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# Atherosclerosis Morphology and Pathogenesis

## Contents



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## Abstract

Atherosclerosis is a very common and important disease being the most important cause of mortality in Brazil. Indeed, in 1995, 23.3% of deaths, all ages, in our country, were the consequence of atherosclerosis. This percentage grows to 26.3% for S. Paulo and 32.7% for Rio Grande do Sul.

Morphologically, there are 3 main types of lesions: fatty streaks, fibrous plaques, and complicated lesions. **Fatty streaks** are inocuous and occur early in life. In some persons, with age, they change into **fibrous plaques** that may lead to stenosis. They also may become complicated by erosion, calcification, hemorrhage and thrombosis.

Atherosclerosis is initiated by endothelial functional alterations responsible for increase in permeability to macromolecules, adhesion, and migration of monocytes-macrophages and lymphocytes plus recruitment of platelets and smooth-muscle medial cells.

Adhesion molecules, cytokines, growth factors, and free radicals are locally synthesized, favoring proliferation of extracellular matrix and progression of the lesion.

Experimental, clinical, and epidemiological evidence point to the importance of lipids, mainly cholesterol-rich low-density lipoprotein (LDL), as one of the most important molecules involved in the genesis and progression of atherosclerosis. Patients with a genetic disorder of cholesterol metabolism (familial hyperlipidemia), caused by a decrease in the availability of receptors for LDL, develop severe atherosclerosis early in life.

A series of other factors, such as age, diabetes melitus, diet, hypertension, lack of exercise, elevated hemocysteinemia, immunological disorders, and coagulation instability, are related to the progression of atherosclerosis. All of them are capable of altering the endothelium or increasing the offer of LDL.

All the above-mentioned factors are systemic; but atherosclerosic lesions are focal, located at preferential sites such as the emergence of colaterals, bifurcations, and curvatures of arteries, all areas in which the laminar flow is disturbed.

In these areas shear stress is diminished favoring the prolongation of permanence time of lipid particles, cells, cytokines, growth factors, etc., in the vicinity of the endothelium. Moreover, the endothelium has "sensors" that act as transducers of mechanical forces in biological responses. Experimental data demonstrate that the number and quality of adhesion molecules, cytokines, and growth factors synthetized, as well as the local production of radicals, and pro and anticoagulation factors may change with shear stress favoring or not the local establishment and progression of atherosclerotic lesions.

**Key-words:** Atherosclerosis, Morphology, Pathogenesis.

## Introduction

Atherosclerosis has always been considered as a disease of the rich countries of the Northern Hemisphere. More recently, epidemiological studies in some of the less-developed countries have shown that this is not always so. In 1968 Puffer and Griffith showed that the adjusted death rate for 100,000 inhabitants varies greatly among American countries, and that in some of the Latin American cities, atherosclerosis was one of the leading causes of death. Indeed, even when the death rate per 100,000 was higher for cities, like S. Francisco (191.2 for men), it was also important in Caracass (98.5) or S. Paulo (95.0). For women the differences were smaller: 55/100,000 in S. Francisco and 40/100,000 in S. Paulo.

In more recent years, reliable data on mortality from Brazil have been published by the Brazilian Ministry of Health (Brasil, 1999); in 1995 atherosclerosis was responsible for 23.3% of deaths, among all ages, in Brazil. This proportion increases to 26.3% in São Paulo and 32.7% in Rio Grande do Sul.

Now, we have information showing that atherosclerosis is the most important cause of death in Brazil and may kill one third of the population in certain states. Myocardial infarcts, one of the more common and severe consequences of atherosclerosis, killed 22,727 persons in São Paulo and almost 120,000 Brazilians in 1995 (Brasil, 1999).

Since atherosclerosis is such an important disease and since information about it is superficial in the non-medical population, discussing its characteristics and possible causes appears to be useful.

## Morphology

The lesions of atherosclerosis begin to appear very early in life. Indeed, the first alteration observed, the **fatty streaks**, are common findings in children and teenagers all over the world. Fig. 1 shows the histology of the inner aspect of the aorta in a 7-month-old child from Botucatu, SP, Brazil, where several cells containing fat (stained red) are present. Fig. 2 shows the aorta of a teenager where several isolated or confluent red streaks are seen; they are fat deposits also stained red.

Fatty streaks are found in all American populations studied by McGill et al. (1968), and their extension did not vary much. In certain populations, however, a few years later a second lesion, the **fibrous plaques**, begins to appear and increases sharply with time. Fig. 3 is from the aorta of a middle-aged white American from New Orleans; it shows white, prominent plaques, close to the origins of the intercostal arteries. Histologically (Fig. 4), these plaques are made of a loose center containing abundant fat, stained red, covered by a fibrous cap. In Fig. 5 we have an ulcerated and thrombosed, **complicated lesion**.

Lesions may also be complicated by calcification.

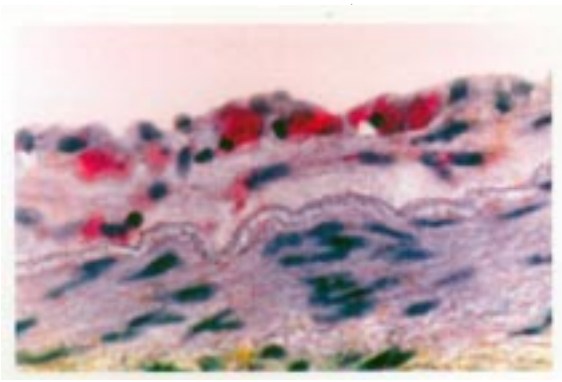

**Figura 1:** Microphoto of the aorta of a 7 months old Brazilian. Sudan IV. Numerous fat ladden (red) macrophages in the slightly thickened intima.

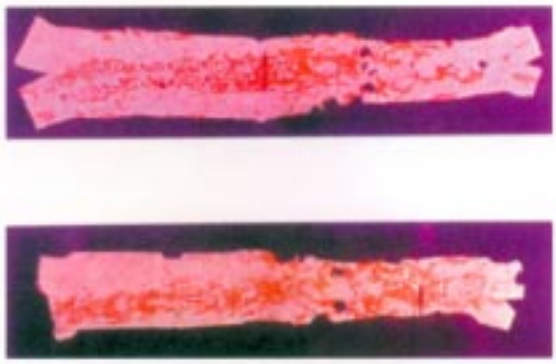

**Figura 2:** Aortas of young Brazilian teenagers. Sudan IV. Note isolate and confluent red **fatty streaks**.

**Figura 3:** Aorta of a middle-aged North American. Sudan IV. Note several white **fibrous plaques** located at the emergence of the intercostal arteries. The surface between the plaques is stained red (fat).

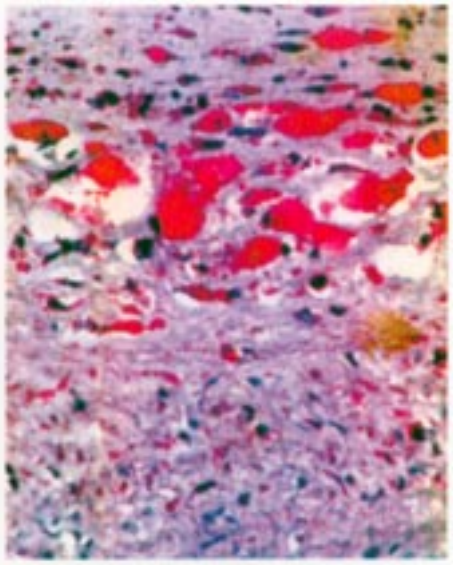

**Figura 4:** Microphoto of aorta of an old adult Brazilian. Sudan IV. **Fibrous plaque**; note the numerous red (fat) macrophages in the center

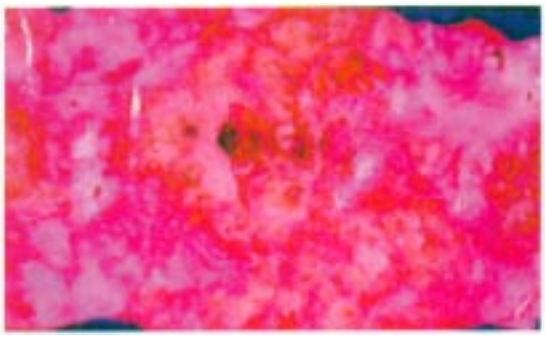

**Figura 5:** Aorta of a middle-aged North American. Sudan IV. The surface is stained red. There are several **fibrous plaques**, some related to the emergence of intercostal arteries. In the center there is a **complicated lesion**, ulcerated and thrombosed.

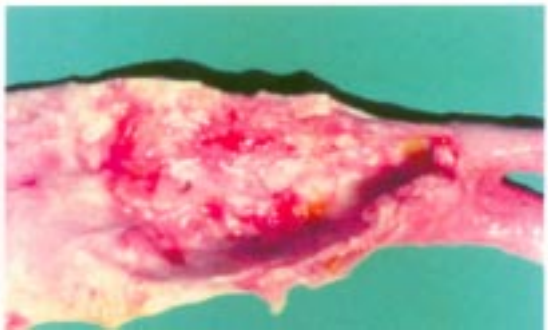

**Figura 6:** Aorta of an old Brazilian. The aorta is dilated (fusiform aneurysm), and the inner surface covered by atherosclerotic fibrous plaques, some of them covered with mural thrombi.

Fatty streaks, fibrous plaques, and complicated lesions may occur in large and mid-sized arteries, as the aorta, the carotids, iliacs, and femurals, coronaries, and cerebral arteries.

The consequences of atherosclerosis are of 3 types:

a) The plaques, especially in the coronary arteries, impinge into the lumen of the vessel that becomes stenosed. The amount of blood that passes through the stenotic areas may be sufficient for the basal nutrition of the tissues irrigated by the artery, but as soon as there is an increase in the demand, such as during exercise, the flow becomes insufficient and symptoms may appear. This is what happens in certain types of "angina pectoris"; the patient feels severe chest pain during exercise and the pain is relieved either by the use of vasodilator drugs or by the end of the exercise. A similar situation occurs when a leg artery is stenosed and the patient suffers severe cramps after walking a few meters; the pain disappears after the exercise is suspended.

b) A second, very important consequence of atherosclerosis is the sudden increase in the size of the plaque, leading to acute, severe reduction or obstruction of the lumen.

In the heart, since colateral circulation is absent or inefficient the sudden reduction of the flow leads to myocardial isquemia and necrosis — myocardial infarct — one of the leading causes of disease and mortality in the modern world. The same occurs in the cerebral arteries causing cerebral infarcts.

According to the American Heart Association Committee on Vascular Lesions (Fuster et al., 1999), the progression of atherosclerosic plaques may be subdivided into five phases and different lesion types.

The fatty streak represents a balance of the entry and exit of lipoproteins in the intima and is accompanied by local increase in extracellular matrix. If there is a decrease in the level of lipoprotein as a result of diet or treatment, exit predominates and the lesions remain stable or even may regress. But if the level of lipoprotein increases, entry of lipids predominate over exit, the plaque increases in size, and fibrosis occurs, resulting in vulnerable plaques containing abundant lipid, separated from the lumen by a cap of connective tissue, the **fibrous cap**.

The fibrous cap may be thin or thick with variable amounts of inflammatory cells. These differences in composition explain the variation in vulnerability to hemorrhage, erosion and rupture of the plaque with the consequent thrombosis and partial or total acute obstruction.

Thin caps, observed in lipid rich plaques, in which influx exceeds outflux of lipids, are much more vulnerable. Thick caps are more resistant, but other factors as the presence of inflammatory cells, (macrophages and lymphocytes), by the local release of their enzymes and cytokines, may became vulnerable.

Today, with the aid of intravascular ultra sound and magnetic-resonant imaging, it is possible to evaluate the vulnerability of the plaques during life and offer the possibility of controlling the drastic consequences of sudden obstruction (Fuster et al., 1999).

In the past, atherosclerosic lesions were thought to be relatively stable. Now we know that on the contrary, they are dynamic lesions that grow when excess lipid increases, regress when the level decreases, and complicate when erosed, hemorrhagic, or ruptured (Fuster et al., 1999).

Besides lipids, other factors such as smoking, diabetes, hypertension, and other circumstances that we will discuss later, are also important in the natural history of atherosclerosis.

c) The third consequence of atherosclerosis is dilatation of the arteries with the formation of **aneurysms**. Nutrition of the wall of large arteries, such as the aorta, in part depend on diffusion of nutrients from the blood through the intima. The thickening of the intima caused by atherosclerosis diminishes diffusion. The undernourished media lose elasticity and the vessel dilates. Additionally, the atherosclerosic plaques may grow into the media, both factors cooperating to form aneurysms (Fig. 6).

## Pathogenesis

The extension of atherosclerosis in the arteries varies among different populations (Tejada et al., 1968). White North Americans and Scandinavians have much more extensive lesions when compared with the white population of São Paulo, the Indian populations of Guatemala and Peru being much less affected. There are important differences in the extension of atherosclerosis between white and black "Paulistas", but these differences do not occur in Puerto Ricans (Tejada et al., 1968). The same occurs between genders. While in white populations of developed cities like New Orleans and Oslo, males are more affected than females, in the Negro population of New Orleans, these differences disappear and are much less marked in the populations of S. Paulo or Santiago (McGill et al., 1968; Montenegro & Iabuki, 1968). These observations point to the importance of environmental factors in the progression of this disease.

### Experimental Data

As early as the end of last century, the presence of fat in the lesions called the attention of pathologists to the importance of lipids in atherosclerosis. Virchow proposed that the lesions were related to insudation of lipid-rich plasma into the intima followed by proliferation of connective tissue. Rokitansky, another German pathologist, however, proposed that the lesions were the consequence of small mural thrombi that, being organized, lead to thickening of the wall.

Early in this century, Anitschkoff gave important experimental support for the "lipid" theory; he produced lesions very similar to human atherosclerosis by feeding rabbits a diet rich in cholesterol.

A very large series of similar experiments in monkeys, pidgeons, chickens, pigs, and other animals gave further support for the lipid theory.

Ultrastructural studies of the arteries of these animals has shown that a short period after the commencement of the diet, mononuclear cells adhere to the

endothelium, send pseudopods through the intercellular spaces, pass between the endothelium cells, and end up reaching the intima. There, these macrophages engulf lipoproteins and become filled with cholesterol. The lesions are indentical with the human fatty streaks (Massuda & Ross, 1990[a]).

With time, the "activated" macrophages secrete enzymes and several cytokines, some of them capable of digesting the above-situated endothelium and the intercellular matrix of the intima; additionally they have chemotatic activities, attracting more macrophages and lymphocytes from the blood and smooth-muscle cells from the media.

The aggression of the endothelium favors the adherence platelets that secrete cytokines and platelet-derived growth factor (PDGF). PDGF increases the proliferation and migration of the smooth muscle cells from the media to the intima. Arterial-smooth muscle cells are capable of synthesizing all the components of the extracellular matrix, such as various types of colagen and elastin, are responsible for the formation and growth of the fibrous cap (Massuda & Ross, 1990[b]).

It seems that, at least experimentally, it is possible to reproduce the atherosclerotic lesions by an exaggerate offer of lipids, mainly cholesterol, in the diet (Massuda & Ross, 1990[a, b]). Furthermore, if the excess lipids are taken out of the diet, the lesions regress (Vessilinovitch et al., 1976).

## Human Data

There is a large amount of epidemiological, clinical, and laboratory informations indicating a very important role for lipids and cholesterol in human atherosclerosis.

Many epidemiologic studies have demonstrated a linear correlation between the level of cholesterol in the blood of a population and the mortality rate from myocardial infarcts.

When the levels of cholesterol are lowered by diet or drugs, the atherosclerotic lesions stop growing and may regress (Waters & Lesperance, 1990).

Populations eating diets rich in cholesterol and saturated fats, like egg yolk, animal fats, and dairy products, have a greater risk of dying of myocardial infarcts and ischemic cerebral lesions.

Dietary replacement of diets rich in cholesterol and saturated fats by mono or polyunsaturated fats, such as vegetable oils or fish meat rich in omega-3 fatty acids, decrease atherosclerosis; the lesions regress and the risk of myocardial infarct diminishes (Waters & Lesperance, 1990).

## Lipid Metabolism

Lipids enter the body with food. In the small intestine they are absorbed and enter the circulation as "kilomycrons", lipoprotein molecules rich in triglycerides. In the capillaries of fat tissue and muscle, by the action of lipoproteic lipases,

triglycerides are removed and the remnants of the circulating kilomycrons are taken by receptors in the liver. The liver synthesizes and secretes into the blood very low-density lipoproteins (VLDL) rich in triglycerides; lipoprotein lipases cleave VLDL into IDL (intermediate density lipoproteins) that, through the loss of triglicerydes, are enriched in cholesterol. About 50% of the IDL is taken by receptors in the liver, where it is used for synthesis of VLDL. The other 50% of IDL ends up losing triglicerydes by the action of lipases and become LDL (low density lipoprotein) very rich in cholesterol. LDL is removed from the plasma by receptors found in almost all the living cells. Once the receptors and LDL are internalized, LDL is bound to lysosomes where it is dissociated from the receptors; these are recycled to the membrane. LDL then is degraded and free cholesterol passes into the cytoplasm where it is used by the cell. This process is controlled and no new receptors are recycled if there is sufficient cholesterol in the cell (Brown & Goldstein, 1983).

This simplified description of lipid metabolism is necessary for the understanding of a genetic disorder — familial hyperlipidemia — associated with accelerated atherosclerosis.

## Familial Hyperlipidemia

Persons affected by familial hyperlipidemia develop, early in life, high levels of circulating LDL and may die of myocardial infarction in their teens. There are several forms of this disorder, but in all of them a decrease of receptors for low density lipoprotein in the cell membranes is the culprit. Cells need cholesterol to build their membranes and to function properly. The metabolism of cholesterol, however, is controlled and as soon as the cell has received the cholesterol sufficient for its functions, there is inhibition of the synthesis of receptors. In other words, the cells cannot absorb excess cholesterol. In familial hyperlipidemias, the insufficiency of receptors for LDL explain the increase in circulating LDL and its precocious deposition in the intima of arteries (Brown & Goldstein, 1983).

Macrophages have receptors for LDL in their membranes, but they also have receptors for modified lipoproteins (Brown & Goldstein, 1983). These "scavenger" receptors, however, are not supressed when excess LDL is offered. In all types of hypercholesterolemia (dietetic, familial, diabetic, etc.), excess LDL transverses the intima being modified. Macrophages in the intima engulf modified LDL's and become lipid-laden, vacuolated "foam cells", very imporant in the iniciation and progression of atherosclerosic lesions.

In hyperlipidemia, the process is accelerated by the continuous excess of LDL.

## The Role of Endothelium

Besides hyperlipidemia, various others factors influence the development of atherosclerosis: age, sex, hypertension, smoking, diabetes melitus, elevated plasma homocystein (Welch & Loscalzo, 1998), thrombosis, lack of exercise, obesity, and associations of factors, such as obesity, diabetes, and hypertension.

Hyperlipidemia, products of smoking, excess homocystein, or hypertension may alter the endothelium; and alteration of the endothelium is essential for the initiation of atherosclerotic lesions.

I am using "alter" the endothelium to imply that the alteration usually is functional and does not modify the morphology of the endothelial cells.

Hyperlipidemia makes the endothelium more permeable to macromolecules, stimulates synthesis of adhesion and chemotactic molecules by the endothelial cells, and facilitates the penetration of macrophages into the intima. Lipoproteins that have passed through the endothelial cells are modified and avidly endocytozed by the scavenger receptors of macrophages in the intima.

Products of cigarette smoking also injure the endothelium, facilitating the formation and progression of atherosclerotic lesions. Increased homocysteinemia (a recently described metabolic error) (Welch & Loscalzo, 1998) also alters endothelium; hypertension increasing the shear stress of the blood into the wall also alters endothelium (Gimbrone, 1999).

Trauma, experimental or not, physically modifies endothelial lining, and in the presence of hyperlipidemia, may initiate atherosclerosis.

## The Role of Thrombosis

Atherosclerotic plaques may impinge into the lumen of middle-sized arteries, promoting stenosis and its consequences. But, thrombosis is another complicating element in the progression of this disease. Vulnerable plaques may develop fissures; soft, lipid-rich plaques may rupture. Once the endothelium is ruptured, subendothelial connective tissue is exposed, platelets adhere, and thrombosis occurs. The thrombus may be thin and end up being organized and incorporated into the intima that becomes thicker, as sugested by Rokitansky more than one hundred years ago.

But, frequently the thrombus grows rapidly and occludes the lumen of the vessel causing infarcts.

Plaques are vascularized and hemorrhage from their vessels may also lead to a sudden increase in its size and favor secondary thrombosis.

That is why, besides the variety of factors involved in the pathogenesis of atherosclerosis another one, changes in the coagulability of blood, must also be considered.

## The Inflammation Theory

A few years ago, Russel Ross, one of the leading scholars devoted to the study of atherosclerosis, proposed that atherosclerosis is the consequence of "various forms of insult to the endothelium and smooth muscle of the vessel wall...". The several publications by Ross and his group have proposed that atherosclerosis is a "response to injury" and belongs to the group of inflammatory diseases (Russel, 1999).

Arguments in favor of Ross are the presence of various subtypes of lymphocytes and other inflammatory cells in the lesions, the central role of macrophages and platelets, the demonstration of the presence of several cytokines and growth factors, all elements found in inflammations.

The propositon of Ross is highly acceptable and permits the understanding of a series of peculiarities in the evolution of atherosclerosic lesions.

The presence in the atherosclerotic plaques of *Chlamidia pneumoniae*, herpes virus (Libby et al., 1997), and *Cytomegalovirus* (Speir et al., 1994) has been used to infer that these agents play a role in the progression of the lesions, but, since they are ubiquitous agents, present in other tissues and organs, their presence in the plaques, for the present, may better be considered as fortuitous.

**The Role of Shear Stress**

Hyperlipidemia, diabetes, hypertension, products of cigarette smoking, and hyperhomocysteinemia are all systemic and should affect diffusely the endothelium. However, atherosclerosic lesions occur preferentially in certain segments of the arteries such as those close to the emergency of colaterals, bifurcations and curvatures. In these segments, disturbances of the laminar flow of the blood, turbulences, and complex dynamic alterations of shear stress occur.

Unbranched, tubular arterial segments, where flow is uniform, are characteristically resistant to atherosclerotic lesions.

Recent experimental evidence (Gimbrone, 1999; Zand et al., 1999) has shown the interactions between changes in shear stress and the initiation and progression of atherosclerotic lesions.

Areas where shear stress is diminished, as in bifurcations, may result in a decrease of the blood velocity leading to a longer contact of lipids with the endothelium, also resulting in local accumulation of cytokines, growth factors, and reactive oxygen molecules. Moreover, endothelial cells are sensitive to alterations in shear stress and answer with modifications of their shape and function. "In vitro" tests have shown that endothelial monolayers exposed to variation in shear stress may alter their metabolic and synthetic activities, including the production of prostacyclin, nitric oxide, cytokines, growth factors, and vasoactive mediators.

Endothelial cells, then, are capable of transducing physical stimuli into molecular, biological events. How this transduction modifies the gene expression in the endothelial nucleus is being studied. Today we know that differences in temporal and spatial characteristics of the flow may induce several changes in endothelial function. Steady laminar flow enhances survival of the endothelium by supressing

apoptosis. The pattern of adhesion molecules expressed by endothelial cells is also dependent on the variations of shear stress.

## Concluding Remarks

Our objective was to give a general view of the morphology and pathogenesis of atherosclerosis to be read by non specialists.

The subjects have been summarized and excess detail was avoided. Nevertheless, all the important facts have been expressed. Readers interested in more detailed information about pathogenesis should read Fuster et al. (1999), Gimbrone (1999), Russel (1999), and Stary et al. (1995).

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