

## STATE-OF-THE-ART PAPER

# Atherothrombosis and High-Risk Plaque

## Part I: Evolving Concepts

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Atherothrombosis is a complex disease in which cholesterol deposition, inflammation, and thrombus formation play a major role. Rupture of high-risk, vulnerable plaques is responsible for coronary thrombosis, the main cause of unstable angina, acute myocardial infarction, and sudden cardiac death. In addition to rupture, plaque erosion may also lead to occlusive thrombosis and acute coronary events. Atherothrombosis can be evaluated according to histologic criteria, most commonly categorized by the American Heart Association (AHA) classification. However, this classification does not include the thin cap fibroatheroma, the most common form of high-risk, vulnerable plaque. Furthermore, the AHA classification does not include plaque erosion. As a result, new classifications have emerged and are reviewed in this article. The disease is asymptomatic during a long period and dramatically changes its course when complicated by thrombosis. This is summarized in five phases, from early lesions to plaque rupture, followed by plaque healing and fibrocalcification. For the early phases, the role of endothelial dysfunction, cholesterol transport, high-density lipoprotein, and proteoglycans are discussed. Furthermore, the innate and adaptive immune response to autoantigens, the Toll-like receptors, and the mechanisms of calcification are carefully analyzed. For the advanced phases, the role of eccentric remodeling, vasa vasorum neovascularization, and mechanisms of plaque rupture are systematically evaluated. In the final thrombosis section, focal and circulating tissue factor associated with apoptotic macrophages and circulatory monocytes is examined, closing the link between inflammation, plaque rupture, and blood thrombogenicity. (J Am Coll Cardiol 2005;46:937–54) © 2005 by the American College of Cardiology Foundation

## NOMENCLATURE AND EVOLVING ASSESSMENT OF DISEASE

In the 19th century, there were two major hypotheses to explain the pathogenesis of atherosclerosis: the incrustation hypothesis, proposed by von Rokitansky in 1852, and the lipid hypothesis, proposed by Virchow in 1856 (1,2). These hypotheses focused on fibrin deposition, lipid accumulation, and extracellular matrix formation. In addition, Virchow used for the first time the name *endarteritis deformans*, linking inflammation to the disease and forming the basis of the response-to-injury hypothesis of Ross more than a century later (3–5). Lipoprotein retention (6) and chronic inflammation are intimately related to the early phases of the disease. Furthermore, inflammation also plays a role in plaque rupture and thrombosis (7–11). Therefore, the integration of these hypotheses can be unified under the term atherothrombosis.

Atherothrombosis is a systemic arterial disease originally involving mostly the intima of large- and medium-sized systemic arteries including the carotid, aorta, coronary, and

peripheral arteries. The main components of atherothrombotic plaques are (12–18): 1) connective tissue extracellular matrix, including collagen, proteoglycans, and fibronectin elastic fibers; 2) crystalline cholesterol, cholesteryl esters, and phospholipids; 3) cells such as monocyte-derived macrophages, T-lymphocytes, and smooth-muscle cells; and 4) thrombotic material with platelets and fibrin deposition. Varying proportions of these components occur in different plaques, thus giving rise to a heterogeneity or spectrum of lesions. These components mainly affect the intima, but secondary changes also occur in the media and adventitia, (19) including growth of vasa vasorum (20–23). Atherosclerosis progresses through lipid core expansion and macrophage accumulation at the edges of the plaque, leading to fibrous cap rupture, as shown in Figure 1.

To establish clinical risk factors for plaque rupture, Burke et al. (24) examined 113 men with coronary artery disease complicated with sudden cardiac death. Plaque rupture was associated with increased total cholesterol/high-density lipoprotein (HDL) ratio, but not with smoking or hypertension. Of note, ruptured plaques showed fibrous cap thickness (mean  $\pm$  SD) of  $23 \pm 19 \mu\text{m}$ ; 95% of ruptured caps measured  $64 \mu\text{m}$  or less. As a result, vulnerable plaque was defined as a plaque with a fibrous cap  $<65 \mu\text{m}$  thick with an infiltrate of macrophages ( $>25$  per high-magnification [0.3-mm diameter] field) (24), as shown in Figure 2.

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#### Abbreviations and Acronyms

ACS	= acute coronary syndrome
AHA	= American Heart Association
apo	= apolipoprotein
CAM	= cell adhesive molecule
CRP	= C-reactive protein
HDL	= high-density lipoprotein
IEL	= internal elastic lamina
LDL	= low-density lipoprotein
MMP	= matrix metalloproteinase
NCP	= non-collagenous bone-associated protein
OPN	= osteopontin
TCFA	= thin-cap fibroatheroma
TF	= tissue factor
TLR	= toll-like receptor

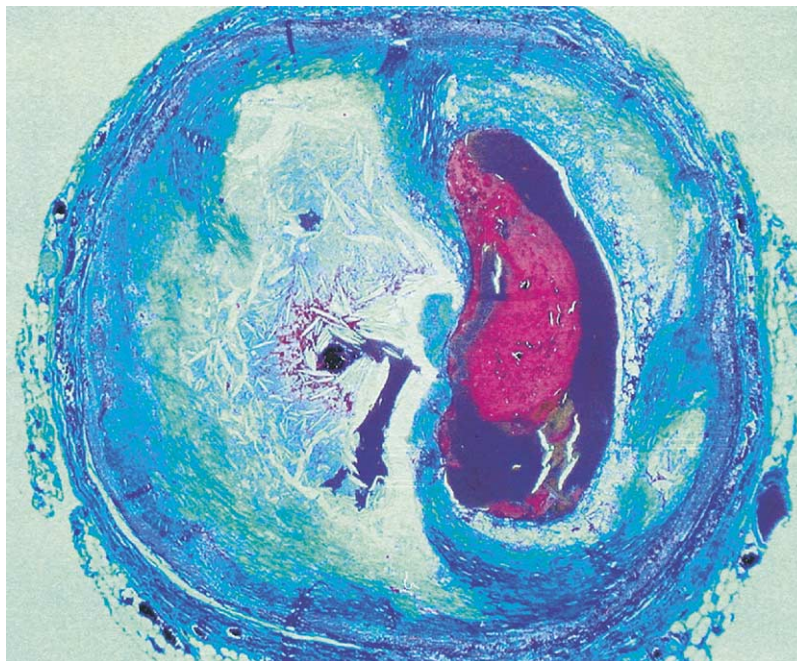
Plaque rupture is the most common substrate for coronary thrombosis in humans. However, 30% to 40% of coronary thrombosis occurs at sites at which plaque rupture cannot be identified. In a landmark publication, Farb *et al.* (25) described 50 consecutive cases of sudden cardiac death attributable to coronary thrombosis, in which 22 had superficial erosion of a proteoglycan-smooth muscle cell-rich plaque. No site of cap rupture could be identified. To establish clinical and histologic characteristics, the remaining 28 cases of plaque rupture served as controls. Eroded plaques were more frequently seen in pre-menopausal women. Of note, eroded plaques were less stenotic, had lower macrophage infiltration, and had a much lower incidence of calcification, as shown in Figure 3.

Therefore, two different mechanisms, plaque rupture and erosion, can give rise to arterial thrombosis. The terms “high-risk” or “vulnerable” can be used as synonyms to describe plaques with an increased risk of thrombosis (26). In addition to these terms, other terms, including culprit lesion, inflamed thin-cap fibroatheroma (TCFA), calcific nodule, thrombosed plaque, and vulnerable patient, have been used. This multiple terminology has created confusion and, therefore, has required standardization. To properly define adequate terminology and avoid confusion, a written consensus from a group of experts properly standardized these terms, providing definitions for proper implementation (26), as summarized in Table 1.

#### PHASES OF ATHEROTHROMBOSIS

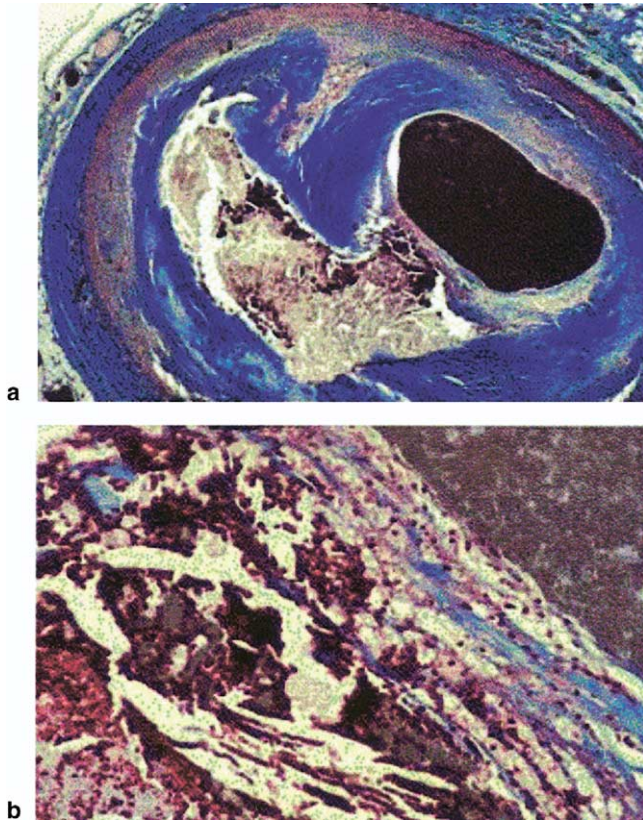
According to a simplified modification of the criteria previously set forth by the American Heart Association (AHA) Committee on Vascular Lesions (14), and more recently by Stary (27), plaque progression can be subdivided into five pathologically/clinically relevant phases, as shown in Figure 4.

**Phase 1 (early).** Lesions are small, commonly seen in young people, and categorized into three types as follows: type I lesions, consisting of macrophage-derived foam cells that contain lipid droplets; type II lesions, consisting of both macrophages and smooth-muscle cells and mild extracellular lipid deposits; and type III lesions, consisting of smooth-muscle cells surrounded by extracellular connective tissue, fibrils, and lipid deposits.



**Figure 1.** Cross-sectioned coronary artery containing a ruptured plaque with a non-occlusive platelet-rich thrombus superimposed. The actual defect in the fibrous cap is not seen in this section but is located nearby, documented by the presence of extravasated radiographic contrast medium (postmortem coronary angiography) in the soft, lipid-rich core just beneath the thin, inflamed fibrous cap. Trichrome stain, rendering thrombus red, collagen blue, and lipid colorless. Adapted with permission from Falk E, Shah PK, Fuster V. Atherogenesis and its determinants. In: Hurst's the Heart. 11th edition. Fuster V, *et al.*, editors. McGraw-Hill, 2004:1123-39.





**Figure 2.** Histologic example of a high-risk, vulnerable plaque. (a) Large lipid-rich core with a thin fibrous cap. The lumen contains contrast medium injected postmortem. (b) Higher magnification showing macrophages (>25 per high-power field) beneath a very thin cap (<65  $\mu\text{m}$  in thickness). Extravasated erythrocytes with plaque hemorrhage within the core indicate plaque rupture nearby. Trichrome stain, rendering lipid colorless, collagen blue, and erythrocytes red. Adapted with permission from Schaar JA, et al. *Eur Heart J* 2004;25:1077-82.

**Phase 2 (advanced).** Lesions, although not necessarily stenotic, may be prone to rupture because of their high lipid content, increased inflammation, and thin fibrous cap. These plaques are categorized morphologically as one of two variants: type IV lesions, consisting of confluent cellular lesions with a great deal of extracellular lipid intermixed with normal intima, which may predominate as an outer layer or cap; or type Va lesions, possessing an extracellular lipid core covered by an acquired fibrous cap. Phase 2 plaques can evolve into the acute phases 3 and 4.

**Phase 3.** These lesions are characterized by acute complicated type VI lesions, originating from ruptured (type IV or Va) or eroded lesions, and leading to mural, non-obstructive thrombosis. This process is clinically silent, but occasionally may lead to the onset of angina (10).

**Phase 4.** These lesions are characterized by acute complicated type VI lesions, with fixed or repetitive occlusive thrombosis. This process becomes clinically apparent in the form of an acute coronary syndrome (ACS), although not infrequently it is silent (28,29). About two-thirds of ACS are caused by occlusive thrombosis on a non-stenotic plaque, although in about one-third, the thrombus occurs on the surface of a stenotic plaque (7). In phases 3 and 4,

changes in the geometry of ruptured plaques, as well as organization of the occlusive or mural thrombus by connective tissue, can lead to the occlusive or significantly stenotic and fibrotic plaques.

**Phase 5.** These lesions are characterized by type Vb (calcific) or Vc (fibrotic) lesions that may cause angina; however, if preceded by stenosis or occlusion with associated ischemia, the myocardium may be protected by collateral circulation and such lesions may then be silent or clinically inapparent (30,31).

The AHA classification falls short of identifying plaque erosion or the TCFA. A different classification including these two categories has been proposed by Virmani et al. (32), as shown in Figure 5.

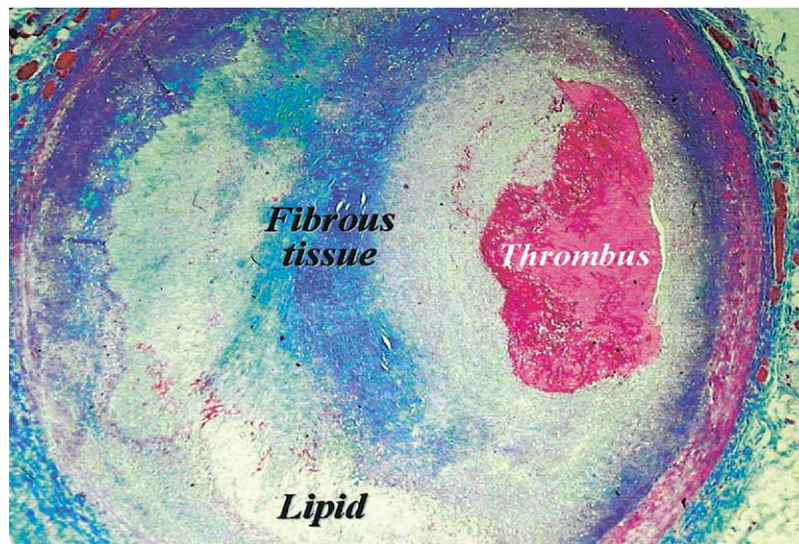
## EARLY ATHEROTHROMBOSIS

**Endothelial dysfunction.** The endothelium is a dynamic autocrine and paracrine organ that regulates anti-inflammatory, mitogenic, and contractile activities of the vessel wall, as well as the hemostatic process within the vessel lumen (33) (Fig. 6). A single molecule, nitric oxide (NO), is responsible for these regulatory processes (34).

A dysfunctional endothelium, characterized by decreased NO synthesis, facilitates vessel wall entry and oxidation of circulating lipoproteins, monocyte entry and internalization or inflammation, smooth cell proliferation and extracellular matrix deposition, vasoconstriction, as well as a prothrombotic state within the vessel lumen (35,36) (Fig. 7).

Endothelial dysfunction, traditionally known as the earliest manifestation of atherothrombosis, is often the result of a disturbance in the physiological pattern of blood flow—flow reversal or oscillating shear stress—at bending points and near bifurcations. (37,38). In addition to biomechanical shear forces enhanced by hypertension (39), the coexistence of other biohumoral risk factors such as hypercholesterolemia, advanced glycation end-products in diabetes and in elderly people, chemical irritants in tobacco smoke, circulating vasoactive amines, and immunocomplexes, have been associated with endothelial dysfunction (40-42) (Fig. 7).

Endothelial cells respond to changes in local shear rates by modulating the induction and/or repression of several genes. A common mechanism of action of the gene modulation in part seems to be mediated via shear stress responding elements located in the genes (43). Thus, as a response to reversal or oscillatory shear stress, endothelial cell activation is characterized by the expression of cell adhesive molecules (CAMs) (Fig. 7) from the selectin superfamily (E- and P-selectins). These proteins facilitate the homing (margination and adhesion) of the circulating monocytes to the activated endothelial cells. The expression of the selectins is regulated by the transcriptional nuclear factor (NF)-kappa-B (44) and is followed by the expression of other CAMs (i.e., intercellular and vascular adhesive molecules-1). These proteins will facilitate the internaliza-



**Figure 3.** Plaque erosion. Cross section of a coronary artery containing a stenotic atherosclerotic plaque with an occlusive thrombosis superimposed. The endothelium is missing at the plaque-thrombus interface, but the plaque surface is otherwise intact. Trichrome stain, rendering thrombus red, collagen blue, and lipid colorless. Courtesy of Dr. Erling Falk, Aarhus, Denmark.

tion of the adhered monocytes into the arterial wall, contributing to atherogenesis. Furthermore, clinical studies have associated high plasma levels of these proteins with an increased risk for coronary events (45–48).

**Lipoprotein transport and proteoglycans.** Low-density lipoproteins (LDLs) infiltrate through the arterial endothelium into the intima (49) (Fig. 7). This binding seems to relate to an ionic interaction of apolipoprotein (apo) B with matrix proteins including proteoglycans, collagen, and fibronectin (50). Proteoglycans are macromolecules composed of a core protein and long-chain carbohydrates called glycosaminoglycans. Proteoglycans along with other extracellular matrix proteins are located between the basement membrane of the endothelial cell and the internal elastic lamina (IEL). The interactions between oxidized LDL and proteoglycans are crucial in early atherosclerosis, mostly related to lipoprotein retention (6), intravascular aggregation of LDL leading to chemical modification, and induction of inflammation (50).

Another important feature of lipoprotein transport is related to the effect of HDL. Classically known as the antiatherogenic lipoprotein, HDL promotes reverse cholesterol transport from the arterial wall, specifically from lipid-laden macrophages (51–55). The first experimental evidence supporting this theory was reported by our group in the hypercholesterolemic rabbit model. Once-per-week administration of HDL inhibited progression and induced regression of macrophage-rich aortic lesions (56,57). Further inhibition of atherosclerosis was then obtained in apoE-null mice using the Apo A-I (Apo A-I<sub>Milano</sub>) complex (58–60). These beneficial effects were recently reproduced in human coronary lesions using once-per-week administration of synthetic HDL made from Apo A-I<sub>Milano</sub> in patients with symptomatic coronary artery disease (61). The HDL sub-fractions may play a role in these beneficial

effects, with HDL<sub>2</sub> being the most important for reverse lipid transport. Despite its protective effects, patients with high HDL plasma levels still can present with ACS, probably related to elevations in HDL<sub>3</sub> rather than in HDL<sub>2</sub> (62). Furthermore, the concomitant use of antioxidant supplements blocks the beneficial effects of niacin and statins and may play a role in recurrent symptoms in patients with high HDL levels and coronary artery disease (63).

**Innate and adaptive immune response to auto-antigens.** The important role of inflammation in atherothrombosis has focused attention on the immune system. Development of atherosclerosis is influenced by innate and adaptive immune responses (64,65). Innate immunity represents the first inflammatory response to microorganisms and pathogens. It is based on detection by pattern recognition on macrophages and dendritic cells (66). Several pattern recognition receptors bind a wide range of proteins, carbohydrates, lipids, and nucleic acids. The most important receptors for innate immunity in atherothrombosis are the scavenger receptors and the toll-like receptors (TLRs) (67).

In the first line of innate immunity, the scavenger receptors SR-A and CD-36 are responsible for the uptake of oxidized LDL, transforming the macrophage into a foam cell. (68,69). Furthermore, this pathway activates the NF-kappa-B nuclear transcriptional factor, triggering a potent chemoattractant cycle of monocyte migration and macrophage/foam cell formation (i.e., monocyte chemoattractant protein [MCP]-1, leukotriene LTB<sub>4</sub>, and monocyte-colony stimulating factor [M-CSF]) (68–70). Macrophage/foam cells produce cytokines that activate neighboring smooth-muscle cells, resulting in extracellular formation and fibrosis (18).

The second line of innate immunity, the TLRs, has gained significant recognition recently. For example, the

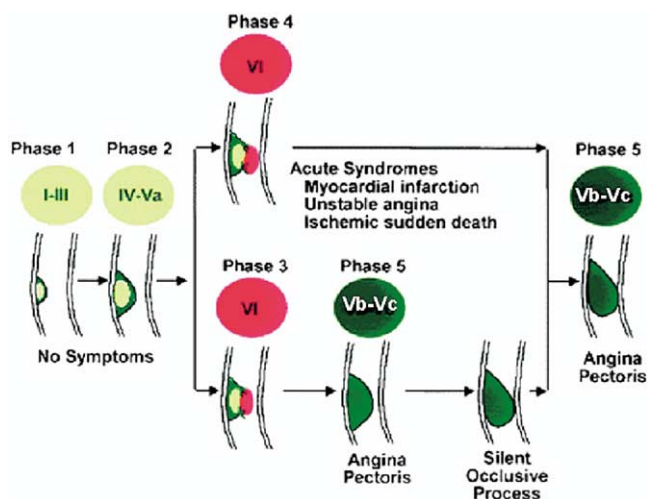


**Table 1.** Definitions for Terminology Commonly Used in Atherothrombosis and Acute Coronary Syndromes

Culprit lesion	A lesion in a coronary artery considered, on the basis of angiographic, autopsy, or other findings, to be responsible for the clinical event. In unstable angina, myocardial infarction, and sudden coronary death, the culprit lesion is often a plaque complicated by thrombosis extending into the lumen.
Eroded plaque	A plaque with loss and/or dysfunction of the luminal endothelial cells leading to thrombosis. There is usually no additional defect or gap in the plaque, which is often rich in smooth muscle cells and proteoglycans.
High-risk, vulnerable, and thrombosis-prone plaque	These terms can be used as synonyms to describe a plaque that is at increased risk of thrombosis and rapid stenosis progression.
Inflamed thin-cap fibroatheroma	An inflamed plaque with a thin cap covering a lipid-rich, necrotic core. An inflamed thin-cap fibroatheroma is suspected to be a high-risk/vulnerable plaque.
Plaque with a calcified nodule	A heavily calcified plaque with the loss and/or dysfunction of endothelial cells over a calcified nodule, resulting in loss of fibrous cap, that makes the plaque at high-risk/vulnerable. This is the least common of the three types of suspected high-risk/vulnerable plaques.
Ruptured plaque	A plaque with deep injury with a real defect or gap in the fibrous cap that had separated its lipid-rich atheromatous core from the flowing blood, thereby exposing the thrombogenic core of the plaque. This is the most common cause of thrombosis.
Thrombosed plaque	A plaque with an overlying thrombus extending into the lumen of the vessel. The thrombus may be occlusive or non-occlusive.
Vulnerable patient	A patient at high risk (vulnerable, prone) for experiencing a cardiovascular ischemic event due to a high atherosclerotic burden, high-risk vulnerable plaques, and/or thrombogenic blood.

Adapted with permission from Schaar JA, et al. Eur Heart J 2004;25:1077-82.

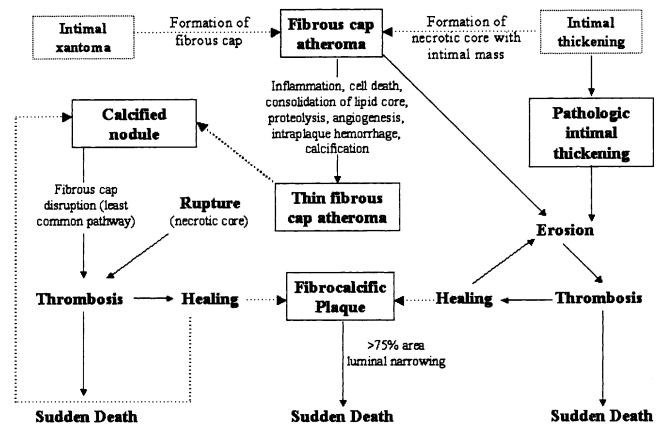
receptor for bacterial lipopolysaccharides, TLR4, is known to recognize cellular fibronectin and heat shock proteins, endogenous peptides produced during tissue injury that may act as auto-antigens early in the disease (71-73). The TLR4 co-localizes with fibroblasts and macrophages in the adventitia and the intima of human coronary atherothrombosis. Stimulation of TLR4-induced activation of NF-kappa-B and increased mRNAs of various cytokines (74). Furthermore, adventitial TLR4 activation augmented neointima



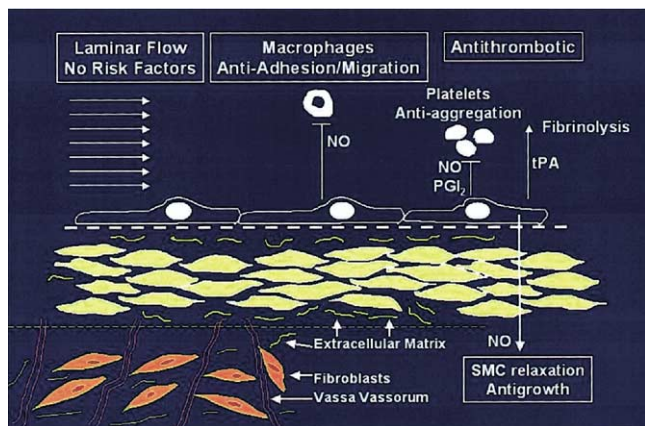
**Figure 4.** Clinicopathologic correlation of asymptomatic atherosclerosis leading to symptomatic atherothrombosis. Modified from Corti R, Fuster V. J Thromb Thrombolysis 2004;17:35-44.

formation in a mouse model, suggesting a link between the immune receptor TLR4 and intimal lesion formation (74). More recently, TLR4 has been shown to be involved not only in the initiation but also in progression and expansive remodeling of atherothrombosis (75,76).

Adaptive immunity is much more specific than innate immunity but may take several days or even weeks to be fully mobilized. It involves an organized immune response leading to generation of T and B cell receptors and immunoglobulins, which can recognize foreign antigens. This type



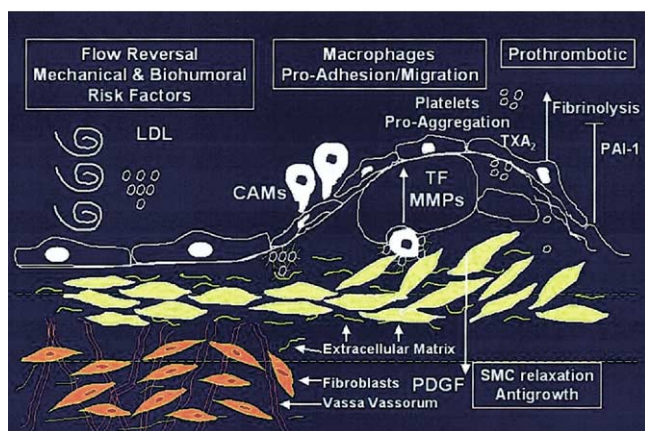
**Figure 5.** Simplified scheme representing seven categories of lesions. Dashed lines reflect controversy regarding etiology. The processes leading to lesion progression are listed between categories. Reproduced with permission from Virmani R, et al. Arterioscler Thromb Vasc Biol 2000; 20:1262.



**Figure 6.** Healthy endothelium under laminar flow conditions and no risk factors. A single molecule, nitric oxide (NO), is involved in multifactorial pathways preventing monocyte adhesion, platelet aggregation, and smooth muscle cell proliferation. PGI<sub>2</sub> = prostacyclin 2; SMC = smooth muscle cell; tPA = tissue plasminogen activator.

of immunity may provide the basis for great advances in the near future, such as immunization and immunosuppressive drugs, which usually target adaptive immune responses. There is certainly a long way to go, but current efforts are setting the foundation to one day produce a vaccine against atherothrombosis (77).

**Mechanisms of calcification.** In addition to immunity, the mechanisms of atherosclerotic calcification have gained significant relevance within the last few years. Coronary calcification is composed of both hydroxyapatite and organic matrix, including type I collagen and non-collagenous bone-associated proteins (NCPs) (78). Collagen-associated crystal deposition initiates mineralization within matrix vesicles, leading to the concept that dystrophic calcification is an active, regulated process rather than passive accumulation of mineral. In addition to collagen, NCPs also play a major role. The most relevant NCPs associated with vascular calcification include osteopontin (OPN), os-



**Figure 7.** Diseased endothelium with non-laminar flow, low-density lipoprotein (LDL) deposition, cell adhesion molecule (CAM) expression, macrophage migration, tissue factor (TF), and matrix metalloproteinase (MMP) expression leading to smooth muscle cell (SMC) proliferation and vasa vasorum neovascularization. PDGF = platelet-derived growth factor; PAI-1 = plasminogen activator inhibitor-1; TXA<sub>2</sub> = thromboxane A<sub>2</sub>.

**Table 2.** Non-Collagenous Proteins Associated With Dystrophic Calcification

Non-Collagenous Proteins	Mechanism of Action	Expression
Osteopontin	<ul style="list-style-type: none"> <li>Bone formation and calcification</li> <li>Increases cell adhesion</li> <li>Chemotactic for smooth muscle cells</li> </ul>	<ul style="list-style-type: none"> <li>Intimal macrophages</li> <li>Smooth muscle cells</li> <li>Adventitial cells</li> <li>Increased in diabetes</li> </ul>
Osteonectin (secretory protein acidic and rich in cysteine)	<ul style="list-style-type: none"> <li>Bone mineralization</li> <li>Increases plasminogen activator inhibitor in endothelial cells</li> <li>Increases matrix metalloproteinases in macrophages</li> <li>Colocalizes with fibrin</li> </ul>	<ul style="list-style-type: none"> <li>Intimal macrophages</li> <li>Platelets</li> <li>Fibroblasts</li> </ul>
Osteoprotegerin	<ul style="list-style-type: none"> <li>Inhibits osteoclast formation</li> </ul>	<ul style="list-style-type: none"> <li>Vascular tissue</li> </ul>
Matrix glycoproteins	<ul style="list-style-type: none"> <li>Inhibits dystrophic calcification</li> </ul>	<ul style="list-style-type: none"> <li>Vascular smooth muscle cells</li> <li>Chondrocytes</li> </ul>

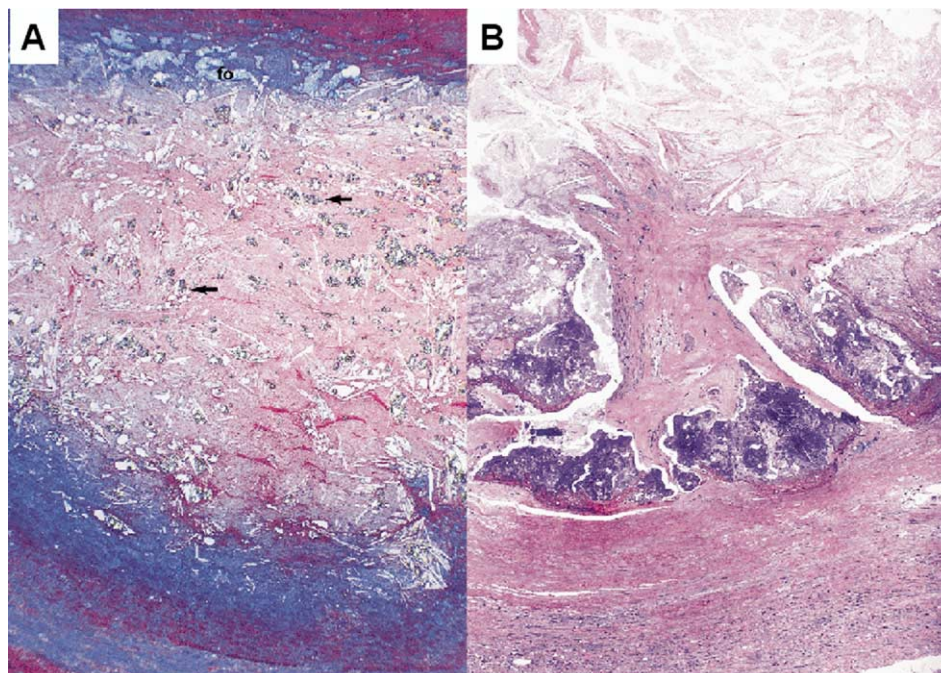
Reproduced with permission from Moreno PR. Calcium deposition in vulnerable atherosclerotic plaques: pathophysiologic mechanisms and implications for acute coronary syndromes. In: Assessing and Modifying the Vulnerable Atherosclerotic Plaque. Fuster V, editor. American Heart Association, Futura Publishing Company, 2002:347-64.

teonectin, osteoprotegerin, and matrix Gla protein, as shown in Table 2.

The most studied NCP in atherothrombosis is osteopontin, which was identified by immunohistochemistry in atherosclerotic plaques (79) and is highly expressed by macrophages in the intima of human arteries (80). The role of OPN mRNA is up-regulated in calcific aortas of LDL receptor-deficient mice fed either high-fat diabetogenic diet (81). Osteopontin expression was detected in peri-aortic adventitial cells, aortic vascular smooth muscle cells, and macrophages of the intimal atheroma. This suggests that OPN-mediated vascular calcification can occur independently of atheroma formation, and that vascular calcification can originate from an osteoprogenitor cell population in the adventitia. Hence, the identification of vascular calcification does not necessarily imply growing atheroma. On the other hand, the functional role of OPN after vascular injury was tested in the rat carotid model two weeks after catheter denudation. The use of anti-OPN antibody decreased intimal areas and cell numbers by 33% and 31%, respectively (82). The OPN promotes vascular cell adhesion and is chemotactic for smooth muscle cells (83). Furthermore, OPN expression is up-regulated by glucose levels, suggesting an active role for OPN in diabetic vasculopathy (84,85).

As it relates to calcification of human atheroma, early microcalcifications can be observed in transitional lesions (AHA classification III and IV), as shown in Figure 8A. As





**Figure 8.** Examples of human atherosclerotic calcification. (A) Microcalcifications identified by light microscopy within the lipid core of a transitional (type IV) coronary plaque (black arrows). (B) Coarse calcification seen in an advanced (type Vb) coronary plaque. Reproduced with permission from Stary HC. *A Slide Atlas of Atherosclerosis Progression and Regression*. New York, NY: Parthenon Publisher Group Inc., 1999.

lesions fibrose, calcification becomes dense, as seen in advanced atherosclerosis, seen in Figure 8B.

### ADVANCED ATHEROTHROMBOSIS

Continuous exposure to the systemic, pro-atherogenic milieu will increase chemotaxis of monocytes leading to lipid accumulation, necrotic core, and fibrous cap formation, evolving into advanced atherosclerosis. Well-established patterns of inflammation and metalloproteinase expression extensively described within the last decade leads to plaque rupture, often found at the shoulder of large lipid-rich plaques (9–11). More recently, new structural and functional features characterizing these lesions have been identified, including eccentric plaque growth with compensatory enlargement of the vessel wall, also known as vascular remodeling, and vasa vasorum neovascularization leading to lipid core expansion and intra-plaque hemorrhage, and lipid core expansion.

**Eccentric vascular remodeling.** Eccentric growth of atheroma involving the inner components of the vessel wall before obstructing the lumen is also known as vascular remodeling. Described by Glagov *et al.* in 1987 (86), remodeling has been consistently identified in atherosclerotic lesions responsible for unstable coronary syndromes. Furthermore, atherosclerotic plaques undergoing remodeling are characterized by a larger lipid core, fewer smooth muscle cells, and increased macrophage infiltration (87). As the plaque grows eccentrically within the vessel wall rather than concentrically into the lumen, remodeling triggers crucial changes within the tunica media and the adventitia.

Several studies have shown increased macrophage-derived matrix metalloproteinase-2 and -9 expression within the intimal-medial interface of remodeled plaques (88). The increased activity of metalloproteinases digests the IEL, modulating the process of remodeling. More recently, our group identified disruption of the IEL as an independent predictor of plaque rupture (19). A strong association between the histologic evidence of IEL disruption and fibrous cap rupture was identified in 598 human aortic plaques. In addition, increased inflammation, fibrosis, and atrophy within the tunica media were documented. Furthermore, adventitial inflammation was increased in ruptured plaques when compared with non-ruptured plaques (19). Concordantly, Burke *et al.* (89) showed that marked expansion of the IEL occurred in plaque hemorrhage with or without rupture. On the contrary, shrinkage of the IEL was found in plaque erosion and total occlusions. Using multivariate analysis, the plaque components most strongly associated with eccentric remodeling were macrophage infiltration, calcification, and lipid core area, linking the concept of remodeling with plaque vulnerability. Therefore, structures such as the IEL, tunica media, and adventitia, involved in the process of eccentric remodeling and historically considered inactive structures in the pathogenesis of atherothrombosis, seem to be actively involved in the development and complications of atherosclerotic disease and may even play a role in precipitating acute coronary syndromes.

**Vasa vasorum neovascularization.** Nourishment of normal blood vessels is accomplished by oxygen diffusion from the lumen of the vessel or from adventitial vasa vasorum.

When vessel wall thickness exceeds the effective diffusion distance of oxygen, vasa vasorum proliferates in the inner layers of the vessel wall, where it is normally absent. Macrophages, attracted by oxidized LDL, are responsible for cytokine production driving neovessel growth (90,91). Therefore, intimal disease is considered a prerequisite for vessel wall and plaque neovascularization. Recent observations have identified increased neovessel density in the outer layers of the artery as the vessel wall undergoes eccentric remodeling. In the Glagov *et al.* (86) seminal work, the first step of remodeling was characterized by overexpansion of the vessel wall in preparation for plaque growth. This crucial observation received almost no attention until recently, when experimental studies documented extensive coronary vasa vasorum neovascularization simultaneously with overexpansion of the vessel wall within the first two to four weeks of a hypercholesterolemic diet in the swine model (92). Of note, increased neovascularization was present in animals with normal endothelial-dependent vasodilation, which became impaired only after 6 to 12 weeks of a high-cholesterol diet (92). Therefore, vasa vasorum neovascularization may play a crucial role in the pathogenesis of atherosclerosis (93-101).

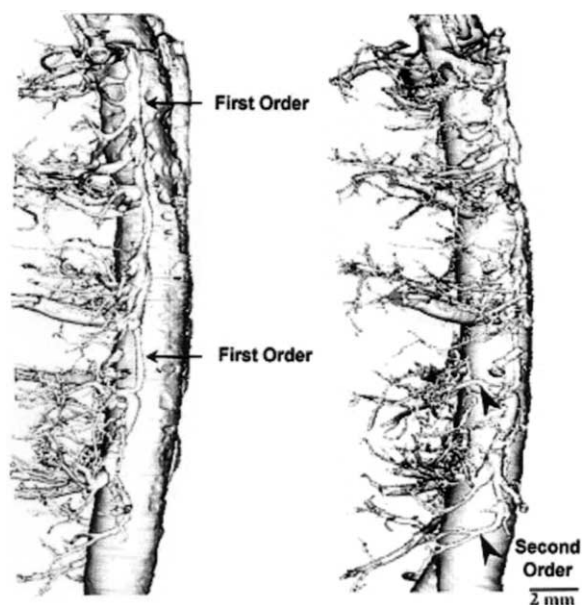
Vasa vasorum surrounds and penetrates the adventitia and outer media of large vessels, including the aorta and the coronary, femoral, and carotid arteries (102). Vasa vasorum can originate from several different sites. In the coronary arteries, vasa vasorum originates from bifurcation segments of epicardial vessels; in the ascending aorta, vasa vasorum originates from coronary and brachiocephalic arteries; in the descending thoracic aorta, vasa vasorum originates from intercostal arteries; and in the abdominal aorta, vasa vaso-

rum arises from the lumbar and mesenteric arteries (95). There are two anatomically distinct patterns of vasa vasorum; first-order vasa vasorum run longitudinally to the lumen of the host vessel, whereas second-order vasa vasorum are arranged circumferentially around the host vessel (Fig. 9). Their main function is to nurture the vessel wall with a number that remains constant throughout life (103). However, atherosclerotic vasa vasorum can proliferate, leading to extensive neovascularization involving the tunica media and directed towards lipid-rich atheroma (104,105).

Our group evaluated the role of vasa vasorum in complex atherothrombosis comparing neovessel content in ruptured and non-ruptured plaques. Double immunohistochemistry was used to identify neovessels, macrophages, and T cells (Fig. 10). Neovessel content was significantly increased in ruptured plaques when compared with non-ruptured plaques in the human aorta (20). We identified neovascularization with monocyte-rich inflammation and disruption of the IEL (presumably as a result of monocyte-released MMPs), as significant contributors to plaque rupture.

More recently we have identified increased microvessel content in atherothrombotic lesions from patients with diabetes mellitus (106). Furthermore, ruptured plaques from patients with diabetes mellitus have increased neovascularization when compared with ruptured plaques from patients without diabetes (107). Of note, microvessels are associated with macrophages and T cell lymphocytes (108). When analyzing diabetes neovascularization, microvessel morphology is characterized by a complex morphology including sprouting, red blood cell and monocyte extravasation with macrophage erythrophagocytosis (109). Furthermore, histologic evidence for atherothrombotic neovascularization as a pathway for macrophage infiltration was documented (110), as shown in Figure 11.

Vasa vasorum may also be involved in the process of plaque regression. When compared with lipid-rich plaques, fibrocalcific lesions with reduced lipid area, also known as regression type lesions, had the lowest microvessel content (111). Most importantly, fibrocalcific, regression-type lesions from diabetic patients are no longer vascularized, suggesting that microvessel involution may be a marker for plaque stabilization (112). Of clinical relevance, Corti *et al.* (113) recently documented for the first time the morphologic pathway for plaque regression occurring from the adventitia. Therefore, vasa vasorum may serve as a potential pathway for reverse lipid transport. As cholesterol exits the plaque, neovascularization and the outer layers of the vessel wall experience regression, as documented experimentally in non-human atherosclerotic primates with documented plaque regression (96).

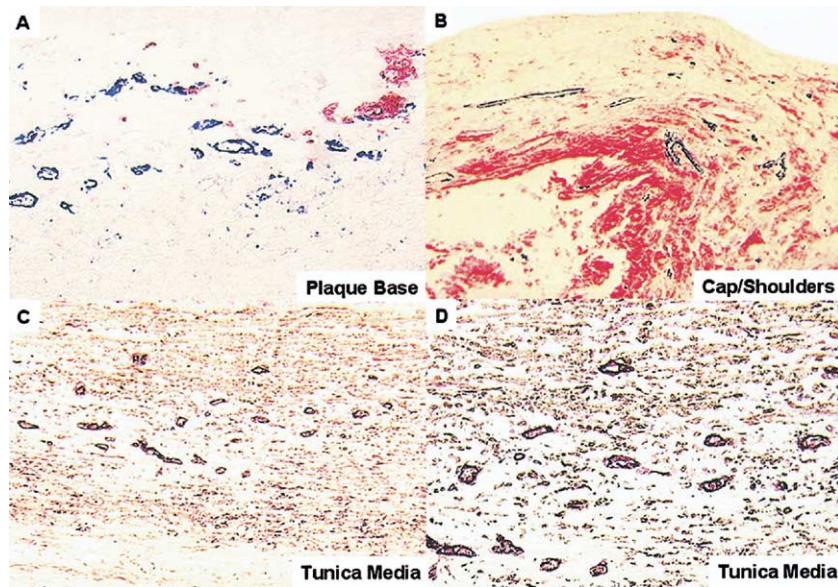


**Figure 9.** Coronary neovessels from adventitial vasa vasorum nurture the vessel wall through the first order (parallel) and the second order (perpendicular). Reproduced with permission from Kwon HM, *et al.* J Clin Invest 1998;101:1551-6.

## PLAQUE RUPTURE

Two mechanisms independently or in conjunction trigger plaque rupture. The first one is related to physical forces and occurs most frequently where the fibrous cap is thinnest,





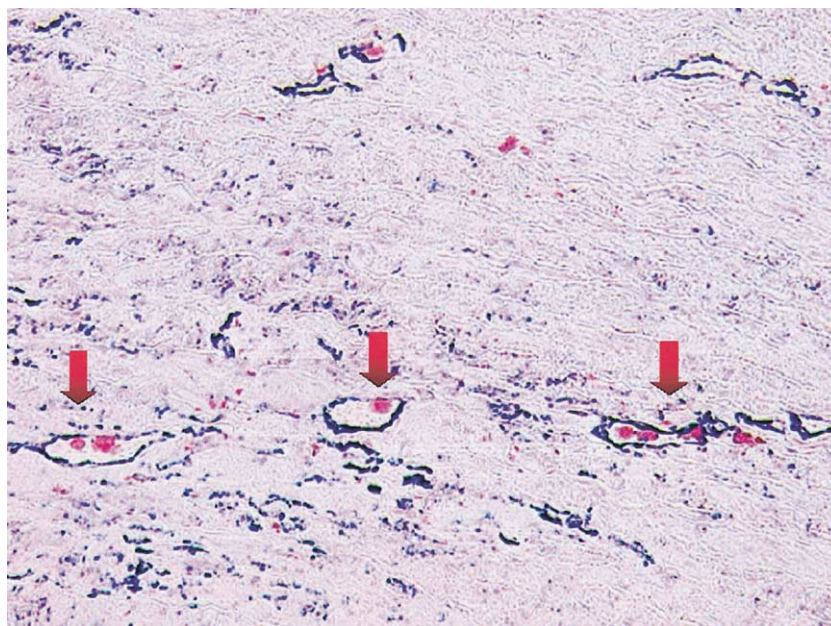
**Figure 10.** (A) High-power image of microvessels identified with the monoclonal endothelial cell marker CD34 linked to a blue chromogen. (B) High-power image from the plaque shoulder region showing CD34-positive microvessels in **blue** contrasting sharply with CD68/CD3-positive inflammatory cells linked to a **red** chromogen. (C) Microvessels at the tunica media (**purple** chromogen) contrasting with smooth muscle cells linked to a **brown** chromogen using alpha-actin marker. (D) Corresponding high power from C, showing disarray of smooth muscle cells in **brown** and microvessels in **purple**. Reproduced with permission from Moreno PR, et al. *Circulation* 2004;110:2032-8.

most heavily infiltrated by foam cells, and therefore weakest (Figs. 2 and 12).

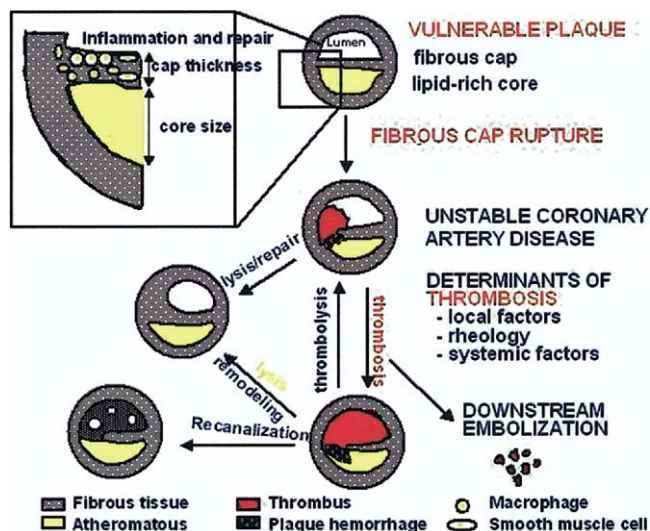
For eccentric plaques, this is often the shoulder or between the plaque and the adjacent vessel wall (114). Specifically, pathoanatomic examination and in vitro mechanical testing of isolated fibrous caps indicated that vulnerability to rupture depends on several factors (7,32,114), circumferential wall stress or cap fatigue; location, size, and consistency of the atheromatous core; and

blood flow characteristics, particularly the impact of flow on the proximal aspect of the plaque (i.e., configuration and angulation of the plaque).

The second mechanism involves an active process within the plaque leading to rupture. Atherectomy specimens from patients with ACS reveal areas very rich in macrophages (11) and mast cells (115). These cells are capable of degrading extracellular matrix by phagocytosis or secretion of proteolytic enzymes; thus, enzymes such as plasminogen



**Figure 11.** Microvessels as a pathway for macrophage entry/exit to atherosclerotic plaques. High-power image showing CD34-positive microvessels (**blue**) and intraluminal, monocyte-derived macrophages (**red**) circulating within the plaque, as highlighted by the **red arrows**. Courtesy of Dr. Purushothaman, Mount Sinai Hospital, 2005.



**Figure 12.** Plaque vulnerability, disruption, and thrombosis: anatomical changes leading to acute coronary syndrome and subsequent plaque remodeling. An element of vasoconstriction is usually present. Modified with permission from Theroux and Fuster (136).

activators and matrix metalloproteinases (MMPs), including collagenases, elastases, gelatinases, and stromelysins, by degrading the components of extracellular matrix, may weaken the fibrous cap and predispose it to rupture (116,117). In *in vitro* conditions, human monocyte-derived macrophages have been observed to degrade collagen of the fibrous cap while simultaneously expressing MMP-1 (interstitial collagenase) and inducing MMP-2 (gelatinolytic) activity in the culture medium—actions that can be prevented by MMP inhibitors (7,116). Certain MMPs observed in human coronary plaques and foam cells may be particularly active in destabilizing plaques (118,119). Furthermore, quantification of certain MMPs and their inhibitors in blood has been correlated with the degree of atherogenesis in humans (120).

The MMPs are also involved in several non-atherosclerotic processes within the heart (121–126). Most importantly, as previously mentioned, disruption of the IEL as a result of the adventitia/media infiltrated by monocytes, which release MMPs mostly at areas of neovascularization. This appears to contribute significantly to plaque rupture (19,88).

The continuing entry, survival, and replication of monocytes/macrophages within plaques are partly dependent on factors such as CAMs, MCP-1, and M-CSF (48,68,127–129). Cytokines regulate macrophage uptake of modified lipoprotein by way of scavenger receptors. Most importantly, interferon-gamma, tumor necrosis factor-alpha, and interleukin-1 activate macrophage apoptosis (68,130). Thus, macrophages, following what appears to be a defensive mission to protect the vessel wall from lipoprotein accumulation, may eventually undergo apoptotic death (68,70,131). This phenomenon leads to the shedding of membrane microparticles, causing exposure of phosphatidylserine on the cell surface, a major contributor for arterial thrombosis after plaque rupture (70,131). Recent work by

our group seems to indicate apoptosis as the common link between inflammation and thrombosis. Thus, Hutter et al. (132,133) have shown an excellent correlation between macrophage density, apoptosis markers, and tissue factor (TF) expression in human and mouse atherosclerotic lesions.

Other inflammatory cells found in intact and disrupted plaques include mast cells present in the shoulder regions but in fairly low densities (115). They can secrete powerful proteolytic enzymes such as trypsin and chymase that subsequently activate the proenzymatic form of MMPs. Finally, the role of neutrophils is less clear (18,117,134). They are rare in intact plaques, and it is likely that they enter shortly after rupture.

## THROMBOTIC COMPLICATIONS

**Acute coronary thrombosis.** Rupture of a high-risk vulnerable plaque changes plaque geometry and triggers coronary thrombosis (7). Such a rapid change in plaque geometry may result in acute occlusion or subocclusion with clinical manifestations of unstable angina or other ACS (135,136). More frequently, however, the rapid changes seem to result in mural thrombus without evident clinical

**Table 3.** The Virchow Triad of Thrombogenicity

Local vessel wall substrates
Atherosclerosis
Degree of plaque disruption (i.e., erosion, ulceration)
Vessel wall inflammation
Components of plaque (i.e., lipid core)
Macrophages and generation of microparticles (i.e., tissue factor content)
Post-interventional vessel wall injury
Plaque disruption after percutaneous transluminal coronary angioplasty, atherectomy, or stenting
Injury of smooth-muscle cells (i.e., rich in thrombin)
Rheology
High shear stress
Severe stenosis (i.e., change in geometry with plaque disruption, residual thrombus)
Vasoconstriction (i.e., serotonin, thromboxane A <sub>2</sub> , thrombin, dysfunctional endothelium)
Oscillatory shear stress
Bifurcation of arteries, plaque irregularities
Post-intervention slow blood flow/local stasis (i.e., dissecting aneurysm)
Systemic factors of the circulating blood
Metabolic or hormonal factors
Dyslipoproteinemia [triglycerides, increased low-density lipoprotein or oxidized low-density lipoprotein cholesterol, decreased high-density lipoprotein cholesterol, lipoprotein(a)]
Diabetes mellitus (i.e., glycosylation)
Catecholamines (i.e., smoking, stress, cocaine use)
Renin-angiotensin system (i.e., high-renin hypertension)
Plasma variables of hemostasis
Tissue factor, factor VII, factor VII, fibrinogen, thrombin generation (fragments 1 and 2), thrombin activity (fibrinopeptide A), plasminogen activator inhibitor-1, tissue plasminogen activator
Infectious (i.e., <i>Chlamydia pneumoniae</i> , cytomegalovirus, <i>Helicobacter pylori</i> ) and cellular blood elements (i.e., monocytes and white blood cells)



symptoms. Thrombus organization mediated by repaired collagen (type III) heals the rupture site, but increases plaque volume, contributing to the progression of atherothrombosis (135,136). More specifically, a number of factors—plaque-dependent thrombogenic substrate, rheology, and systemic procoagulant activity—may influence the magnitude and stability of the resulting thrombus and thus, the severity of the coronary syndrome (24,137), as shown in Table 3.

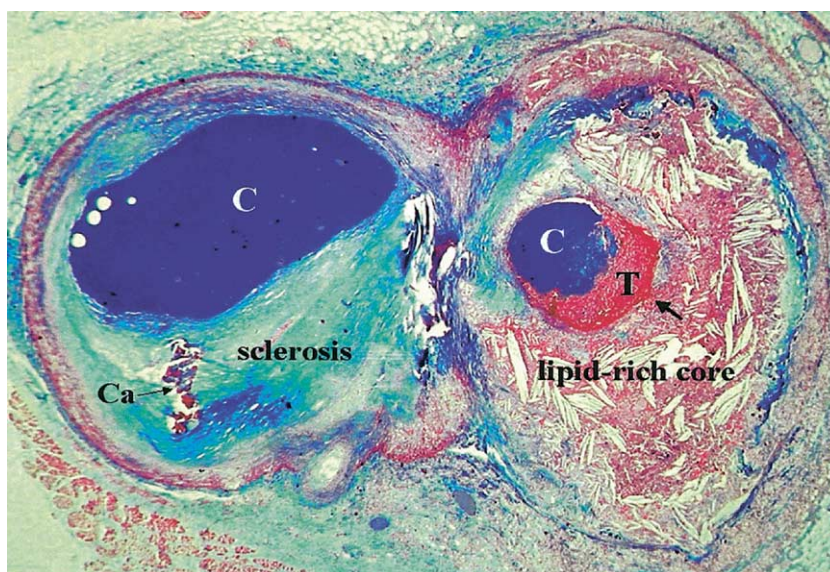
**PLAQUE-DEPENDENT THROMBOGENIC SUBSTRATE.** Exposure of a thrombogenic substrate is a key factor in determining thrombogenicity at the local arterial site (Table 3). Heterogeneity of plaque composition varies even within the same subject, as shown in Figure 13.

Data on the thrombogenicity of ruptured atherosclerotic lesions are limited. Using an original perfusion chamber, we exposed different types of human aortic plaques to flowing blood and their thrombogenicity was assessed. Lipid-rich plaques were by far the most thrombogenic of all, which explains why rupture of lipid-rich plaques is the most frequent cause of coronary thrombosis in ACS. In addition, thrombogenicity was modulated by TF content, mostly located in macrophage-rich areas. (138-140). Residual mural thrombus in itself was also highly thrombogenic, presumably as a result of monocyte/TF-related activation (141,142) with generation of thrombin (143,144).

Tissue factor, a small-molecular-weight glycoprotein, initiates the extrinsic clotting cascade and is believed to be a major regulator of coagulation, hemostasis, and thrombosis (145). Tissue factor forms a high-affinity complex with coagulation factors VII/VIII; TF/VIIa complex activates factors IX and X, which in turn leads to thrombin generation, as shown in Figure 14 (141,146).

Co-localization analysis of coronary atherectomy specimens from patients with unstable angina showed a strong relation between TF and macrophages (147). This relation suggests a cell-mediated thrombogenicity in patients with unstable angina and ACS. Furthermore, TF is particularly present in apoptotic macrophages, highlighting the role of local TF in ACS (70,131,148). In addition, specific inhibition of vascular TF by the use of r-tissue factor pathway inhibitor was associated with a significant reduction of acute thrombus formation in human lipid-rich plaques (149) and in pig injured plaques (150). Conversely, native tissue factor pathway inhibitor degradation after thrombolysis may enhance procoagulant activity at these sites of TF expression, thus contributing to early reocclusion after thrombolysis in myocardial infarction (151,152). Such observations document the active role of TF in coronary thrombosis and open a new therapeutic strategy in the prevention of ACS (153).

**RHEOLOGY AND THROMBOSIS.** The degree of stenosis caused by the ruptured plaque and the overlying mural thrombi are also key factors for determining thrombogenicity at the local arterial site (Table 3). Specifically, shear rate is directly related to flow velocity and inversely related to the third power of the lumen diameter. Thus, acute platelet deposition after plaque rupture is highly modulated by the degree of narrowing after rupture. Changes in geometry may increase platelet deposition, whereas sudden growth of thrombus at the injury site may create further stenosis and thrombotic occlusion. Most platelets are deposited at the apex of a stenosis, where the highest shear rate develops (154,155). Furthermore, mural thrombus formation may contribute to vasoconstriction originated from platelets—serotonin and thromboxane A<sub>2</sub> (156)—increasing shear force-dependent platelet deposition (135,157,158).



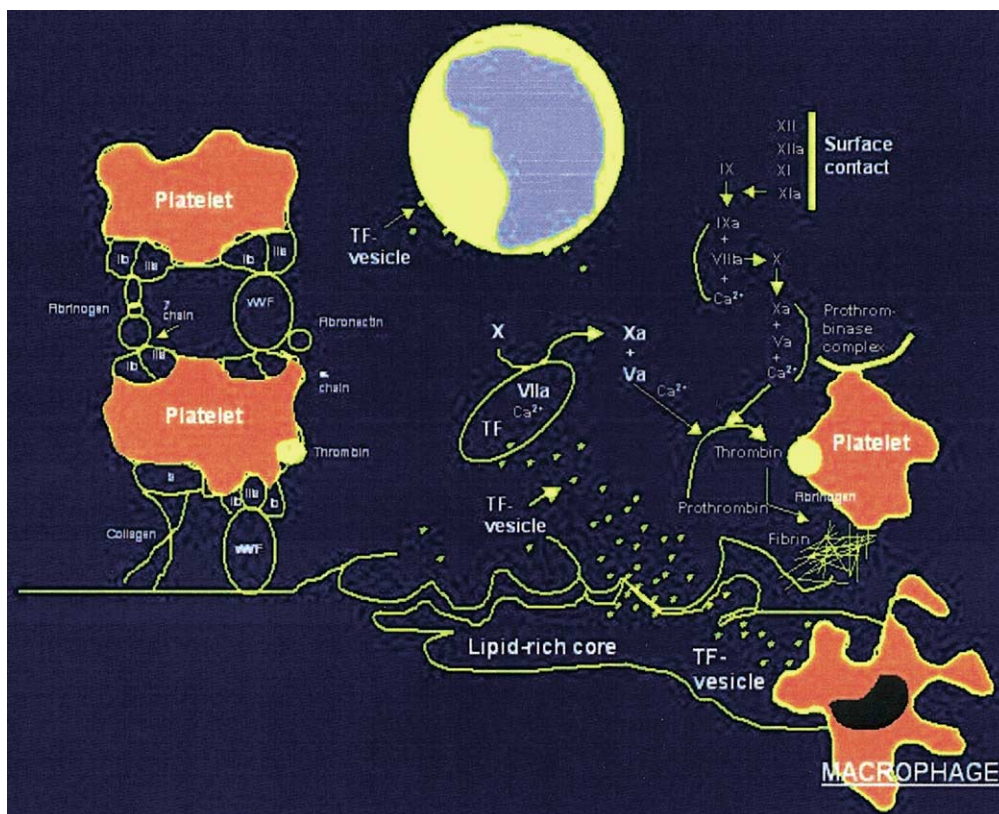
**Figure 13.** Atherothrombosis: a variable mix of chronic atherosclerosis and acute thrombosis. Cross-sectioned arterial bifurcation illustrating a collagen-rich (blue-stained) plaque in the circumflex branch (left) and a lipid-rich and ruptured plaque with a non-occlusive thrombosis superimposed in the obtuse branch (right). C = contrast in the lumen; Ca = calcification; T = thrombosis. Adapted from Falk E, Prediman S, Fuster V. Coronary plaque disruption. *Circulation* 1995;92:657-71.

**SYSTEMIC PROCOAGULANT ACTIVITY.** As previously discussed, 30% of coronary thrombosis occurs at sites of superficial erosion of a fibrotic plaque (Fig. 3) (24,25,32). Thus, complicated thrombi in such cases may well be dependent on a hyper-thrombotic state triggered by systemic factors (2). Two major pathways are deeply involved in systemic procoagulant activity: coronary risk factors and circulating tissue factor.

Changes in lipid metabolism, cigarette smoking, hyperglycemia, hemostasis, and others are associated with increased blood thrombogenicity (141,159-162) (Table 3). Elevated LDL cholesterol levels increase blood thrombogenicity and growth of thrombus under defined rheology conditions (163,164). Reducing LDL cholesterol levels using statins decreased thrombus growth by approximately 20% (164). Smoking increases catecholamine release, potentiating platelet activation (165) and increasing fibrinogen levels (166). Catecholamine-dependent effects may explain the increased incidence of sudden death and acute cardiovascular events after emotional and physical stress (141,167). Patients with diabetes, especially those with poorly controlled diabetes, have increased blood thrombogenicity (168-170). Platelets from patients with diabetes have increased reactivity and hyper-aggregability and expose a variety of activation-dependent adhesion proteins (169-171); such abnormal platelet function is reflected by increased platelet consumption and increased accumulation of

platelets on the altered vessel wall (171-173). Recent observations indicate that the thrombogenic state associated with high LDL cholesterol, cigarette smoking, and diabetes may share a common biological pathway. That is, an activation of leukocyte-platelet interactions associated with release of TF and thrombin activation has been observed in these conditions (141,170), being more particularly studied in the diabetic population (120-124). Furthermore, reversal of such risk factors may alter such cell-cell interactions, being particularly studied with the statins (174-176).

Recent studies showed increased levels of circulating TF antigen in patients with cardiovascular disease (177) and coagulation disorders, such as disseminated intravascular coagulation (178,179). Circulating TF antigen has been associated with increased blood thrombogenicity in patients with ACS (177,180) and chronic coronary artery disease (181). Furthermore, Increased TF-positive procoagulant microparticles are present in the circulating blood of patients under pathophysiologic conditions (182). Thus far, the cellular origin of TF-positive microparticles in the circulating blood has not been established. As described, atherosclerotic plaques have been shown to contain TF that is associated with macrophages within the lesion (147). High levels of shed apoptotic microparticles are found in extracts from atherosclerotic plaques (70,131). These microparticles with increased TF activity seem to be of monocytic origin, suggesting a causal relationship between



**Figure 14.** Interactions between platelet activation, tissue factor (TF) vesicle expression from plaque macrophages, and activation of the coagulation cascade.  $Ca^{2+}$  = calcium; vWF = von Willebrand factor.



shed membrane microparticles and procoagulant activity of plaque extracts. In addition, TF has also been identified within thrombi formed in coronaries (140,147). Immunoelectron microscopy showed TF in thrombi within 5 min of formation, mainly localized on membrane vesicles attached to platelets and fibrin strands (183,184). Neutrophils and monocytes have been isolated from the circulating blood using anti-TF antibodies (183). Thus, aside from apoptotic macrophages and microparticles from atherosclerotic plaques, activated monocytes in the circulating blood seem to be a source of TF microparticles and may represent the result of the activation by the aforementioned risk factors and others, so contributing to thrombotic events (140,141), as shown in Figure 14. Indeed, the predictive value of C-reactive protein (CRP) and CD40L may in part be a manifestation of such systemic phenomena; CRP, like fibrinogen, is a protein of the acute-phase response and a sensitive marker of low-grade inflammation. It is produced in the liver as a result of mediators such as interleukin-6 generated by inflammation in the vessel wall (i.e., macrophages) or extravascularly (i.e., circulating monocytes) (185). Increased levels of CRP have been reported to independently predict acute coronary events (186) even in people whose blood lipid values are below the median levels in the population (187,188). Furthermore, statin therapy prevented coronary events in individuals with high CRP and relatively normal LDL cholesterol values (187). Of interest, the lowering effect of statin on CRP values was independent of its effect on lipid levels. Whether CRP reflects the inflammatory component of atherosclerotic plaques or of the circulating blood and whether it is a surrogate marker or a biologically active element in the process of plaque development or thrombus formation is not known (185,189). However, recent studies support that CRP is an activator of blood monocyte and vessel wall endothelial cells (189–192). This encourages further investigation into the effect of certain risk factors in the activation of inflammation of the vessel wall and circulating blood, probably leading to an active role of TF, CRP, and perhaps CD40 (193,194) as local and systemic key factors in the process of atherothrombosis.

**Acute thrombosis and emboli of non-coronary arteries (Table 4).** Thrombosis and thromboemboli originated in carotid plaques are frequently the result of rupture or dissection of a heterogenous plaque, presumably as a result

of the impact of the systemic high-energy blood flow against the resistance offered by the plaque (195,196). Intra-plaque hemorrhage caused by the rupture of vasa vasorum may play a significant role. Plaque rupture with exposure of lipid-rich material has also been documented as a common form of stroke (197–202). Thrombosis and thromboemboli from the thoracic aorta is also a consequence of plaque rupture (114,143,144), probably related to mechanisms similar to those described in about two-thirds of acute coronary thrombosis (114). Thrombosis of the peripheral arteries is most frequently observed in the surface of stenotic and fibrotic plaques, as described in about one-third of acute coronary thrombosis (203,204). Peripheral atherothrombosis is predominantly the consequence of a thrombogenic systemic blood associated with certain risk factors described previously (i.e., smoking, diabetes, hyperlipidemia) (203,205–207). Finally, acute occlusion of the peripheral vasculature frequently results from thromboemboli of cardiac or abdominal aortic origin (203,206,207).

## CONCLUSIONS

Atherothrombosis is a complex disease in which cholesterol deposition, inflammation, and thrombus formation play a major role. High-risk, vulnerable plaque is responsible for acute coronary thrombosis, leading to clinical manifestations of unstable angina, acute myocardial infarction, and sudden cardiac death. Plaque rupture is the most common trigger of thrombosis. However, plaque erosion also plays a significant role. Atherothrombosis can be classified according to histologic criteria, most commonly known as the AHA classification. However, this classification does not include plaque erosion or the thin-cap fibroatheroma. As a result, new classifications have emerged. The disease is asymptomatic during a long period, and dramatically changes its course when complicated by thrombosis. This is summarized in five phases, from early lesions to plaque rupture, thrombosis and plaque healing, followed by fibrocalcification. Recent studies have documented increased neovascularization and intra-plaque hemorrhage in complex atherothrombosis. Tissue factor, the most potent trigger of the coagulation cascade, seems to be critical for plaque thrombogenicity. Circulating tissue factor microparticles seem also associated with circulating monocytes, closing the link between inflammation, plaque rupture, and thrombogenicity.

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**Table 4.** Atherothrombosis—Complicated Lesions

Location	Suggested Predominant Mechanisms		
	Plaque Rupture		Blood Thrombogenicity
	Lipid Rich	Non-Lipid Rich	
Coronaries	+	±	+
Carotids	±	+	–
Thoracic aorta	+	–	–
Peripheral	–	–	+

+ = predominant; ± = non-predominant; – = no mechanism.

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