Wim Martinet

Additional information is available at the end of the chapter

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Abstract

Food restriction is a promising therapy for many age-associated pathologies as it stimulates the health-supportive mechanism autophagy. Because atherosclerosis is an inflammatory, age-related disease, dietary modification can be an important strategy in preventing atherosclerotic plaque development. A cholesterol-supplemented diet, used to induce plaque formation in rabbits, induces a pronounced hypercholesterolemia, which can be reversed after 4 weeks of normal diet. However, food restriction induces a further increase in circulating LDL cholesterol. These elevated cholesterol levels are associated with the induction of autophagy. Although neither a short-term normal diet nor food restriction alters plaque size, rabbits fed a normal diet show signs of increased plaque stability such as elevated collagen content and decreased expression of vascular cell adhesion molecule (VCAM)-1. Surprisingly, these favorable effects are not present after 4 weeks of food restriction. On the contrary, atherosclerotic plaques of food-restricted rabbits displayed enhanced apoptosis, a process known to further undermine plaque stability. In conclusion, severe short-term food restriction in rabbits prevents stabilization of atherosclerotic plaques as observed after regular cholesterol withdrawal via a normal diet.

Keywords: atherosclerosis, plaque stability, food restriction, cholesterol, autophagy

1. Introduction

Atherosclerosis is an inflammatory disease characterized by the formation of plaques in the large- and medium-sized arteries. Despite current pharmacological therapies, atherosclerosis remains the leading cause of death and morbidity among adults in the Western world [1]. Because a diet rich in calories, together with a sedentary lifestyle, contributes to the development

of atherosclerosis, dietary change is considered an important strategy in the prevention of atherosclerosis [2]. Moreover, dietary modification has shown to play an important role in several age-associated pathologies and in aging itself. Moderate calorie restriction results in a lifespan expansion of different species including yeast, fruit flies, nematodes, fish, rodents, and rhesus monkeys [3]. Besides favorable effects on longevity, long-term as well as short-term caloric restriction improves the cardiovascular disease risk profile in humans [4, 5]. Consistent with this finding, animal studies showed that dietary restriction attenuates atherosclerotic plaque development and decreases endothelial dysfunction [6, 7].

Starvation, as an extreme form of food restriction, is also one of the most important stimuli for autophagy induction [8]. Autophagy is a subcellular degradation pathway for long-lived proteins and damaged organelles. Under normal conditions, autophagy is a homeostatic process that is found in all cell types. However, under stress conditions, it functions as an important cell survival mechanism through nutrient recycling and the generation of energy [9]. Growing evidence indicates that autophagy deficiency plays a crucial role in plaque growth and destabilization [10–12]. Moreover, autophagy induction is suggested as a novel strategy for the prevention and treatment of atherosclerosis [13, 14].

2. Food restriction induces hypercholesterolemia

Cholesterol withdrawal by feeding atherosclerotic rabbits a normal diet for 4 weeks significantly reduces LDL cholesterol in serum (**Table 1**). In contrast, cholesterol withdrawal by severe food restriction (only 20% of normal diet) leads to elevated LDL cholesterol levels and a significant loss of bodyweight (**Table 1**), which confirms previous studies showing hypercholesterolemia in healthy subjects after fasting or moderate caloric restriction [15–17] as well as in patients with eating disorders such as anorexia nervosa [18]. Several mechanisms may account for hypercholesterolemia including downregulation of the hepatic LDL receptor leading to decreased LDL uptake in the liver, lipolysis in adipose tissue, or increased cholesterol synthesis [15, 17, 19].

	Weeks	Baseline	Normal diet	Restricted diet
LDL cholesterol (mg/dL)	20	1026 ± 147	589 ± 98	702 ± 13
	24	/	250 ± 105$^{\#\#}$	1101 ± 177$^{\#, ***}$
Triglycerides (mg/dL)	20	92 ± 29	53 ± 13	56 ± 9
	24	/	49 ± 7	44 ± 9
Bodyweight (kg)	20	4.0 ± 0.2	4.3 ± 0.1	4.0 ± 0.1
	24	/	4.4 ± 0.1	3.2 ± 0.1$^{\#\#\#}$, ***

Data are expressed as mean ± SEM.

$^{\#}P < 0.05$.

$^{\#\#}P < 0.01$.

$^{\#\#\#}P < 0.001$ versus 20 weeks (paired sample t-test, n = 10).

$^{***}P < 0.001$ versus normal diet (independent sample t-test, n = 10).

Table 1. Serum lipid values and body weight in cholesterol-fed rabbits (baseline, 20 weeks of cholesterol) followed by dietary lipid lowering for 4 weeks (normal diet) or a restricted diet for 4 weeks (restricted diet).

Despite increased levels of circulating LDL, there is no difference in lipid accumulation in the liver or aorta of rabbits undergoing severe food restriction. Both normal diet and food restriction do not affect serum triglycerides (**Table 1**).

3. Hypercholesterolemia induced by food restriction is associated with autophagy induction

LDL cholesterol levels are negatively correlated with SQSTM1/p62 protein levels in the liver (**Figure 1**), suggesting stimulation of autophagy as an alternative mechanism for the increase

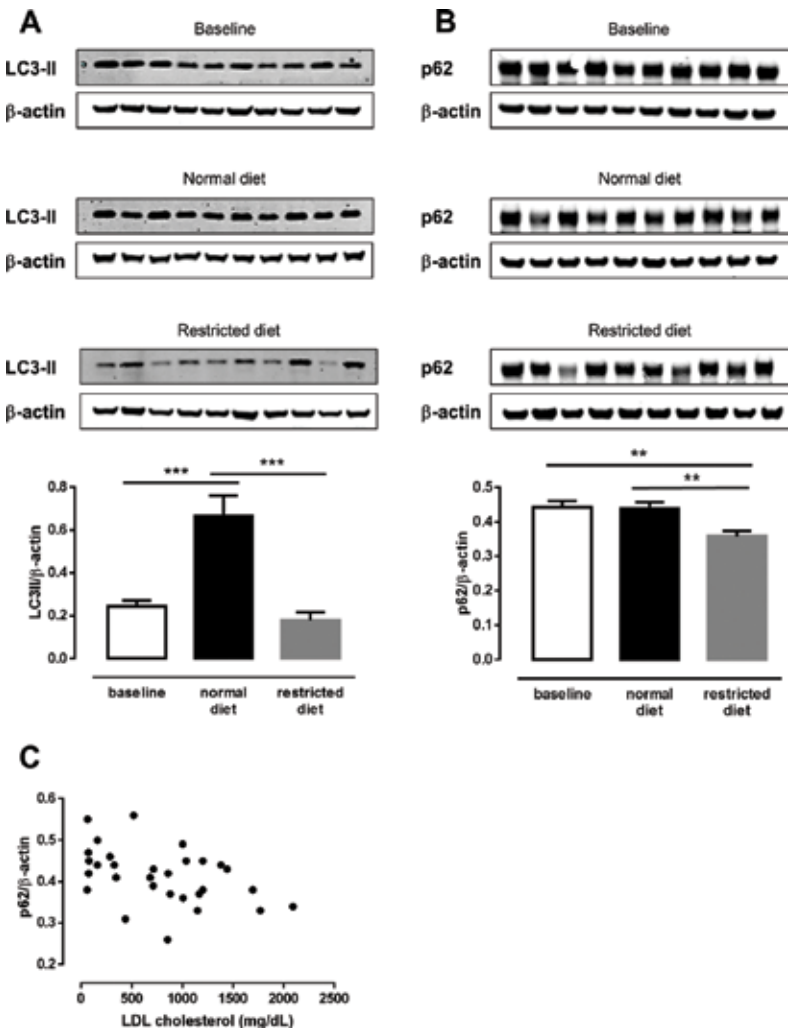

Figure 1. Induction of autophagy in liver of rabbits that were fed 0.3% cholesterol for 20 weeks (baseline), followed by cholesterol withdrawal for 4 weeks either via a normal diet or a restricted diet (20% of normal diet). Liver samples of ten rabbits per group were analyzed by Western blotting for the expression of autophagy marker proteins LC3-II (A) and p62 (B). **P < 0.01, ***P < 0.001 (One-way ANOVA with post-hoc LSD, n = 10 in each group). (C) Serum LDL-levels show an inverse correlation with liver p62 protein levels (Pearson Correlation Coefficient −0.44, P<0.05).

in serum lipids. SQSTM1/p62 is a scaffold protein that binds directly to the autophagosomal marker Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates via autophagy. Nutrient deprivation is a powerful autophagy-inducing condition [20]. Rabbits that undergo cholesterol withdrawal via a normal diet show high LC3-II levels but unaltered amounts of SQSTM1/p62 (**Figure 1A**), indicating moderate induction of autophagy. In contrast, rabbits undergoing severe food restriction show low levels of LC3-II and a clear reduction of SQSTM1/p62 (**Figure 1B**), which points to strong autophagy stimulation. It has been described that autophagy is strongly involved in managing intracellular lipids [21, 22]. Lipid droplets are taken up by lysosomes, where lysosomal acid lipases hydrolyze cholesteryl esters to generate free cholesterol for ATP-binding cassette transporter 1 (ABCA1)-mediated cholesterol efflux [21]. Impairment of autophagy in macrophages reduces reverse cholesterol transport [21], a condition that refers to net cholesterol flux from the peripheral tissues to the liver (for excretion via the bile). Conversely, pharmacological activation of the autophagy pathway attenuates lipid accumulation [23] and in some conditions (e.g., after treatment with mTOR inhibitors) triggers hypercholesterolemia [24].

4. Cholesterol withdrawal increases plaque stability

Numerous studies indicate that dietary modification is an important strategy for the prevention of cardiovascular disease [2, 25]. However, studies examining the effects of food restriction on atherosclerosis are scarce. Although short-term cholesterol withdrawal (4 weeks) does not alter plaque size (**Table 2**), a normal unrestricted diet results in a more stable plaque phenotype. Indeed, collagen content of the atherosclerotic plaques is increased, mainly due to an increase in type I collagen (**Figure 2**), which is essential for plaque stability. Moreover, VCAM-1 expression in endothelial cells declines (**Figure 3**). VCAM-1 is important for leucocyte recruitment and thereby contributes to plaque inflammation and macrophage accumulation. However, despite a decrease in VCAM-1 expression, the total amount of macrophages in the plaque does not alter within 4 weeks of cholesterol withdrawal (4 weeks). Indeed, only prolonged cholesterol withdrawal (12–24 weeks) results in a dramatic loss of plaque macrophages [26–28].

	Baseline	**Normal diet**	**Restricted diet**
Plaque area (mm^2)	3.3 ± 0.8	3.6 ± 0.6	3.6 ± 1.0
Macrophages (%)	22 ± 3	24 ± 4	34 ± 6
Smooth muscle cells (%)	26 ± 4	23 ± 2	26 ± 4
Fibrous cap thickness	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1

Data are expressed as mean ± SEM.

Table 2. Plaque area and cellular composition in the proximal ascending in cholesterol-fed rabbits (baseline, 20 weeks of cholesterol) followed by dietary lipid lowering for 4 weeks (normal diet) or a restricted diet for 4 weeks (restricted diet).

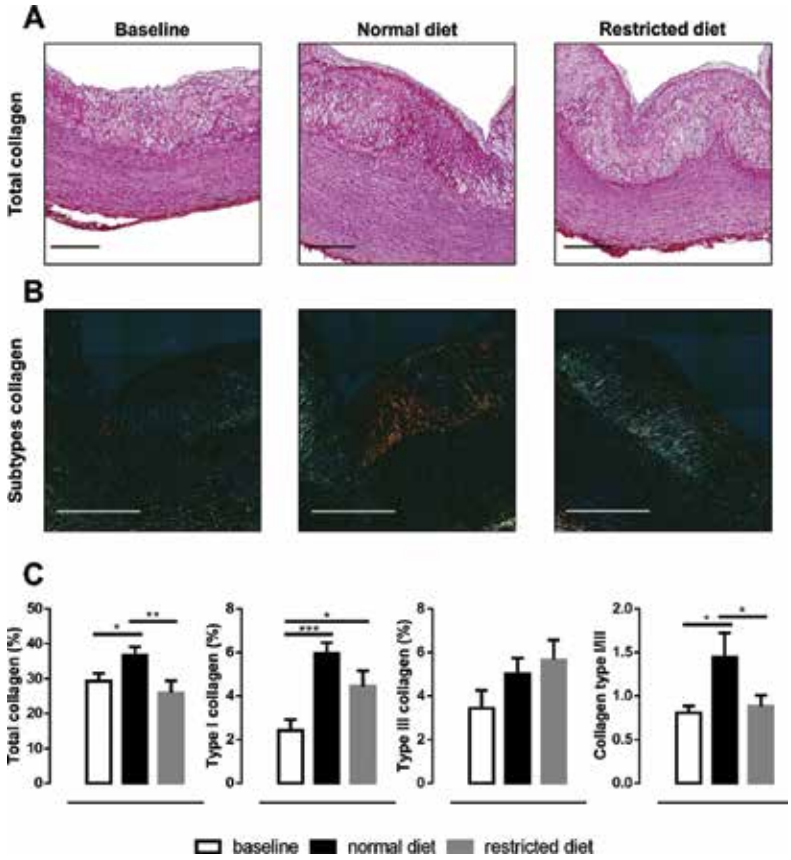

Figure 2. Collagen content of atherosclerotic plaques in rabbits that were fed 0.3% cholesterol for 20 weeks (baseline), followed by cholesterol withdrawal for 4 weeks either via a normal diet or a restricted diet (20% of normal diet). (A) Sections of the proximal ascending aorta were stained with Sirius red for total collagen determination. Scale bar = 500 μm. (B) Analysis of Sirius red staining via polarized light microscopy. Collagen type I is displayed in red, type III in green. Scale bar = 500 μm. (C) Quantification of total collagen, type I and type III collagen as well as the type I/III collagen ratio in Sirius red stained sections. *P < 0.05, **P < 0.01, ***P < 0.001 (One-way ANOVA with post-hoc LSD, n = 8–10 in each group).

5. Effect of food restriction on plaque development is controversial

In contrast with a normal unrestricted diet, severe food restriction does not promote beneficial effects such as increased collagen synthesis and decreased VCAM-1 expression. On the contrary, plaques of rabbits undergoing food restriction reveal an increase in apoptosis (**Figure 3**). Depending on the cell type and stage of the plaque, apoptosis could be detrimental for plaque stability [29]. Moreover, apoptosis can stimulate the release of inflammatory cytokines and chemotactic factors, thereby further aggravating plaque inflammation [30].

Given that food restriction stimulates autophagy, a well-known cellular survival mechanism, increased apoptosis may seem surprising. However, autophagy induction after intensive nutrient deprivation may be insufficient to counteract apoptosis.

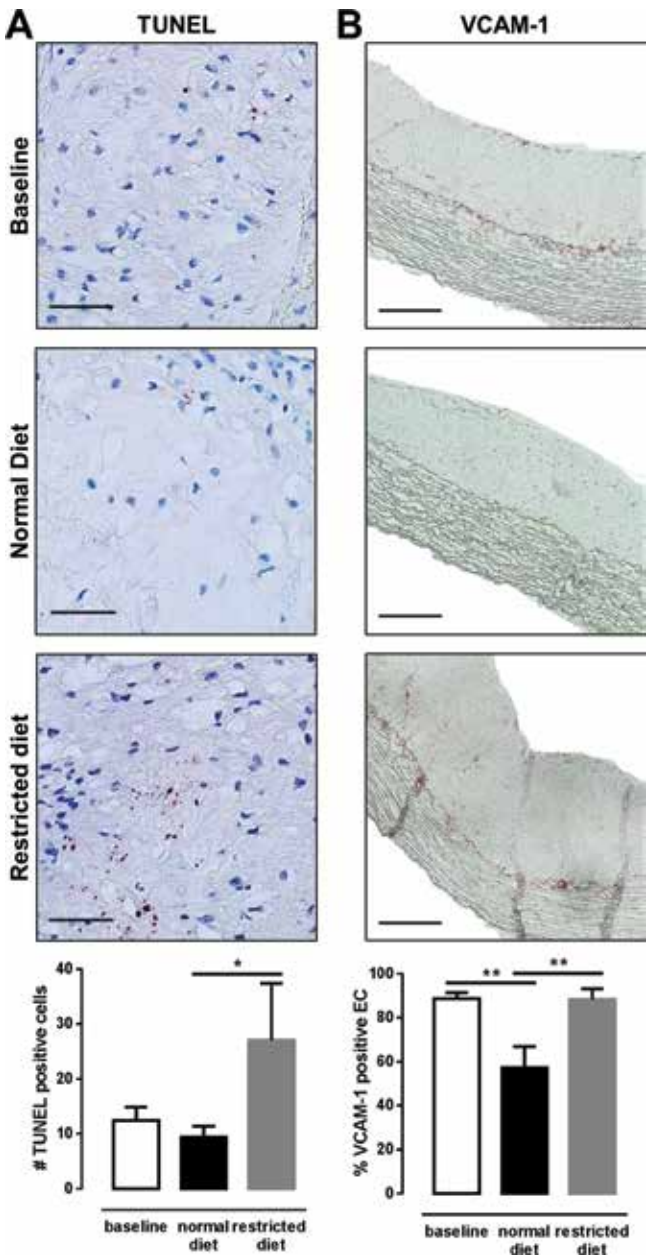

Figure 3. Atherosclerotic plaque composition of rabbits that were fed 0.3% cholesterol for 20 weeks (baseline), followed by cholesterol withdrawal for 4 weeks either via a normal diet or a restricted diet (20% of normal diet). (A) Sections of the proximal ascending aorta were TUNEL stained for the detection of apoptosis and the number of TUNEL positive cells in each group was quantified. Scale bar = 50 μm. *P < 0.05 (One-way ANOVA with post-hoc LSD, n = 10 in each group). (B) Sections of the proximal ascending aorta were immunohistochemically stained for VCAM-1 expression on endothelial cells. The number of VCAM-1 positive endothelial cells in each group was quantified. Scale bar = 500 μm. **P < 0.01 (One-way ANOVA with post-hoc LSD, n = 8–10 in each group).

The abovementioned findings are in agreement with previous studies in rabbits showing increased plaque development after a 50% reduction in food intake [31], even though Lacombe et al. [16] reported that aggravated atherosclerosis only occurs in rabbits when dietary restriction is combined with cholesterol feeding. Prenatal under nutrition is also known to program a pro-atherosclerotic phenotype and to accelerate plaque development in young adult offspring [32, 33]. Nonetheless, a large body of evidence indicates that food restriction is associated with a range of positive effects on cardiovascular health. Dietary restriction in apolipoprotein E-deficient mice, for example, results in the development of smaller and less advanced atherosclerotic lesions [7, 34]. A lower incidence of atherosclerotic plaque development is also seen in genetically obese rats consuming a low calorie diet, as compared to rats fed ad libitum [35]. Studies in humans clearly describe a reduction in cardiovascular risk factors but often fail to demonstrate a direct effect on atherosclerotic plaque development [4, 5]. Still, the incidence of atherosclerosis was decreased during the years following World War I and World War II, which supports the general benefit of food deprivation [36]. Importantly, at least two main differences in the experimental design or setup of different studies should be mentioned that may explain a different outcome. First, differences might be related to the severity of food restriction (50% food restriction = moderate, 80% food restriction = severe). Accordingly, severe food restriction holds a higher risk of vitamin deficiency that should be taken into account. Indeed, vitamin D deficiency may contribute to atherosclerosis [37], and also vitamin C and vitamin E depletions are demonstrated to aggravate plaque development [38]. Second, the time span of dietary restriction could be an important factor. Four weeks of food restriction is relatively short in comparison with other studies showing beneficial effects of food restriction. Fontana et al. [4], for example, reported a reduced risk for atherosclerosis in individuals who had been on food restriction for an average of 6 years.

In conclusion, severe short-term food restriction seems to counteract the plaque stabilizing benefits of cholesterol withdrawal in rabbits.

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Section 3

Organ Manifestations

Chapter 6

Atherothrombosis as a Leading Cause of Acute Coronary Syndromes and Stroke: The Main Killers in Developed Countries

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Additional information is available at the end of the chapter

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Abstract

Worldwide, cardiovascular incidents are estimated to cause 17.5 million deaths, 80% of which are ischemic strokes or acute coronary syndromes. Cardiovascular disease results in a significant financial burden for healthcare system—namely, in 2009, it was 9% of the gross health service expenditure in the European Union. Therefore, the development of the knowledge about atherosclerosis—initially thought to be solely degenerative disorder but now considered a multifactorial inflammatory state—is essential. Acute coronary syndrome (ACS) is usually a manifestation of severe reduction in coronary blood flow caused by atherosclerotic plaque and thrombus. The pathology of the atherosclerotic plaque is complex. Essentially, it is disease of the arterial intima that, through subsequent stages, results to luminal narrowing. Over the years, various theories regarding the genesis growth and vulnerability of atherosclerotic lesions have been promoted, usually focusing on endothelial injury, smooth muscle cell proliferation, lipid accumulation, and, more recently, inflammatory reactions.

Keywords: atherothrombosis, atherosclerotic plaque, intravascular thrombus, acute coronary syndrome, cardiovascular events, ischemic heart disease, vulnerable plaque, plaque erosion

1. Introduction

Ischemic heart disease, despite the significant progress of drug therapy as well as coronary revascularization techniques, still represents the most common cause of death in developed countries [1]. Social significance of the problem is reflected by analyzing the results of the

randomized controlled trials (POLSCREEN, EURO-ACTION, POLCARD, DART, GISSI). The above-mentioned trials, as well as other carried out so far, including autopsy studies and clinical and experimental studies, have shed new light on the pathogenesis of atherosclerosis, which for years was believed to be a disease solely degenerative. It is now known that it is a condition characterized by systemic low-grade inflammation [2, 3]. This process begins perinatally [4]. Lipid disorders that occur in the mother increase the sensitivity of the fetus to the risk factors for atherosclerosis. Moreover, the low birth weight correlates positively with an instance of the metabolic syndrome in adulthood [5, 6].

Inflammation affects the compliance of the arteries. It is a response of vascular walls to agitation and injury of vascular endothelium (*response-to-injury hypothesis*) [2, 7]. It has been shown that endothelial cells are the main component of the vessel wall that is responsible for empowerment of the process of atherogenesis. It has also been suggested that they participate in various stages of development, destabilization and cause for plaque rupture [8]. Under physiological conditions, the cells of proper endothelium produce substances that regulate vascular smooth muscle tension, adhesion and aggregation of platelets and the migration of monocytes and polymorphonuclear leukocytes. Damage to the vascular endothelium, also considered for activation of the inflammation, is characterized by a reduced bioavailability of endothelial vascular distending substances (mainly nitric oxide [NO] and prostacyclin [PGI]), increased permeability of plasma lipoprotein vessel intima and changing the properties from anti-adhesive to pro-adhesive [9, 10]. The state of endothelial cells depends, among other things, on vascular endothelial NO formed under the influence of L-arginine NO synthase [10]. This is the endothelial substance responsible for the anti-atherosclerotic, vascular distention, anti-inflammatory and anti-coagulant activity of vascular endothelium [9, 11]. Factors responsible for endothelium dysfunction include elevated LDL cholesterol, high homocysteine, hypoxia, diabetes, oxidative stress (due to excessive formation of free radicals of oxygen), bacterial and viral infections (Chlamydia pneumoniae, Helicobacter pylori, Herpes virus, Cytomegalovirus). These components of atherogenesis cause mainly functional but also morphological damage. Moreover, shear stress variability in hypertension causes mechanical injury of endothelial cells [9, 12]. Increased sensitivity to the damage is shown in the endothelium of the diabetic patients, as its cells can be stimulated, under the influence of the increased concentration of glucose and the accumulation of advanced glycation end-products. These substances, acting through the receptors for glycation end products, may induce proinflammatory molecule expression in endothelial cells [13].

2. Epidemiology and impact of life style on atherothrombosis

Atherothrombosis is a complication of atherosclerosis. The essence of this process consists of closing or narrowing vessel lumen, which is caused by a clot formation following exposure of thrombogenic, lipid rich necrotic core, of the ruptured plaque. Depending

on the affected vascular bed, it can manifest as a heart attack or unstable coronary artery disease, transient ischemic episode or stroke, as intermittent claudication or acute limb ischemia [14].

About 80% of deaths from cardiovascular events occur as a result of a stroke or a heart attack. Approximately 17.5 million people die every year due to cardiovascular disease, which is approximately 31% of general mortality in the world. Atherothrombosis is the main cause of mortality due to cardiovascular diseases (CVD). Approximately 75% of the cases of heart attack and approximately 90% of strokes associated with carotid arteries atherosclerosis are caused by thrombosis [15].

CVD is a big financial burden for healthcare systems. In 2009, CVD-related costs totaled 106 billion euros, which was approximately 9% of the total expenditure on health care in the European Union [16]. There exists global trend towards the increase in the incidence of life-style diseases and a decrease in cases of premature death as compared to years on disability. In the context of lost years of life and life years on disability, ischemic heart disease (IHD) and stroke are, respectively, in the first and third place in the world [17]. About 85–90% of strokes are of ischemic etiology [18].

In accordance with meta-analysis, based on an analysis of studies involving a total of more than 250,000 people, the risk of death due to CVD in the course of lifetime is approximately 30%, and taken into account the risk of death and all cardiovascular events dating back to it, 50% for both sexes, in each age group [19]. Among diabetics, most of whom die due to CVD, 8 of 10 deaths are due to atherothrombosis [20].

According to the findings of the Global Burden of Disease Study from 2010 onwards, adjusted for age, the mortality due to CVD has fallen approximately 20% during the last 80 years of the twentieth century [21]. The success of the reduction of mortality due to CVD is associated with the development of methods of treatment and better organization of healthcare, as well as preventive activities, including non-pharmacological interventions. To the above, one can also add changes in tobacco legislation, which can lead to a 15% reduction in the risk of hospitalization and a 16% reduction in mortality from coronary heart disease and stroke [22]. Not less important are the lifestyle changes, including eating habits. It has been demonstrated that appropriate physical activity and dietary intervention can contribute to approximately 35% reduction in the risk of death already accepted with adjustment of pharmacologic medication [23]. Proper diet contributes to the reduction of cardiovascular events (CVE) in patients after 55 years of age diagnosed with diabetes or a history of CVE irrespective of the use of drugs in secondary prevention [24].

In terms of cardiovascular risk reduction, the introduction of statin therapy was the pharmacological milestone. This has proven effective in reducing CVE and mortality due to CVD [25]. What's more, their use in low-risk populations decreased by approximately 30% the relative risk in this population. In addition, for patients intolerant of statins or for those who despite optimal therapy fail to achieve their therapeutic goal, Ezetimibe or

Evolucumab can be currently used, new drugs of proven efficacy and safety of therapy [26, 27]. Ezetimibe connects with Niemann-Pick C1-like 1 (NPC1L1) proteins preventing absorption of cholesterol from the gastrointestinal tract. Used together with simvastatin, it significantly reduced the risk of mortality compared to statin monotherapy. Evolocumab is a monoclonal antibody interacting with enzyme PCSK9 (proprotein convertase subtilisin kexin type-9) and significantly lowering LDL-cholesterol and total cholesterol and reducing CVE rate in combination with a statin as compared to statin monotherapy [26, 27].

Very important element of therapy is patient's compliance. Adherence of the patient affects the effectiveness of the therapy. The review of approximately 20 studies involving a total of over 375,000 patients showed only 42–61% of adherence to treatment in patients receiving cardiovascular drugs as primary prevention and 62–76% adherence in secondary prevention [28].

There are some differences in CVD mortality among different races. Black people have a higher risk of death from coronary heart disease and 2–4 times higher risk of ischemic stroke than white people. The Asian race and the people of the Pacific Islands are at the highest risk for hemorrhagic stroke [29].

Appropriate prevention would reduce the CVD cases by 80% [19, 30]. Unfortunately, there are still inequalities between countries. About 80% of deaths from CVD take place in countries with low-to-moderate prosperity, in which the frequency of multiple risk factors, especially obesity and diabetes mellitus (DM), tends to increase significantly [16]. Interestingly, despite the general decline in the consumption of tobacco products, there currently exists three times higher risk for smoking in women because of the trend to start the habit at a younger age.

Among patients with CAD, the most common manifestations of atherothrombosis are myocardial infarctions with ST segment elevation (STEMI) and non-ST segment elevation myocardial infarction (NSTEMI). In-hospital mortality in STEMI varies, according to a variety of records, around 6–14%. Despite the development of pharmacotherapy and the invasive therapy, the mortality rate in 6 months after STEMI is still approximately 12%. Over the last decade, the proportion of STEMI has been reduced as compared to NSTEMI. Although, in the early years, the population of patients with NSTEMI acute coronary syndrome is characterized by lower mortality; after about 2 years, it is similar as in patients with STEMI [31].

3. The role of inflammation in the process of the formation of atherosclerosis

Atherosclerosis is a chronic inflammatory disease [9, 32–34]. Due to the damage to the vascular endothelium and the development of inflammatory lesions in the vessel wall, there is a tendency to create blood clots leading to the occurrence of the thromboembolism evolving from atherosclerotic plaques or closing of the artery lumen at the site of inflamed plaque rupture, i.e. thrombotic vessel occlusion. Acute coronary syndromes (ACS) are in the form of unstable coronary artery disease (unstable angina—UA) and acute myocardial infarction

(AMI)—which includes STEMI and NSTEMI or sudden cardiac death (SCD). Atherosclerosis in carotid arteries can lead to transient ischemic attacks (TIA) or ischemic stroke. The cause for these complexes, known as cardiovascular syndromes, is a blood thrombosis, forming on the surface of the damaged endothelium, a narrowed coronary artery or carotid artery, most frequently internal carotid artery (ICA) [9, 32].

Pathophysiological studies have shown that the most common cause of formation of a blood clot is rupture of the fibrous cap, which separates the contents of the plaque from the blood [9, 13, 33, 35]. This was confirmed in intravascular ultrasound (IVUS) with virtual histology (VH-IVUS), optical coherence tomography (OCT) and magnetic resonance imaging (MRI) [36, 37]. This mechanism applies to approximately 55–60% (in some studies dating back to the 80%) cases of ACS [33, 35, 36]. Other mechanisms are damage of endothelial cells known as erosion on the surface of atherosclerotic plaque (plaque erosion) consisting of 30–35% of the ACS and of approximately 2–7% endovascular calcifications (calcified nodules) [33, 35]. Inflammatory changes ongoing in the atherosclerotic plaque cause a loss of stability making it vulnerable to rupture, the so-called unstable atherosclerotic plaque (vulnerable plaque). Unstable plaque is characterized by a thin fibrous cap (thin cap fibroatheroma—TCFA) covering the big necrotic core around which revolves the inflammatory process and positive remodeling of the artery [9, 32, 33, 35]. A similar transformation in the atherosclerotic plaque has also been observed in the ICA. This location is responsible for TIA and strokes [9]. Positive remodeling of the artery proves that narrowing of its lumen does not have to be relevant, and it may not exceed 50–70% [9, 33]. The widespread use of statin drugs, especially atorvastatin and rosuvastatin, also likely ACE inhibitors, and lifestyle changes in developed countries have resulted in better control of inflammatory changes ongoing inside atherosclerotic plaque. This results in reduction in the incidence of strokes (primary stroke prevention) and ACS in the form of STEMI, but higher prevalence of NSTEMI and UA [9, 13, 32]. As a result, this has led to decreased cardiovascular mortality in many countries. It cannot be excluded that control of inflammation in the vessel wall by commonly used statins lowers the incidence of share in causes for destabilizing plaque rupture leading to occlusion of the artery. Because the total number of ACS and cerebral ischemic events remains at a similar level, this finding probably reveals other mechanisms leading to intravascular thrombosis with a smaller share of acute inflammation within the plaque rupture-induced accumulation of oxygen-modified LDL cholesterol (oxy-LDL) leading to destabilization. These mechanisms include endothelial injury by vascular flow disorders caused by artery stenosis, abnormal healing processes of the damaged endothelium, infectious pathogens as well as autoimmune responses against modified plaque components [9, 13, 35, 38, 39].

4. The evolution of stable coronary artery disease to ACS

4.1. Vulnerable plaque

Endocrine endothelial function, which consists in the synthesis and secretion of NO and PGI, is a prerequisite for the preservation of its integrity and correct relationship between

it and flowing blood. Known atherosclerotic risk factors may interfere with this function of encouraging the penetration of lipoproteins, monocytes and lymphocytes into the vessel wall. Currently, there is no doubt that atherosclerosis is a chronic inflammatory disease involving many immunological processes. Some researchers of these medical conditions compare them with other chronic inflammatory disorders, in which immune and autoimmune reactions play an important role [40, 41].

Inflammation in connexion with the accumulation of cholesterol, principally oxygen-modified LDL cholesterol (oxy-LDL), causes the proliferation of monocytes from peripheral blood, which is then converted into macrophages. It also stimulates the recruitment of myofibroblasts producing proteoglycans—the main substrate of extracellular matrix. Changes are conducive to the occurrence of the vasoconstriction and activation of the endovascular inflammatory and prothrombotic mechanisms. Gradual increase of the volume of the emerging plaque leads to abnormal blood flow, which in turn causes the oscillating shear stress and intensifies the atherothrombotic mechanisms stimulating further plaque growth [35, 42, 43]. The resultant atherosclerotic plaque is made of fibrous cap of smooth muscle cells and connective tissue. The cap separates the lumen of vessels from the contents in which necrotic core is surrounded by inflammatory infiltrates containing macrophages, foam cells and lymphocytes. The core of the plaque also contains the oxy-LDL cholesterol, free cholesterol crystals and calcium deposits [13, 43–46]. A part of the atherosclerotic plaques also contains foci of hemorrhage arising from the damage to the proliferating blood vessels, stimulated by inflammation [37, 47, 48]. Angiogenesis plays an important role in the pathophysiology of plaque instability and plaque rupture. New blood vessels rarely penetrate from the main lumen, but more often from the vasa vasorum [47, 48]. They lack the cells constituting the vessel wall which are fragile and porous, so that they become a source of local extravasation plasma protein and blood cells. Such bleedings in the plaque are frequent, may increase the volume of necrotic core and cause sudden progression of artery stenosis [49]. A central role both in the development and destabilization of plaque was attributed to macrophages, which, through their surface scavenger receptors, absorb oxy-LDL and transform into foam cells. Cytokines produced by macrophages infiltrates would lead to the degradation of the connective matrix tissue and smooth muscle cell necrosis and, consequently, to fibrous cap rupture. It leads to a thrombus formation responsible for acute ischemic syndromes. It has been repeatedly described for years as a mechanism of emergence and rise of the plaque volume, as well as its destabilization, is simplistic and does not actually translate the complex changes taking place in its interior.

Currently, a greater role in initiation of changes is attributed to lymphocytes and mutual relations between lymphocytes and macrophages, which cause varying degrees of inflammation activity. The main antigen that initiates and maintains inflammation in the vessel wall is oxy-LDL [13, 32, 41, 50]. It is toxic to the vessel wall cells causing the immune system to try eliminating it. The presence of oxy-LDL-derived antigens on the surface of the dendritic cells, macrophages and different types of lymphocytes regulates the activity of inflammation [13, 41, 50, 51]. Antigen can also be protein of bacterial cells, viral, heat shock protein 60 or β2glycoprotein 1 [3].

Naïve T-cells maturating in the thymus gland under the influence of natural antigens differentiate into the cells of total immune memory, slow reacting (central memory—T_{CM}) long remaining in circulation and storing the memory of antigens (e.g. cancer, HIV) and effector cells (effector memory—T_{EM}) rapidly responsive and likely to produce cytokines [52]. The T_{CM} have the molecule CD27 on their surface. After contact with nominal antigens, they irreversibly lose surface molecule and can settle in the lymph nodes where they acquire the characteristics of T_{EM} [52]. These cells differentiate into the lymph nodes in the direction of various cell lines—helper (Th) and regulatory (Treg) [53]. Antigen-presenting cells (APC) play key role in direction of differentiation of T cells by stimulating their surface receptor (T-cell receptor—TCR). Under their influence, functionally differentiated Th cells arise, which are classified according to the type of produced cytokines, surface markers and expression of lineage specifying transcription factors. The direction of the differentiation of T cells depends on the antigen quantity and intensity of TCR stimulation and on cytokine-inducing specific types of cells [39, 53]. It has been shown that circulating T lymphocytes, CD4+ and CD8+, differentiate preferentially in the direction of Th_1, Th_2 and Th_{17}, which promotes the transformation from stable atherosclerotic plaque into unstable vulnerable plaque [9, 50, 51, 54]. Arising Th cells produce different cytokines, including interleukin 2 (IL-2), which controls immune processes by influencing the maturation of lymphocytes Treg demonstrating immunosuppressive reactivity [13, 42, 50, 51, 53, 54]. It is now suspected that cell response (Th_1, Th_{17}) and its mediators: tumor necrosis factor-α (TNFα), interferon-γ (INFγ) and interleukins (IL)—IL-1β, IL-12, IL-17, IL-18 are responsible for promoting the development of atherosclerosis, whereas humoral immune response (Th_2) and its mediators: IL-2 IL-4, IL-5, IL-10, IL-13 have an inhibitory effect on this process [3]. Propagators of the ongoing inflammation are increased levels of proinflammatory cytokines (i.e. TNF-α, IL-6); soluble adhesion-intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), L-selectin and so-called acute-phase proteins—inflammation C-reactive protein (CRP), amyloid A and fibrinogen [2].

The mechanism of lymphocytes penetration into the arterial wall is not exactly known. Probably this is done with the participation of chemokines and L-selectin [50]. Because most chemokine receptors are found on different cell types, research to clarify the mechanisms of lymphocytes homing in the atherosclerotic plaque is mostly inconclusive.

Th_1 and Th_{17} cells produce large amounts of IFNγ that activates inflammatory processes and expresses the transcription factor β (T-bet). These factors play a decisive role in the destabilization of atherosclerotic plaque. IFNγ activates APCs and macrophages, reduces collagen synthesis and increases production of cytokines degrading extracellular matrix. An important role in the degradation of the extracellular matrix is also played by matrix metalloproteinases (MMP) [9, 55, 56]. A strong factor in boosting the activity of MMP-9 (an enzyme that breaks down collagen type IV, which is component of the fibrous cap) is TNFα (**Figure 1**) [57].

Treg cells inhibit the inflammatory reactions by IL production such as IL-10 and similarly acting transforming growth factor beta (TGFβ) [9, 13, 39, 50, 60]. They reinforce simultaneously the fibrous cap by stimulating the proliferation of smooth muscle cells and the production

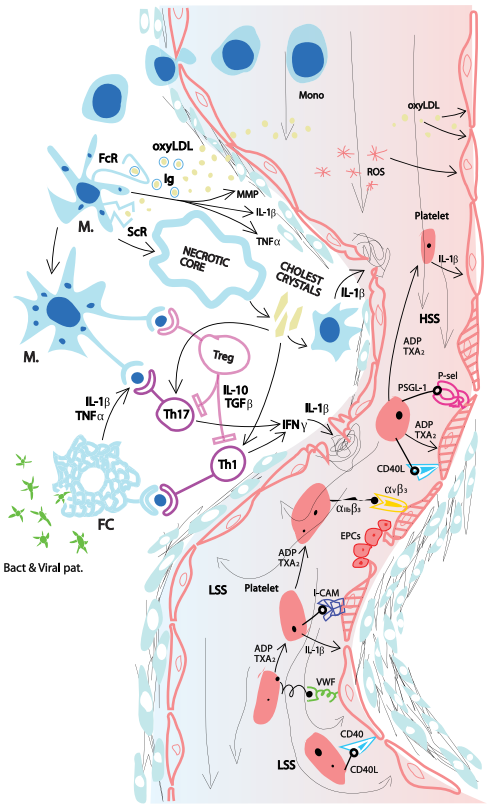

Figure 1. Possible mechanisms of plaque vulnerability (left) and plaque endothelial erosion (right). *Vulnerability*: oxy-modified LDL (oxy-LDL) causes inflammation by the proliferation of monocytes from peripheral blood, which is then converted into macrophages (M). The excess oxy-LDL in the blood penetrating into plaque results in the formation of the complexes with immunoglobulin (Ig). These complexes combine with Fc receptors (FcR) on the surface of macrophages stimulate the secretory activity for MMP, IL-1β and TNFα. Presentation of the oxy-LDL on the surface of macrophages stimulates Treg lymphocytes, which slows the inflammation by reducing the activity of the Th lymphocytes. Cell response (Th_1, Th_{17}) and its mediators—tumor necrosis factor-α (TNFα), interferon-γ (INFγ) and interleukins (IL-1β)—are responsible for promoting the development of atherosclerosis and activating inflammatory processes. IFNγ activates macrophages, reduces collagen synthesis and increases production of cytokines degrading extracellular matrix. Treg cells inhibit the inflammatory reactions by IL production (IL-10) and similarly acting transforming growth factor beta (TGFβ). The binding of oxy-LDL to scavenger receptor (ScR) located on macrophages, which is not subject to feedback, results in overloading of these cells leading to their death, releasing the free cholesterol and increasing volume of necrotic core. Such antigens, when released, trigger mechanisms of the vicious circle by stimulation of the Th_1 and Th_{17} instead of Treg lymphocytes. *Endothelial erosion*: both low (LSS) and improperly high shear stress (HSS) interfere with endothelial functions and can produce prothrombotic state. Shear stress fluctuations increase tendency of endothelial cells covering the atherosclerotic plaque to apoptosis. Damage to the endothelial cells activates endothelial progenitor stem cells (EPCs) derived from bone marrow, which proliferate at the place of damage, and may prevent the described processes that leading to the thrombus formation. Endothelial cells increase their adhesion properties in relation to the platelets which by releasing granulation substances (IL-1β) activate the endothelial cells creating a mutual feedback. Reactive oxygen species (ROS) and circulating lipoprotein (oxy-LDL) also express endothelial surface adhesion proteins as selectins (P-sel) and von Willebrand factor (VWF), which supports the mutual relationships between them and platelets. Platelet CD40 ligand (CD40L) binds CD40 on endothelial cells, resulting in upregulation of adhesive molecules (ICAM-1, VCAM-1, P-sel), cytokines, and TF release, leading to reduction in NO synthesis. Surface platelets glycoprotein-1 receptors for P-selectin (PSGL-1) allow for adhesive bond and rolling platelets but not strict binding to the endothelium. Glycoprotein surface receptor GPVI and αvβ3 bind vascular walls collagen causing the activation of receptors αIIbβ3 and release of ADP and thromboxane A2 (TxA2). The combination of glycoprotein platelet receptors αIIbβ3 with VWF and fibrinogen stabilizes the platelet clot. This initiates a platelet thrombus protruding into artery lumen and is essential for the later stages of thrombus formation.

of collagen [39]. Functional balance between these T-cell types provides the stability of atherosclerotic plaque. The excess oxy-LDL in the blood penetrating into plaque results in the formation of the complexes with immunoglobulin. These complexes combine with Fc receptors (FcR) on the surface of macrophages and stimulate the secretory activity for MMP, IL-1β and TNFα. At the same time, the presentation of the oxy-LDL on the surface of macrophages stimulates Treg lymphocytes, which slow the inflammation down by reducing the activity of the Th lymphocytes. Such control system works well in people with low levels of risk factors. The binding of oxy-LDL to scavenger receptor located on macrophages, which is not subject to mentioned feedback, results in overloading of these cells leading to their death, releasing of free cholesterol and increasing volume of necrotic core [13, 58]. The release of such antigens triggers the mechanisms of the vicious circle by stimulation of the Th_1 and Th_{17} instead of Treg lymphocytes [9, 13, 39, 42, 50].

Lately, attention is focused on the role of receptor programmed target death protein-1 (PD-1) presented on the naive CD8+ cells. It bears the responsibility for the so-called immune exhaustion observed in chronic inflammatory states (tbc, HIV) and cancer. There are suggestions that chronic stimulation of TCR by oxy-LDL leads to increased presentation of PD-1. The presence of PD-1 correlates inversely with the level of IL-2 produced by Th_2. This can interfere with the CD8+ cell differentiation in the direction of Treg and potentiate their apoptosis leading to competitive advantage mechanisms acting as pro-inflammatory and destabilizing factors in the plaque [41].

Probably within a plaque, there are three subtypes of macrophages. The most common are classically activated macrophages M1 induced by INFγ or Th_1 and Th_{17} lymphocytes cytokines. The second group are macrophages M2 induced by cytokines of helper lymphocytes Th_2 (IL-4 and IL-13). They produce the anti-inflammatory acting cytokines—IL-10 and TGFβ [59]. Probably, there is a third group of macrophages presenting CD163+, activated by hemoglobin, which do not produce pro-inflammatory cytokines and have reduced ability to produce inducible nitric oxide synthase (iNOS). A decrease in the levels of intracellular iron ions within the macrophage probably plays a leading role in the transcription of genes protecting these security cells from the accumulation of lipids. This is done by increase in the levels of ferroportin-1 leading to reduction of free radicals (-OH) production as result of iron ions accumulation and depletion. One of the key regulators of atherosclerotic plaque stability may prove to be hepcidin, responsible for ferroprotein-1 degradation, resulting in the accumulation of iron ions, the accumulation of intracellular lipids and apoptosis of macrophages. Hepcidin blockage inhibits the development of atherosclerosis by regulating ATP-binding protein subfamily G [59, 60].

A significant role in the weakening of the fibrous cap, consequently causing it to rupture, is played by T-cells CD4 + $CD28^{nul}$. They produce a significant amount of INFγ and TNFα, strongly stimulating macrophages. They also have cytotoxic properties in relation to fibrous cap, smooth muscle cells and are apoptosis resistant. These cells are presenting cytotoxic immunoglobulin (killer immunoglobulin) on their cell membrane that acts as cytotoxic receptors (Ig-like receptors).

They also produce cytolytic enzymes against the endothelial cells that directly kill them, such as perforins, granzyme A and granzyme B, which are usually present in killer T cells (KTC) and natural killer cells (NK) [13, 39, 61, 62]. It has been shown that the number of these cells in the circulation is an important prognostic for occurrence and course of ACS [61].

Production of proinflammatory proteins, such as IL-1β, chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-C motif) ligand 3 (CCL3), E-selectin (SELE), ICAM-1, MMP-3 and the MMP-9, involved in the process of destabilizing atherosclerotic plaque denotes a genetic profile connected with polymorphism of many genes. Polymorphism of this plays an important role in the susceptibility to risk factors for atherosclerosis and to changes in already existing atherosclerotic plaque. What's more, single nucleotide polymorphisms located within the regions of functional genes for these proteins may affect their concentration and activity causing further clinical implications [63].

By examining the mechanisms leading to the development of atherosclerosis, it was shown that in these processes, beyond the stimulated endothelial cell and cells of the immune system, also vascular smooth muscle cells (VSMC) are involved [64]. VSMC function is not just limited to the production of extracellular matrix in the vessel wall. It has been shown that in response to a stimulus, these cells may change the type of produced extracellular matrix and thus affect the lipid content in the vessel wall and the multiplication of other cells. Under specific conditions, they can also take over the function of other cells, for example macrophages, and due to the expression of the relevant receptors acquire absorption capacity of fat by mimicking foam cells. While taking over some functions of endothelial cells, they can produce cell adhesion molecules, VCAM-1 or ICAM-1. In addition, being a component of atherosclerotic plaque, they can also produce cytokines—platelet-derived growth factor (PDGF), TGFβ, IFN and monocyte chemoattractant protein 1 (MCP-1) [64].

Under the influence of these cytokine, extracellular matrix degradation occurs into fibrous cap. It is weakened further due to result of the apoptosis of smooth muscle cells and cell death due to primary necrosis (oncosis) [9, 44].

Contact of the flowing blood with the content of the ruptured plaque activates processes of coagulation, which can occur rapidly. A large amount of tissue factor (TF) liberated by inflammation tissue activates plasma factor VII, which runs the enzymatic coagulation cascade. TF forms a complex with factor VII, activating it to active form (VIIa). The complexes TF/VIIa activate factors IX and X, leading to thrombin generation. The consequence of this cascade of activation is rapid formation of the intravascular thrombus [9, 35, 65].

4.2. Erosion on the surface of the atherosclerotic plaque as a cause of ACS and stroke

Epidemiological studies have shown that myocardial infarction may occur in people with normal levels of LDL cholesterol. In addition, as demonstrated by pathomorphological and clinical studies using optical coherence tomography, 30-40% of patients with vascular thrombosis atherosclerotic plague have no inflammatory features [13, 45, 46]. The morphology of such plaques is completely different from the above, subjected to the inflammatory changes. The blood clot formed on its surface is in direct contact with the intima at a place completely devoid of the endothelium. Fibrous cap is well demarcated and includes numerous smooth muscle cells and an extensive connective tissue forming an extracellular matrix [33, 46]. The interior of the well-demarcated plaque contains few macrophages and lymphocytes. As well, the profile of patients with ACS, who have been found to have this

type of plaque, differed from the profile of patients who suffered vulnerable plaque. In available reports, these patients were younger, 80% of these were premenopausal women, and frequent tobacco smokers [9, 33, 45]. The mass of the plaque, which was the basis of thrombosis, was less than in the case of plaque rupture and often it was nonconcentric [9, 13, 33, 45]. In contrast to the inflammatory plaques that show positive remodeling, arteries affected by erosion are characterized by negative one [45]. Demonstrated characteristics suggest a different mechanism in formation of a blood thrombus, which, like in the case of plaque rupture, can cause both the closure of an artery and peripheral embolism, more often associated with such morphology of the intravascular changes [9, 13, 33, 45]. However, the mechanism of the formation of this type of inter arterial thrombosis has not been fully understood. It is suspected that a decisive role in its formation plays abnormal blood flow due to arterial plaque stenosis, which causes changes in shear stress, endothelial dysfunction that covers plaque affecting its anti-inflammatory and antithrombotic signals of the endothelium [9, 13]. Laminar flow disorders more often occur in places of bifurcation and in the folds of the arteries. The correct endocrine function of the endothelium creates normal shear stress, which is the force of friction between the flowing blood and cellular layer covering the interior surface of the vessel. Both low and improperly high shear stress interfere with endothelial functions and can produce prothrombotic state. In the case of atherosclerotic narrowing of the artery, both pre- and poststenosis flow are slowed down—shear stress is low, whereas at the apex of the plaque, the flow is accelerated—shear stress is abnormally high. This creates conditions conducive to damaged endothelium. Low shear stress and turbulent blood flow facilitate the accumulation of lipids, the recruitment of inflammatory cells and increased expression of adhesion molecules and proteases [59]. The correct vascular flow—valid shear stress induces the enzyme systems that prevent the expression of pro-inflammatory and pro-thrombotic genes and at the same time promote the layout security by activating the endothelial NO synthase. Simultaneously, high shear stress stimulates the synthesis of several types of microRNAs that interact with Krüppel-like factor 2 (KLF2) and nuclear factor erythroid cell-specific 2-related factor2 (Nrf2), which support the interaction of anti-inflammatory and anti-thrombotic pathways [13]. Accelerated flow of blood within the largest narrowing and supra-physiologically high shear stress suppresses these systems, which prevent inflammation and activate the prothrombotic processes, and may be the reason for damage to the endothelial cells and the activation of inflammation with further consequences of thrombosis [9, 13]. These biomechanical force fluctuations associated with shear stress are particularly apparent in people with hypertension and their effects are intensified under the influence of other atherosclerotic risk factors, such as hypercholesterolemia, advanced glycation end-products in diabetes, tobacco smoking, vasoactive amines and immune complexes. These factors in terms of alternating shear stress can lead to endothelial dysfunction [35].

In areas of damaged vascular endothelium and high shear stress, there are abnormal interactions between thrombocytes and endothelial cells, which is the basis for the pathogenesis of endovascular thrombosis and activates the processes leading to instability of the atherosclerotic plaque. Another suggested mechanism that can coexist with described above is increased tendency of endothelial cells covering the atherosclerotic plaque to apoptosis. It is

associated with the possibility of presentation by so-called pattern recognition receptor—toll-like receptor 2 (TLR2). It contains a hyaluronian molecules in its structure, identical to that found in Gram+ bacteria, which can result in the recognition of these cells as foreign by the immune system, leading to their destruction, and thus initiating thrombotic processes. This may lead to identification of these cells by the immune system as foreign, leading to damage and thus initiating the processes of thrombosis [9].

In the event of existence of some pro-inflammatory agents, endothelial cells increase their adhesion properties in relation to the platelets which by releasing granulation substances activate the endothelial cells creating a mutual feedback. Platelets associated with endothelial cells become very effective in recruiting leukocytes in blood by promoting their adhesion and transmigration within the atherosclerotic plaque. They play such an important role due to the interaction of numerous cell types such as endothelial cells, neutrophils, monocytes, dendritic cells and cytotoxic T-cells. Under physiological conditions, platelets circulate in the blood remaining in close contact with the endothelial cells. However, their adhesion is prevented by a specific phenotype of endothelium cells controlled by three intracellular ways: way of NO, trace ectoADPase/CD39/NTPDase and eicosanoids-arachidonic acid-prostacyclin pathway (PGI2), which inhibit the activation of platelets on the way of synthesis of cAMP and cGMP stimulation [59]. Impairment of these three mechanisms causes the expression of cell adhesion particles and then a multistep process coagulation cascade activation involving bondage, translocation and strict adhesion of platelets to the inner layer of the vessel [65]. Activated blood plate increases its volume, which denotes cardiovascular risk, and is used in the clinical trial identification of inflammation and accompanying prothrombotic state [66, 67].

Activation of endothelial cells, arising not only under the influence of flow disorders but also under the influence of reactive oxygen species (ROS), and circulating lipoprotein leads to the expression of surface adhesion proteins—P-i L-selectin and von Willebrand factor (VWF), which support the mutual relationships between endothelial cells and platelets [65]. Endothelial cells present selectin on their surface, such as selectin-P, which stimulate platelets to produce glycoproteins, including glycoprotein-1. Complex selectin-P-glycoprotein-1 (PSGL-1) allows platelet adhesion and rolling but not tight binding of endothelial cells. Glycoprotein surface receptors GPVI and $\alpha v\beta 3$ bind vascular walls collagen, which activates the channels for intracellular calcium flux, causing the activation of receptors $\alpha IIb\beta 3$ and release of ADP and thromboxane A2 (TxA2). This initiates a platelet thrombus protruding into artery lumen and is essential for the later stages of thrombus formation. The combination of glycoprotein platelet receptors $\alpha IIb\beta 3$ with VWF and fibrinogen stabilizes the platelet clot, which in clinical practice becomes the target of preventing thrombolysis by pharmacological interventions (**Figure 1**) [65]. Impregnation of so-formed platelets conglomerate on the inner surface of the artery by fibrin finally decides the formation of a stable thrombus [9, 65].

Mutual activation of endothelium and platelets largely depends on the IL-1β, accumulated in granules of platelets and activated by mRNA already several hours after thrombin stimulation or adhesion-dependent integrins. Stimulation of platelets by IL-1β induces secretion of IL-6 and IL-8 and the expression of surface adhesion molecules-ICAM-1, $\alpha v\beta 3$ and hemotactic monocyte's protein-1 (MCP-1). Due to these mechanisms, platelets are

capable of recruiting monocytes and neutrophils from blood and then causing them to migrate and participate in the above described pathophysiology of atherosclerotic plaque vulnerability. Presented mechanisms ensure the presence of activated thrombocytes in the center of the pathophysiology of the process, not only with the thrombus formation but also as an important part in the activation and maintenance of the inflammatory process. Human platelets are capable of producing all types of toll-like receptors (TLR). It has been shown that higher TLR expression in women may be responsible for differences in cardiovascular risk profile, as well as the tendency for a higher incidence of ACS in the superficial thrombosis mechanism, without active inflammatory features in the atherosclerotic plaque [9, 59, 65].

Damage to the endothelial cells activates endothelial progenitor stem cells (EPCs) derived from bone marrow, which proliferate at the place of damage and may prevent the described processes that leading to the thrombus formation. These repair mechanisms are defective in patients with diabetes, characterized by a general weakness of repair capacity of damaged tissues [13, 33].

4.3. Artery calcification

Calcification in the arteries is especially visible in the carotid arteries. The relationship of calcification with instability of atherosclerotic plaques was not proven and is rather dubious [33]. It is suspected that calcifications are the result of increased apoptosis of smooth muscle cells and bleeding inside the atherosclerotic plaque [33].

Previously, it was thought that the calcification in the arterial walls increases the risk of cardiovascular complications [68]. Research in recent years has not shown; however, that calcifications increase the risk of plaque destabilization, and even reversely, calcified atherosclerotic plaques are now considered to be more stable [66, 67, 69, 70].

It has been shown that in the process of vascular calcification same changes occur as in the process of mineralization of bone tissue [33, 68]. Calcification of arteries in atherosclerosis is an active process and a complicated arrangement of mediators and calcification inhibitors is involved in it. This process includes participation of many cells (monocytes/macrophages, smooth muscle cells, vascular endothelial cells) and a variety of substances and transcription factors that are specific to bone rebuilding [71].

Emphasis is placed on separate pathomechanism of calcification of the arteries in patients with DM2 as compared with non-diabetic patients [2]. It has been proved that serum proteins like glycosylated albumin, through nuclear factor kappa-lightchain-enhancer of activated B cells (NFkB), mitogen-activated protein kinase (MAPK) and p38 kinase MAPK, leads to activation of vascular smooth muscle cells, which leads to the induction of inflammatory response, proliferation and migration of cells [72].

The dependence of calcifications on age and their predominance in men gender is underlined. The current prevailing opinion is that the presence of calcification demonstrates the extent and progress of atherosclerosis and identifies the risk of the patient generally associated with atherosclerosis and not directly related to the acute cardiovascular risk [70].

5. Summary

Progress within understanding the causes of the described disorders and the mechanisms leading to the formation of a blood clot opens up new therapeutic possibilities now and in the future to prevent acute cardiovascular incidents. The importance of the anti-inflammatory activity of statins as shown in many studies proving their clinical efficacy has already been mentioned. Similar importance has been demonstrated for other forms of therapy to lower LDL cholesterol with the help of ezetimibe or evolocumab. Controlling inflammation with patient behavior and statin drugs administration reveals other mechanisms leading to destabilization of atherosclerotic plaques. It cannot be ruled out that statins act on different levels of ongoing inflammation. In patients with HIV, there exist demonstrated beneficial effects of rosuvastatin, which reduced the presentation of PD-1 receptor on the surface of naïve-CD8+ [41]. It cannot be ruled out that it may be an indirect effect of its activity, by lowering levels of LDL cholesterol. Similar effects, including other pathways, leading to increased production of IL-2 and the intensification of differentiate naïve-CD8+ lymphocytes in the direction of Treg can restore the immunological balance and prevent destabilization of inflammatory atherosclerotic plaque [41].

Understanding of the immunological mechanisms, vulnerability of atherosclerotic plaque creates the chance to obtain antibodies against antigens involved in this process. The multitude of these antigens operating at different stages of development is currently an important difficulty. There exist attempts to gain effective antibodies against oxy-LDL, the key antigen for activation of inflammation and atherosclerosis. Research is directed into the efficacy and safety of antibodies directed against interleukins active in generating plaque instability. Some hopes are in the direction of tocilizumab, that is, IL-6 antagonist. The disadvantage to the trials that use anti-inflammatory medications is due to accompanying increase in metabolic disorders of the lipid fraction which can negatively affect the progression of atherosclerotic lesion [34]. In the last published results of CANTOS study, it has been shown to reduce the risk of several percent in recurrent cardiovascular events after the monoclonal antibody—canakinumab application, which is the antagonist of IL-1β, and reduce the level of highly sensitive C-reactive protein without affecting the level of cholesterol. The result of this study was considered inflammatory confirmation theory of atherosclerosis and a new perspective in its treatment [73, 74]. An interesting suggestion for therapy is in trying to influence the MMP family. Some of them, like MMPs-8; 10; 12; 13, are the enzymes responsible for the destruction of the fibrous cap as well as plaque rupture, its stabilization and reconstruction. MMPs are an interesting target for therapy of acute coronary syndromes; however, their general inhibition may lead to opposite effects [75].

Strong anti-inflammatory and anti-atherogenic effects have apoA-I, which is a protein component of high-density lipoprotein HDL. HDL is responsible for the reverse transport of cholesterol contained in the atherosclerotic plaque into the bloodstream. Change in aspect ratio of apoB/apoA-I can change the course of the atherosclerotic process. Significant in this regard are clinical studies [35].

The chances of stabilizing atherosclerotic plaques also connect with the future possibility of affecting the subpopulations of T lymphocytes. Controlling activity of Th by increasing the impact of immunosuppressive Treg may foster chronicity process atherosclerosis and avoid exacerbations associated with inflammation [34].

Another investigation directed towards controlling the course of the disease is the effect on macrophages. Control of their pro-inflammatory function could prevent destabilization of atherosclerotic plaque by reducing their in-plaque activity [34].

Recognizing the importance of external antigens (bacterial and viral infections) in activating inflammatory process, it is proposed to use vaccination as a prevention of exacerbations [13, 34].

Ongoing attempts to intensify healing processes within using pluripotent stem cells and activate endothelial progenitor cells derived from bone marrow. Getting progress in this regard would be an opportunity to control endothelial dysfunction in the early stages of atherosclerosis, particularly in patients with diabetes mellitus [13].

Above mentioned studies, as well as other ongoing multidirectionally experimental and clinical studies, offer hope that in the future one will be able to better understand and control the processes leading to the initiation and progression of atherosclerosis and mechanisms of activation and worsening inflammatory changes that lead to intravascular thrombosis—direct causes of acute cardiovascular syndromes.

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Chapter 7

Cerebrovascular Atherosclerosis: Cognitive Dysfunction Progress and Autophagic Regression

Kyoung Joo Cho and Gyung Whan Kim

Additional information is available at the end of the chapter

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Abstract

As the aging of society, metabolic disorders have become a major concern and a major cause for cardio- and neurovascular diseases such as atherosclerosis, stroke, and even cognitive decline. This chapter shows the progressive plaque formation mechanisms and regression under autophagic flow in both experimental and clinical side. Atherosclerotic plaque formation is not irrevocable. Clinical and experimental reports accept that atherosclerosis can regress after statin treatment. This chapter focuses on autophagic roles in atherosclerotic plaque formation, progression, and regression. Another focus is on the relationship between atherosclerosis and an increased risk of cognitive decline and further conversion from mild cognitive impairment (MCI) to dementia. There has been broad and strong support on the relationship between atherosclerotic severity and cognitive function. Ultrasound findings such as intima-media thickness (IMT) and plaque numbers could potentially be useful in identifying individuals with a higher risk of progression from cognitive decline according to morphological criteria. This also suggests the possibility as a predictive indicator of MCI and dementia by considering the presence of atherosclerotic changes. Focusing on therapeutics, this chapter provides mechanisms for regressing atherosclerotic plaques. Autophagy suggests therapeutic possibilities for atherosclerosis and it consequently paves the way for preventing cognitive impairment.

Keywords: atherosclerosis, autophagy, mild cognitive impairment, dementia, neurovascular

1. Pathogenesis of atherosclerosis

Cardio and cerebrovascular disease are leading causes of mortality worldwide. Mainly, they are caused by atherosclerosis, which is a chronic inflammatory disease of blood vessels. Cardiovascular events in the arterial wall result from the interactions between lipoproteins

and macrophages [1]. Accumulated lipoproteins bound to infiltrated macrophages to form a fibrous cap composed mostly of collagen in vascular smooth muscle cells, and this develops into an atherosclerotic plaque [2]. The developed plaque can cause stenosis, which can lead to ischemic conditions in surrounding tissues. When a plaque ruptures, in the worst-case scenario, thrombogenic materials are exposed, platelets are aggregated, and they form a thrombus. The detached thrombus becomes an embolus capable of blocking blood flow. Thus, atherothrombosis has the capability to cause ischemic stroke and myocardial infarction.

Lipoproteins transport cholesterol through the blood. Low-density lipoprotein (LDL) consists of esterified cholesterol and triglycerides that contain phospholipids, free cholesterol, and apolipoprotein B100 (ApoB100) [3]. Cellular LDL uptake is well-regulated. Their feedback mechanisms systemically limit excessive uptake and lipid overload in cells [4]. In contrast, oxidized LDL (oxLDL) mostly bypasses the feedback system, and results in intracellular lipid accumulation as foam cells present in atherosclerotic plaques [5]. Generally, the scavenger receptor-mediated uptake system misses the modified lipoproteins such as oxidized forms of LDL. During oxidation, physico-chemical properties such as lipid charge, size, and content are changed. Because oxidation modifies the LDL particles, oxLDL particles have already undergone physico-chemical changes. The oxLDL is technically different from natural LDL. The components of oxLDL can activate endothelial cells, and also induce the expression of adhesion molecules (E-selectin and VCAM-1) on the endothelial surface of the artery [2]. With endothelial activation by these oxidized lipids, oxLDL help macrophages to infiltrate into tissues and to produce chemokines as well as adhesion molecules [6].

The role of macrophages is critical in the development of atherosclerosis. Macrophages infiltrate to the arterial intima in response to oxLDL in the vessel. Macrophages engulf various lipids containing oxLDL and show a changed phenotype in comparison to lipid-laden foam cells. Spontaneously, they progress to a pro-inflammatory state. This is an early event in forming atherosclerotic lesion plaques. Macrophages secrete pro-inflammatory cytokines and recruit additional macrophages into the artery, and continuously increase atherosclerotic plaque size and complexity [7]. The early events of atherosclerosis induce additional immune cell infiltration and a progressive dysfunction to initiate a cell death pathway [5]. When atherosclerotic lesions develop, apoptotic as well as necrotic cell death occur. Cell debris and cholesterol form a necrotic core in the lesion covered by a fibrous cap of variable thickness [8]. Atherosclerosis forms under chronic exposure to cellular stressors, which promotes accumulated lipid degrading cascades and consequently dysfunction. It has been revealed that macrophage autophagy is linked to lipid metabolism [7]. In atherosclerotic plaques, there is intracellular accumulation of LDL as well as damaged tissue and misfolded/aggregated proteins. Biologically, these extra-accumulating materials are dealt via autophagy. Through the use of adapter proteins, the cells undergo autophagy. The process involves selective events rather than random bulk cleavage [9]. The selective autophagy can be described as: mitophagy, handling mitochondria; pexophagy, charging on peroxisomes; lipophagy, dealing with lipids; aggrephagy, taking care of aggregated proteins; and xenophagy, treating microorganisms. Among them, lipophagy is

the initiating event of autophagy by mediating a cholesterol efflux [10]. It has slowly become clear that an atherosclerotic macrophage can induce and degrade cargo lipids by selective autophagy. In the case of the chaperone protein p62/SQSTM1 in chaperone-mediated autophagy (CMA), p62/SQSTM1 can hold and transfer poly-ubiquitinated cargo to autophagosomes for degradation. This machinery performs degrading events through the ubiquitin-binding domain (UBD) and the LC3-interacting region (LIR) [11]. The dysregulation of this autophagic pathway results from the markedly elevated p62/SQSTM1 protein levels in macrophages of the atherosclerotic plaque [12].

It is apparent that inflammatory factors and inflammatory reactions play critical roles in atherothrombotic disease. However, this chapter will focus on the autophagic pathway and dysregulated autophagy among contributors (members) to atherothrombosis.

2. Autophagic regulation and dysfunction in atherosclerosis

Autophagy literally means "to eat oneself" and originated in Greek. It is an evolutionary conserved mechanism, that is, a catabolic process to degrade cytoplasmic contents such as cellular proteins and organelles through lysosomes for recycling and use in downstream metabolism [13]. Biomolecules degrade and generate free fatty acids, amino acids, and nucleotides, which can be reused by the cell to maintain energy production and protein synthesis [13]. Degradation of intracellular molecules occurs through two distinct systems: the ubiquitin-proteasome system and the lysosome-autophagy system [14, 15]. In mammals, autophagy is the major pathway used to degrade abnormal products besides the ubiquitin-proteasome system. Autophagy is primarily used for the removal of damaged organelles, abnormal proteins, and protein aggregates [16], and this housekeeping function is particularly essential in the heart and brain. When autophagy-specific genes are lacking, a severe cardiomyopathy or neurodegeneration occurs [17].

There are several types of autophagy according to the method of delivery of the cargo to lysosomes: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) [18]. Macroautophagy is the predominant mechanism among these three types. Macroautophagy starts with the formation of double-membrane vesicles, autophagosomes. Autophagosomes fuse with lysosomes and finally progress to autolysosomes. Physiological stress conditions such as starvation upregulates autophagy. Identified genes and molecules involved include around 30 genes and they are called autophagy-related genes (ATGs) required for autophagic pathways [19]. Among them, ATG5 and ATG12 are involved in the first step, controlling autophagy with two ubiquitylation-like reactions. ATG12 links to ATG5 requiring ATG7 (serves as an ubiquitin-activating enzyme, E1) and ATGT10 (serves as an ubiquitin-activating enzyme, E2). Then, the ATG5-ATG12 complex is involved in autophagosome formation. Autophagosomes randomly formed in the cytoplasm are trafficked along microtubules to lysosomes in a way that is dynein-dependent. Autophagosomes, then, are fused with lysosomes. The SNARE proteins of yeast are thought to be involved in this fusion [20, 21].

Microautophagy raises the possibility of direct cytoplasmic engulfment by the lysosome in mammals or the vacuole in plant and fungi [22]. In macroautophagy, a double- or multi-membrane-surrounded autophagosome forms, which fuse with lysosomes in a non-specific way for degradation [22]. In contrast to macroautophagy, in microautophagy, the lysosomal/vacuolar membrane is randomly engulfed and differentiates into the autophagic tube enclosing the cytosolic portion [23]. Microautophagy starts with making membrane knobs into the surface of the lysosome, and constructs small smooth areas that are able to degrade. The invaginations move laterally and also can shrink, which are specified into particular tubular shape "autophagic tubes" [23]. This characteristic is unique and gives them an autophagic function. After that, a dramatic decrease occurs along with the autophagic tube intramembranous proteins toward the top of the tube. Collectively, microautophagy performs degradation of cargo lipids and proteins in the following order: vesicle formation, vesicle expansion, vesicle scission, and eventual vesicle degradation and recycling [22].

Another case of autophagy is chaperone-mediated autophagy (CMA). Cargo recognition in macroautophagy has a non-selective process, because soluble cytosolic proteins cannot be selected as single protein molecules and are targeted for degradation through this pathway [24]. CMA targets only single proteins. In CMA, proteins are identified one by one, and the identified proteins are degraded by using a cytosolic chaperone system that delivers them to the surface of the lysosomes [25]. Selectivity in CMA uses a pentapeptide amino acid sequence motif in the substrate proteins. When the substrate proteins are recognized by a cytosolic chaperone, it results in targeting substrates to lysosomes [26]. CMA proceeds in sequential multi-steps: (i) recognition of substrate proteins; (ii) binding and unfolding of substrates; (iii) translocation of substrates inside the lysosomes; and (iv) degradation of substrates in the lysosomal lumen through its cellular functions [27].

CMA-targeting motifs are generated through posttranslational modifications with KFERQ on the targets. This pentapeptide was first reported to be critical in the degradation of RNase A [28], and it is shared by all identified substrate proteins to date [24]. The proteins carrying the KFERQ motif are targeted by a constitutive chaperone, the heat shock-cognate protein of 70 kDa (Hsc70). Hsc70 is the only chaperone to interact with the substrate via regulated ATP/ADP binding cycles [29]. The chaperone Hsc70 combines the proteins with a KFERQ motif, and binds with the cytosolic tail of the single-span membrane protein lysosome-associated membrane protein type 2A (LAMP-2A19, LAMP-2A), which shuttles the chaperone complex and the targeted protein into the lysosomal lumen [29]. Hsc70 also interacts with protein aggregates, and has mediated their degradation by macroautophagy, which is called a chaperone-assisted selective autophagy (CASA) [30]. This reaction works the same way on a responsible disassembly of clathrin from coated vesicles, and is needed to fold the unfolded cytosolic proteins upon recognition of exposed hydrophobic regions [28]. Once the substrate is translocated into the lysosomal lumen, LAMP-2A is rapidly dissembled from the complex into monomers, which endows LAMP-2A to bind with other substrates again [27]. LAMP-2A is one of the three splice variants of the *lamp2* gene [9], and is a single-span membrane protein. There is a very heavily glycosylated luminal region and a short (12-amino acid) C-terminus

tail in LAMP-2A. When they are exposed on the surface of the lysosomes, substrate proteins bind to them [25].

2.1. Autophagy in VSMC of atherosclerotic plaques

The general architecture and cellular composition of blood vessels have basic components in the wall of blood vessels: endothelial cells (EC), vascular smooth muscle cells (VSMC), and extracellular matrix (ECM), including elastin, collagen, and glycosoaminoglycans. Each vessel is composed of the three concentric layers intima, media, and adventitia [31]. In normal mature blood vessels, VSMC predominantly exist in a contractile or differentiated phenotype that regulates blood flow and blood vessel diameter with vasodilation and vasoconstriction. The contractile VSMCs are surrounded by their own basement membrane, some macrophages, and fibroblasts. When damaged, VSMC generates intimal vascular lesions. The VSMC layers of the nearest vessel lumen receive oxygen as well as nutrients by direct diffusion from the vessel lumen.

VSMC in atherosclerosis consists of aberrantly proliferated VSMC to promote plaque formation. However, VSMC in advanced plaques is involved in preventing rupture of the fibrous cap [32]. The tensile strength of the protective cap relies on structural properties that are determined by the number of VSMCs and the collagen [33]. This is the reason why loss of VSMC leads to plaque destabilization and rupture. In advanced plaques, disintegrating VSMCs in the fibrous cap undergo programmed cell death, which is not apoptosis but autophagy. This has been shown in electron-microscopy imaging as formation of myelin figures [34], accumulation of ubiquitinated inclusions in the cytosol [13], and severe vacuolization [34]. In this stage, macrophages actively induce SMC death [13].

VSMCs loading a lot of cholesterol activate multiple pro-inflammatory genes and are altered to form macrophage-like cells driven by lipid accumulation in the plaque, and are then induced to perform phagocytic activity [32]. In the fibrous cap of advanced human plaques, VSMCs die showing ubiquitinated inclusions indicating they are undergoing autophagic death [13, 19]. Actually, the fibrous cap is a thick layer of basal lamina. It may be easier to let these "caged" cells undergo autophagy. Also, it has been suggested in *in vitro* studies that caged cells can trigger autophagy in atherosclerotic plaques. It has been reported in human plaques that lipid-laden VSMCs increase the expression of death-associated protein (DAP) kinase, a pro-apoptotic mediator, and regulate the formation of autophagic vesicles [35].

Generally, autophagy is well-recognized as a survival mechanism under starvation and not as a death pathway [36]. In atherosclerotic plaques, autophagy most likely plays a safeguarding role for plaque cells against oxidative stress, a hallmark of advanced atherosclerotic lesions. The successful autophagy in the atherosclerotic plaque is anti-apoptotic and eventually contributes to cellular recovery. However, it becomes another story under acute or persistent oxidative stress. In this case, over-produced intracellular ROS can be harmful to the lysosomal membrane. When autophagy does not work in the oxidative stress response in atherosclerotic plaques, or when oxidative injury overwhelms the cellular defenses, cells might undergo apoptosis.

2.2. Autophagy in macrophages of atherosclerotic plaques

Macrophages are immune cells having a strong phagocytic potential. They migrate into tissues derived from the differentiation of monocyte precursors in blood [7]. They are primarily involved in the phagocytosis against extracellular pathogens. They are also responsible for treating cellular debris, antigen presentation, and activation of the adaptive immune system. Macrophages secrete either pro- or anti-inflammatory cytokines according to their activation state [8]. Monocytes are recruited to the vessel intima, and they are initiated by chemokines secreted from endothelial cells, which are activated by excess lipoprotein accumulation [21]. These events show a profound effect on the reduction of atherosclerotic plaque burden through a lower number of circulating monocytes or to prevent their interactions with the endothelium via chemokine/chemokine receptor blockage [37].

In atherosclerotic plaques, macrophages contribute to cytokine production, the maintenance of vessel wall inflammation, and finally atherosclerotic progression [38]. Inflammatory signaling is a general and major event in atherosclerosis. Several other pathways, besides inflammation signals, are triggered by macrophages involved in wreaking havoc on the plaque [7]. They are over-expressed reactive oxygen intermediates containing myeloperoxidase-induced reactive nitrogen species. Under oxidative stress, secreted cathepsins and matrix metalloproteinases (MMPs) worsen the sub-endothelial environment. This toxic environment results in a vicious cycle of lipoprotein oxidation, enhanced lipoprotein uptake, and increased inflammatory signaling [7]. When the membrane cholesterol content of macrophages exceeds their handling capacity, a lipid droplet is formed. Cells with lipid droplets are defined as foam cells in the atherosclerotic lesion. A primary response to such lipid overload is the efflux of excess cholesterol out of macrophages with the help of high-density lipoproteins (HDLs). This process occurs in the cytoplasm where a family of cholesteryl ester hydrolases releases cholesterol from macrophage lipid droplets. This is followed by ATP-binding cassette transporter ABCA1 (ABCA-1), known as the cholesterol efflux regulatory protein (CERP). Finally, exogenously derived cholesteryl esters are hydrolyzed in lysosomes [7]. After that, the free cholesterols are distributed to different cellular membrane compartments. In addition to cholesterol, the focal lipid substrate and other lipid species may affect macrophage lysosomal function [7].

Under physiological states in contrast to pathological states, most cells turn on compensatory mechanisms for handling such insults. Autophagy is one of the responses to toxic intermediates found in the atherosclerotic plaque, and autophagic processes concomitantly increase in macrophages [14]. Lipophagy was first discovered in the liver where a specific mechanism handles lipids [39]. It has also been evaluated in foam cell macrophages. Autophagic uptake of lipid droplets is subsequently subjected to lysosomal acid lipase (LAL)-dependent degradation of cholesteryl esters in lysosomes. This is also an alternative mechanism of generating free cholesterol for ABCA1-mediated efflux to HDL [14]. It can be concluded that autophagy deficiency in macrophages increases macrophages' susceptibility to foam cell formation. It is undoubtedly true that macrophage autophagy has an essential role in the atherosclerotic process. In mice lacking *Atg*5, atherosclerotic plaques are enlarged and overloaded with lipids, there is extensive pro-inflammation, and the atherosclerotic core is filled with apoptotic and necrotic cells [7]. Recently, autophagy has been implicated in regulating cholesterol

efflux, suppressing inflammasome activation, and improving apoptosis in atherosclerotic macrophages.

2.3. Autophagy in vascular endothelial cells

Endothelial cells are arranged in many layers in large blood vessels in which they form a tough wall as connective tissue. The endothelial cells in mature vessels send signals to the surrounding connective tissue, and take on an important part in regulating the vessel's function and structure [40]. For regulating roles, the endothelial cells mediate fluid filtration, hormone trafficking, neutrophil recruitment, and finally maintaining hemostasis [41]. Endothelial cell dysfunction (ECD) in the artery is the first detectable change of a forming atherosclerotic lesion [42]. The changes in the sub-endothelial area contain: the focal permeation and trapping, and the physiological and chemical modification of circulating lipoprotein particles [43].

The term endothelial dysfunction has already entered the lexicon of modern cardiovascular medicine [40]. However, the concept has not been developed to our present understanding of the cellular and molecular mechanisms of atherosclerosis. Under atherogenesis, the earlier characterization of endothelial dysfunction was focused on whether anatomical integrity of the intima was intact. The simplest definition of endothelial dysfunction is a lack of nitro oxide (NO), which is involved in various disease states: atherosclerosis, diabetes mellitus, coronary artery disease, hypertension, and hypercholesterolemia [41]. Endothelium-derived NO can modulate leukocyte adhesion. Endothelium-derived NO prevents leukocyte recruitment to the vascular wall via the anti-inflammatory effects of NO. Endothelium-derived NO suppresses the expression level of VCAM-1, ICAM-1, and E-selectin, which respond to pro-inflammatory cytokines. The cellular adhesion molecules mediate activation of the transcription factor NF-κB, and NF-κB inhibited by endothelial NO prevents endothelial cell activation [44]. It has been found that inhibition of basal eNOS activity rapidly induces VCAM-1 and also increases monocyte adhesion [45]. This linkage could induce or enhance endothelial cell activation. The mediators of endothelial dysfunction such as hypercholesterolemia or oxidative stress can lead to increasing vasoconstriction, smooth muscle proliferation, platelet aggregation, leukocyte adhesion, LDL oxidation, and MMP activation. In the vessel wall when there is turbulent flow, endothelial cell activation and atherosclerosis may occur more readily because there is less endothelium-derived NO. With support, atherosclerotic lesions develop more frequently at vascular branching sites when exposed to turbulent flow rather than laminar flow. In animal studies, eNOS deleted mice develop increased atherosclerosis and vascular inflammation [46].

In contrast to endothelial dysfunction, endothelial cell activation is defined by the endothelial cell surface adhesion molecules, such as VCAM-1, ICAM-1, and endothelial leukocyte adhesion molecule (E-selectin) [41]. Endothelial cell activation is typically induced by pro-inflammatory cytokines, such as TNF-α and IL-6. When endothelial cells are activated, they facilitate the recruitment and attachment of circulating leukocytes to the vessel wall. Progressive structural remodeling of developing lesions starts with the formation of a fibrous cap. The lateral edges of these complicated atherosclerotic plaques contain a rich population of inflammatory cells such as activated macrophages, T-cells, natural killer T-cells (NK T-cell), and dendritic

cells. These inflammatory cells further modulate the endothelial cells into a pro-inflammatory phenotype, and have the endothelial cells work on structural instability of the plaque by modifying the proteolytic activity of extracellular matrix components [47].

Because of the above-mentioned characteristics, exogenous NO has been implicated as a therapeutic target. NO has benefits in vascular inflammatory diseases, and some researchers have tried to ameliorate atherosclerosis and other vascular diseases with NO donor therapy [41]. The therapeutic use of NO therapy has been reported to ameliorate atherosclerosis [48]. It is an important aspect of therapy whether atherogenesis initiates the formation of endothelial dysfunction or activation. Although it is unclear how endothelial cells recruit inflammatory cells, it is clear that inflammatory cytokines secretion of endothelial cells is tightly linked to eNOS expression. This relationship gives us hints for therapy. Also, vascular endothelium-derived NO has a protective role extending to endothelial-leukocyte interactions, leukocyte trafficking to hinder platelet activation, and smooth muscle contraction and proliferation. Statins (HMG-CoA reductase inhibitors) restore endothelial function, and protect vessels by boosting endothelium-derived NO. Endostatin has been reported to induce autophagic cell death in human endothelial cells (EA.hy926) [49]. When human endothelial cells are exposed to oxLDL, autophagy in the cells is increased to deal with plaque components. It is accepted that endostatin induces damaged endothelial cells by overloaded lipid through autophagic cell death pathways [50].

3. Atherothrombosis stabilization and regression mechanism

Atherosclerotic plaque lesions are generally asymptomatic for years with slowly evolving in restricting blood flow around lesion [51]. The transition between stable and unstable is decided by the development of a large necrotic core resulting from cell death within the plaque and failure to clearing dead cells. Macrophages are key players in the transition from stable to unstable lesions [52].

Primarily, autophagy is recognized as a survival mechanism and not as one of the cell death pathways. This renders the role of autophagy in atherosclerosis to be equivocal [53]. Successful autophagy generally contributes to cellular survival by acting anti-apoptosis and cellular recovery by supplying biomaterials. Autophagy serves as safeguards for atherosclerotic plaque cells against cellular oxidative stress by polarizing mitochondria not to release cytochrome c [54]. In this reason, autophagy of VSMCs of the fibrous cap in advanced atherosclerotic lesion is important to plaque stabilization. Autophagic death in VSMCs results from excessively stimulated autophagy, and results in plaque destabilization [53]. Autophagic death in endothelial cells affects to maintain the structure of the thrombotic plaques. In view of stabilizing plaque on the rupture-prone lesion, induction of autophagic macrophages might be a promising strategic role in plaque which is not obstructive into lumen but prone to rupture [55]. Dysfunctional autophagy stimulates accumulation of damaged mitochondria, ROS over-expression, and ceroid in human plaque. Continuously and excessively stimulated autophagy can initiate autophagic VSMCs death resulting in plaque destabilization because

collagen synthesis in reduced and also the fibrous plaque cap gets thinning. Of course, autophagic cell death is triggered in endothelial cells, which is detrimental role in the sustaining structure of the atherosclerotic plaque. It is an acute clinical event promoting thrombosis on the atherosclerotic lesion.

Lipid modification such as LDL oxidation brings about a range of modifications with various physiological and biochemical properties [8]. Modified lipids in macrophage cells are able to induce lysosomal dysfunction which can result in the accumulation of intra-lysosomal cholesteryl esters [56]. A number studies have shown that uptake of modified lipids induces a lysosomal lipid storage disease-like condition [5]. Accumulated lipids in lysosomes cause lysosomal dysfunction and affects the intracellular transport machinery. When macrophages are exposed to oxLDL and cholesterol, so-called atherogenic or modified lipids, lysosomal dysfunction occurs [16]. The oxLDL-derived cholesteryl esters form cholesterol crystal when oxLDL-derived cholesteryl esters are inefficiently hydrolyzed and transported in lysosomes [57]. Through CD36-dependent mechanisms, oxLDL is moved to macrophage lysosomes; cholesterol crystals accumulate in the lysosomes. Cholesterol crystals beyond the dealing range initiate lysosomal damage and result in leaking lysosomes [57]. As an example, phagocytosis of apoptotic cells (efferocytosis) is detected in plaque progression and is regarded as a critical feature of increasing plaque complexity [5]. PRPs, cell surface receptors and also scavenger receptors, recognize modified lipids (oxLDL) and pathogens. Plasma levels of soluble CD36, one of scavenger receptors, are higher in the context of risk factors for the development of atherosclerosis such as diabetes [58]. The altered "eat-me" signals can also affect efferocytosis and the targets of apoptotic cells. For example, mice lacking complement factor C1q exhibited efferocytosis dysfunction and atherosclerotic plaque burden [59]. In human atherosclerotic plaques, efferocytosis is impaired and also shaded phagocytic receptors, which impedes phagocytic capacity of macrophages and involves activation of the inflammatory response [60]. The LDLR-related protein 1 (LRP1) is one of the important receptors interacting with C1q for opsonizing.

Prolonged oxidative damage induces protein misfolding and the accumulation of dysfunctional proteins to be degraded [61]. Large protein aggregates are ubiquitinated, and the poly-ubiquitinated protein aggregates are shuttled to the autophagosome. This is generally performed via chaperone proteins such as p62/SQSTM1 [11]. The reason for inflammasome activation in the plaque is not currently unclear, but two mechanisms have been suggested. One is that inefficient mitophagy clearing of damaged mitochondria results in increasing reactive oxygen species (ROS), which induces inflammasome activation. However, the level of protein oxidation and superoxides are augmented in autophagy-deficient macrophages and atherosclerotic plaques [12]. The other mechanism is that overloaded oxLDLs and cholesterol crystals destabilize the lysosomal membrane, resulting in inflammasome activation by producing IL-1β [7]. In the atherosclerotic context, it has been shown that aggregated proteins activate inflammasomes and aggravate atherosclerosis in autophagy-deficient systems [12].

Atherosclerosis progression presents the features of impaired autophagy. Autophagy is sequential events called as autophagic flux (autophagosome formation, cargo sequestration, and autolysosomal fusion), and unfortunately, hard to assess the flux in vivo. When p62/SQSTM1, a

chaperone shuttling protein aggregates from cytosol to autophagosomes, is combined to protein aggregates and degraded, increased level of p62/SQSTM1 indicate defective in autophagic flux autophagy [62]. Correspondingly, deficient autophagy of macrophage can facilitate atherosclerotic plaque progression. *Atg*5 knock-out mice with ApoE-null background showed that western diet for 2 months increased the level of p62/SQSTM1 in the vessel with similar level of control mice whereas atherosclerotic lesion was bigger than control both in aortic root and whole aorta [62]. Using animals with experimental atherosclerosis, ApoE-null mice, recent study proposed that plaque formation expands when macrophagic autophagy is completely disrupted and not partially disrupted. Partially disrupted autophagic condition induces rather macrophagy inflammation and excess IL-1beta, because cholesterol crystal of atherosclerotic plaques is potent stimuli to activate inflammasome [62].

Cholesterol efflux is induced to balance the level of macrophage storing lipid by transferring increased cholesterol from peripheral tissues to the liver. The primary cholesterol efflux mechanism has been thought that cholesterols are hydrolyzed cholesteryl esters cytosolic hydrolases; free cholesterols are moved to the plasma membrane; finally free cholesterols are delivered to the periphery by ATP-binding cassette transporters (ABCA1 or ABCG1) [63]. Autophagic malfunction of macrophages abrogates this cholesterol efflux when macrophages are faced to hinder autophage by chemically (chloroquine) or genetically (*Atg*5-deficiency). Furthermore, inhibitors of lysosomal acid lipase also diminish cholesterol efflux. These showed that cholesterol hydrolysis as well as autophagic delivery is a critical step in atherosclerotic plaque progression and regression. Although lipid-laden macrophages induce lipophagy and also trigger a counter regulatory mechanism are unclear, it is clear that lipophagy-mediated efflux plays an important role in cholesterol transport in vivo [7]. Therefore, efficient cholesterol metabolism and efflux considered athero-protective mechanisms against accumulated lipid-laden atherogenic condition [64].

4. Cognitive impairment after atherosclerosis

Aging is a major risk factor for neurodegenerative disease associated with atherosclerosis [65]. Previous studies have demonstrated a strong association between aging and vascular diseases. Recent clinical investigations have focused on the relationship between levels of circulating adhesion factors in peripheral blood and cerebrovascular diseases [66]. Platelets and leukocytes play a major role in atherothrombosis, aggregates of which result in the formation of atherosclerotic plaques [67]. Although other factors associated with vascular disease can influence the cognitive state, few studies have utilized flow cytometry to investigate platelet and leukocyte markers in older adults with cognitive decline. Research has demonstrated a correlation between circulating adhesion molecules in patients with atherosclerosis and atherosclerosis factors such as intima-media thickness (IMT) and the number of plaques, which may assist in determining the presence and/or extent of cognitive decline [68]. To determine the potential usefulness of this correlation for determining diagnoses/prognoses, blood factor analysis is required. Based on the pathophysiological mechanism underlying dementia, most relevant studies have aimed to identify molecular markers based on drug responses [69]. As

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such, little is known regarding the potential role of circulating adhesion molecules in patients with vascular diseases during the early and later stages of cognitive dysfunction.

Many definitions have been proposed for the transition point when healthy aging with a slight cognitive decline progresses to dementia [70]. Mild cognitive impairment (MCI), which was first proposed by a group of investigators from the Mayo Clinic in the late 1990s [70], was defined to be based on a memory problem. This section provides our results about assessing the relationship between changes in blood factors and ultrasound findings in patients with MCI and dementia who were also exhibiting signs of atherosclerosis.

4.1. Atherosclerosis and dementia

Carotid atherosclerosis severity is assessed by considering the plaque number, proportions, and location as well as the presence of carotid stenosis that is caused by plaques. Additionally, the severity of carotid stenosis is determined according to the blood flow velocities, residual rumen diameter, and carotid artery flow velocities ratio to internal carotid artery versus the common carotid artery [71]. For AD, it is generally accepted that vascular risk factors have an epidemiological effect on dementia [72]. It has been reported that a narrowed carotid lumen is a risk factor for cognitive impairment in steno-occlusive carotid artery disease patients [72]. Revascularization procedures may have some benefit in the alleviation of dementia, but not for all of these patients [72]. In cases of mild AD with severe asymptomatic intra-carotid artery (ICA) stenosis, cognitive decline progressed even though they have not experienced cerebral ischemia [72]. One possible explanation of this relationship is that insufficient cerebrovascular flow causes cerebral atrophy. Another one is vascular factors that are promoting the degenerative changes of AD [72].

The available studies have identified factors associated with aging and vascular dysfunction that exhibit a cross-sectional relationship with mental status based on the Mini-Mental State Examination (MMSE) score. Recent studies have reported that carotid artery atherosclerosis is associated with a subsequent risk of new or recurrent cerebrovascular diseases, such as stroke, post-stroke vascular dementia, and MCI [66, 73, 74]. Furthermore, chronic hypoperfusion caused by carotid stenosis has been reported to play a role in cognitive decline [75]. Dementia represents a major public health concern [68], as accumulating evidence has demonstrated that the incidence and prevalence of dementia increases rapidly with advancing age. Although it has been difficult to investigate changes in the incidence and prevalence of dementia due to variations in diagnostic criteria and methods, a recent epidemiological study indicated that the dementia prevalence and incidence have decreased in some countries. Moreover, the number of patients with dementia has remained stable in the aging population of these countries [76]. Some evidence has suggested that vascular risk factors are associated with the onset and progression of AD [77]. There are increasing concerns that microvascular disease and tau deposition are found concomitantly and it is thought that treating vascular risk factors is as important as preventing cognitive decline [66]. Although the association between anterior cerebral artery (ACA) plaques and dementia has not been fully determined for the number and the location of plaques, it can be used as a better indicator of disease progression and severity.

In addition, increased cerebrovascular risk has been associated with more severe dementia and a higher MCI incidence [78]. Considering the role of vascular blood factors in patients with MCI, such factors may also influence the progression of cognitive decline [79]. However, there are currently no markers for the prediction of prognosis or the risk of conversion from MCI to dementia. Therefore, it is necessary to develop noninvasive diagnostic methods for the assessment of vascular status [80]. This aspect is discussed in more detail in the next subsection with my results.

4.2. Neurosonological findings of atherosclerosis and dementia

In our recent study, we demonstrated that alterations in IMT and plaque number are associated with an increased risk of cognitive decline as well as a risk of dementia. Our results suggest that ultrasound findings may aid in identifying older individuals at increased risk for the progression of cognitive decline when morphological impairment of cerebrovascular structures has been identified. Moreover, our findings suggest that the presence of atherosclerotic changes and changes in blood factors such as p-selectin glycoprotein ligand (PSGL, CD162), platelet-leukocyte aggregation (PLA), and platelet-monocyte aggregation (PMA) can be used to predict MCI and dementia.

Our study showed that levels of p-selectin in circulating platelets, PSGL, and circulating platelet-monocyte aggregates were significantly increased in patients with MCI relative to controls. The changes in circulating blood factors have been reported to relate with vascular diseases such as ischemic stroke or atherosclerosis [81]. Based on this association, several noninvasive measures for evaluating subclinical atherosclerosis have received intense attention in clinical and research settings for the predictive diagnosis of cerebrovascular diseases. Researchers have suggested a relationship between atherosclerotic severity and circulating adhesion blood factors and atherosclerotic severity and cognitive decline in the above-mentioned reports. With one step further linked between them, our findings provide insight into the use of blood factor analysis (using FACS) as well as ultrasonographic evaluation of vessel status in both clinical and research settings. Changes in platelet activation and monocyte distribution are observed in the early stages of atherosclerosis. Such changes are strongly associated with stroke onset, as demonstrated by various studies [82]. The monocyte receptor CD14 and leukocyte antigen CD45 are best known for their crucial role in immunity. In addition, CD14 and CD16 are well-known biomarkers for atherosclerotic disease progression [67]. Research has also suggested that PSGL is a pro-atherogenic marker of vascular disease progression [67].

The present study shows that increased IMT was more frequently observed in patients with MCI, whereas increased numbers of carotid plaques were more frequently observed in patients with dementia. The patients with MCI in our study comprise 32% of all patients with atherosclerosis, and all patients of the MCI group in the present study had been diagnosed with carotid vascular stenosis or atherosclerosis. These findings suppose that vessel damage is followed by MCI. A lot of findings in previous studies suggest that greater degrees of carotid atherosclerosis are associated with the progression from MCI to dementia [66, 68, 78]. A recent study reported that up to 50% of patients develop vascular stenosis, and that ACA

plaques are associated with dementia even after controlling for vascular risk factors [66]. Other researchers have suggested that atherosclerosis plays a role in cognitive impairment, particularly in older adults [83]. Such research has further demonstrated a converging relationship between degenerative vascular dysfunction and cognitive dysfunction. In our study, most patients with MCI exhibit atherosclerotic vessel abnormalities, such as increased IMT and plaque numbers, increasing the risk for progression to dementia. An estimated 15–42% of people over the age of 65 years exhibit some form of MCI, and approximately 5–15% of patients with MCI go on to develop dementia [70]. Recent evidence has revealed that vessel dysfunction contributes to AD as well as vascular dementia [84]. In this previous study, the authors reported an IMT cutoff value of 0.805 for the prediction of MCI development (baseline: 0.825 mm) [84]. Diagnosis of dementia in such patients is required in order to ensure the appropriate therapeutic guidelines and treatments are utilized [76].

Our results indicated that intima thickness and plaque number are associated with higher levels of p-selectin, supporting the evidence that platelets are engaged in the formation of PLAs [85]. In the dementia group of the present study, which included individuals with dementia, plaque numbers corresponded strongly with levels of PSGL-positive platelets. Control of plaque numbers with appropriate therapy such as statin treatment may thus delay or prevent the progression of cognitive decline to dementia. Our findings also indicated that carotid atherosclerosis correlates with MCI as well as increased numbers of PSGL-expressing platelets. Analysis of blood factors using ultrasonography may aid clinicians in determining the most appropriate treatment strategy for patients with cognitive decline with vessel disease. Our simple assessment of vascular risk factors does not seem to be a fully satisfactory approach for adequately counteracting the risk of developing dementia, when compared to other large-scale studies [86]. Nevertheless, we suggest that analysis of circulating adhesion factors may aid in predicting the risk of progressive cognitive impairment. Additionally, aggressive treatments for vascular disease should be considered for individuals with a predisposition toward dementia. Despite these limitations, our findings provide a basis for a future study regarding biomarkers of both cerebrovascular disease and cognitive dysfunction.

In conclusion, our findings demonstrate that circulating adhesion molecule levels and interaction between factors present significant differences in patient with MCI or dementia. Alterations in IMT and plaque number are associated with an increased risk of cognitive decline as well as conversion from MCI to dementia. These results suggest that ultrasound findings may aid in identifying older individuals at increased risk for the progression of cognitive decline when there is cerebrovascular damage. Moreover, our findings suggest that the presence of atherosclerotic changes and changes in blood factors such as p-selectin, PSGL, PLA, and PMA can be used to predict the progression of MCI and dementia.

4.3. Prevalence and incidence of MCI and dementia in atherosclerosis

The World Alzheimer Report 2015 announced the estimate that 46.8 million people worldwide have dementia, and this number is expected to increase to 74.7 million by 2030 and 131.5 million by 2050 [76]. Accordingly, due to concerns about the increasing incidence of dementia, dementia is predicted to be 'epidemic' and a consequent economic burden. The G8 dementia

summit in 2013 and the WHO Ministerial Conference in 2015 decided to engage in a global action against dementia. The Atherosclerosis Risk in Communities (ARIC) study performed in 1987–1989 enrolled 15,792 individuals: they were a bi-racial group, with an age range from 45 to 64 years, from 4 US communities. Cognitive assessments were performed in the second ARIC examination in 1990–1992 [74]. A comprehensive dementia study, Atherosclerosis Risk in Communities Neurocognitive Study (ARIC-NCS), was used as the fifth ARIC examination in 2011–2013. They evaluated what happens to participants with extensive cardiovascular disease and cognitive dysfunction after a long history of ARIC and for the participants who died. This is a longitudinal cohort depicting the association of cognitive function, cardiovascular condition, cerebrovascular condition, and mortality. In ARIC-NCS, they reported that the overall prevalence of dementia in living ARIC participants is similar to the estimate of the World Alzheimer Report 2015. Although the prevalence of MCI in ARIC-NCS and the prevalence of MCI have been reporting to be at a similar level, there is a variation in MCI prevalence because of different MCI definitions [74]. Therefore, longitudinal studies of incident dementia in this cohort are required for validation of the MCI definition.

From some studies, it is obvious that the prevalence of dementia is related to stroke, heart disease, hypertension, and diabetes. These results are limited because many medications used to treat cardiovascular disease and other vascular diseases have been observed to have an effect on dementia prevalence and incidence. This is why no single factor has been identified to fully explain the changes in dementia prevalence and incidence. However, it is important to identify multiple risk factors and protective factors throughout a personal whole life-course relating to physical, mental, and cognitive health. In particular, atherosclerotic and vascular risk factors need to be well-controlled for reducing the risk of dementia in later life.

5. Prospects of therapy

For developing new therapeutic strategies for atherosclerosis, it is important to understand the cause of the disease pathogenesis and its progression [33]. A number of studies have shown that cell deaths produce different patterns depending on the stage of the plaque and types of cells involved in cell death. In summary, atherosclerosis is an inflammation-related arterial intima disease. A number of pharmacologic approaches have developed the way to stabilize rupture-prone atherosclerotic lesion by applying macrophage autophagic death. For therapeutic approach in atherosclerosis, it should be implicated for plaque stability and selective depletion of macrophages by modulating sterols. This section will introduce them.

5.1. Harnessing macrophagic autophagy as a therapy for atherosclerosis

One such pathway regulating the initiating autophagy involves mammalian target of rapamycin (mTOR). Blocking mTOR using rapamycin has an effect on cell proliferation both *in vitro* and *in vivo* [87]. Inhibition of mTOR leads to autophagic cell death through some ATG protein pathways [88]. ATG13 is rapidly dephosphorylated when the mTOR pathway is inhibited. By stimulating affinity to ATG1, the ATG1-ATG13 complex is involved in autophagosome

formation. When the rapamycin derivative everolimus is delivered from a stent in atherosclerotic plaques, in the lesion site of cholesterol-fed rabbits, autophagic macrophage death occurred due to macrophage reduction. In contrast, the amounts of VSMCs were sustained without change [89]. An mTOR-mediated pathway induces dephosphorylation of p70 S6 kinase, which is responsible for selective induction of macrophage death. On the other hand, the protein synthesis inhibitor cycloheximide induced selective macrophage death in plaques of cholesterol-fed rabbits. In this case, apoptosis occurred not only via autophagy, because plaque macrophages might be highly metabolic active and vulnerable to protein synthesis inhibitors relative to SMCs [90].

As another therapeutic case of rapamycin, inhibited translation of VSMCs leads to upregulation of smooth muscle B-actin, calponin, and myosin heavy chain, which modulates VSMCs to be differentiated, quiescent, and have a contractile phenotype. As a result, VSMCs undergo cell death. It has been suggested that restricted protein translation might selectively induce macrophage cell death rather than cell death protein expression. mTOR gene silencing is another therapeutic approach. Transfection of mTOR-specific small interfering RNA selectively induces macrophage cell death [89]. The recent evidence shows that the transcription factor TFEB works as a transcriptional activator for a network of autophagy and lysosomal genes [91]. The studies show that macrophage specific gene activator of TFEB has the ability of lysosomal biogenesis, recovering lysosomal dysfunction via atherogenic lipids. It allows some functions such as increasing cholesterol efflux, inhibiting inflammation activation, and clearing abnormal protein aggregate [16]. This study provides the possibility that overexpressed TFEB in macrophages alleviates atherosclerosis [16].

Lipid reduction is the one of the well-known ways to eradicate macrophages from atherosclerotic plaques [36]. One of recent studies using a rabbit atherosclerosis model suggested that the lower levels of lipid led not to macrophage apoptosis but instead monocyte recruiting impairment because of a decrease in macrophage replication. Statins are generally used in myocardial infarction patients. In SMCs treated with the autophagy inducer 7-ketocholesterol, fluvastatin failed to activate caspases. It has been suggested there is a possibility that activation of autophagy interferes with the statin-induced apoptotic pathway [36]. Another suggestion that has been proposed is that defective mitochondria are engulfed by autophagosomes, which limits the relocation of pro-apoptotic molecules from mitochondria into the cytosol or nucleus [36].

5.2. Treating atherosclerosis as a therapy for cognitive impairment

Currently, statin drugs are major therapeutics to prevent acute coronary events. Statins inhibit cholesterol biosynthesis, reduce LDL receptors (LDLRs), and consequently trigger a reduction in blood cholesterol levels [92]. Statins work at multiple stages in atherosclerotic plaque formation. Among these stages, statins have effects at earlier atherosclerosis development stages because they hinder cholesterol accumulation, monocyte infiltration, and inflammation in arteries [92]. Carotid atherosclerosis is measured by two distinct characteristics: carotid intima-media thickness (cIMT) and carotid plaque burden quantified by plaque presence or localization. A recent study suggests that plaque burden may act as a predictor of cardiovascular

disease other than cIMT, although cIMT has been better represented in preventative measures [68]. It has been suggested that plaque numbers and cIMT may be involved in cognitive impairment and dementia [66]. These studies report early intervention of atherosclerosis to prevent cognitive impairment [72]. It is obvious that carotid atherosclerosis can be a potential target for early intervention and risk management for those at risk for cognitive decline. A recent study was performed under the hypothesis that cognitive performance in the dominantly affected domain would be related to carotid plaque burden and cIMT, and cognitive decline would be shown differently in the racial and ethnical diverse Northern Manhattan Study (NOMAS) [93]. They indicated that elderly individuals with a larger cIMT have a higher future risk of progression to MCI or dementia. It is required to monitor patients for earlier detection of cognitive dysfunction [93]. Additionally, they identified a cutoff value for predicting cognitive impairment progression (0.825 mm of cIMT), which corresponds to the cutoff values for predicting stroke and CVD in the previous report [93].

Considering the role of vascular blood factors in patients with MCI, some blood factors suggested in our previous study may also influence the progression of cognitive decline [94]. However, there are currently no markers for prognostication or the risk of conversion from MCI to dementia. Therefore, it is necessary to develop noninvasive diagnostic methods for the assessment of vascular status [80]. This aspect is discussed further in the next section along with my results. Our results may provide a route for determining the most appropriate treatment strategy for patients with MCI or multiple diagnoses.

According to recent research, trends of dementia prevalence and incidence have been reported, which are based on healthcare and insurance databases, clinical records, and meta-analysis. These studies have not currently provided how to control the recent trends of cognitive function about diagnosis, clinical details, or public awareness for it. Nonetheless, researchers and clinicians are agreeing that long-term determinants are needed for both healthy and unhealthy aging in the most of society. Furthermore, it goes on the efforts to reduce risk of dementia by maintaining health with age.

6. Conclusions

Clinically and pathologically, atherosclerosis is an important disease in a worldwide aging society. It has been shown that innate immune factors and adaptive immune factors are associated with the atherosclerotic process since inflammatory mechanisms are identified as major causes in patients. A number of studies have identified several potential targets for therapy. Unfortunately, however, inflammation is an independent risk factor for atherosclerosis progression in humans. Researchers have tried to evaluate the mechanism of immune-related therapies in atherosclerotic cardiovascular disease. Along with inflammation-related mechanisms, autophagy in atherosclerosis is also responsible for the foam cell formation and insoluble oxLDL uptake and clearance in human atherosclerotic lesions. Autophagic macrophages produce pro-inflammatory cytokines such as TNF-beta and interleukin-6, and these cytokines are not immunologically silenced during the autophagic process. Lipid droplets

are spilled from lipid-overloaded macrophages in the plaques. Based on previous research, future studies are focusing on therapeutic advantages of autophagic microphages in unstable atherosclerotic plaques.

Despite efforts to develop strong therapeutic targets, it is not feasible to establish respective contributors to degeneration and vascular disease onset and progression in each patient. Today, in our aging society, dementia has becoming an important issue in the public heath, economics, and social aspects, and also in political fields. With careful converging and treating on vascular risk factors containing atherosclerosis, it would get available therapeutic strategies for prognosis and diagnosis in patients with progressive dementia.

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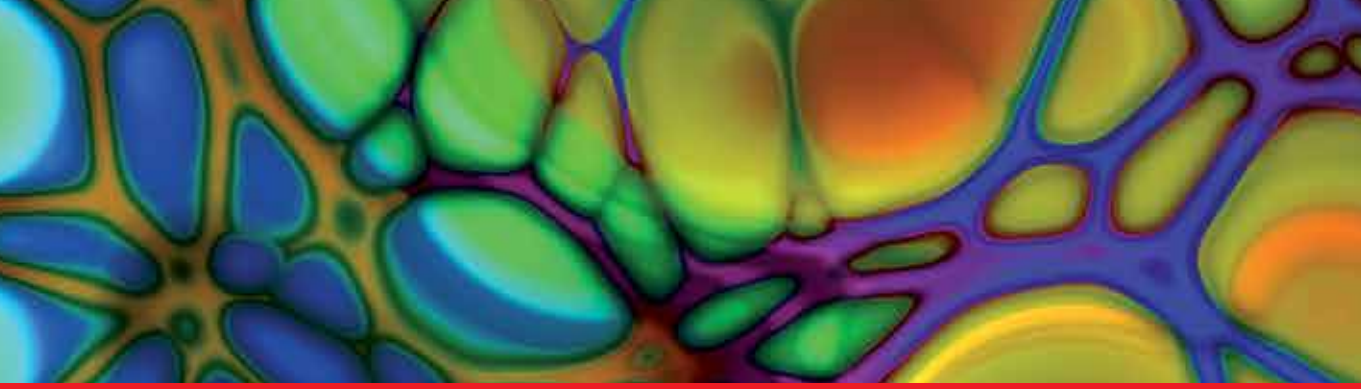

Edited by Luigi Gianturco

Atherosclerosis is a subject of enormous contention for cardiologists and in general for all medical doctors. With this publication we have given you a concise "state-of-the-art" look at the world of atheroma.

Many other elements could be included and so it is only a brief analysis of "today" (the preventive medicine era) and "tomorrow" (transforming the *cure* medicine era into the *care* medicine era) but also remembering "yesterday" (the *ex-cathedra* medicine era).

Let's hope our arteries are free from atherosclerotic events: have a good read!

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