Mechanisms of Plaque Formation and Rupture

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Abstract: **Atherosclerosis causes clinical disease through luminal narrowing or by precipitating thrombi that obstruct blood flow to the heart (coronary heart disease), brain (ischemic stroke), or lower extremities (peripheral vascular disease). The most common of these manifestations is coronary heart disease, including stable angina pectoris and the acute coronary syndromes. Atherosclerosis is a lipoprotein-driven disease that leads to plaque formation at specific sites of the arterial tree through intimal inflammation, necrosis, fibrosis, and calcification. After decades of indolent progression, such plaques may suddenly cause life-threatening coronary thrombosis presenting as an acute coronary syndrome. Most often, the culprit morphology is plaque rupture with exposure of highly thrombogenic, red cell–rich necrotic core material. The permissive structural requirement for this to occur is an extremely thin fibrous cap, and thus, ruptures occur mainly among lesions defined as thin-cap fibroatheromas. Also common are thrombi forming on lesions without rupture (plaque erosion), most often on pathological intimal thickening or fibroatheromas. However, the mechanisms involved in plaque erosion remain largely unknown, although coronary spasm is suspected. The calcified nodule has been suggested as a rare cause of coronary thrombosis in highly calcified and tortious arteries in older individuals. To characterize the severity and prognosis of plaques, several terms are used. Plaque burden denotes the extent of disease, whereas plaque activity is an ambiguous term, which may refer to one of several processes that characterize progression. Plaque vulnerability describes the short-term risk of precipitating symptomatic thrombosis. In this review, we discuss mechanisms of atherosclerotic plaque initiation and progression; how plaques suddenly precipitate life-threatening thrombi; and the concepts of plaque burden, activity, and vulnerability.** (*Circ Res*. 2014;114:1852-1866.)

Key Word: atherosclerosis

Coronary heart disease (CHD) and other manifestations of atherosclerosis were not among the most common causes of death until the beginning of the 20th century, but thereafter a dramatic increase was observed in industrialized countries, including Western Europe and the United States, peaking around 1960 to 1980.[1] Comparable increases in the incidence of CHD have later occurred or are currently occurring in many other parts of the world mainly because of population growth and an increased average life span.[2] Atherosclerosis is today globally the leading cause of

Nonstandard Abbreviations and Acronyms	
ACS	acute coronary syndrome
CHD	coronary heart disease
LDL	low-density lipoprotein
MI	myocardial infarction
SMC	smooth muscle cell
TCFA	thin-cap fibroatheroma
VH	virtual histology

death and disability with the bulk of disease in the developing world.[2]

In affluent countries, the age-adjusted mortality from CHD has declined substantially in recent decades. However, this decline is partly explained by improved survival after myocardial infarction (MI), and as more individuals become at risk of developing CHD because of an aging population, the prevalence and economic burden of CHD are likely to increase.[3–5] Thus, the task is as important as ever for cardiovascular researchers and clinicians to work toward its prevention, especially with strategies that can be applied around the world.

Causes

An increased blood concentration of apolipoprotein B–containing lipoproteins, of which low-density lipoprotein (LDL) usually is the most prevalent form, can be a sufficient cause of atherosclerosis, such as in familial hypercholesterolemia (FH) and other genetic hyperlipidemias (monogenic disease). More often, however, the disease develops at lower levels of LDL in combination with other risk factors that facilitate atherosclerosis (multifactorial disease).[6,7] These include smoking, hypertension, diabetes mellitus, male sex, and a complex genetic susceptibility to the disease (family history). The fact that CHD can be prevented in several different ways, for example, by statins and antihypertensive drug usage, smoking cessation, or other lifestyle modifications, is a reflection of its multifactorial basis.

Persons with exceedingly low LDL generally do not develop clinically relevant atherosclerosis irrespective of the presence of other risk factors.[8] Furthermore, Mendelian randomization studies investigating the potential effect of lifelong LDL reduction have shown protective effects of inherited low LDL levels, underscoring the central importance of LDL as a causal factor also for the common, multifactorial form of the disease.[9] Together, the known modifiable risk factors explain >90% of the occurrence of MI in populations around the world.[6]

Although the central pathophysiological mechanisms are assumed to be the same irrespective of the set of etiologic factors in a particular patient, the presence of individual risk factors adds nuances to disease presentation. For instance, cigarette smoking increases the risk more for MI than for stable angina,[10] hypertension is an exceptional powerful risk factor for stroke,[11] and smoking and diabetes mellitus account for most of the risk of developing peripheral vascular disease.[12] The level of LDL seems to be less important for stroke and peripheral vascular disease than for CHD.[12,13]

Basis of Knowledge

What we know today about atherosclerotic disease mechanisms stems mainly from descriptive pathology of human autopsies and experimental pathology in animal models with severe hypercholesterolemia. In this introductory review, we will not discuss how each piece of the information was derived and the strengths and limitations of the underlying studies, but we will point out some general caveats of our knowledge base. Animal models are the cornerstone for the understanding of a complex disease like atherosclerosis, but models are only available for asymptomatic lesion development and not for the processes that lead to thrombosis. Another and possibly related issue is that current models, of which the genetically modified mouse is the most widely used, are essentially modeling homozygous familial hypercholesterolemia with an accelerated mode of disease progression over months, whereas in life, the disease progresses over many decades. The pathology of atherosclerosis in the extremely small subgroup of patients with homozygous familial hypercholesterolemia seems to differ from the common multifactorial disease process in several important aspects, including a lower frequency of thrombosis as a cause of death.[14] Therefore, it may not be surprising that the morphological characteristics and fate of lesions are quite different in animal models and humans.

These limitations for research are mirrored in what we know about the disease. There is a greater in-depth, mechanistic knowledge of how LDLs cause atherosclerotic lesion formation, but considerably less is known about how other important risk factors, such as hypertension, smoking, or diabetes mellitus, are involved, and why some plaques, but not others, finally cause devastating thrombotic complications.

Mechanisms of Plaque Formation

The disease mechanisms elicited by LDL and the other causal factors are multifaceted as discussed below, involving lipoprotein retention, inflammatory cell recruitment, foam cell formation, apoptosis and necrosis, smooth muscle cell (SMC) proliferation and matrix synthesis, calcification, angiogenesis, arterial remodeling, fibrous cap rupture, thrombosis, and more. There is a complex interaction between these processes and a variable importance of each process in the development of single plaques leading to unpredictable progression rates, heterogeneous plaque morphology, and variable clinical outcomes. Most plaques remain asymptomatic (subclinical disease), some become obstructive (stable angina), and others elicit acute thrombosis and may lead to an acute coronary syndrome (ACS).

Lesion Classification

The pathogenesis of atherosclerotic lesions has been inferred from microscopic analysis of arteries in different age groups. This area of research has been extensively reviewed by Stary et al[15–17] in a series of articles that remain one of the richest resources for microscopic descriptions of atherosclerosis. This work also led to a proposed histological classification of lesions (American Heart Association classification, types I–VIII).[18] An alternative and simpler classification, which emphasizes the link between lesion morphology and clinical disease, was later introduced by Virmani et al[19] and is used in the present article. The main lesion types recognized in this classification are displayed in Figure 1. A single patient with
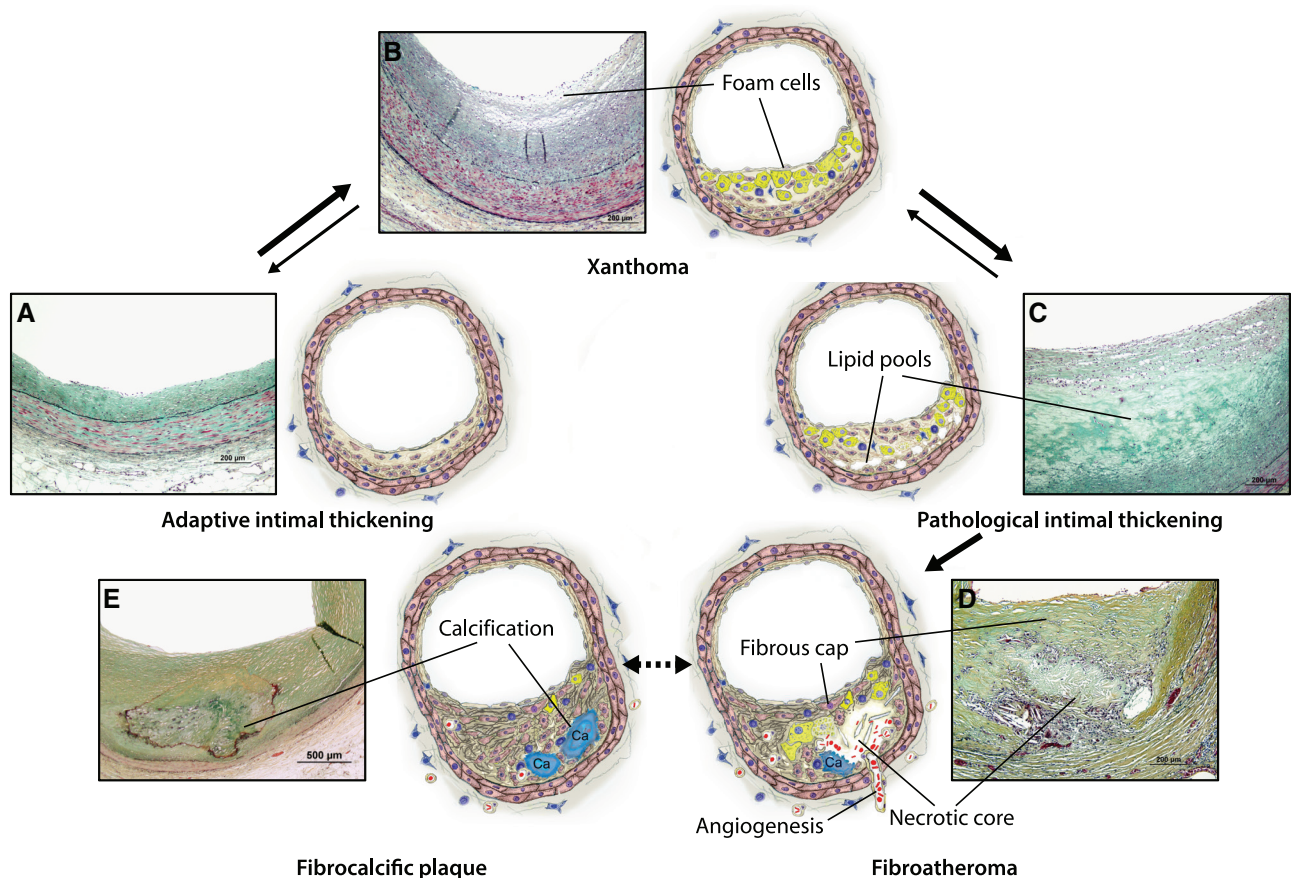
Figure 1. Lesion types of atherosclerosis and a proposed sequence of their development. A, Adaptive intimal thickening characterized by smooth muscle cell accumulation within the intima. **B**, Intimal xanthoma corresponding to the accumulation of foam cell macrophages within the intima. Pathological intimal thickening in **C** denotes the accumulation of extracellular lipid pools in the absence of apparent necrosis. **D**, Fibroatheroma indicating the presence of a necrotic core. The necrotic core and surrounding tissue may eventually be calcified, which forms fibrocalcific plaque shown in **E**. Because some of the advanced lesion types (fibroatheromas and fibrocalcific plaques) evolve simultaneously in life, their interrelationships are difficult to resolve in autopsy studies. Movat pentachrome stain.

advanced disease will typically harbor many different lesion types at different sites of the arterial tree.

Predilection Sites

Atherosclerosis is a multifocal disease that attacks reproducible regions of the arterial tree. Sites with low or oscillatory endothelial shear stress, located near branch points and along inner curvatures, are most susceptible,[20] and the abdominal aorta, coronary arteries, iliofemoral arteries, and carotid bifurcations are typically the most affected. Before development of atherosclerosis, these predilection sites are characterized by changes in endothelial turnover and gene expression,[20] presence of subendothelial dendritic cells,[21] and, in humans, by the presence of adaptive intimal thickening (Figure 1A).[15,22] Adaptive intimal thickenings develop spontaneously after birth and may grow to be as thick as the underlying media.[15] They may provide a soil for initial lesion development,[22] and the rate of progression remains higher here than at other arterial sites. However, with time, the disease spreads to adjacent intima,[23] and in elderly patients dying from MI, most of the epicardial coronary arteries are affected by plaques.[24]

Experiments in animals indicate a causal relationship between low wall shear stress and disease initiation,[25] but the mediating mechanism is not fully understood. As plaque develops and the arterial wall remodels, local flow patterns change. This dynamic interplay between flow and the vessel wall may influence the progression of the disease and ultimately the fate of lesions.

Lipoprotein-Driven Inflammation

LDLs cause atherosclerosis by accumulating in the arterial intima where they may be modified by oxidation and aggregation.[26] The modified LDLs, and oxidized lipid moieties deriving from them, in turn act as chronic stimulators of the innate and adaptive immune response. They induce endothelial cells and SMCs to express adhesion molecules (eg, vascular cell adhesion molecule–1 and intercellular adhesion molecule–1), chemoattractants (eg, monocyte chemoattractant protein-1), and growth factors (eg, macrophage colony-stimulating factor and granulocyte-macrophage colony-stimulating factor) that interact with receptors on monocytes and stimulate their homing, migration, and differentiation into macrophages and dendritic cells.[27,28]

Binding of LDL to intimal proteoglycans is an important step for disease initiation,[29] which may potentially explain the atherosclerosis proneness of adaptive intimal thickenings. As

the disease evolves, however, the endothelium becomes more leaky, and the expression of lipoprotein-binding molecules in the plaque may further promote the ability to retain LDL.[30] This suggests that higher LDL levels are needed to induce the disease than to sustain progression once lesions have formed, an idea consistent with the strong relationship between LDL levels during young adulthood and the risk of developing CHD later in life.[30–32]

The recruited macrophages express several different polarization phenotypes and exert manifold effects in lesion development.[33] Some macrophages attain a proinflammatory M1-like phenotype, possibly through binding of modified LDL to pattern-recognition receptors (eg, Toll-like receptors), and they secrete proinflammatory cytokines (eg, interleukin-1β and tumor necrosis factor-α), enzymes, and reactive oxygen species that promote further retention and modification of LDLs (eg, myeloperoxidase) as well as many other mediators that have been shown to play a role in atherosclerosis (eg, plasminogen activators, cathepsins, and matrix metalloproteinases).[33] Others have an M2-like phenotype and may secrete factors (eg, transforming growth factor β and proresolving lipids) that favor the resolution of inflammation.[33–36]

The adaptive immune system recognizes modified LDL and other autoantigens related to the atherosclerotic process, and immune cells participate in the development of lesions.[27] T helper 1 cells appear in the human intima coincident with foam cells and secrete proinflammatory cytokines (interferon-γ and tumor necrosis factor-α) that accentuate vascular inflammation in mouse models,[37] but other immune cell types, such as regulatory T cells and possibly B cells, ameliorate it.[38]

Both macrophages and dendritic cells serve as deposits of lipids from the insudating lipoproteins and become foam cells by mechanisms that remain incompletely understood in vivo.[39] It may involve scavenger receptor–mediated uptake of oxidized LDL, hydrolysis of free cholesterol from aggregated LDL complexes in extracellular lytic compartments, direct uptake of native LDL or remnant lipoproteins, or indeed a combination of these and other proposed pathways.[40–42] Notably, SMCs also take up lipids in human atherosclerosis and accumulate droplets of cholesteryl esters, possibly by similar mechanisms.[43]

Foam cells, easily recognizable by light microscopy, are telltales of lipoprotein-driven inflammation occurring in the vascular wall. They initially accrue within the proteoglycan layer of the intima, and when several layers have formed, they are visible to the unaided eye as yellow-colored xanthomas or fatty streaks (Figure 1B). Xanthomas are harmless and fully reversible if the stimuli that caused their formation dissipate. They are present already in some fetal aortas and infants in the first 6 months of life, probably reflecting risk factors of the mother,[44] but their number declines in subsequent years.[45] At adolescence, they reappear in atherosclerosis-prone regions of the coronary arteries and the aorta in most people.

Necrotic Core Formation

Many xanthomas do not progress further, but some, especially those occurring at predilection sites, develop into progressive atherosclerotic lesions. The defining characteristic is the accumulation of acellular, lipid-rich material in the intima. Smaller lipid pools are initially seen beneath the layers of foam cells

without gross disruption of the normal structure of the intima.[16,19,46] This type of lesion is termed pathological intimal thickening (Figure 1C) and is commonly observed from 20 to 30 years of age in the coronary arteries.[16,47] In some lesions, the isolated lipid pools grow into confluent necrotic cores (or synonymously lipid-rich core) through the invasion by macrophages. This process irreversibly disrupts the normal structure of the intima and leaves behind a matrix-devoid gruel of lipids and cell debris.[46] The necrotic core may be characterized as early or late with the former showing some presence of hyaluronan and versican with macrophage infiltration, whereas in the latter, no matrix at all is observed.[48] When a necrotic core is present, the lesion is a fibroatheroma (Figure 1D).

Apoptosis and secondary necrosis of foam cells and SMCs are thought to be an important cause for necrotic core development.[49,50] Many factors that are capable of inducing apoptosis in vitro are present in plaques,[51,52] and it is reasonable to assume that several of these co-operate to cause apoptosis in vivo.[49] Apoptotic macrophages and SMCs become detectable coincident with the occurrence of lipid pools in human lesions,[53] and cell death, both apoptotic and other forms, can be seen at the margin of the necrotic core.[54] The presence of free apoptotic remnants in lesions, that is, not associated with phagocytic cells, indicates that impaired removal of apoptotic remnants (efferocytosis) contributes to the growth of the necrotic core.[36,55]

This theory for necrotic core development is supported by experiments in mice using genetic engineering to induce or protect against apoptosis in macrophages and SMCs. In early stages of atherosclerosis, selective inhibition of macrophage apoptosis increases foam cell number, indicating that apoptosis, although undetectable at this stage because of efficient efferocytosis, balances the ongoing recruitment and proliferation of macrophages.[56] However, when experimentally induced and after necrotic foci have begun to form, macrophage and SMC apoptosis increases necrotic core formation and plaque inflammation,[56,57] possibly because neighboring phagocytes no longer efficiently remove the apoptotic remnants.[36] Instead, they are left to undergo secondary necrosis and the lipid-rich cargo is deposited in tissue, where it incites further inflammation.[36,56,57]

The chemical composition of the necrotic core indicates that other sources of lipid may be important cocontributors, including direct accumulation of cholesterol esters from insudating LDL[58] and free cholesterol-rich erythrocyte membranes deriving from intraplaque hemorrhages.[48] It is also possible that macrophage-catalyzed extracellular hydrolysis of aggregated LDL contribute to the high content of free cholesterol in the necrotic core.[59]

Why necrosis occurs in some, but not other lesions, is not known, and apparently, the causal factors are at least partly dissociated from those that cause xanthomas. For instance, men and women develop similar amounts of coronary xanthomas early in life, but men have many more progressive atherosclerotic lesions by the age of 30.[60]

Plaque Angiogenesis and Intraplaque Hemorrhage

Neovessels, mainly originating from the adventitial vasa vasorum, grow into the base of progressive atherosclerotic lesions and provide an alternative entry pathway for monocytes

and immune cells of unknown quantitative importance.[61] The plaque neovessels lack supporting cells and are fragile and leaky, giving rise to local extravasation of plasma proteins and erythrocytes.[62] Such intraplaque bleedings are common in fibroatheromas[63] and may expand the necrotic core and promote inflammation.[48] Another common source of plaque hemorrhage is extravasation of blood through a ruptured fibrous cap (see below).[64]

Plaque hemorrhage is associated with macrophages of the hemoglobin-induced phenotype (M(Hb)) that express CD163, a scavenger receptor that takes up haptoglobin-bound hemoglobin and thereby protect against the cytotoxic effects of free hemoglobin.[65] These macrophages lack typical markers of M1 macrophages (tumor necrosis factor-α and inducible nitric oxide synthase), express the mannose receptor typical of M2-like macrophage differentiation, and are resistant to foam cell formation.[65]

Emerging data indicate that the inflammatory response to intraplaque hemorrhage is accentuated in patients with impaired haptoglobin function caused by the common Hp2-2 genotype, including in particular patients with diabetes mellitus,[66] and this may contribute to the increased risk of CHD in individuals with elevated glycosylated hemoglobin.[67]

Fibrosis and Calcification

The connective tissue of lesions is initially that of the normal arterial intima or adaptive intimal thickening, but gradually this loose fibrocellular tissue is replaced and expanded by collagen-rich fibrous tissue, which often grows to become the quantitatively dominant component of plaques.[68] Tissues that lie intertwined between a necrotic core and toward the luminal surface of the plaque (fibrous cap) are fibrous with a high content of type I collagen (Figure 1D).

The collagen, elastin, and proteoglycans of the fibrous matrix are mainly produced by SMCs, and this secretory function of lesional SMCs is reflected by their ultrastructural phenotype. Plaque SMCs are characterized by an abundant rough endoplasmic reticulum and Golgi complex and only sparse myofilaments.[69] This phenotype has been termed synthetic in contrast to the contractile phenotype of medial SMCs. Few synthetic SMCs are present already in the normal intima, but their number increases substantially during lesion development,[17] probably both by local proliferation and by migration of medial SMCs that subsequently undergo phenotypic modulation to the synthetic phenotype. Notably, the number of SMCs in plaques at all stages may be grossly underestimated because many synthetic SMCs do not contain detectable levels of the contractile proteins routinely used to recognize SMCs in tissue sections (eg, smooth muscle α-actin and smooth muscle myosin heavy chain).[70,71]

The ability of contractile SMCs to undergo phenotypic modulation has been recognized for decades,[72] and the migration of medial SMCs from the arterial media into intima has been directly demonstrated in mouse models.[70,73] Over the years, however, several additional or alternative origins of plaque SMCs have been reported, including circulating progenitor cells, subpopulations of synthetic-type SMCs in the arterial media, or multipotent stem cells in the media or adventitia.[74,75] Some of these findings have subsequently been refuted or contested,[71,76] but exhaustive tracking of the origin of human lesional SMCs has yet to be performed.[70]

Calcifications are common in progressive atherosclerotic lesions and increase with age. Apoptotic cells, extracellular matrix, and necrotic core material may act as nidus for microscopic calcium granules, which can subsequently expand to form larger lumps and plates of calcium deposits.[77,78] The necrotic core can completely calcify with time and calcifications can constitute most of plaque volume.[17] Osseous metaplasia is sometimes seen in human lesions (versus chondroid metaplasia in mouse models),[78,79] but these are rare and only occur in arteries that are already heavily calcified.[77,80]

Many plaques at autopsy exclusively consist of fibrous and sometimes calcified tissue without extracellular lipid pools or a necrotic core. The genesis of these fibrocalcific plaques is not fully understood (Figure 1E). Some pathologists think that the development of a necrotic core is the prerequisite of fibrosis[18] and, indeed, decalcification and sequential sectioning often reveal that a necrotic core is present at the site of calcification or in the upstream or downstream vicinity of the section within fibrocalcific plaque.

Arterial Remodeling

During atherogenesis, the local vessel segment tends to remodel in such a way that the lumen area is usually not compromised until plaques are large (expansive remodeling).[81] Thereafter stenosis formation may occur through continued plaque growth or shrinkage of the local vessel segment (constrictive remodeling) or a combination of the 2 processes.[81,82] Expansive remodeling is more often seen with fibroatheromas, and the extent of enlargement is positively correlated to plaque inflammation, medial atrophy, and the size of the necrotic core.[83,84] Constrictively remodeled segments often contain lesions rich in fibrous tissue.[84]

The mode and extent of remodeling is at least as important as plaque size in determining stenosis severity. Therefore, imaging of the lumen of an artery by angiography is not useful for diagnosing the presence of atherosclerotic plaque, and vice versa, microscopic examination of plaques cannot estimate the degree of luminal stenosis in vivo (see below).

Mechanisms of Plaque Rupture, Erosion, and Thrombosis

Atherosclerosis alone may obstruct coronary blood flow and cause stable angina pectoris, but this is rarely fatal in the absence of scarring of the myocardium, which can elicit an arrhythmia presenting as sudden cardiac death. ACS are nearly always caused by a luminal thrombus or a sudden plaque hemorrhage imposed on an atherosclerotic plaque with or without concomitant vasospasm.[85] In ST-segment elevation myocardial infarction, the thrombus is mostly occlusive and sustained, whereas in unstable angina and non–ST-segment elevation myocardial infarction, the thrombus is usually incomplete and dynamic, or even absent. Also in victims of sudden coronary death, acute or organized thrombus is often found; the rest die with severe coronary disease in the absence of thrombosis with or without myocardial scarring.[19,85] Rare causes of ACS include emboli, artery dissection, vasculitis, cocaine abuse, tunnel coronary arteries, and trauma.

Plaque rupture is the most frequent cause of thrombosis.[85,86] In plaque rupture, a structural defect—a gap—in the
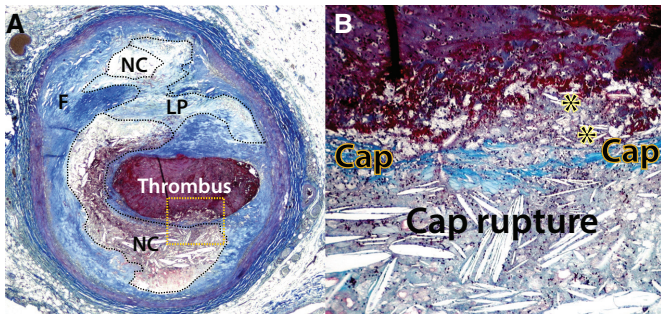
Figure 2. A, Thrombosis caused by plaque rupture. The culprit plaque shown in **A** is a fibroatheroma consisting of fibrous tissue (F), areas dominated by extracellular lipid pools (LP), and fully developed necrotic cores (NC). **B**, Large magnification of the orange inset in A. The thin and inflamed fibrous cap covering the large necrotic core has ruptured and core material, including cholesterol crystals (*), has been propelled into the lumen where it can be found at the base of the thrombus. Elastin–trichrome stain (collagen blue).

fibrous cap exposes the highly thrombogenic core to the blood (Figures 2 and 3). Dislodged plaque material is sometimes found within the thrombus, indicating that rupture and thrombosis coincided and thereby supporting its causal relationship. Plaque rupture is a well-defined term, described in a consensus statement from 2004,[87] whereas other terms, such as plaque disruption and fissuring, are used ambiguously in the literature.[88]

In rare cases, nodular calcifications (calcified nodule) are found protruding into the lumen through a ruptured fibrous cap, and this has been suggested as a separate precipitating mechanism of thrombosis.[19] When no plaque rupture can be identified despite a thorough microscopic search, the term plaque erosion is used (Figure 4). This term was chosen because the endothelium is typically absent beneath the thrombus, but whether this is the precipitating mechanism remains unknown.[19] Both pathological intimal thickening and fibroatheromas may be complicated by plaque erosion.

In a recent compilation of data from autopsy studies around the world, the majority of fatal coronary thrombi was associated with plaque rupture regardless of clinical presentation (MI, 79%; sudden coronary death, 65%), age (>60 years, 77%; <60 years, 64%; unknown, 73%), sex (men, 76%; women, 55%), and continent (Europe, 72%; United States, 68%; Asia, 81%).[88] Plaque rupture is also the most common substrate for thrombi causing MI in those who survive.[89] The sex differences seem noteworthy and, interestingly, plaque rupture has been found to be particularly infrequent in premenopausal women, who however constitute an extremely small group of heart attack victims.[90] Some studies have reported that diabetes mellitus, smoking, and the level of hyperlipidemia are associated with the mechanism of thrombosis in ACS but, except for sex and menopause, no consistent relationships have been demonstrated.[88] Smoking seems to promote coronary thrombosis, regardless of plaque type.[91] A recent clinical study using intravascular optical coherence tomography indicated that the occurrence of plaque ruptures may be higher in ST-segment elevation myocardial infarction than in those with non–ST-segment elevation myocardial infarction.[92]

Mechanisms of Plaque Rupture

Plaque rupture occurs where the cap is thinnest and most infiltrated by foam cells (macrophages). In eccentric plaques, the weakest spot is often the cap margin or shoulder region,[86] and only extremely thin fibrous caps are at risk of rupturing. Assessed by microscopic examination in an autopsy study of sudden cardiac death, the average thickness of ruptured caps was found to be only 23 μm and 95% of ruptured fibrous caps were below 65 μm.[91] Based on these observations, Virmani et al[19] introduced the term thin-cap fibroatheromas (TCFAs) for coronary fibroatheromas with a fibrous cap thickness of <65 μm, which can thus be assumed to encompass the majority of plaques at risk for rupture (Figure 5).[19]

Thinning of the fibrous cap probably involves 2 concurrent mechanisms. One is the gradual loss of SMCs from the fibrous cap. Ruptured caps contain fewer SMCs and less collagen than intact caps,[93] and SMCs are usually absent at the
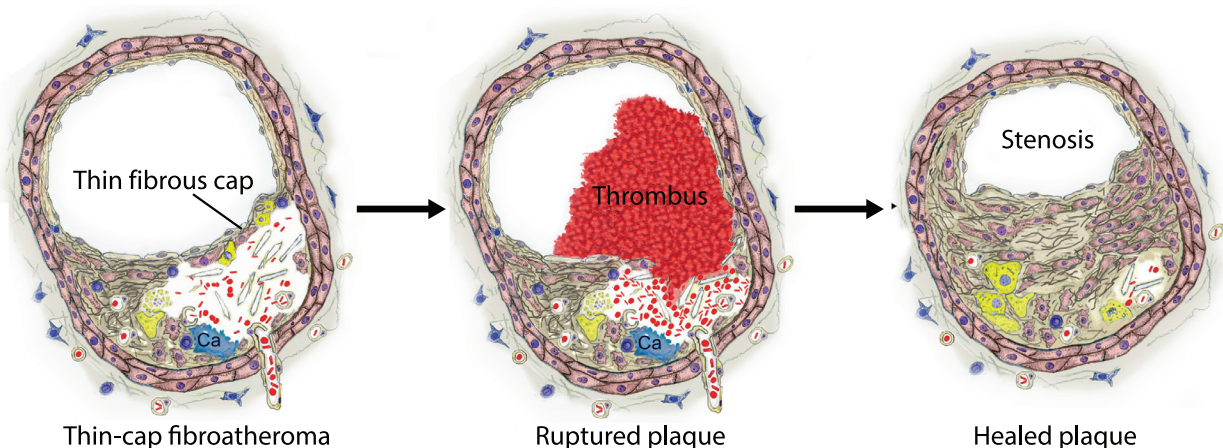
Figure 3. Plaque rupture and healing. Rupture of a thin-cap fibroatheroma with nonfatal thrombus and subsequent healing with fibrous tissue formation and constrictive remodeling.
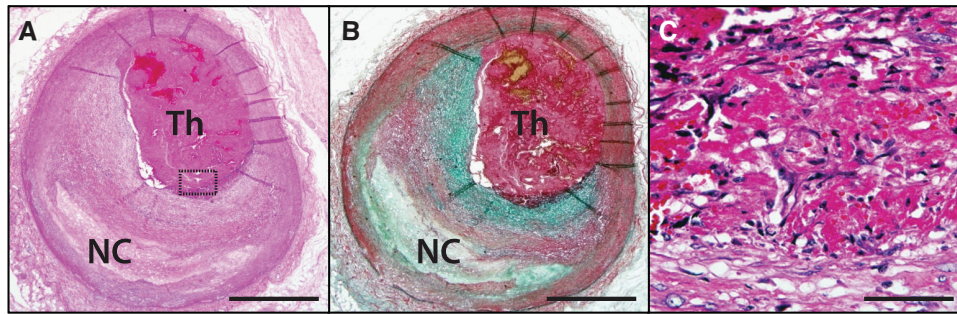
Figure 4. Plaque erosion. A and **B**, Plaque erosion with occlusive luminal thrombus (Th) and underlying fibroatheroma with early necrotic core (NC). A high-power image in **C** shows invading cells resembling smooth muscle cells and endothelial cells. Hematoxylin–eosin (A and C) and Movat pentachrome stains (**B**). Scale bars 1 mm (**A** and **B**) and 50 μm (**C**). Reproduced with permission from Kramer et al[100] with permission of the publisher. Copyright 2010, the American College of Cardiology Foundation.

actual site of rupture.[93,94] At the same time, infiltrating macrophages degrade the collagen-rich cap matrix. The ability of statin therapy to protect rapidly against coronary events indicates that this type of inflammation is also lipoprotein driven. Ruptured caps examined at autopsy are usually heavily infiltrated by macrophage foam cells,[64,94] which secrete proteolytic enzymes such as plasminogen activators, cathepsins, and matrix metalloproteinases. The matrix metalloproteinases are secreted as latent zymogens that require extracellular activation, after which they are capable of degrading virtually all components of the extracellular matrix. As a proof of principle, macrophages that overexpress a constitutively active mutant form of matrix metalloproteinase-9 caused plaque ruptures in a mouse model of atherosclerosis.[95] Whether thinning of the fibrous cap takes decades to evolve or is much more dynamic is not known. However, the fact that fibroatheromas are commonly seen from 30 years of age,[17,47] where ACS is exceedingly rare, seems to indicate that it is a slow, smoldering process.

Rupture of a thin cap and subsequent thrombosis may occur spontaneously, but in some cases, a temporary increase in emotional or physical stress provides the final triggering of the event. Recognized triggers include physical and sexual activity, anger, anxiety, work stress, earthquakes, war and terror attacks, temperature changes, infections, and cocaine use.[96] Also simple daily activities or the circadian rhythm of biological pathways may determine the onset of ACS, which are most frequent in the morning.[97] The triggering pathways may include activation of the sympathetic nervous system with increased heart rate and blood pressure, leading to plaque rupture, or increased coagulability and platelet reactivity, leading to an accentuated thrombotic response on already ruptured plaques.[96–98] It is important to note that even though triggers temporarily increase the relative risk of ACS in susceptible persons, this means little for absolute risk because the exposure is transient and often relatively infrequent.[96] Furthermore, some studies of population-wide triggers, such as the Northridge earthquake in Los Angeles in 1994, indicate that coronary events that are not precipitated by a trigger often would have occurred anyway in the absence of triggers within a few weeks.[99]

Mechanisms of Plaque Erosion

The mechanism leading to thrombus without rupture is one of the most important unresolved questions within atherosclerosis research. The surface endothelium under the thrombus is usually absent, but no distinct morphological features of the underlying plaque have been identified. Eroded and thrombosed
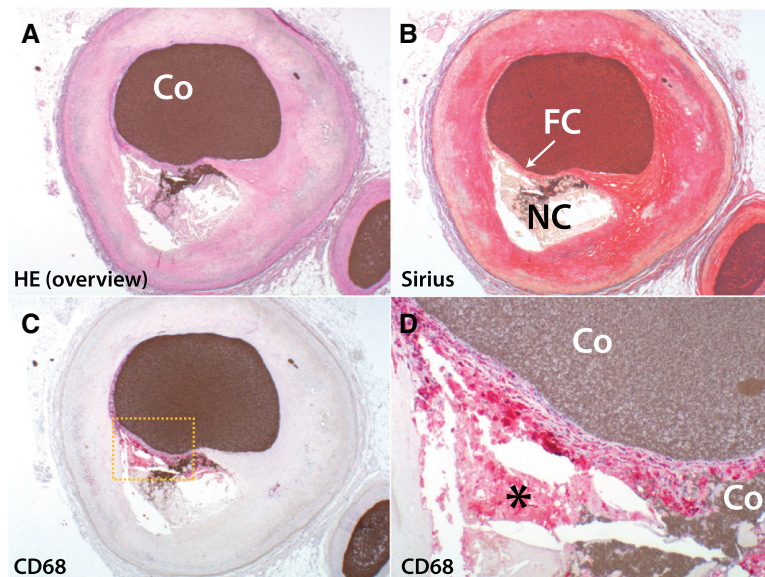
Figure 5. Thin-cap fibroatheroma with inflammation in the cap. A and **B**, The plaque is predominantly composed of fibrous tissue and a necrotic core (NC) with an extremely thin fibrous cap (FC). **C**, Staining for CD68 (macrophages red) visualizes abundant macrophages in the fibrous cap, but other parts of the plaque are not inflamed. **D**, Larger magnification of inset in **C**. The cap contains few SMCs (not shown). Dead macrophages without nuclei are seen within the necrotic core (*). Penetration of the contrast medium (Co) injected postmortem from the lumen into the necrotic core reveals that in this particular case a cap rupture is present in an adjacent segment. HE indicates hematoxylin–eosin.

plaques causing sudden cardiac death are often scarcely calcified, often associated with negative remodeling, and less inflamed than ruptured plaques.[19,100] Some, but not others, have reported focused inflammation immediately beneath the superimposed thrombus.[19,94] Vasospasm has been suggested as a cause of the endothelial damage and subsequent thrombosis.[101] Consistent with this hypothesis, plaque erosion lesions typically show intact internal and external elastic laminas and a well-developed media with contractile SMCs unlike lesions of plaque rupture where the intact internal lamina is often disrupted and the underlying media thin and disorganized.[102]

Interestingly, morphology identical to that of plaque erosion (endothelial denudation over pathological intimal thickening and fibroatheromas with thick cap) can often be found in sections up- or downstream of plaque rupture with a fatal superimposed thrombus.[19] This illustrates the need for tightly spaced sectioning to diagnose erosion (by ruling out rupture). It might also suggest that loss of endothelium can sometimes occur secondarily to thrombus formation, provided that one assumes that the neighboring rupture in these cases is the sole precipitating cause. Autopsy studies indicate that only a minority of ruptures leads to clinical symptoms, whereas the others heal silently with only mural thrombus.[103–105] Hypothetically, loss of the antithrombotic properties of the plaque surface, which in its extreme may present as plaque erosion, could be a determining factor between these outcomes, together with circulating thrombogenic factors.

Thrombosis

The magnitude of the thrombotic response on ruptured or eroded plaques is extremely variable, and only occasionally does a major and life-threatening luminal thrombus evolve. Probably the determinants are those of the classic triad of Virchow: (1) thrombogenicity of the exposed plaque material, (2) local flow disturbances, and (3) systemic thrombotic propensity. Cigarette smoking predisposes to CHD at least partly through increasing systemic thrombotic propensity.[91,106]

With plaque rupture, cap collagen and the highly thrombogenic lipid core, enriched in tissue factor–expressing apoptotic microparticles, are exposed to the thrombogenic factors of the blood.[107,108] Plaque erosion is probably a weaker thrombogenic stimulus, and thus, other factors of the triad may be particularly important in this setting. Consistently, fatal thrombi associated with plaque erosion seem to be longer in the making than those precipitated by rupture.[100]

The time relationship between plaque rupture and syndrome onset is not easily assessed because rupture in itself is asymptomatic and the following thrombotic process is highly unpredictable. Plaque material is sometimes found interspersed in the thrombus,[64,94] indicating that severe thrombosis followed immediately after plaque rupture. In other cases, the thrombotic response is dynamic: thrombosis and thrombolysis, often associated with vasospasm, tend to occur simultaneously causing intermittent flow and the formation of a layered thrombus developing over days.[109]

Thrombi obtained by thrombectomy or analyzed postmortem can be partly degraded or organized, indicating that they were initiated either several days or up to 1 week before ACS presentation.[100,110] It is conceivable that the thrombus during this period, as in thrombosis-induced inflammation of veins,[111] could lead to endothelial sloughing, and neutrophil and
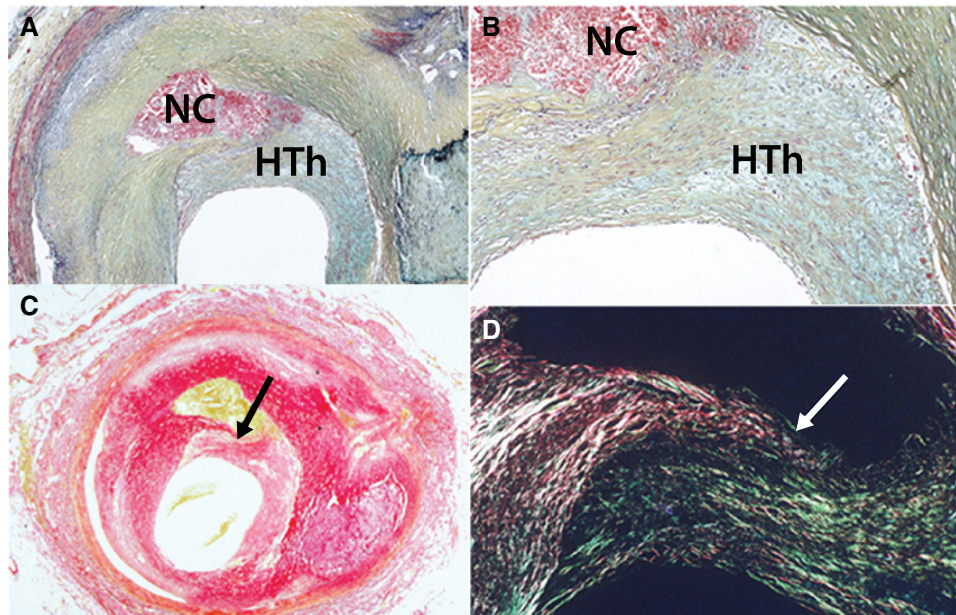
Figure 6. Healed plaque rupture. A, Fibroatheroma with hemorrhage in a late necrotic core (NC) is seen underlying a healed thrombus (HTh). Movat pentachrome stain. **B**, Higher magnification of the healed thrombus shows extensive smooth muscle cells within a collagenous proteoglycan-rich neointima and clear demarcation from the fibrous plaque region to the right. **C**, Layers of collagen shown by Sirius red staining. Note the area of dense, dark red collagen surrounding the necrotic core presumably corresponding to the old fibrous cap. **D**, Image of the same section taken with polarized light. Dense collagen (type I) in the old fibrous cap is lighter reddish yellow and is disrupted (arrow), with newer greenish type III collagen on right and above rupture site. Reproduced with permission from Burke et al[105] with permission of the publisher. Copyright 2001, the American Heart Association.

monocyte/macrophage infiltration in the underlying vascular wall, which might mistakenly be assumed to have existed before thrombus initiation.

Although blood flow continues over the culprit lesion, microemboli of plaque material and thrombus may be washed away, leading to distal embolization of the myocardium.[109,112] Such emboli have been reported to be more frequent in erosions (74%) than ruptures (40%) in individuals presenting as sudden coronary death in the absence of any coronary intervention,[113] which may itself produce iatrogenic embolization.[112] The distal emboli from either source may cause microvascular obstruction that prevents myocardial perfusion despite a recanalized infarct-related coronary artery.

Healed Plaques and Incorporated Thrombi

A special nonuniform pattern of dense type I (older) and loosely arranged type III (younger) collagen, judged to indicate healed plaque rupture, can be identified in many coronary plaques, particularly in those that cause chronic high-grade stenosis (Figure 6).[104] Often several healed rupture sites are present, and the number of healed ruptures correlates with the severity of stenosis.[105] Other plaques may have a multilayered appearance consistent with a history of incorporation and organization of thrombus on eroded plaques.[19] Although these findings are observational, they indicate that silent plaque ruptures and erosions are important for plaque growth and chronic stenosis formation (Figure 3). Furthermore, scar-like contraction of the healing fibrous tissue has been proposed as a cause of the constrictive remodeling often seen with severely stenotic plaques.[84,114] A central role of plaque healing in stenosis formation may explain why chronic coronary stenosis often develops in a phasic rather than linear manner, forming at sites that were only insignificantly narrowed in an antecedent angiography.[104,115]

Plaque Burden

In research and clinical practice, there is a need to characterize atherosclerosis or individual plaques for a few important and measurable characteristics that convey the status of the disease process and the risk of progression. Such variables can be used as end points for clinical trials and as risk prediction tools to guide decisions about therapies. Some of the terms used in this area are plaque burden, activity, and vulnerability.

Plaque burden is a measure of the extent of atherosclerosis in the body or in a particular vascular bed irrespective of the cellular composition and activity of plaques. It can be measured as plaque volume, arterial surface covered with lesions, or by some correlated proxy, such as the measurement of coronary calcium score by computed tomography, intima-media thickness and plaque area in the carotid bed by ultrasound, and ankle-brachial pressure index in peripheral vascular disease. Because atherosclerosis is a multifocal disease affecting the entire vasculature, having a high plaque burden in one territory, for example, the carotids or lower limbs, may also be a marker for advanced disease elsewhere, especially in the coronary arteries because of their high susceptibility.[47,116–118] Thus, the presence of a carotid stenosis detected by ultrasound or peripheral vascular disease detected by a low ankle-brachial index carries a risk for MI comparable to that of patients with CHD and therefore are treated as CHD risk equivalents.[119]

Similar thinking applies to the prospect of finding plasma biomarkers correlated to atherosclerosis. Because the bulk of atherosclerosis is located in the abdominal aorta and iliofemoral arteries, any plasma biomarker of atherosclerosis would be expected to reflect the extent of atherosclerosis here, which then by a similar argument as above would indicate the risk of having concomitant coronary atherosclerosis.

Consistent with plaque being composed predominantly of hypocellular fibrotic tissue with slow turnover,[68,120] plaque burden changes only slowly and modestly even with therapy that substantially lowers the risk of ACS.[121] Of the different components of progressive atherosclerotic lesions, lipids and macrophages seem to be the most amenable for regression.[122,123]

Plaque Activity

The activity of the disease or individual plaques is an important, but difficult, concept. It is important because the ability to measure disease activity faithfully, for example, by non-invasive imaging or a circulating plasma biomarker, would be an important tool for the discovery of causal factors and for demonstrating efficacy of therapies in small clinical trials. Furthermore, not having to rely on clinical end points to measure effect would pave the way for research and possibly preventive treatment at early stages of the disease, where we seem to know more about the pathophysiological processes and where the disease may potentially be more modifiable.

It is a difficult concept because disease or plaque activity does not have a simple, defined meaning. Often it is taken to mean inflammation, measured for instance as the density of macrophages in plaques. This is reasonable given the central role of vascular inflammation in plaque development. However, there is a fundamental difference between inflammation of an early atherosclerotic lesion and the focused inflammation of a fibrous cap that may lead to rupture and thrombosis.[124] Several other processes in atherosclerotic plaques could be included under the heading of plaque activity, including intimal necrosis, which constitute the perhaps most detrimental activity of the disease. Angiogenesis, leaky endothelium, and plaque bleeding/hemorrhage often accompany inflammation and constitute other potential biomarkers of disease activity.

Plaque Vulnerability

To predict which plaques are at risk of precipitating thrombosis and to understand the mechanisms leading to their formation, much effort has been put into recognizing the pathological features of vulnerable plaques (or synonymously thrombosis-prone or high-risk plaques): those plaques at high short-term risk of thrombosis.[87]

Notably, the presence of thrombosis is not the same as the occurrence of ACS. As discussed in the preceding sections, many, perhaps the majority, of ruptures and erosions are asymptomatic in the short term although they may sometimes lead to gradual coronary narrowing.[103–105] The vulnerable patient is a term used to describe patients at high short-term risk of an acute clinical event.[125] This depends on plaque burden,

Table.   Features of Ruptured Plaques*

Thrombus

Large necrotic core

Fibrous cap covering the necrotic core

    Thin (thickness usually <65 μm)

    High macrophage density

    Few smooth muscle cells

Expansive remodeling preserving the lumen

Neovascularization from vasa vasorum

Plaque hemorrhage

Adventitial/perivascular inflammation

Spotty calcification

*The same features, except cap rupture and luminal thrombus, are assumed to characterize vulnerable plaques of the rupture-prone type.

plaque vulnerability, systemic thrombotic propensity, and the myocardial susceptibility to ischemia and arrhythmia.

The vulnerable plaque is used sometimes as a concept comprising plaques at high-risk of thrombosis by any mechanism (rupture, erosion) and sometimes to describe a set of histological features that by association are assumed to increase the risk of imminent rupture and thrombosis. The latter usage explains why the vulnerable plaque term is also used in research with mouse models, where the occurrence of thrombosis on plaques is exceedingly rare.

The Table outlines the features that have been found to characterize ruptured plaques in autopsy studies. By inference, the same features, except thrombus and cap disruption, are assumed to mark vulnerable (rupture-prone) plaques. The prototypical rupture-prone plaque is a TCFA with a large necrotic core and macrophage infiltration in the cap (Figure 5).[86,93] Although the macrophage density in ruptured caps is usually high,[93] whole-plaque macrophage density rarely exceeds a few percent because ruptured caps are small,[124] and thus, it is a misconception that ruptured plaques are always highly inflamed. Other associated features include big plaque size, expansive remodeling mitigating luminal obstruction (mild stenosis by angiography), neovascularization, plaque hemorrhage, adventitial inflammation, and a spotty pattern of calcifications.[124]

In the next sections, we will briefly discuss some of the individual features of the rupture-prone plaque. The other types of vulnerable plaques predisposing to thrombosis with erosion or possibly calcified nodule remain poorly understood.

Thin Fibrous Cap

TCFAs are the likely precursors of the majority of fatal coronary plaque ruptures.[88,91] They tend to cluster in the proximal segments of the major coronary arteries, where most plaque ruptures and thrombi are seen,[126] and rarely more than a few TCFAs exist simultaneously.[127,128]

The absence of TCFAs in a patient indicates a low imminent risk for plaque rupture and thrombosis. An attempt to measure the risk conferred by their presence was done in the recent PROSPECT (Providing Regional Observations to Study Predictors of Events in the Coronary Tree) study. Of 595 virtual histology (VH)-TCFAs identified in 313 of 623 patients examined by VH-intravascular ultrasound, only 26 led to coronary events (mostly progressive angina) at a median follow-up of 3.4 years.[129] Another recent serial VH-intravascular ultrasound study found that VH-TCFAs arise and disappear more dynamically than what was previously thought.[130] Both these studies weaken the rationale for a targeted therapeutic approach to rupture-prone plaques. However, it is important to note that the performance and resolution of VH-intravascular ultrasound do not permit the identification of TCFAs as pathologists define them,[131,132] and the use of more precise methods in future studies may lead to different results.

Necrotic Core

If no necrotic core is present, there is no overlying fibrous cap to rupture. Consistently, not having necrotic cores among nonculprit lesions in the proximal coronary arteries indicates
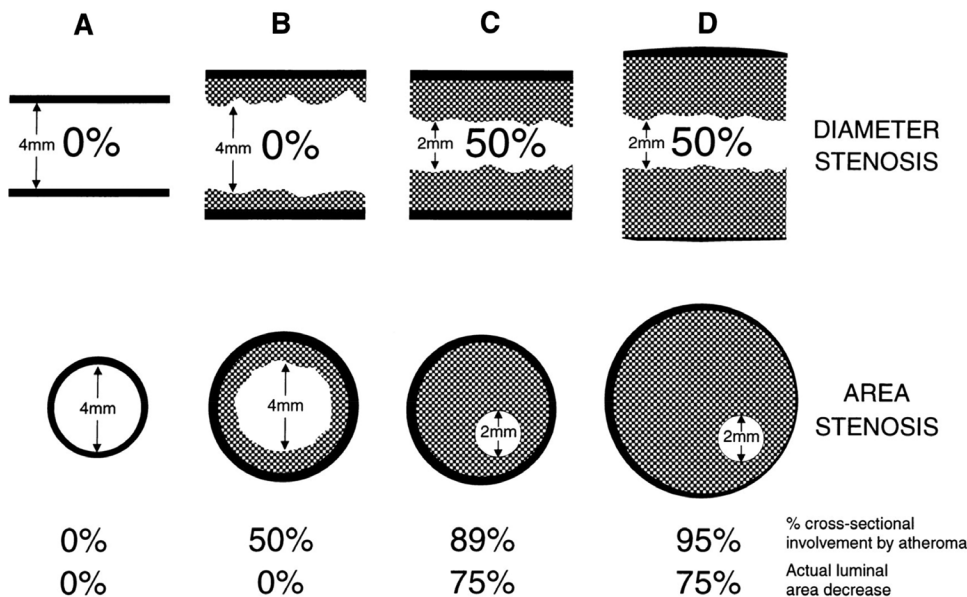
Figure 7. Angiographic and cross-sectional evaluations of the arterial lumen. **Top row**, angiographic views. The examples (**A–D**) illustrate how angiography cannot be used to determine the presence or size of atherosclerotic plaques because of arterial remodeling. **Bottom row**, cross-sectional views. The same segments seen under the microscope illustrating how the lumen stenosis cannot be determined because of arterial remodeling. A ≤50% angiographic stenosis may look severely obstructed. Thin-cap fibroatheromas and ruptured plaques are usually large and associated with expansive remodeling, as shown in example **D**. Reproduced with permission from Fishbein and Siegel[135] with permission of the publisher. Copyright 1996, the American Heart Association.

a favorable prognosis after ACS.[133] However, a larger necrotic core also confers greater risk than a small one.[101,134] The importance of necrotic core size for plaque stability is comprehensible because the expansion of the core may erode the fibrous cap from below and because the total lack of supporting collagen in the lipid-rich core confers greater tensile stress to the overlying fibrous cap. A large necrotic core may also increase the thrombogenecity of the plaque material and hence the risk of a clinical event in case of plaque rupture.[107]

Plaque Size and Severity of Stenosis

Retrospective angiographic studies and recently the PROSPECT study have shown that plaques that cause stenosis have a higher risk of producing a clinical event than plaques that do not.[86,129] However, most ACS are precipitated by plaques that did not cause significant stenosis on angiography weeks or months before, simply because such plaques are much more prevalent than the few stenotic plaques even if they have a lower per-plaque vulnerability.[86,129] The mild preexistent stenosis in these cases is explained by expansive remodeling because ruptured plaques are on average large.[135,136]

Recent studies have described significant angiographic narrowing in the days before MI.[137,138] Furthermore, autopsy data show that TCFAs are smaller than ruptured plaques, and the latter are nearly always large and seem severely obstructive when studied under the microscope.[139] These observations have challenged the long-held notion that mild to moderate obstructive coronary lesions are responsible for the majority of MIs.[137,138]

It may, however, not come as a surprise that an angiographic examination performed close to an MI may reveal a severe coronary lesion or that the size of a ruptured plaque exceeds that of its precursor. Plaque rupture is followed not only by dynamic luminal thrombosis (±vasospasm) but also by hemorrhage from the lumen into the plaque through the ruptured surface (bleeding into the necrotic core from vasa vasorum may have preceded rupture),[88] giving rise to rapid plaque expansion unresponsive to thrombolysis and aspiration thrombectomy, and the temporal relationship between plaque rupture and ACS is often protracted.[110] Furthermore, it is important to consider that cross-sectional area stenosis determined from formalin-fixed, paraffin-embedded lesions does not equate to luminal diameter stenosis determined by angiography, due to arterial remodeling, mathematical conversion between diameter and area, and shrinkage of tissue from fixation and dehydration (Figure 7).[135]

Other Associated Features of Plaque Rupture

Additional plaque features are more common in ruptured plaques than in intact plaques, including increased neovascularization and adventitial inflammation.[140,141] Furthermore, culprit lesions for ACS are generally less calcified than plaques responsible for stable angina, and the pattern of plaque calcification also differs.[80,142] These features are not independently associated with plaque rupture, however, and if there is a causal link, it is probably through modulation of the thickness or inflammation of the fibrous cap or the size of the necrotic core. The importance of these features, however, lies in the fact that they may be targets for noninvasive imaging.

Perspectives for Prevention

Most of our mechanistic knowledge of atherosclerosis relates to the development of atherosclerotic plaques, including the driving influence of LDLs on vascular inflammation, but targeted primary prevention are rarely initiated before the first clinical complications of atherosclerosis have arisen.[143] At this stage of the disease, comparatively little is known about the relevant biological processes against which it may be possible to intervene. Knowledge of what causes fibrous cap thinning and plaque rupture is incomplete, and major gaps remain in our understanding of the mechanisms leading to thrombosis of nonruptured plaques. Furthermore, plaques in ACS patients are relatively hypocellular and inert with low tissue turnover,[68,120] which may altogether diminish the potential for changing their fate by pharmacological intervention.

Measurements of plaque burden in middle-aged persons may improve risk stratification enabling preventive therapy to be initiated earlier in more persons at risk.[144] However, in pursuing improvements in individualized prevention strategies, one must not forget the continued importance of public health approaches,[145] such as the promotion of diet quality, smoking cessation, physical activity, and weight control through information and legislation. During recent decades, risk factor levels have declined substantially in many developed countries, especially for smoking and cholesterol,[3] and plaque burden among young US service members is now apparently only a fraction of what it was during the Korean War.[146] The quality of continued public health initiatives, however, could be considerably improved by the development of noninvasive techniques to measure atherosclerotic disease activity in asymptomatic persons enabling recommendations for lifestyle habits to be rooted in clinical studies rather than epidemiological associations alone.

Disclosures

None.

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