Atherosclerosis and its clinical consequences are the leading cause of death in Western nations [1]. Several factors have been implicated in the evolution, progression, and destabilization of atherosclerotic plaque highlighting its multifaceted nature. Atherosclerosis, now considered a chronic inflammatory disease, begins at a young age and progresses slowly for decades [2–4]. The clinical symptoms of atheroma occur in adults and usually involve plaque rupture and thrombosis [5–7].

While several advances have helped curb some of the complications resulting from atherosclerosis, this disease still represents an ongoing challenge with several new insights raising optimism that help to improve clinical outcomes is at hand. This chapter reviews the pathogenesis of atherosclerosis and the inflammatory cascades leading to plaque progression and destabilization. New coronary imaging modalities and developments in computer modeling are critiqued as tools to help improve the understanding of cardiovascular diseases.

Pathogenesis of atherosclerosis
Atherosclerosis is an inflammatory fibro-proliferative process in which plaque forms in the intima, bringing about stenosis or thrombosis and hence ischemia [8–10]. Though the exact initiator of plaque formation remains unknown, there is a general consensus that the triggering episode is endothelial damage, which could be caused by factors such as cigarette smoke toxins, hypertension, or immune injury [4,11–15]. Damaged cells become more permeable, ultimately causing subendothelial macrophages to consume circulating low density lipoproteins (LDL) which are altered in the intima to induce further endothelial damage [8,9,16]. More macrophages are then recruited, after which they remain in the intima as lipid-rich foam cells [9,10,17–19]. Meanwhile, in an attempt to restore endothelial function, smooth muscle cells migrate from the media to the intima to proliferate and generate a connective tissue matrix to cap the lipid core, further thickening the lesion [8,19,20]. Plaques enlarge as the process becomes chronic, classified as stable or unstable (Figures 1.1 and 1.2), either of which can lead to clinical sequelae [8,17,21].

Clinical features
The first indication of coronary artery disease (CAD) may be sudden death, or patients can present with silent ischemia, stable angina, or an acute coronary syndrome (ACS) [22]. ACSs comprise a range of syndromes resulting from atherosclerotic plaque disruption or rupture and are divided into unstable angina (UA), non-ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI) [21,23,24]. An unstable plaque, characterized by a large lipid core covered by a thin and unstable fibrous cap, is prone to rupture [21,25–27]. The sudden rupture can cause thrombus formation, in turn leading to ACS (Figure 1.2) [26,28,29]. Conversely, a stable plaque has a thick fibrous cap which is not easily ruptured (Figure 1.1), causing the chronic condition of stable angina through episodes of ischemia experienced upon physical exertion [25,27,30].

Consequences of atherosclerosis
The risk of major thrombotic and thromboembolic complications of atherosclerosis appears to be related more to the stability of atheromatous plaques than to the extent of disease [31,32]. Stable angina is associated with smooth fibrous coronary artery plaques (stable plaque), whereas unstable angina, acute myocardial infarction (AMI), and sudden cardiac death are almost invariably associated with destabilization of plaques [29]. Similarly, in patients with carotid artery atherosclerotic disease, plaque irregularity and rupture are closely associated with cerebral ischemic events, and patients with irregular or ulcerated plaque demonstrate a higher risk of ischemic stroke irrespective of the degree of luminal stenosis [33].

Much attention has been placed on trying to identify plaques at high risk of disruption leading to thrombosis. Such “vulnerable plaques” have also been areas of intense research using novel intracoronary imaging modalities: optical coherence tomography (OCT) [6,29]. OCT offers the advantages over intravascular ultrasound or angiography of ultra-high resolution and superiority in imaging the vessel wall and lumen interface [34–36].
PART I Principles and Techniques
SECTION I Basic Knowledge

Figure 1.1 Stable atherosclerotic plaque characterized by the presence of a low inflammatory infiltrate. This type of lesion is constituted by a lipid core (extracellular lipid, cholesterol crystals, and necrotic debris) covered by a thick fibrous cap consisting principally of smooth muscle cells (SMC) in a collagenous–proteoglycan matrix, with varying degrees of infiltration by macrophages and T lymphocytes. HDL, high density lipoprotein.

Figure 1.2 Unstable atherosclerotic plaque characterized by the presence of a thin fibrous cap rich in inflammatory macrophagic foam cells and T lymphocytes. Rupture of the fibrous cap at the shoulder region has resulted in thrombus formation.
Considerable data exist to sustain the hypothesis that several morphologic and molecular markers identifying unstable plaques could be expressed during plaque vulnerability. As shown by a number of anatomical and clinical studies, these vulnerable plaques are more often associated with rupture and thrombosis than stable plaques covered by a thin fibrous cap and show an extensive inflammatory infiltrate [28,37].

Unlike the stable plaque that shows a chronic inflammatory infiltrate, the vulnerable and ruptured plaque is characterized by features of acute inflammation [37,38]. There are a large number of studies showing that “active” inflammation mainly involves T lymphocytes and macrophages which are activated toward a pathway of inflammatory response, secrete cytokines and lytic enzymes which in turn cause thinning of the fibrous cap, predisposing to plaque rupture. Recent research has furnished new insight into the molecular mechanisms that cause transition from a stable to an unstable phase of atherosclerosis and points to inflammation as the playmaker in the events leading to plaque destabilization and suggest that alterations in shear stress may also play a pivotal part [39,40].

A current challenge is to identify morphological and molecular markers able to discriminate stable plaques from vulnerable ones allowing the stratification of “high risk” patients for acute cardiac and cerebrovascular events before clinical syndromes develop. Bearing this aim in mind, this chapter focuses on cellular and molecular mechanisms affecting plaque progression and serum markers correlated to plaque inflammation.

**Insights from coronary imaging**

Traditionally, coronary angiography has been the gold standard to detect extent and severity of CAD. These findings form the foundation of the interventionist’s clinical decision-making process and whether to proceed to percutaneous therapy. It is widely acknowledged, however, that angiography has several limitations. First, it maintains a relatively low image resolution. Second, it represents a luminogram of the artery and stenosis. Therefore little detail is provided as to the composition of the underlying plaque causing the stenosis and, finally, it is a 2D imaging method used to assess what are complex 3D structures.

**Intravascular ultrasound**

Intravascular ultrasound (IVUS) utilizes ultrasound waves that reflect off vascular tissues to yield real-time images [41,42]. While angiography only portrays a luminal silhouette [41], IVUS, with a resolution of 100–150 μm, captures details not retrievable with angiography—cross-sections of the lumen and vessel wall, even a differentiation of its layers [43–46]. Thus, IVUS enables study of the atherosclerotic process through the visualization of plaque in the vessel wall [41,47–49]. Indeed, the technology has demonstrated a greater prevalence of atherosclerosis than initially claimed with angiography [44].

**Optical coherence tomography**

OCT, the optical analog of IVUS, employs the reflection of near-infrared (NIR) light instead of sound. Initially applied in ophthalmology, advancement in the technology has now enabled OCT to capture non-transparent tissues such as coronary vessels [50,51]. OCT offers real-time, in vivo and in situ cross-sectional imaging of vascular structures with a resolution 10-fold that of IVUS (15 μm versus 150 μm) and a penetration depth similar to that of histology [34,43,50–53].

By virtue of its superior resolution, OCT can provide near-histological analysis of atherosclerotic plaques in real time (Figure 1.3). OCT definition of thin cap fibroatheroma (TCFA) follows the findings of autopsy studies of sudden death patients that had revealed the presence of fibrous caps <65 μm in the majority of plaques that had ruptured. These thin ruptured caps were also found to have an infiltrate of macrophages [54]. Whereas OCT is well placed in precisely defining the thinness of fibrous cap, macrophage infiltration seen as punctate, signal-rich spots at the junction of fibrous cap and lipid pool has been described less consistently. Previous autopsy studies had also shown that plaque rupture, erosion, and calcified nodules were the three leading underlying mechanisms for luminal thrombosis with a frequency of 65%, 30%, and 5%, respectively [25]. In recent years OCT has enabled this type of information to be obtained in vivo and has confirmed similar prevalence of plaque morphologies in patients presenting with STEMI and NSTEMI [55].

Plaque rupture on OCT is identified by a clear-cut disruption in the signal-rich thin fibrous cap overlying a signal-poor necrotic core resulting in extrusion of highly thrombogenic material into the lumen. Plaque erosion on the other hand is identified by the presence of luminal thrombus adjacent to a plaque that has an irregular but intact, thicker fibrous cap. Such plaques are mostly devoid of necrotic core. Calcified nodules are the least common etiology in ACS and are less well defined. They are recognized by sharp nodules protruding into the lumen causing discontinuation of the fibrous cap (Figure 1.4).

In patients with stable CAD, coronary imaging can provide lesion level information and help to show the changes in plaque microstructure in response to pharmacotherapies. Kataoka et al. [56] evaluated 293 and 122 lipid and fibrous plaques in 280 stable statin-treated patients with CAD and reported that patients with LDL-C levels <50 mg/dL were less likely to have lipid plaques, and had more features of plaque stability such as thicker fibrous caps and smaller lipid arcs.

**The vulnerable plaque**

Atherosclerotic lesions, according to the classification of the American Heart Association modified by Virmani et al. [29], are divided in two groups: (i) non-atherosclerotic intimal lesions and (ii) progressive atherosclerotic lesions which include stable, vulnerable, and thrombotic plaques.

The different pathologic characterization of atherosclerotic lesions largely depends on the thickness of the fibrous cap and its grade of inflammatory infiltrate, which is in turn largely constituted by macrophages and activated T lymphocytes. Typically, the accumulating plaque burden is initially accommodated by an adaptive positive remodeling with expansion of the vessel external elastic lamina and minimal changes in lumen size [57,58]. The plaque contains monocyte-derived macrophages, smooth muscle cells, and T lymphocytes. Interaction between these cells types and the connective tissue appears to determine the development and progression of the plaque itself, including important complications such as thrombosis and rupture.

The lesions classified as vulnerable or TCFA identify a plaque prone to rupture and thrombosis characterized by a large necrotic core containing numerous cholesterol clefts. The overlying cap is thin and rich in inflammatory cells, macrophages, and T lymphocytes with few smooth muscle cells [28,29,59]. Burke et al. [54] identified a cut-off value for cap thickness of 65 μm to define a vulnerable coronary plaque. Despite the predominant hypothesis...
focusing on the responsibility of a specific vulnerable atherosclero
tic plaque rupture [5,7] for acute coronary syndromes, some
pathophysiologic, clinical, and angiographic observations seem to
suggest the possibility that the principal cause of coronary instabil
ity is not to be found in the vulnerability of a single atherosclerotic
plaque, but in the presence of multiple vulnerable plaques in the
entire coronary tree, correlated with the presence of a diffuse
inflammatory process [37,38,60,61].

Within this context, recent angiographic studies have demo
strated the presence of multiple vulnerable atheromatous plaques in
patients with unstable angina [20,62] and in those affected by trans
mural myocardial infarction [61]. Recently, by means of flow

Figure 1.3 Stable coronary plaques seen on optical coherence tomography (OCT). (a) Calcified plaque is seen at 7- to 10 o’clock position. It is
classified by sharply delineated borders and heterogeneous core. (b) Calcified plaque is outlined with white dotted line. (c) Lipid-rich plaque
marked by white lines. It is characterized by dark, signal-poor core with ill-defined margins and a bright thick fibrous cap (>65 μm). As the light
rapidly attenuates through the necrotic core, OCT cannot be used to measure the depth of such plaques.

Figure 1.4 Unstable coronary plaques seen on OCT. (a) Plaque erosion: intact fibrous cap with irregular luminal surface and superficial calcium.
(b) Plaque rupture with luminal thrombus. At the 11 o’clock position a thin cap fibro-atheroma (TCFA) is seen (fibrous cap thickness measured
40 μm, marked with small white bar).
cytometry Spagnoli et al. [38] have demonstrated the presence of an activated and multicentric inflammatory infiltrate in the coronary vessels of individuals who died of AMI. Similar results have been obtained by Buffon et al. [60], who, through the determination of the neutrophil myeloperoxidase activity, have proved the presence of a diffuse inflammation in the coronary vessels in individuals affected by unstable angina. These results have been confirmed by a morphological study which demonstrated the presence of a high inflammatory infiltrate constituted by macrophages and T lymphocytes activated in the whole coronary tree, also present in the stable plaques of individuals who died of AMI. These plaques showed a two- to fourfold higher inflammatory infiltrate than aged-matched individuals dying from non-cardiac causes with chronic stable angina (SA) or without clinical cardiac history (CTRL), respectively [37]. Moreover, it has also been demonstrated that activated T lymphocytes infiltrate the myocardium both in the peri-infarcted area and in remote unaffected myocardial regions in patients who died of a first myocardial infarction [63].

The simultaneous occurrence of diffuse coronary and myocardial inflammation in these patients further supports the concept that both coronary and myocardial vulnerabilities concur in the pathogenesis of fatal AMI.

AMI—at least associated with unfavorable prognosis—is therefore likely to be the consequence of a diffuse “active” chronic inflammatory process which determines the destabilization of both the entire coronary tree and the whole myocardium, not only the part of it affected by infarction. The causes of the diffuse inflammation associated with myocardial infarction are scarcely known. The presence of activated T lymphocytes suggests the “in situ” presence of an antigenic stimulus which triggers adaptive immunity.

**Role of inflammation in the natural history of atherosclerosis**

**Inception of the plaque**

Endothelial injury has been proposed to be an early and clinically relevant pathophysiologic event in the atherosclerotic process [4,32]. Patients with endothelial dysfunction have an increased risk for future cardiovascular events including stroke [64]. Endothelial dysfunction was described as the ignition step in atherogenesis. From this point on, an inflammatory response leads to the development of the plaque.

Endothelial damage can be caused by physical and chemical forces, by infective agents or by oxidized LDL (ox-LDL). Dysfunctional endothelium expresses P-selectin (stimulation by agonists such as thrombin) and E-selectin (induced by IL-1 or TNF-α). Expression of intercellular adhesion molecule-1 (ICAM-1) by both macrophages and endothelium and vascular adhesion molecule-1 (VCAM-1) by endothelial cells is induced by inflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-1 (TNF-α), and γ-interferon (IFNγ).

Monocytes recalled in the subintimal space ingest lipoproteins and morph into macrophages. These generate reactive oxygen species (ROS), which convert ox-LDL into highly oxidized LDL. Macrophages upload ox-LDL via scavenger receptors until foam cells form. Foam cells with leukocytes migrate at the site of damage and generate the fatty streak. The loss of biologic activity of endothelium determines nitric oxide (NO) reduction together with increased expression of prothrombotic factors, proinflammatory adhesion molecules cytokines, and chemotactic factors. Cytokines may decrease NO bioavailability increasing the production of ROS. ROS reduces NO activity both directly, reacting with endothelial cells, and indirectly via oxidative modification of eNOS or guanyl cyclase [65]. Low NO bioavailability can upregulate VCAM-1 in the endothelial cell layer that binds monocytes and lymphocytes in the first step of invasion of the vascular wall, via induction of nuclear factor κB (NFκB) expression [66]. In addition, NO inhibits leukocyte adhesion [67] and NO reduction results in induction of monocyte chemotactic protein-1 (MCP-1) expression which recruits monocytes [68]. NO is in a sensitive balance with endothelin-1 (ET-1) regulating vascular tone [69]. Plasma ET-1 concentrations are increased in patients with advanced atherosclerosis and correlate with the severity of the disease [70,71]. In addition to its vasoconstrictor activity, ET-1 also promotes leukocyte adhesion [72] and thrombus formation [73]. Dysfunctional endothelium expresses P-selectin (stimulation by agonists such as trombin) and E-selectin (induced by IL-1 or TNF-α) [74]. The expression of both ICAM-1 by macrophages and endothelium, and VCAM-1 by endothelial cells is induced by inflammatory cytokines such as IL-1, TNF-α, and IFNγ. Endothelial cells also produce monocyte chemotactic protein-1 (MCP-1), monocyte colony-stimulating factor, and IL-6 which further amplify the inflammatory cascade [75]. IL-6 production by smooth muscle cells represents the main stimulus for C-reactive protein (CRP) production [3]. Recent evidence suggests that CRP may contribute to the proinflammatory state of the plaque both mediating recruitment of monocytes and stimulating monocytes to release IL-1, IL-6, and TNF-α [76]. The damaged endothelium allows the passage of lipids into the subendothelial space. Fatty streaks represent the first step in the atherosclerotic process.

**Evolving fibro-atheromatous plaque**

The atheroma evolution is modulated by innate and adaptive immune responses [3,77,78]. The most important receptors for innate immunity in atherothrombosis are the scavenger receptors and the toll-like receptors (TLRs) [79]. Adaptive immunity is much more specific than innate immunity but may take several days or even weeks to become fully mobilized. It involves an organized immune response leading to generation of T- and B-cell receptors and immunoglobulins, which can recognize foreign antigens [80].

**Stable plaque**

Macrophages take up lipid deposited in the intima via a number of receptors, including scavenger receptor-A, and CD36. Deregulated uptake of modified LDL through scavenger receptors leads to cholesterol accumulation and “foam cell” formation. The lipid laden macrophages (foam cells) forming the fatty streak secrete proinflammatory cytokines that amplify the local inflammatory response in the lesion, matrix metalloproteinasises (MMPs), tissue factor into the local matrix, as well as growth factors, which stimulate the smooth muscle replication responsible for lesion growth. Macrophages colony-stimulating factor (M-CSF) acts as the main stimulator in this process, next to granulocyte-macrophage stimulating factor (MGGM-CSF) and IL-2 for lymphocytes [81]. Lymphocytes enter the intima by binding adhesion molecules: VCAM-1, P-selectin, ICAM-1, MCP-1 (CCL2), IL-8 (CXCL8) [75]. Such infiltrate constituted mainly by CD4+ T lymphocytes recognize antigens bound to MHC class II molecules involved in antigen presentation to T lymphocytes thus provoking an immune response [2]. The major histocompatibility complex molecules (MHC II) are expressed by endothelial cells, macrophages, and vascular smooth muscle cells in proximity to activated T lymphocytes in the atherosclerotic plaque. Proinflammatory cytokines manage a central transcriptional control
point mainly mediated by NFκB. Macrophage/foam cells produce cytokines that activate neighboring smooth-muscle cells (SMCs), resulting in extracellular matrix production [2].

Repeated inflammatory stimuli induce foam cells to secrete growth factors that induce proliferation and migration of SMCs into the intima. The continuous influx of cells in the subintimal space convert the fatty streak in a more complex and advanced lesion in which inflammatory cells (monocytes/macrophages, lymphocytes), SMCs, necrotic debris mainly resulting from cell death, ox-LDL elicit a chronic inflammatory response by adaptive immune system. SMCs form a thick fibrous cap that cover the necrotic core and avoid the exposition of thrombogenic material to the bloodstream. The volume of lesion grows and protrudes into the arterial lumen causing variable degrees of lumen stenosis. These lesions are advanced complicated “stable” atherosclerotic lesions, asymptomatic and often unrecognized [82,83].

Vulnerable plaque: a shift toward Th1 pattern
Early phases of the plaque development are characterized by an acute innate immune response against exogenous (infectious) and endogenous non-infectious stimuli. Specific antigens activate adaptive immune system leading to proliferation of T and B cells. A first burst of activation might occur in regional lymph nodes by dendritic cells (DCs) trafficking from the plaque to the lymph node. Subsequent cycles of activation can be sustained by interaction of activated/memory T cells re-entering in the plaque by selective binding to endothelial cell surface adhesion molecules with plaque macrophages expressing MHC class II molecules. In this phase of the atherogenic process the selective recruitment of a specific subtype of CD4+ cells play a major part in determining the future development of the lesion. Two subtypes of CD4+ cells have a juxtaposed role: Th1 and Th2 cells [84].

Th1 cells secreting proinflammatory cytokines, such as IFNγ, promote macrophage activation, inflammation, and atherosclerosis, whereas Th2 cells (cytokine pattern IL-4, IL-5, and IL-10) mediate antibody production and generally have anti-inflammatory and antiatherogenic effects [64]. Therefore the switch to a selective recruitment of Th1 lymphocyte represents a key point toward plaque vulnerability and disruption. T cells in the plaque may encounter antigens such as ox-LDL. Moreover, T-cell response can be triggered by heat shock proteins of endogenous or microbial origin [85]. It is still unknown why the initial inflammatory response becomes a chronic inflammatory condition. However, when the plaque microenvironment triggers the selective recruitment and activation of Th1 cells they in turn determine a potent inflammatory cascade.

The combination of IFNγ and TNF-α upregulates the expression of fractalkine (CX3CL1) [86]. IL-1 and TNF-α-activated endothelium express also fractalkine (membrane bound form) which directly mediates the capture and adhesion of CX3CR1 expressing leukocytes providing a further pathway for leukocyte activation [87]. This cytokine network promotes the development of the Th1 pathway which is strongly proinflammatory and induces macrophage activation, superoxide production, and protease activity.

Role of inflammation as vulnerability factor
Homeostasis of plaque “microenvironment” (i.e., the balance between cell migration and cell proliferation, extracellular matrix production and degradation, macrophages and lymphocytes interplay) appears strictly related to the transition of a stable plaque into a vulnerable one. A limited number of T cells, following the Th1 pathway, initiates the production of large amounts of molecules downstream in the cytokine cascade orchestrating the transition from the stable to unstable plaque [77,88].

Within the plaque, inflammatory cells such as foam cells and monocyte-derived macrophages are induced to produce matrix-degrading enzymes, cytokines, and growth factors strictly implicated in extracellular matrix homeostasis. In particular, cytokines such as INFγ suppress collagen synthesis, a major component of the fibrous cap [75]. Moreover, infiltration of mononuclear cells results in release of proteases which causes plaque disruption [89]. The production of ROS within the atherosclerotic plaque has important implications for its structural integrity [65]. Deregulated oxidant production has the potential to promote the elaboration and activation of matrix degrading enzymes in the fibrous cap of the plaque. Moreover, impaired NO function coupled with oxidative excess can activate MMPs [90], namely MMP-2 and MMP-9, which weaken the fibrous cap. Another mechanism that can determine the thinning of the fibrous cap is the apoptosis of smooth muscle cells. In fact, there is evidence for extensive apoptosis of SMCs within the cap of advanced atherosclerosis, as well as those cultured from plaques [32,91].

A very important role, not yet well studied, is that of dendritic cells, namely cells specialized in antigen presentation with a key role in the induction of primary immune response and in the regulation of T-lymphocyte differentiation, as well as in mechanisms of central and peripheral tolerance aiming at the elimination of T lymphocytes that are potentially self-reactive toward self-antigens [92,93]. A characteristic of dendritic cells is also the ability to polarize T-cell responses toward a T-helper phenotype (Th1) in response to bacterial antigens. Molecules expressed by activated T lymphocytes, like CD40L, OX40, stimulate the release from dendritic cells of chemokines (fractalkines) able to attract other lymphocytes toward the inflammation site, amplifying the immune response [94].

Patients with ACS are characterized by the expansion of an unusual subset of T cells, CD4+CD28null T cells, with functional activities that predispose for vascular injury [95,96]. CD4+CD28null T cells are a population of lymphocytes rarely found in healthy individuals. Disease-associated expansions of these cells have been reported in inflammatory disorders such as rheumatoid arthritis. CD4+CD28null T cells are characterized by their ability to produce high amounts of IFNγ [96]. Equally importantly, CD4+CD28null T cells have been distinguished from classic Th cells by virtue of their ability to function as cytotoxic effector cells. Possible targets in the plaque are SMCs and endothelial cells, as recently shown [97]. In vivo, CD4+CD28null cells have a tendency to proliferate with the frequent emergence of oligoclonality, raising the possibility of continuous antigenic stimulation, as it is the case in certain autoimmune disorders and in chronic infections. The demonstration of oligoclonality within the CD4+CD28null T-cell subsets and sharing of T-cell receptor sequences in expanded T-cell clones of patients with ACS strongly support the notion that these cells have expanded and are activated in response to a common antigenic challenge [98]. CD4+CD28null T cells are long-lived cells. Clonality and longevity of these cells are associated with defects in apoptotic pathways [99]. Moreover, CD28 is relevant for the expansion of naïve T cells, thus the absence of this molecule contributes to the senescence of lymphocytes. The excessive expansion of a pool of senescent T lymphocytes might compromise the efficacy of the immune responses direct against exogenous antigens as well as determinate autoimmune responses.
Recently, a subpopulation of T CD4+ cells, expressing IL-2 receptor, CD25 membrane marker, has been pointed out. Such lymphocytes represent 7–10% of T CD4+ cells and their homeostasis is due to some co-stimulatory molecules, such as CD28 receptor expressed by T cells and B7 molecules expressed by dendritic cells [100]. The current knowledge of the role of this specific subset of T cells in human atherogenesis is still incomplete, even though a very recent study carried out on mice has demonstrated an antiatherogenic effect of T CD4+CD25+ cells [101].

Th1 cells and T regulatory 1 cells have been demonstrated to play opposite roles in rupture of atherosclerotic lesion. The role of novel subset of T regulatory cells, known as CD4+CD25−Foxp3 T cells, has been recently studied in CAD. Han et al. [102] found that the reduction of CD4+CD25−Foxp3 T lymphocytes was consistent with the expansion of Th1 cells in patients with unstable CAD. The reversed development between CD4+CD25+ Tregs and Th1 cells might contribute to plaque destabilization.

### Serum markers correlated to plaque inflammation

In recent years, a number of studies have correlated different serologic biomarkers with cardiovascular disease [4,103] leading to a rapid increase in the number of biomarkers available (Table 1.1). These biomarkers are useful in that they can identify a population at risk of an acute ischemic event and detect the presence of so-called vulnerable plaques and/or vulnerable patients [104,105]. Ideally, a biomarker must have certain characteristics to be a potential predictor of incident or prevalent vascular disease. Measurements have to be reproducible in multiple independent samples, the method for determination should be standardized, variability controlled, and the sensitivity and specificity should be good. In addition, the biomarker should be independent from other established risk markers, substantively improve the prediction of risk with established risk factors, be associated with cardiovascular events in multiple population cohorts and clinical trials, and the cost of the assays has to be acceptable. Finally, to be clinically useful a biomarker should correctly reflect the underlying biological process associated with plaque burden and progression.

Traditional biomarkers for cardiovascular risk include LDL cholesterol and glucose. However, 50% of heart attacks and strokes occur in individuals who have normal LDL cholesterol, and 20% of major adverse events occur in patients with no accepted risk factors [106]. Therefore, in light of changing atherosclerotic models, vulnerable blood may be better described as blood that has an increased level of activity of plasma determinants of plaque progression and rupture.

In this context, proposed biomarkers fall into nine general categories: inflammatory markers, markers for oxidative stress, markers of plaque erosion and thrombosis, lipid-associated markers, markers of endothelial dysfunction, metabolic markers, markers of neovascularization, and genetic markers. The last six biomarker categories are not treated in this chapter but only listed in Table 1.1. Some of these markers may indeed reflect the natural history of atherosclerotic plaque growth and may not be directly related to an increased risk of cardiovascular events. On the contrary, other markers are more related to complex plaque morphological features and may reflect an active process within the plaque which is in turn related to the onset of local complications and onset of acute clinical events.

However, it is important to emphasize that, in any individual patient, it is not yet clear how these biomarkers relate to quantitative risk of major adverse cardiovascular events. The best outcomes may be achieved by a panel of markers that will capture all of the different processes involved in plaque progression and plaque rupture, and that will enable clinicians to quantify an individual patient’s true cardiovascular risk. In all likelihood, a combination of genetic (representing heredit) and serum markers (representing the net interaction between heredit and environment) will ultimately be the ones that should be utilized in primary prevention. Finally, different non-invasive and invasive imaging techniques may be coupled with biomarkers detection to increase the specificity, sensitivity, and overall predictive value of each potential diagnostic technique.

### Markers of inflammation

Markers of inflammation include CRP, inflammatory cytokines soluble CD40L (sCD40L), soluble vascular adhesion molecules (sVCAM), and TNF.

CRP is a circulating pentraxin that has a major role in the human innate immune response [107] and provides a stable plasma biomarker for low grade systemic inflammation. CRP is produced predominantly in the liver as part of the acute phase response. However, CRP is also expressed in SMCs within diseased atherosclerotic arteries [108] and has been implicated in multiple aspects of atherogenesis and plaque vulnerability, including expression of adhesion molecules, induction of NO, altered complement function, and inhibition of intrinsic fibrinolysis [109]. CRP is considered to be an independent predictor of unfavorable cardiovascular events in patients with atherosclerotic disease. Beyond the ability of CRP to predict risk among both primary and secondary prevention patients, interest in it has increased with the recognition that statin-induced reduction of CRP is associated with less progression in adverse cardiovascular events that is independent of the lipid-associated changes [110] and that the efficacy of statin therapy may be related to the underlying level of vascular inflammation as detected by high-sensitivity CRP (hs-CRP). Among patients with stable angina and established CAD, plasma levels of hs-CRP have consistently been shown associated with recurrent risk of cardiovascular events [111,112]. Similarly, during acute coronary ischemia, levels of hs-CRP are predictive of high vascular risk even if troponin levels are non-detectable, suggesting that inflammation is associated with plaque vulnerability even in the absence of detectable myocardial necrosis [113,114]. Despite these data, the most relevant use of hs-CRP remains in the setting of primary prevention. To date, over two dozen large-scale prospective studies have shown baseline levels of hs-CRP to independently predict future myocardial infarction, stroke, cardiovascular death, and incidence of peripheral arterial disease [115,116]. Moreover, eight major prospective studies have had adequate power to evaluate hs-CRP after adjustment for all Framingham covariates, and all have confirmed the independence of hs-CRP [117]. Despite this evidence, it is important to recognize that there remain no firm data to date that lowering CRP levels per se will lower vascular risk. Further, as with other biomarkers of inflammation, it remains controversial whether CRP has a direct causal role in atherogenesis [118], and ongoing work with targeted CRP-lowering agents are required to fully test this hypothesis. However, the clinical utility of hs-CRP has been well established, and on the basis of data available through 2002, the Centers for Disease Control and Prevention and the American Heart Association endorsed the use of hs-CRP as an adjunct to global risk prediction, particularly among those at “intermediate risk” [119]. Data available since 2002 strongly reinforce these recommendations and suggest...
expansion to lower risk groups, as well as those taking statin therapy. Perhaps most importantly, data for hs-CRP provides evidence that biomarkers beyond those traditionally used for vascular risk detection and monitoring can have important clinical roles in prevention and treatment.

Cellular adhesion molecules can be considered potential markers of vulnerability because such molecules are activated by inflammatory cytokines and then released by the endothelium [120]. These molecules represent the one available marker to assess endothelial activation and vascular inflammation. The Physicians’ Health Study evaluated more than 14,000 healthy subjects and demonstrated ICAM-1 expression positive correlation with cardiovascular risk and showed that subjects in the higher quartile of ICAM-1 expression showed 1.8 times higher risk than subjects in the lower quartile [121]. Furthermore, soluble ICAM-1 and VCAM-1 levels showed a positive correlation with atherosclerosis disease burden [122]. IL-6 is expressed during the early phases of inflammation and it is the principal stimulus for CRP liver production. In addition, CD40 ligand, a molecule expressed on cellular membrane, is a TNFα homologue which stimulates activated macrophages proteolytic substances production [123]. CD40 and CD40L have been found on platelets and several other cell types in functional-bound and soluble (sCD40L) forms. Although many platelet-derived factors have been identified, recent evidence suggests that CD40L is actively involved in the pathogenesis of ACS. CD40L drives the inflammatory response through the interaction between CD40L on activated platelets and the CD40 receptor on endothelial cells. Such interactions facilitate increased expression of adhesion molecules on the surface of endothelial cells and release of various stimulatory chemokines. These events, in turn, facilitate activation of circulating monocytes as a trigger of atherosclerosis.

Beyond known proinflammatory and thrombotic properties of CD40L, experimental evidence suggests that CD40L-induced platelet activation leads to the production of reactive oxygen and nitrogen species, which are able to prevent endothelial cell migration and angiogenesis [124]. As a consequence of inhibiting endothelial cell recovery, the risk of subsequent coronary events may be greater. Clinical studies have supported the involvement of CD40L in ACS and the prognostic value in ACS populations. Levels of sCD40L have been shown to be an independent predictor of

**Table 1.1** Serologic markers of vulnerable plaque/patient.

<table>
<thead>
<tr>
<th>Reflecting metabolic and immune disorders</th>
<th>Reflecting hypercoagulability</th>
<th>Reflecting complex atherosclerotic plaque</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal lipoprotein profile (i.e., high LDL, low HDL, lipoprotein [a], etc.)</td>
<td>Markers of blood hypercoagulability (i.e., fibrinogen, α-dimer, factor V Leiden)</td>
<td>Morphology/structure</td>
</tr>
<tr>
<td>Non-specific markers of inflammation (hs-CRP, CD40L, ICAM-1, VCAM, leukocytosis and other immuno-related serologic markers which may not be specific for atherosclerosis and plaque inflammation)</td>
<td>Increased platelet activation and aggregation (i.e., gene polymorphism of platelet glycoproteins IIb/IIIa, Ia/IIa, and Iib/IX)</td>
<td>• Cap thickness</td>
</tr>
<tr>
<td>Serum markers of metabolic syndrome (diabetes or hypertriglyceridemia)</td>
<td>Increased coagulation factors (i.e., clotting of factors V, VII, VIII, XIII, von Willebrand factor)</td>
<td>• Lipid core size</td>
</tr>
<tr>
<td>Specific markers of immune activation (i.e., anti-LDL antibody, anti-HSP antibody)</td>
<td>Decreased anticoagulation factors (i.e., proteins S and C, thrombomodulin, antithrombin III)</td>
<td>• Percentage stenosis</td>
</tr>
<tr>
<td>Markers of lipid peroxidation (i.e., ox-LDL and ox-HDL)</td>
<td>Decreased endogenous fibrinolysis activity (i.e., reduced tissue plasminogen activator, increased type I PAI, PAI polymorphisms)</td>
<td>• Remodeling (positive vs. negative)</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>Prothrombin mutation (i.e., G20210A)</td>
<td>• Color (yellow, red)</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>Thrombogenic factors (i.e., anticalcidiolin antibodies, thrombocytosis, sickle cell disease, diabetes, hypercholesterolemia)</td>
<td>• Collagen content vs. lipid content</td>
</tr>
<tr>
<td>Circulating apoptosis markers (i.e., Fas/Fas ligand)</td>
<td>Transient hypercoagulability (i.e., smoking, dehydration, infection)</td>
<td>• Calciﬁcation burden and pattern</td>
</tr>
<tr>
<td>ADMA/DDAH/ Circulating NEFA</td>
<td></td>
<td>• shear stress</td>
</tr>
</tbody>
</table>

**Activity/function**

- Plaque inflammation (macrophage density, rate of monocyte and activated T-cell inﬁltration)
- Endothelial denudation or dysfunction (local nitric oxide production, anti/procoagulation properties of the endothelium)
- Plaque oxidative stress
- Superﬁcial platelet aggregation and fibrin deposition
- Rate of apoptosis (apoptosis protein markers, microsatellite)
- Angiogenesis, leaking vasa vasmor, intraplaque hemorrhage
- Matrix metalloproteinases (MMP-2, -3, -9)
- Microbial antigens (Chlamydia pneumoniae)

**Temperature**

- Pan arterial
  - Transcoronary gradient of vulnerability biomarkers
  - Total calcium burden
  - Total coronary vasoreactivity
  - Total arterial plaque burden (intima media thickness)
adverse cardiovascular events after ACS [125], with increased levels portending a worse prognosis [126]. Importantly, specific therapeutic strategies have shown to be beneficial in reducing risk associated with sCD40L [127]. IL-18 is a proinflammatory cytokine mostly produced by monocytes and macrophages, which acts synergistically with IL-12 [105]. Both these interleukines are expressed in the atherosclerotic plaque and they stimulate IFNγ induction which, in its turn, inhibits collagen synthesis, preventing a thick fibrous cap formation and facilitating plaque destabilization. Mallat et al. [128] examined 40 stable and unstable atherosclerotic plaques obtained from patients undergoing carotid endarterectomy and highlighted how IL-18 expression was higher in macrophages and endothelial cells extracted from unstable rather than stable lesions and it correlated with clinical (symptomatic plaques) and pathological (ulceration) signs of vulnerability.

Pregnancy-associated plasma protein-A (PAPP-A) is a high molecular weight, zinc-binding metalloproteinase, typically measured in women's blood during pregnancy and later found in macrophages and SMCs inside unstable coronary atherosclerotic plaques. This protease cleaves the bond between insulin like growth factor-1 (IGF-1) and its specific inhibitor (IGFBP-4 e IGFBP-5), increasing free IGF-1 levels. IGF-1 is important for monocytes–macrophages chemotaxis and activation in the atherosclerotic lesion, with consequent proinflammatory cytokine and proteolytic enzyme release, and stimulates endothelial cell migration and organizational behavior with consequent neangiogenesis. Hence, IGF-1 represents one of the most important mediators in the transformation of a stable lesion into an unstable one [129]. Bayes-Genis et al. [130] demonstrated that PAPP-A is more often expressed in the serum of patients with acute coronary syndromes (UA, MI), than subjects presenting with SA. In particular, PAPP-A serum levels >10 mIU/L recognize patient vulnerability with a specificity of 78% and a sensitivity of 89%. It has also been demonstrated that PAPP-A histological expression is higher in complex, vulnerable/ruptured carotid plaques than stable lesions [131]. As PAPP-A serum levels can be easily measured today by means of enzyme-linked immunosorbent assay (ELISA), this protease could represent an easily quantifiable marker of vulnerability, with a reproducible method, allowing the identification of a patient subgroup with a high cerebrovascular risk before its clinical manifestation.

Jaffer et al. [132] have published a detailed review on different techniques for detection of vulnerable plaque based on several biomarkers that have been implemented in recent years. In this context, plaques with active inflammation can be identified directly by extensive macrophage accumulation [133]. Possible intravascular diagnostic techniques [134] based on inflammatory infiltration determination within the plaque include thermography [135], contrast-enhanced MRI [136], fluorodeoxyglucose positron emission tomography [137], and immunochemistry [138]. In addition, non-invasive techniques include MRI with superparamagnetic iron oxide [139,140] and gadolinium fluorine compounds [141,142].

Oxidative stress markers

Oxidative stress has a very important role in atherogenesis. Evidence shows that activation of vascular oxidative enzymes leads to lipid oxidation, foam cell formation, expression of vascular adhesion molecules and chemokines, and ultimately atherogenesis. Myeloperoxidase (MPO) is a heme peroxidase that is present in and secreted by activated phagocytes at sites of inflammation. MPO can generate several reactive, oxidatively derived intermediates, all mediated through a reaction with hydrogen peroxide, to induce oxidative damage to cells and tissues [143]. Oxidation products from MPO are found at significantly increased rates (up to 100-fold higher than circulating LDL) on LDL isolated from atherosclerotic lesions [144] and lead to accelerated foam cell formation through nitrated apoB-100 on LDL and uptake by scavenger receptors [145]. Accumulating evidence suggests that MPO may have a causal role in plaque vulnerability [146]. Sugiyama et al. [147] showed that advanced ruptured human atherosclerotic plaques, derived from patients with sudden cardiac death, strongly expressed MPO at sites of plaque rupture, in superficial erosions and in the lipid core, whereas fatty streaks exhibited little MPO expression. In addition, MPO macrophage expression and HOCl were highly co-localized immunochemically in culprit lesions of these patients. Several inflammatory triggers, such as cholesterol crystals and CD40 ligand, induced release of MPO and HOCl production from MPO-positive macrophages in vitro. Consistent with the potential role for MPO in the atherosclerotic process, genetic polymorphisms resulting in MPO deficiency or diminished activity are associated with lower cardiovascular risk, although the generalizability of these findings is uncertain [148]. In parallel with the effects of MPO on nitric oxide, LDL oxidation, and presence within ruptured plaques, several recent clinical studies have suggested that MPO levels can provide diagnostic and prognostic data in endothelial function, angiographically determined CAD, and ACSs. In a case–control study of 175 patients with angiographically determined CAD, Zhang et al. [149] showed that the highest quartiles of both blood and leukocyte MPO levels were associated with odds ratios of 11.9 and 20.4, respectively, for the presence of CAD compared with the lowest quartiles. Brennan et al. [150] obtained MPO levels in the emergency department in 604 patients presenting with chest pain but no initial evidence of myocardial infarction, and showed that MPO levels predicted the in-hospital development of myocardial infarction, independent of other markers of inflammation, such as CRP. In addition, they showed that MPO levels were strong predictors of death, myocardial infarction, and revascularization 6 months after the initial event. Current data suggest that MPO can serve as both a marker of disease, providing independent information on diagnosis and prognosis of patients with chest pain, and also as a potential marker for assessment of plaque progression and destabilization at the time of acute ischemia.

Biomechanical stress as a trigger for plaque progression and rupture

Despite the exposure of the entire coronary tree to the systemic risk factors and inflammation, spatial distribution of atherosclerotic plaques is often a focal phenomenon [151]. Vascular endothelium is subjected to complex mechanical stresses resulting from its 3D geometry, vessel curvatures, and cardiac motion. These mechanical strains in combination with fluid frictional forces or shear stress gradients inside the arteries can lead to a number of structural and humoral changes in endothelial cells [39,152]. High wall shear stress (>15 dyne/cm²) has been found to induce endothelial quiescence and an atheroprotective gene expression profile, whereas low shear stress (<4 dyne/cm²) stimulates an atherogenic phenotype [152]. It has been shown that the plaques and wall thickening are localized mostly on the outer wall of one or both daughter vessels at bifurcations and along the inner wall of curved segments [151]. In the Prediction study, Stone et al. [153] studied the natural history of plaques in 506 patients with ACS treated with percutaneous coronary intervention, and used reconstructed coronary models from angiography and IVUS. A total of 74% patients had follow-up studies at 6–10 months to relate the effects of local hemodynamic milieu on plaque changes. Authors reported that decrease in lumen area
was independently predicted by baseline large plaque burden and low endothelial shear stress [153]. Other investigators have reported that high wall shear stress is associated with transformation of plaques into high risk phenotypes prone to instability and rupture [154,155].

**Neoatherosclerosis**

The neointimal tissue inside the stents is subject to similar atherogenic forces as the native vessels [156,157]. Neoatherosclerosis is the development of atherosclerosis within this neointima. Histologically, it is recognized by the presence of clusters of lipid-laden foamy macrophages within the neointima with or without necrotic core formation [157,158]. On OCT it is seen as areas of heterogeneous appearance within the neointima with low-intensity lipid-laden regions or well demarcated calcification within stents (Figure 1.5) [159,160]. Although the exact pathogenesis of this phenomenon is yet to be proven, inflammation and endothelial dysfunction have been shown to have a fundamental role [157,158,161]. It has been reported in autopsy and in vivo imaging studies that neoatherosclerosis occurs at an earlier stage and with higher frequency in DES than BMS [157,162]. It is thought to be one important mechanism for late stent failure including in-stent restenosis and very late stent thrombosis [156,158,163].

**Future challenges in the treatment of vulnerable plaques**

With the concept of “vulnerable” plaque not nearly as straightforward as once thought, there are challenges to creating a therapeutic strategy for assessing the risk of rupture of vulnerable plaques in asymptomatic patients.

First, there must be an ability to identify the vulnerable plaque with non-invasive or invasive techniques. It has been demonstrated that coronary plaque composition can be studied with invasive and non-invasive imaging techniques, allowing real-time analysis and in vivo plaque characterization including the identification of TCFA. However, the severity of the inflammatory infiltration of the cap, which certainly has a major role in plaque disruption, cannot be accurately evaluated even with the most advanced in vivo imaging techniques. Moreover, dynamic plaque changes, such as abrupt intra-plaque hemorrhages from vasa vasorum which may be fundamental in predicting the potentiality of a plaque to rupture, will be extremely difficult to identify with real-time imaging techniques. Nevertheless, some promising work has been done in this regard in the SECRITT trial introducing the concept of sealing the non-occlusive, high risk IVUS and OCT-derived TCFA, using a dedicated nitinol self-expanding vShield device. Authors reported an interesting observation of neocap formation in the shielded plaques with an increase in the average cap thickness from 48 ± 12 μm at baseline to 201 ± 168 μm at 6 months’ follow-up [164]. It is hoped that this study may provide the foundation for larger scale trials in future.

A second challenge is that a lesion-specific approach requires that the number of vulnerable plaques in each patient needs to be known and the number of such lesions need to be limited. That is not the case, however. Several pathological studies indicate the presence of multiple “lipid-rich” vulnerable plaques in patients dying after ACS or with sudden coronary death [37,61]. Further complicating the issue, coronary occlusion and myocardial infarction usually evolve from mild to moderate stenosis—68% of the time, according to an analysis of data from different studies.

The third and fourth challenge is that the natural history of the vulnerable plaque (with respect to incidence of acute events) has to be documented in patients treated with patient-specific systemic therapy, and the approach has to be proven to significantly reduce the incidence of future events relative to its natural history. At this time, neither is documented nor proved.

Fifth, we believe that at the current stage it is not possible to know which vulnerable plaques will never rupture. Although we suspect it is the vast majority of them, we may have to shift to a more appropriate therapeutic target. In addition, targeting not only the vulnerable plaque but also the vulnerable blood (prone to thrombosis) and/or vulnerable myocardium (prone to life-threatening arrhythmia) may be also important to reduce the risk of fatal events.

**Conclusions**

Atherosclerosis is now recognized as a diffuse and chronic inflammatory disorder involving vascular, metabolic, and immune system with various local and systemic manifestations. A composite...
vulnerability index score comprising the total burden of atherosclerosis and vulnerable plaques in the coronary, carotid, aorta, and femoral arteries, together with blood vulnerability factors, should be the ideal method of risk stratification. Obviously, such index is hard to achieve with today’s tools. A future challenge is to identify patients at high risk of acute vascular events before clinical syndromes develop. At present, aside from imaging modalities such as IVUS, virtual histology, magnetic resonance, and local Raman spectroscopy that could help to identify vulnerable plaques, highly sensitive inflammatory circulating markers such as hsCRP, cytokines, PAPP-A, pentraxin-3, LpPLA2 are currently the best candidates for diffuse active plaque detection. In order to achieve this aim a coordinated effort is needed to promote the application of the most promising tools and to develop new screening and diagnostic techniques to identify the vulnerable patient.

Interactive multiple choice questions are available for this chapter on www.wiley.com/go/dangas/cardiology

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Natural regulatory T cells control arterial inflammation and fibrosis


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