CHAPTER 1

Atherogenesis and Inflammation

Daisuke Shishikura, Yanti Octavia, Umair Hayat, Vikas Thondapu, and Peter Barlis

The importance of atherosclerosis and its clinical consequences are well known as the leading cause of vascular disease worldwide [1]. The aetiology of atherosclerosis is multifactorial, and it affects various regions of the circulation preferentially resulting in heart attack, stroke, and peripheral vascular disease [2]. Atherosclerosis is considered a chronic immunoinflammatory disease fuelled by lipid, which typically occurs over many years [3]. The growth of atherosclerotic plaques does not occur in a smooth, linear fashion but discontinuously, with a stage of relative 'silent' period, punctuated by a period of rapid evolution which manifests clinically [4].

The formation of atherosclerosis is a complex process involving the interaction of plasma lipids with the vascular wall and immune cells [5]. Multiple independent pathways of evidence pinpoint inflammation as a centre regulatory process that connects various risk factors for atherosclerosis and its complications with altered arterial biology [6]. Abundant research from experimental and clinical studies have lent strong support to unravel the complex pathogenesis and find targeted management of the atherosclerotic disease; this entity still represents an ongoing challenge in clinical practice. This chapter will review the multifaceted pathology of atherosclerosis leading to plaque progression and destabilisation, with a recent update in coronary imaging modalities and novel developments in computer modelling as promising tools to help improve the understanding of cardiovascular diseases.

Pathogenesis of atherosclerosis

Inception of the plaque

Atherosclerosis derives from the Greek word for "gruel" or "porridge", demonstrating the manifestation of the lipid material found in the core of the typical atherosclerotic plague [7]. Though the exact initiator of atherosclerotic plaque formation is still debatable, there is a general consensus that the endothelial denuding injury, which could be triggered by multiple causes, such as smoking, hypertension, hyperglycaemia, immune injury, infection, sets a complex pathogenic sequence into motion [7–9]. From this point on, an inflammatory response leads to the development of the plaque. Endothelial cell dysfunction leads to platelet aggregation and release of platelet factors which subsequently recruit circulating monocytes from the blood into the intima, where they differentiate into macrophages and induce the proliferation of smooth muscle cells in an attempt to restore endothelial function. These cells will expand the extracellular matrix that would entrap and modify lipoproteins to become lipid-rich foam cells, forming a fibromuscular plaque [7,8].

Endothelial dysfunction

Endothelium, the continuous cellular lining of the vascular system, is an essential part of critical regulatory nodes in the homeostatic network [8]. Endothelial denuding injury is well-known to be an early and clinically relevant pathophysiologic event in the atherosclerotic process, a pivotal contributor to the local and systemic manifestations of atherosclerotic cardiovascular disease [10]. Endothelial dysfunction is more likely to occur at the arterial site that subjected to low shear stress and disturbed blood flow [11,12]. Acute inflammation involves the rapid recruitment of neutrophils and the stimulation of the endothelium, both type I and type II activation. Type 1 activation is independent of new genes expression, while type II activation is dependent on new genes expression with slower response [13]. Type I activation is of rapid onset, self-limited and usually do not result in sustained morphological or functional changes. It is typically mediated by ligands that bind to a heterotrimeric G-protein-coupled receptor (GPCR), which triggers the release of Ca^{2+} ions from the endoplasmic reticulum (ER) leading to increased production of arachidonic acid [13]. Arachidonic acid is converted by cyclooxygenase-1 (COX1) and prostacyclin synthase into prostaglandin I2 (PGI2), a potent vasodilator that relaxes vascular smooth muscle [14]. Elevated cytosolic Ca^{2+} ions also bind to the adaptor protein calmodulin and activates endothelial nitric oxide synthase (eNOS) producing NO, which has similar function with PGI2 [15]. The formation of Ca^{2+}–calmodulin complex also leads to the phosphorylation of myosin light chain, which initiates contraction of actin filaments that are attached to tight junction and adherent junction proteins, resulting in the opening of gaps between adjacent endothelial cells, allowing leukocytes to cross through [15, 16]. Altogether, these pathways lead to increase blood flow including leucocyte delivery and enhance the leakiness of venules to the extracellular matrix, that supports the attachment, survival and extravasation of invading neutrophils [17,18].

Different to type II activation of endothelial cells, type I activation typically last for 10–20 minutes due to desensitisation of the receptors, which prevents the restimulation and limiting the degree of

Interventional Cardiology: Principles and Practice, Third Edition. Edited by George D. Dangas, Carlo Di Mario, Holger Thiele, and Peter Barlis.

Companion website: www.wiley.com/go/dangas/cardiology

inflammation and neutrophil migration [19]. Activated leukocytes producing inflammatory cytokines such as interleukin-1 (IIL-1) and tumour necrosis factor α (TNFα), which are the key players in type II activation [13]. The binding of IL-1 or TNFα to its respective receptors initiates various kinase cascades that lead to the activation of the transcription factor-κβ (NF-κβ) and activating protein 1 (AP1) [15]. These factors trigger the transcriptions of various nucleus genes leading to expression of proinflammatory proteins (E-selectin, intercellular adhesion molecule 1 [ICAM1] and vascular cell-adhesion molecule 1 [VCAM1]), chemokines (interleukin-8 [IL-8]), and enzymes (cyclooxygenase-2 [COX2]) [15]. Hence, type II activation requires a longer time due to transcription and translation of new proteins [15]. Similar with type I activation, type II activation induce the leakage of plasma proteins and leukocyte recruitment with slightly different mechanism compared to type I activation. TNFα and IL-1 stimulate venular endothelial cells to reorganise their actin and tubulin cytoskeletons and open up gaps between adjacent endothelial cells [20,21].

Historically, a critical link between the activations and arterial endothelial dysfunction in atherogenesis comes with the discovery of atherosclerosis-associated VCAM-1, which found to have selectivity adhesivity for mononuclear leukocytes and lymphocytes [22,23]. The dysfunctional endothelium overexpressed VCAM-1 and ICAM-1 on its surface, facilitates the adhesion and transendothelial migration of leukocytes [9]. Monocyte migration to extracellular matrix induces expression of various cytokines, such as TNFα, IL-1, IL-6, platelet-derived endothelial cell growth factor, transforming growth factor (TGF)-α and -β, and macrophage colony-stimulating factor which is one of the key players in the differentiation process of monocytes to macrophages in the subendothelial space [9,24,25]. The macrophages ingest oxidised low-density lipoprotein (LDL) via scavenger receptors, forming "foam" cells. Endothelial cells also produce MCP-1, monocyte colony-stimulating factor and IL-6 which further amplify the inflammatory cascade [26]. IL-6 production by smooth muscle cells represents the main stimulus for C-reactive protein (CRP) production [27]. Recent evidence suggests that CRP may contribute to the proinflammatory state of the plaque both mediating monocytes recruitment and stimulating monocytes to release IL-1, IL-6, TNFα [28]. The damaged endothelium allows the passage of lipids into the subendothelial space. Fatty streaks represent the first step in the atherosclerotic process.

Cholesterol

LDL particles are well-known as one of the critical players in the pathogenesis of atherosclerosis, however, the association of LDL amount can cause atherosclerosis remains unclear, even though lowering this lipoprotein reduces atherosclerosis-related cardiovascular events [7, 29–31]. On the other hand, even though both LDL and high-density lipoprotein (HDL) particles are highly heterogenous [32], plasma HDL-cholesterol concentrations are negatively associated with atherosclerosis [29,33]. Interestingly, raising plasma HDL-cholesterol concentrations did not show any benefit on existing atherosclerosis [34], which might suggest that HDL function may play a more pivotal part in the prevention against atherosclerosis by averting endothelial activation and enhance NO production [35-37]. LDL can deposit in the arterial wall due to impaired endothelial barrier function and retain within the intimal layer by extracellular matrix macromolecules [38]. Enormous researches demonstrated that the oxidisation of LDL particles including ROS formation in the intimal wall due to metal ion catalysis could initiate atherogenesis [39,40]. Oxidised LDL is the major modified form of native LDL since structurally it is very prone to oxidative damage [41]. The oxidised LDL particles serves as ligands for the scavenger receptors (SRs) that facilitate macrophage foam cell formation which is the hallmark of early atherosclerotic lesions that subsequently promoting humoral and adaptive immunity leading to plaque formation [7,42,43].

Oxidative stress

Since the 1950s, oxidative stress has a significant role in the pathogenesis of atherosclerosis, and the degree of oxidation correlates with the severity of the diseases [44]. Multiple reactive oxygen species (ROS) generator are present in the vascular wall, including the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, uncoupled endothelial nitric oxide synthase (eNOS), mitochondrial enzymes, and xanthine oxidase (XO), which develops a cross-talk between the enzymes, creating a vicious cycle [5]. The function of the NADPH oxidase enzymes is to catalyse the one-electron transfer from NADPH to molecular oxygen, thereby generating ROS [45] in a regulated way in response to, e.g. calcium, growth factors, and cytokines [46]. Seven isoforms of the catalytic, membrane-spanning NADPH oxidase subunit NOX exist, each encoded by a separate gene, i.e. NOX1–5 and Duox1 and Duox2 [47]. They differ in molecular composition, subcellular localisation, tissue distribution, and expression [48]. Endothelial cells mainly express NOX2 and NOX4, and vascular smooth muscle cells mainly express NOX4 [49]. In vascular smooth muscle cells of resistance arteries, NOX2 expression is relatively high [50], and cardiomyocytes mainly express NOX2 and NOX4 [51], NOX activity contributes to the oxidised LDL production in macrophages [52], overexpression of endothelial adhesion molecules, monocyte infiltration, and VSMC proliferation [53]. Coupled eNOS (the physiological condition) generates NO, which induces vascular smooth muscle relaxation and inhibits platelet aggregation and adhesion [54]. However, under conditions of oxidative stress, eNOS becomes uncoupled, dysfunctional, and generates superoxide instead of NO. Subsequently, low NO bioavailability can up-regulate 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 NFkB expression [55]. Furthermore, NO inhibits leukocyte adhesion [56] and NO reduction results in induction of monocyte chemotactic protein-1 (MCP-1) expression which recruits monocytes [57]. NO is in a sensitive balance with endothelin1 (ET-1) regulating vascular tone [58]. Plasma ET-1 concentrations are increased in patients with advanced atherosclerosis and correlate with the severity of the disease [59,60]. In addition to its vasoconstrictor activity, ET-1 also promotes leukocyte adhesion [61] and thrombus formation [62]. The deficiency of eNOS cofactor tetrahydrobiopterin (BH4), or eNOS substrate ι-arginine, or eNOS S-glutathionylation were found to be the culprit of the eNOS uncoupling [6].3 Endothelial NOS-dependent ROS production has been shown as one of significant contributor in patients with atherosclerosis [64], hypertension [65], hypercholesterolemia [66], diabetes mellitus [67], and chronic smoker [68].

Mitochondrial is a crucial element in physiology vascular cell growth and function; however, mitochondrial dysfunction can result in excessive ROS production. Mitochondrial oxidative stress also found to be associated with atherosclerosis which can occur under

pathological situation because of over ROS production or failure of antioxidant mechanisms [69]. Mitochondrial dysfunction also associated with plaque ruptures, which accelerate the progression of hemodynamically significant atherosclerotic lesions [69], XO is found both in plasma and endothelial cells and generates superoxide anions and hydrogen peroxide by using molecular oxygen as an electron acceptor [70]. The expression and activity of endothelial XO are enhanced in human atherosclerotic plaque [71]. XO stimulates SRs expression on the macrophages and VSMC, namely LOX-1 and CD 36, resulting in ROS production and subsequently causing transformation of macrophages and VSMC into foam cells [52].

Progression of atherosclerotic plaque

Stable plaque

Once established, atherosclerosis develops progressively through continuous accumulation of cholesterol-rich lipids and the accompanying inflammatory response, which can vary considerably among individuals [4]. Early fibroatheroma involves numerous foam cells, extracellular proteoglycans, accumulation of extracellular lipid which progressively distorts the normal architecture of the intima [4]. The extracellular matrix contains collagen, elastin, proteoglycans, and glycosaminoglycans, entrap lipoproteins and promote lipid accumulation which subsequently causes cell necrosis that dominate the central part of the intima, ultimately occupying up to half the volume of the arterial wall and can lead to the formation of flow-limiting lesions [4,7]. Gradually, the loose fibro cellular tissue is replaced and expanded by collagen-rich fibrous tissue, with tissues that lie in between a necrotic core and the luminal surface of the plaque (fibrous cap) are fibrous with a high content of type I collagen [72,73]. Macrophages colony stimulating factor (M-CSF) acts as the main stimulator in this process, next to granulocyte-macrophage stimulating factor (MG-CSF) and IL-2 for lymphocytes [74]. Lymphocytes enter the intima by binding adhesion molecules (VCAM-1, P-selectin, ICAM-1 MCP-1(CCL2), IL-8 (CxCL8) [26]. T cells have been proven as a critical driver in the pathogenesis of atherosclerosis, with different T cells subset can serve as a pro-atherogenic or anti-atherogenic [75]. CD4+ T helper 1 (TH1) cells and natural killer T cells have pro-atherogenic roles, on the contrary, regulatory T (Treg) cells have anti-atherogenic properties [75]. Fernandez *et al.* [76] showed that the majority of CD4+ T cells in the atherosclerotic plaque are TH1 and TH2, and more TH1 cells are found in plaque compared with peripheral blood. Initially, antigen-presenting cells (APCs) process oxidised LDL, and present peptides from apolipoprotein B (ApoB) on major histocompatibility complex (MHC) class II molecules to the naïve T cells, which recognise this complex through their specific T cell receptors (TCRs). This process induces T cells to express various transcription factors that favour the differentiation into distinct TH phenotypes and subsequently will differentiate to the complete phenotype of the effector (Teff) or Treg [76]. Even the pro-atherogenic role for TH1 cells and the anti-atherogenic role of Treg cells have been well established, the role of the other TH cells subtypes, such as TH2, TH9, TH17, TH22 and follicular helper T (TFH) is still controversial and warrant further investigations [75].

The vulnerable plaque

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 remodelling with expansion of the vessel external elastic lamina and minimal changes in lumen size [77,78]. The plaque contains monocyte-derived macrophages, smooth muscle cells, and T cells. 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. Atherosclerotic lesions expand outward radially, in the direction away from the lumen to preserve the lumen calibre [7]. Plaque with limited lipid accumulation and thicker fibrous caps are referred as "stable plaques" [7]. On the other hand, thin-cap fibroatheromas (TCFAs) or "vulnerable plaques" are likely the precursors of plaque ruptures, usually in the proximal segments of the major coronary arteries, where most plaque ruptures and thrombi are seen [79–81]. Burke *et al.* identified a cut-off value for cap thickness of 65 microns to define a vulnerable coronary plaque [80]. Rupture occurs where the cap is thinnest and most infiltrated by foam cells.72 Despite the predominant hypothesis focusing on the responsibility of a specific vulnerable atherosclerotic plaque rupture [82,83] for acute coronary syndromes, some pathophysiologic, clinical and angiographic observations seem to suggest the possibility that the principal cause of coronary instability 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 [84–87]. The gradual loss of SMCs from the fibrous cap and infiltrating macrophages degrade the collagen-rich cap matrix lead to TCFAs formation, as ruptured caps contain less collagen and SMCs with numerous foam cells compared with intact caps [88–90]. Emotional or physical stress, such as anger, anxiety, work stress, earthquakes, war, sexual activity, hyperthermia, infections, and cocaine use, is known to cause plaque rupture [91]. Plaque rupture exposes the contents of the plaque to the blood compartment, where thrombogenic material in the plaque core produced by macrophages and smooth muscle cells can trigger thrombosis, the most dreaded complication of atherosclerosis, resulting in acute coronary syndrome or stroke [7].

Vulnerable plaque: a shift towards Th1 pattern

Immunoinflammation drives the formation, progression, and rupture of atherosclerotic plaques. 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 lymph node. Subsequent cycle 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 role determining the future development of the lesion. Th1 cells secreting proiflammatory 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 [92]. Therefore, the switch to a selective recruitment of

Th1 lymphocyte represents a key point toward plaque vulnerability/disruption. T cells in the plaque may encounter antigens such as oxLDL. Moreover T cell response can be triggered by heat shock proteins of endogenous or microbial origins [93]. The mechanisms of why the initial inflammatory response becomes a chronic inflammatory condition remains unclear. 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 TNFα upregulates the expression of fractalkine (CX3CL1) [94]. Interleukin 1 and TNFα-activated endothelium express also fractalkine (membrane bound form) that directly mediates the capture and adhesion of CX3CR1 expressing leukocytes providing a further pathway for leukocyte activation [95]. This cytokine network promotes the development of the Th-1 pathway which is strongly pro-inflammatory and induces macrophage activation, superoxide production and protease activity.

Plaque erosions

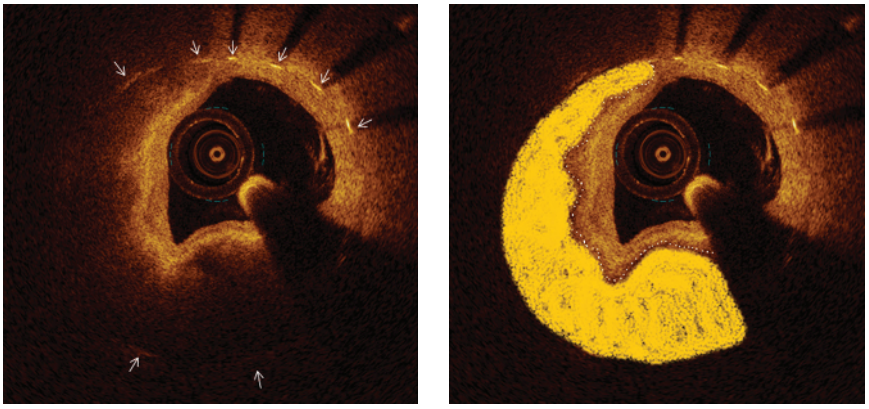
Improvement in anti-atherosclerotic therapy reduces the risk of plaque rupture [7]. Atherosclerotic plaques have become less inflamed and more fibrous, minimising the risk of rupture due to fissure of the fibrous cap [96]. Plaque erosions tend to have a rich extracellular matrix without a thin, friable fibrous cap, with less foam cells and lipid accumulation [97]. It typically shows endothelial denudation with intact internal and external elastic laminas and a well-developed media with contractile SMCs unlike ruptured plaque where internal lamina is disrupted and the underlying media is thin and disorganised [98]. Interestingly, plaque erosions sometimes found in up- or downstream of a plaque rupture with a fatal superimposed thrombus, which might suggest that loss of endothelium can occur secondarily to thrombus formation assuming that the ruptured plaque nearby is the sole precipitating cause.

Neoatherosclerosis

The neointimal tissue inside the stents is subject to similar atherogenic forces as the native vessels [99, 100]. Neoatherosclerosis is the development of atherosclerosis within this neointima following bare metal stent (BMS) and drug eluting stent (DES). See Figure 1.1. Histologically it is recognized by the presence of clusters of lipid-laden foamy macrophages within the neointima with or without necrotic core formation [100,101]. Although the exact pathogenesis of this phenomenon is yet to be proven, inflammation and endothelial dysfunction have been shown to play a fundamental role [100–102]. 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 [100,103], suggesting that mechanisms of failure after BMS and DES implantation are quite different. Indeed, in general, patients with BMS are likely to develop in-stent restenosis (ISR) early due to neointima hyperplasia, whereas patients with DES are likely to develop less neointima in the early period, leading to less frequency of target lesion revascularization (TLR) after one year follow-up compared to BMS. However, patients with DES are likely to develop neoatherosclerosis later, which possibly related to the late catch-up phenomenon and very late stent thrombosis (VLST) [99,101,104].

Insights from coronary imaging

Technological advances enable us to visualise the extent and composition of atherosclerotic plaques in the coronary artery non-invasively and invasively. Intravascular imaging modalities including intravascular ultrasound (IVUS), backscattered radiofrequency (RF) IVUS, optical coherence tomography (OCT) and near-infrared spectroscopy (NIRS) have been currently available for assessing coronary atherosclerotic plaque morphology and composition. The great advantage of intravascular imaging is that it is based on a device that is practical for use in the clinical setting, and it generates a real-time assessment of plaque morphology. In addition, serial intravascular imaging enables us to evaluate the natural history progression of coronary atherosclerosis, which has also been used for assessing the pharmaceutical effects on coronary atherosclerosis. A number of studies have employed these techniques for detecting the factors contributing to cardiovascular events and assessing the efficacy of therapies targeting cardiovascular risk factors. These high-risk or vulnerable atherosclerotic plaques which are defined as precursors to lesions that rupture have specific characteristics, such as large plaque burden, expansive arterial remodelling, thin cap fibroatheroma (TCFA), lipid pools, necrotic core, inflammatory cell



(a) (b)

Figure 1.1 OCT illustration of Neoatherosclerosis. (a) Neo-intimal hyperplasia inside the stent. A uniform layer of neo-intima is seen covering the stent struts (white arrows) from 12 to 4 o'clock. Remainder of the stent circumference is covered by an irregular, very thick tissue layer containing dark, signal poor core consistent with lipid pool and signal rich, thick fibrous cap. (b) Lipid/ necrotic core in the neo-atherosclerotic plaque has been highlighted in yellow.

Table 1.1 Comparison of plaque characteristics evaluation.

	IVUS	VH-IVUS	OCT	NIRS
Imaging technology	Ultrasound	Ultrasound	Infrared	Near-infrared
Resolution (m)	100–200	100–200	< 10	N/A
Penetration (mm)	10.0	10.0	1.0–2.5	1.0-2.0
Plaque volume	++	–	–	–
Remodeling	++	–	–	–
TCFA detection	–	+	++	–
Calcification	++	++	++	–
Thrombus	+	–	++	–
Neovascularization	–	–	+	–
Macrophages	–	–	–	–
Cholesterol crystals	–	–	+	++
Lipid core	+	+	+	–

accumulation and neovascularisation [105]. These markers of vulnerability provide potential targets for high-risk plaque imaging. However, each intravascular imaging device has specific individual advantages and disadvantages in regards to evaluating plaque morphology and plaque compositions, shown in Table 1.1.

Intravascular imaging (IVUS)

The combination of high-frequency ultrasound placed within close proximity to the artery wall generates a high resolution, cross-sectional image of the full thickness of the artery wall. This enables us to visualize the full circumference of the vessel wall and atherosclerotic plaque, to examine arterial remodeling and to measure the area of plaque within each cross-sectional image with quantitative techniques. A series of cross-sectional images throughout a length of the artery is acquired with continuous imaging during catheter withdrawal, extending the quantitation of plaque burden to a volumetric measure. Additionally, intravascular ultrasound (IVUS) provides a unique opportunity to characterize the plaque morphology in the atherosclerotic coronary artery. Although identification of atherosclerotic plaque components is limited, several IVUS signatures have been suggested to be associated with either clinically unstable or a high risk for cardiovascular events in patients with coronary artery disease undergoing percutaneous coronary intervention [106–111]. Indeed, a preliminary pathological study has enhanced these findings with the relationship of the vulnerable IVUS characteristics with lipid contents and necrotic core [112]. These characteristics include atherosclerotic plaques with plaque attenuation characterized by a hypoechoic area with deep ultrasonic attenuation despite the absence of bright calcium (attenuated plaque) (Figure 1.2a), plaque echolucency characterized by an intraplaque zone of absent or low echogenicity (echolucent plaque) (Figure 1.2b), and spotty calcification characterized by the presence of lesions 1 to 4 mm in length containing an arc of calcification of <90° (Figure 1.2c). These coronary plaque characteristics associated with atherosclerotic vulnerability, inherent to IVUS imaging, offer potential advantages in the evaluation of coronary artery disease. Subsequently, IVUS has provided a valuable tool to study the vascular biology of coronary atherosclerosis *in vivo*.

Backscattered radio-frequency (RF) IVUS

Beyond the IVUS, there is considerable interest in the ability to distinguish individual plaque components. Spectral analysis of IVUS radiofrequency backscattered signals, known as virtual histology IVUS (VH-IVUS, Volcano Therapeutics, Inc., Roncho Cordova, Calfornia) or integrated backscatter IVUS (IB-IVUS, TERUMO, Japan), was developed to reconstruct a color-coded tissue map of plaque composition that distinguishes between fibrous, fibrofatty, necrotic core and dense calcific material (Figure 1.3) and provide detailed quantitative information of these individual components. Indeed, it has been shown that the RF IVUS has a 80% to 92% in vitro accuracy when used to identify the four different types of atherosclerotic plaques [113]. Additionally, according to the relative amount of these components, the pathological analysis is conducted to classify as pathological intimal plaque (PIT), thin-cap fibroatheroma (TCFA), thick-cap fibroatheroma (ThFA), fibrotic plaque and fibrocalcific plaque. Anatomical characteristics of vulnerable plaques which are prone to rupture were identified by the histological studies to have fibrous caps that are thin and rich in macrophages overlying a lipid pools [114]. Subsequent studies defined TCFA fibrous cap thickness as <65 μm [80] and showed

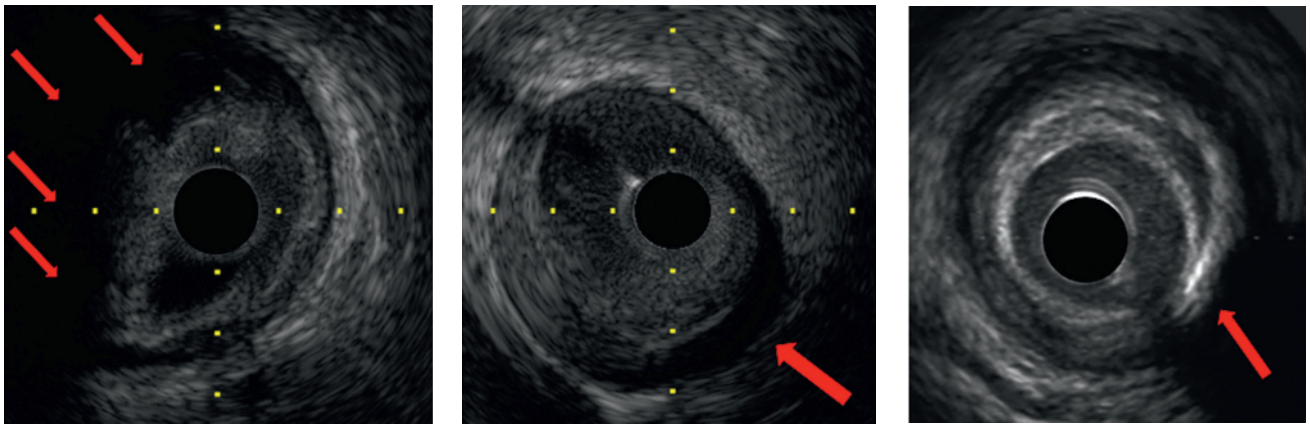


Figure 1.2 IVUS features of vulnerable plaques. (a) Attenuated plaque (red arrows): a hypoechoic area with deep ultrasonic attenuation despite the absence of bright calcium. (b) Echolucent plaque (red arrows): plaque echolucency characterized by an intraplaque zone of absent or low echogenicity. (c) Spotty calcification: the presence of lesions 1 to 4 mm in length containing an arc of calcification of <90°.

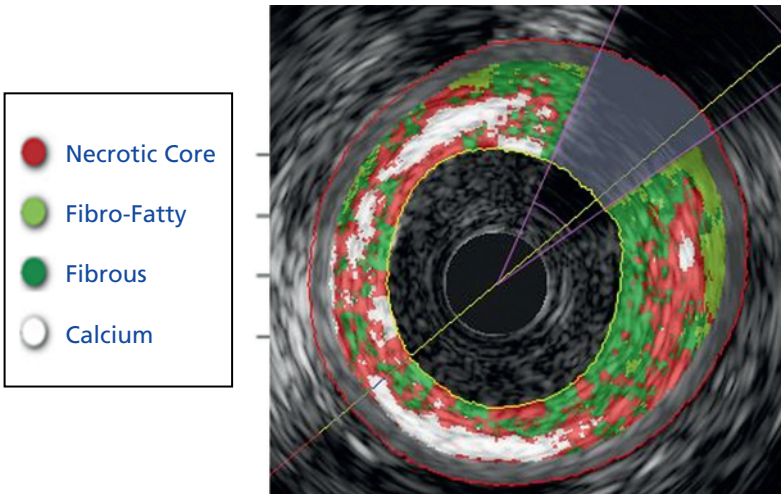


Figure 1.3 VH-IVUS imaging. Red – Necrotic core plaque; Light green – Fibro-fatty plaque; Dark green – Fibrous plaque; White – Calcified plaque.

that the majority of TCFAs had >10% of the plaque area occupied by a lipid-rich NC [115]. Given that the resolution of VH-IVUS is insufficient to directly image a thin fibrous cap, TCFA acquired from VH-IVUS, VH-TCFA, has been defined as the presence of >10% necrotic core volume without obvious overlying fibrous tissue and a total plaque burden of >40% observed within three consecutive VH-IVUS frames. In the clinical settings, it has been shown by a three-vessel VH-IVUS study that patients with ACS have a greater incidence of VH-TCFA than those with stable coronary artery disease [116]. Prospective analyses found that the baseline presence of VH-TCFA was independently predictive of future coronary events in patients admitted with an ACS [117,118]. The Providing Regional Observations to Study Predictors of Events in the Coronary Tree (PROSPECT) study assessed 700 patients with ACS to identify the impact of baseline plaque composition on future coronary events [117], which demonstrated that the highest risk plaque type for recurrent cardiac events was TCFA derived by VH-IVUS with small luminal area and large plaque burden. Furthermore, the European Collaborative Project in Inflammation and Vascular Wall Remodeling in Atherosclerosis-Intravascular Ultrasound (ATHEROREMO-IVUS) study that demonstrated that the predictive value of TCFA derived by VH-IVUS in a non-culprit coronary artery for the occurrence of acute cardiac events, particularly of death and ACS was even stronger [119]. Thus, a color-coded mapping method using IVUS radiofrequency is a promising research tool to identify plaque characteristics across a range of patient populations that may predict adverse outcomes.

Optical coherence tomography (OCT)

Although IVUS is widely used to investigate plaque morphology, including plaque burden and remodeling, the resolution may be insufficient to characterize subtle changes in the vascular wall. Optical coherence tomography (OCT) which uses backscattered

reflections of near-infrared light, with a wavelength of about 1300 nm to generate an axial image of the coronary artery offers unparalleled spatial resolution (<10 micrometer axial; 20–40 micrometer lateral). This modality enables high-resolution characterisation of the vascular layers within a healthy artery, tissue characterization (fibrous, fibrocalcific, and lipid plaque) and the identification of morphological changes to the vessel surface such as plaque rupture (Figure 1.4b), plaque erosion (Figure 1.4a) and calcified nodule (Figure 1.4c), which have been considered to be the most common underlying mechanisms contributing to ACS.115 In addition, this also permits the characterization of vulnerable plaques which are prone to rupture (Figure 1.5), including TCFA macrophage accumulation, cholesterol crystals, and micro-vessels within atherosclerotic plaque.

OCT assessment of culprit lesions with ACS

Plaque rupture

Plaque rupture, defined by an area of fibrous cap disruption whereby the overlying thrombus is in continuity with the underlying necrotic core is the most frequent finding in autopsy studies of patients with sudden cardiac death [115, 120]. Plaque rupture is thought to be consequence of thin-cap atheroma disruption and inflammation is the key regulator of the structural integrity of the plaque. Recently, circumferential biomechanical forces have been focused and recognized as one of the risk factor leading to plaque development and plaque rupture [121]. OCT is currently the most ideal modality to identify and evaluate plaque rupture. Interestingly, in the preliminary study, the area of ruptured cavity was significantly larger in STEMI compared with non-ST-segment elevated ACS (NSTE-ACS), suggesting that the morphological feature of plaque rupture could relate to the clinical presentation in patients with ACS [122] Additionally, another study conducted 3-vessel OCT imaging in 38 patients with ACS demonstrated that ruptured culprit plaques had non-culprit plaques with thinner prevalence of TCFA, wider maximum lipid arc, greater lipid length and thinner fibrous caps compared with non-ruptured culprit plaques, indicating that the patients with ruptured culprit plaques have increased pan-coronary vulnerability in non-culprit plaques which may cause future adverse events [123].

Plaque erosion

Plaque erosion, pathologically characterized by luminal thrombus and absence of the endothelium, without evidence of fibrous cap disruption) [115] is frequently followed by plaque rupture in patients with sudden cardiac death, ranging from 30–40% [124–126] especially more frequently in younger female [126] and smokers [127] Given that OCT does not permit the identification of the endothelial lining, the pathological definition of erosion cannot simply be adapted for the OCT definition. Subsequently, OCT-identified plaque erosion has been defined as the presence of thrombus and an irregular luminal surface in the absence of cap rupture (Figure 1.4) [128]. In contrast to plaque rupture, plaque erosion is distinctly different entity. Firstly, markers of inflammation are significantly lower in plaque erosion with sparse infiltration of macrophages and T lymphocytes within the vessel wall [79, 129]. Consistent with these findings, differential intracoronary cytokine expression between plaque erosion and rupture has been shown by a clinical study that examined 40 STEMI patients who underwent OCT [130]. Secondly, the stenosis of coronary lumen is not always significant in eroded plaques. According to pathological study examined 111 sudden coronary death, the internal elastic area and percent stenosis were significantly smaller in erosions compared with ruptures ($p<0.0001$ and $p=0.02$, respectively), where plaque burden was greater ($p=0.008$) [131]. Thirdly, coronary thrombi exhibit diverse healing phases, depending on the etiology of the underlying culprit plaque. These findings of differences between plaque erosion and plaque rupture attributed to clinical settings in which the frequency of STEMI was significantly higher in the patients with plaque rupture, whereas NSTE-ACS was predominant in patients with plaque erosion [132]. These findings may require tailored therapy in individual plaque features. Recently, in a small number, non-randomized study, a potential alternative treatment strategy for patients with ACS who had OCT-identified plaque erosion without stent implantation has been shown to result in satisfactory clinical outcomes in which none of 12 patients who had plaque erosion treated with thrombectomy and anti-thrombotic agents without stenting required an additional revascularization after two years [133]. More recently, another prospective study demonstrated that anti-thrombotic therapy without stent implantation reduced thrombus volume and enlarged the flow area without re-occlusion of the culprit lesion at one month in ACS patients with OCT derived erosion [134]. These findings may highlight the utility of OCT assessment to optimize a treatment in patients with ACS.

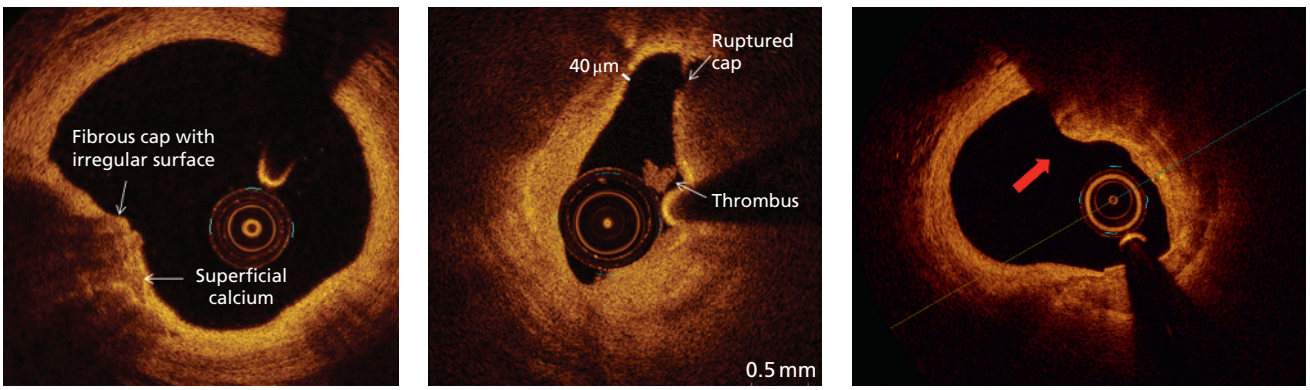


Figure 1.4 OCT appearance of vulnerable plaques (plaque erosion, plaque rupture, calcified nodule). (a) Plaque Erosion: Intact fibrous cap with irregular luminal surface and superficial calcium. (b) Plaque rupture with luminal thrombus. At 11 o'clock a Thin Cap Fibro-Atheroma (TCFA) is seen (fibrous cap thickness measured 40 microns, marked with small white bar). (c) Calcified nodule: the presence of fracture of a calcified plate protruding into the lumen through a disrupted fibrous cap with an overlying thrombus.

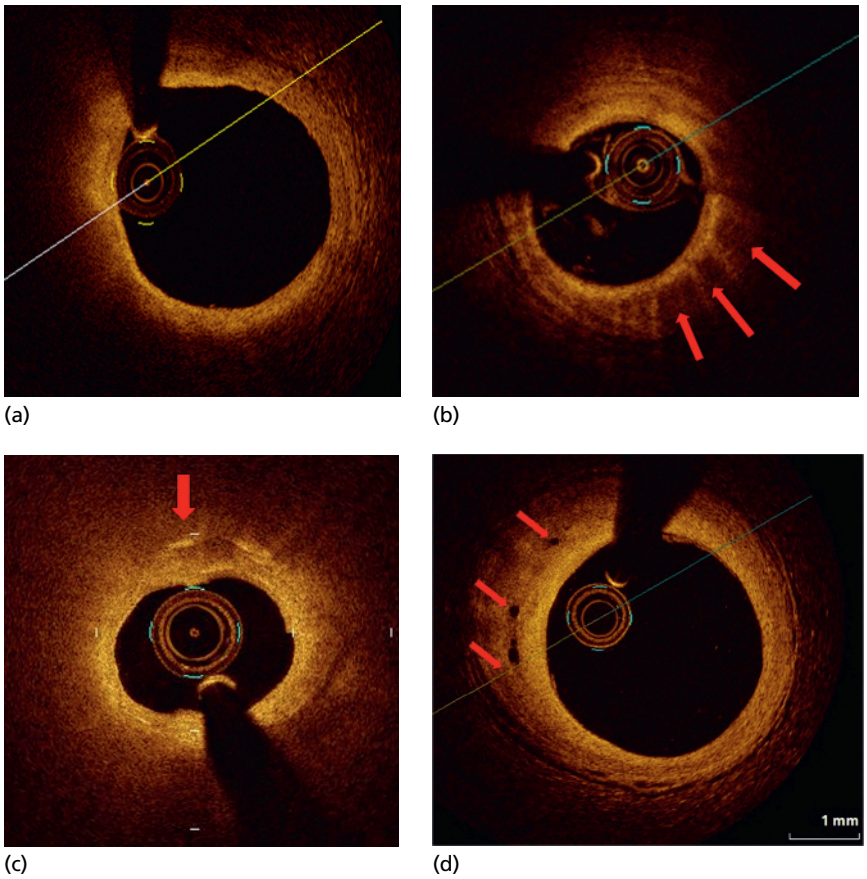


Figure 1.5 OCT appearances of vulnerable plaques (others). (a) Thin-cap fibroatheroma (7-9 o'clock of the plaque seen as red arrow): a presence of thin fibrous cap thickness as <65 μm overlying a signal-poor lesion with diffuse border representing a lipid-rich plaque. (b) Macrophage infiltration (red arrows): signal-rich, distinct or confluent punctuate regions with heterogeneous backward shadows. (c) Cholesterol crystals (red arrows): linear, highly backscattering structures within the plaque. (d) Neovascularization (red arrows): black holes with a diameter of 50-300 μm within plaque that are present on at least 3 consecutive frames.

Calcified nodule

Calcified nodule, pathologically defined as, the presence of fracture of a calcified plate protruding into the lumen through a disrupted fibrous cap with an overlying thrombus (Figure 1.4), is the least frequent pathological finding associated with coronary thrombosis [115]. Consistent with the histological findings, clinical OCT evaluation in a study of 126 patients with ACS found a prevalence of 7.9%, with being more common in older patients [132]. Thus, given that the prevalence of plaque rupture and erosion are higher enough to get details compared with calcified nodule, it remains a poorly understood entity. In pathology series, calcified nodules are more commonly found in older individuals in the mid-right coronary artery or left anterior descending artery where torsion is greatest [115]. Coronary calcification correlates highly with plaque burden and is an independent marker of cardiovascular risk [135]. Furthermore, coronary calcification within a thin fibrous cap can increase the circumferential stress leading to plaque rupture and thrombotic events [136]. OCT is the only tool to depict the thickness of superficial calcium deposits accurately, superior to IVUS [137]. Additionally, a great ability to detect calcified nodules have been reported with a sensitivity of 96% and specificity of 97% [138]. Recently, striking images showing a close correlation between pathological and OCT findings in a human coronary artery were reported [139]. However, more recent studies have raised an important issue with regard to the visualization of intense dorsal shadowing generated by protruding red thrombus and protruding bony calcified spicules [140,141]. Further investigation is required to clarify whether protruding luminal red thrombus emerged directly, or small bony calcified nodules generated intense dorsal shadowing.

OCT-derived Vulnerable Plaques

OCT- derived TCFA

With the high resolution of OCT, this modality might be the best for its reliability as a tool to measure the fibrous cap thickness in vivo. A histology study examined 38 human cadavers and showed a good correlation of the fibrous cap thickness between OCT and histology ($r=0.90$; $p<0.001$) [142]. In the clinical setting, OCT-TCFA was reported to be more frequently observed in the culprit lesion in patients with STEMI compared with those with stable coronary artery disease [143], and NSTEMI [122]. In addition, its distribution in the coronary tree was similar to that in the previous pathological observation demonstrating that TCFAs were more commonly found in the proximal distribution of the coronary artery [88, 144]. Subsequently, serial OCT imaging has been employed to assess the natural history and the impact of lipid modification on

atherosclerotic coronary plaques. OCT-TCFA has been shown to be a predictor of subsequent plaque progression and acute coronary events [145]. An early clinical study reported that in patients with AMI, the use of statins for nine months significantly increased fibrous cap thickness compared with placebo control,146 indicating that its effects of stabilization on vulnerable atherosclerotic coronary plaques. Subsequently, the effect of atorvastatin therapy on fibrous cap thickness in coronary atherosclerotic plaque assessed by optical coherence tomography (EASY-FIT) trial compared the efficacy of 20 mg and 5 mg atorvastatin on plaque microstructures acquired from OCT in 70 patients with unstable angina pectoris. The increase in fibrous cap thickness was significantly greater with the higher dose of atorvastatin (69% vs 17%, p<0.001), along with more favourable changes in lipid arc (–27% vs –8%, p<0.001) and macrophage grade (–38% vs –24%, p<0.001) [147].

Macrophage infiltration

Pathophysiologically, macrophages play a very important role in the development of atherosclerosis and are the predominant inflammatory cell population within the fibrous cap of vulnerable plaques [79]. TCFA manifested by the infiltration of macrophages (average size 20 to 50 μm) has been recognized as contributing to weakening structural integrity of the cap [148–150] and predispose plaques to rupture. They are more frequently found in coronary artery specimens obtained from patients with ACS compared to stable CAD.150 The high contrast and resolution of OCT enables the quantification of macrophages within fibrous caps. OCT visualizes macrophages as signal-rich, distinct or confluent punctuate regions with heterogeneous backward shadows (Figure 1.5). Tearney, *et al.* demonstrated that the OCT-derived macrophage density of plaque fibrous caps correlated strongly (r=0.84, p<0.0001) with macrophage density quantified histolomorphometrically by CD68 immunoperoxidase staining in the corresponding histological samples [151]. They also showed that macrophage density in the fibrous caps correlated with the circulating white blood cell count, which is used in clinical practice as a marker of systemic inflammation and has been shown to be an independent predictor of cardiovascular events, and presence of TCFA [152]. Thus, OCT has been employed to identify and quantify macrophage infiltration.

Cholesterol crystal (CCs)

CCs are formed very early in the process of atherogenesis and activate the proinflammatory cytokines interleukin-1-beta (IL-1β) and IL-18 via activating inflammasomes in neutrophils and monocytes/macrophages [153], which has been postulated to contribute to CC-mediated plaque destabilization. These findings are supported by the fact that demonstrated CCs are often seen abundantly within atheromatous plaques and at sites of plaque disruption [154]. Cholesterol crystallization in plaque is an important trigger for core expansion, leading to intimal injury [155]. Therefore, CCs have been thought to be one of the factors leading to disruption of the plaque cap and overlying intima. With OCT, cholesterol crystals (CCs) are defined as linear, highly backscattering structures within the plaque (Figure 1.5). Preliminary, Kataoka, *et al.* demonstrated that plaques containing CCs exhibited distinct OCT-derived microstructures associated with plaque vulnerability [156]. Recently, in a study that investigated the relationship between CCs and culprit lesion vulnerability in patients with ACS, the frequency of CCs was significantly higher in patients with STEMI compared to NSTEMI (50.8% vs 34.7%, p=0.032). Moreover, culprit lesions with CCs had higher prevalence of macrophage accumulation (77.8% vs 40.0%, p<0.001), microchannels (67.9% vs 24.8%, p<0.001), plaque rupture (58.0% vs 36.0%, p=0.001), thrombosis (66.7% vs 49.6%, p=0.016) and spotty calcification (35.8% vs 10.4%, p<0.001), suggesting that CCs may increase plaque vulnerability and trigger plaque rupture in patients with ACS [157].

Neovascularization

In atherosclerotic plaques, neovascularization proliferates from the adventitia into the arterial wall in order to supply nutrition and inflammatory cell accumulation to the lesion. Also, micro-vessels play a role in plaque haemorrhage associated with lipid core expansion through the accumulation of free cholesterol from erythrocyte membranes [89]. Therefore, pathological neovascularization of the artery wall is a consistent feature of atherosclerotic plaque development and progression of disease. In addition, it has also been recognized as a common feature of plaque vulnerability [89]. Some previous reports showed that micro-vessels are increased in coronary lesions from patients with acute myocardial infarction [158, 159]. Micro-channels are characterized using OCT as black holes with a diameter of 50–300 μm within plaque that are present on at least three consecutive frames (Figure 1.5). According to a study that examined 63 patients with CAD, micro-channels detected by OCT evaluation were associated with a higher incidence of concomitant TCFAs and increased high-sensitivity C-reactive protein levels, which indicates the importance of intraplaque micro-channels as a marker for plaque vulnerability [160].

Neoatherosclerosis

It is seen as diffusely bordered, signal-poor regions with overlying signal-rich bands, while calcific neointima shows well-delineated, signal-poor regions with sharp borders (Figure 1.1).

Near infrared spectroscopy (NIRS)

Advances in catheter-based spectroscopy enables generation of a chemical fingerprint of atherosclerotic plaque. Near infrared spectroscopy (NIRS) is a technique that has been used for decades in the physical sciences to characterize chemical composition for substances [161]. This method could be ideal for identification of lipid-rich and potentially vulnerable atherosclerotic plaques. The initial catheter-based system was LipiscanTM (InfraRedx Inc., Burlington, MA, USA). Subsequently, since NIRS imaging did not provide any volumetric data of plaques, the NIRS/intravascular ultrasound (IVUS) catheter (TVC Imaging SystemTM, InfraRedx Inc., Burlington, MA, USA) was developed. NIRS generates a color map that displays the lipid content of the arterial wall as a "chemogram". Every pixel represents the probability of lipid presence at the given location on a colour scale in which low probabilities of lipids are depicted as red, and high probabilities of lipids are shown as yellow (Figure 1.6). The "block chemogram" is a semiquantitative summary of the results for each 2 mm segment of the imaged artery. The lipid core burden index (LCBI) is the quantification of lipid present in the scanned region and is defined as the fraction of yellow pixels on the chemogram multiplied by 1000. The maximal lipid core burden index (max LCBI) per 4 mm describes the region with the highest lipid burden. ex vivo validation studies support the feasibility of

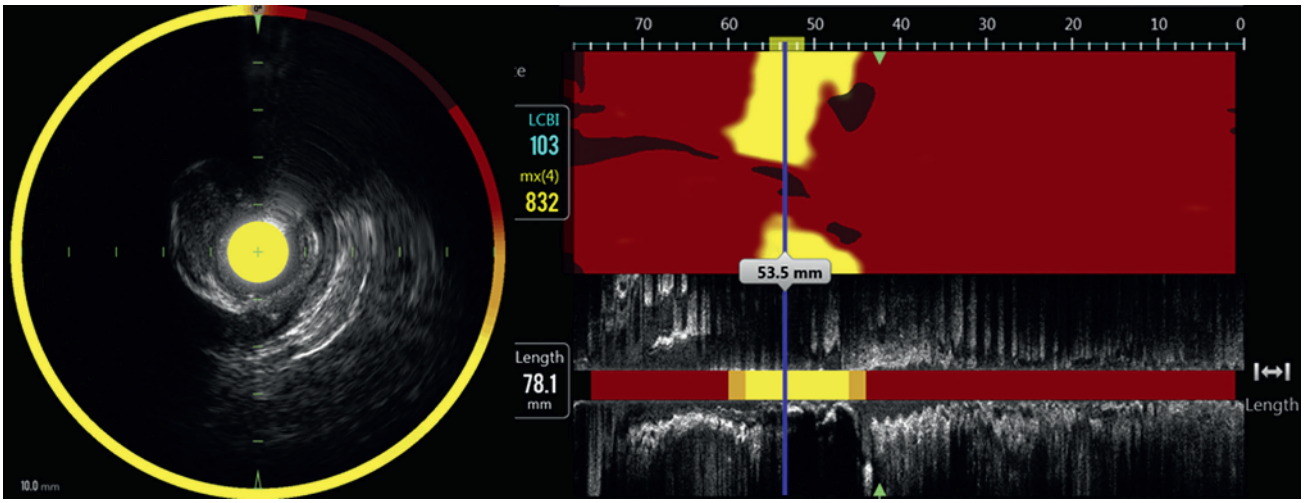


Figure 1.6 NIRS IVUS imaging of vulnerable plaque. Lipid rich plaque (yellow at left and right images): cross sectional image (left hand) and lon-gitudinal pullback image with its chemogram (right hand).

NIRS imaging with reasonable sensitivity and specificity for evaluating the extent of lipidic materials [162, 163]. Signal loss behind calcium due to acoustic shadowing is an important limitation for conventional IVUS, whereas NIRS penetrates effectively through calcium and stents, not affected by lower intensity due to attenuation. Additionally, with inability of IVUS and OCT to visualize or determine actual composition of lipidic plaques due to signal drop-out, NIRS offers a more reliable and quantitative detection of lipid core plaques than other intravascular imaging methods. On the other hand, a potential limitation of NIRS may be its inability to assess the depth of a lipid core and the measurement of lipid volume.

Lipid rich plaques

The common denominator for plaque rupture causing ACS is lipid accumulation, either as a lipid core or lipid pools [115]. This has been proved in a study that compared ACS culprit sites with lesions responsible for stable CAD using NIRS [164]. Subsequently, it has been shown that NIRS-IVUS is highly specific for the identification of ST-elevation myocardial infarction (STEMI) [165] and non-ST-elevation myocardial infarction [166]. According to these studies, a maximum LCBI4mm >400 was the optimal threshold for identification of culprit plaque in patients with both STEMI and non-STEMI. The visualization of lipid rich plaque on NIRS imaging has been also employed to predict PCI procedural risks and optimize coronary stent implantation. A clinical study examined 62 patients with CAD demonstrated that a maximum LCBI4mm >500 as a discriminatory threshold for the occurrence of periprocedural myocardial infarction after PCI [167]. Moreover, the prognostic value of NIRS-derived LCBI has been investigated in a study examined 203 patients with CAD [168]. Patients with an LCBI equal to or above the median of 43.0 had a fourfold risk of adverse cardiovascular events during one year follow-up. Thus, these results indicate the potential of intravascular NIRS imaging for risk stratification of cardiovascular events in patients with CAD. Additionally, the relationship of NIRS-derived LCBI with cardiovascular disease also indicates the potential benefit of pharmacological lipid rich plaque modulation prior to cardiovascular events. The reduction in yellow plaque by aggressive lipid lowering therapy (YELLOW) trial has demonstrated that short-term intensive statin therapy reduced lipid content using NIRS in severely obstructive coronary lesions compared with standard therapy [169]. However, in the integrated biomarker and imaging study 3 (IBIS-3) study, the effect of high intensity Rosuvastatin therapy on coronary plaque composition and LCBI was investigated within non-stenotic, non-culprit coronary segments with a relatively low atheroma burden and failed to demonstrate a significant reduction of necrotic core volume or LCBI under high intensity rosuvastatin therapy during one year [170]. Further investigations are required to better understand lipidic materials within vessel wall.

Serum markers correlated to plaque inflammation

Numerous studies have correlated different serologic biomarkers with cardiovascular disease [171–174] leading to a rapid increase in the number of biomarkers available (Table 1.2). 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 [175,176]. 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 low-density lipoprotein (LDL) cholesterol and glucose. However, 50% of heart attacks and strokes occur in individuals that have normal LDL cholesterol, and 20% of major adverse events occur in patients with no accepted risk factors [177]. Therefore, in light of changing

Table 1.2 Serologic markers of vulnerable plaque/patient.

Reflecting Metabolic and Immune Disorders	Reflecting Hypercoagulability	Reflecting Complex Atherosclerotic Plaque
• Abnormal lipoprotein profile (i.e. high LDL, low HDL, lipoprotein [a], etc.) • 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 • Serum markers of metabolic syndrome (diabetes or hypertriglyceridemia) • Specific markers of immune activation (i.e. anti-LDL antibody, anti heat shock protein (HSP) antibody • Markers of lipid peroxidation (i.e. ox-LDL and ox-HDL) • Homocysteine • PAPP-A • Circulating apoptosis markers (i.e. Fas/Fas ligand) • ADMA/DDAH (i.e. asymmetric dimethylarginine/dimethylarginine dimethylaminohydrolase) • Circulating NEFA (nonesterified fatty acids)	• Markers of blood hypercoagulability (i.e. fibrinogen, D-dimer, factor V of Leiden) • Increased platelet activation and aggregation (i.e. gene polymorphism of platelet glycoproteins IIb/IIIa, Ia/IIa, and Ib/IX) • Increased coagulation factors (i.e. clotting of factors V, VII, VIII, XIII, von Willebrand factor) • Decreased anticoagulation factors (i.e. protein S and C, thrombomodulin, antithrombin III) • Decreased endogenous fibrinolysis activity (i.e. reduced tissue plasminogen activator, increased type I plasminogen activator (PAI), PAI polymorphisms) • Prothrombin mutation (i.e. G20210A) • Thrombogenic factors (i.e. anticardiolipin antibodies, thrombocytosis, sickle cell disease, diabetes, hypercholesterolemia) • Transient hypercoagulability (i.e. smoking, dehydration, infection)	*Morphology/Structure* • Cap thickness • Lipid core size • Percentage stenosis • Remodelling (positive vs. negative) • Color (yellow, red) • Collagen content vs. lipid content • Calcification burden and pattern • Shear stress *Activity/function* • Plaque inflammation (macrophage density, rate of monocyte and activated T cells infiltration) • Endothelial denudation or dysfunction (local nitric oxide production, anti/procoaugulation properties of the endothelium) • Plaque oxidative stress • Superficial platelet aggregation and fibrin deposition • Rate of apoptosis (apoptosis protein markers, microsatellite) • Angiogenesis, leaking vasa vasorum, intraplaque haemorrhage • 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)

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 the past decade, the potential role of different micro-RNAs (miRNAs) in the cardiovascular system biomarkers has been widely recognised. Stable extracellular miRNAs circulate in the bloodstream and might serve as novel diagnostic markers for a wide range of cardiovascular diseases and risk factors, such as myocardial infarction [178,179], heart failure [180], coronary atherosclerotic disease [181], hypertension [182], and type 2 diabetes mellitus [183]. It is to be expected that combining multiple miRNAs into a single miRNA profile may provide improved accuracy.

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 the presented chapter but only listed in Table 1.1. As mentioned earlier, 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. 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 heredity) and serum markers (representing the net interaction between heredity 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 C-reactive protein (CRP), inflammatory cytokines soluble CD40L (sCD40L), soluble vascular adhesion molecules (sVCAM), and tumour necrosis factor (TNF).

C-reactive protein is a circulating pentraxin that plays a major role in the human innate immune response [184] and provides a stable plasma biomarker for low-grade systemic inflammation. C-reactive protein is produced predominantly in the liver as part of the acute phase response. However, CRP is also expressed in smooth muscle cells within diseased atherosclerotic arteries [185] and has been implicated in multiple aspects of atherogenesis and plaque vulnerability, including expression of adhesion molecules, induction of nitric oxide, altered complement function, and inhibition of intrinsic fibrinolysis [186], CRP is considered to be an independent predictor

of unfavourable cardiovascular events in patients with atherosclerotic disease. Beyond CRP's ability 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 [187] and that the efficacy of statin therapy may be related to the underlying level of vascular inflammation as detected by 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 [188, 189]. 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 [190, 191]. 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 incident peripheral arterial disease [192,193]. 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 [194]. The association of plaque morphology prone to rupture with hs-CRP has been also shown in the preliminary study [195]. Subsequently, a number of drug approaches to the inflammatory cardiovascular residual risk have been guided by knowledge of the inflammatory basis of cardiovascular disease. Recently, the CANTOS trial with the anti-IL-1-beta monoclonal antibody canakinumab, demonstrated the reduction of hs-CRP by 35% and reduced the composite of nonfatal-MI, nonfatal stroke or cardiovascular death (hazard ratio (HR) =0.85, 95%CI: 0.74–0.98) [196]. Additionally, canakinumab reduced MACE rates to a similar extent in patients with or without diabetes197 and was associated with a significant reduction in deaths from lung cancer [198].

Colchicine, another anti-inflammatory drug widely used for gout and pericarditis, has been examined to have a beneficial effect on cardiovascular disease, which showed the reduction of ischemic cardiovascular events in patients with MI [199], whereas a further trial investigating the efficacy of another anti-inflammatory drug of methotrexate, most commonly used for arthritis, on patients with coronary artery disease was stopped given that it did not result in the lowering of IL-1beta, IL-6 or hs-CRP compared to placebo and was associated with raised liver enzymes, reduction in leukocytes and hematocrit and a higher incidence of non-basal cell skin cancer compared to placebo [200]. Thus, given unclear the anti-inflammatory protective mechanism further investigation is required to better understand the association between inflammation and atherosclerotic disease.

Cellular adhesion molecules can be considered potential markers of vulnerability since such molecules are activated by inflammatory cytokines and then released by the endothelium.201 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 compared to subjects in the lower quartile [202]. Furthermore, soluble ICAM-1 and VCAM-1 levels showed a positive correlation with atherosclerosis disease burden [203]. IL-6 is expressed during the early phases of inflammation and it is the principle stimuli for CRP liver production. In addition, CD 40 ligand, a molecule expressed on cellular membrane, is a TNF-α homologue which stimulates activated macrophages proteolytic substances production.204 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 acute coronary syndrome (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 [205]. 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 adverse cardiovascular events after ACS [206] with increased levels portending a worse prognosis [207]. Importantly, specific therapeutic strategies have shown to be beneficial in reducing risk associated with sCD40L [208]. IL-18 is a pro-inflammatory cytokine mostly produced by monocytes and macrophages, and it acts synergistically with IL-12 [176]. Both these interleukines are expressed in the atherosclerotic plaque and they stimulate IFN-γ induction which, on its turn, inhibits collagen synthesis, preventing a thick fibrous cap formation and facilitating plaque destabilization. Mallat *et al.* [209] examined 40 stable and unstable atherosclerotic plaques obtained from patients undergoing carotid endarterectomy and they 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 (Figures 1.7 and 1.8).

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 smooth muscle cells 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 pro-inflammatory cytokine and proteolytic enzyme release, and stimulates endothelial cell migration and organizational behaviour with consequent neo-angiogenesis. Hence, IGF-1 represents one of the most important mediators in the transformation of a stable lesion into an unstable one [210]. Bayes-Genis *et al.* [211] demonstrated that PAPP-A is more expressed in the serum of patients with acute coronary syndromes (unstable angina, myocardial infarction), compared to subjects presenting with stable angina. In particular, PAPP-A serum levels > 10 mIU/l recognize patient vulnerability with a specificity of 78% and a sensibility of 89%. It has also been demonstrated that PAPP-A histological expression is higher in complex, vulnerable/ruptured carotid plaques compared to stable lesions [212]. Since PAPP-A serum levels can be easily measured today by means of ELISA, this protease could represent an easily quantifiable marker of vulnerability, with a

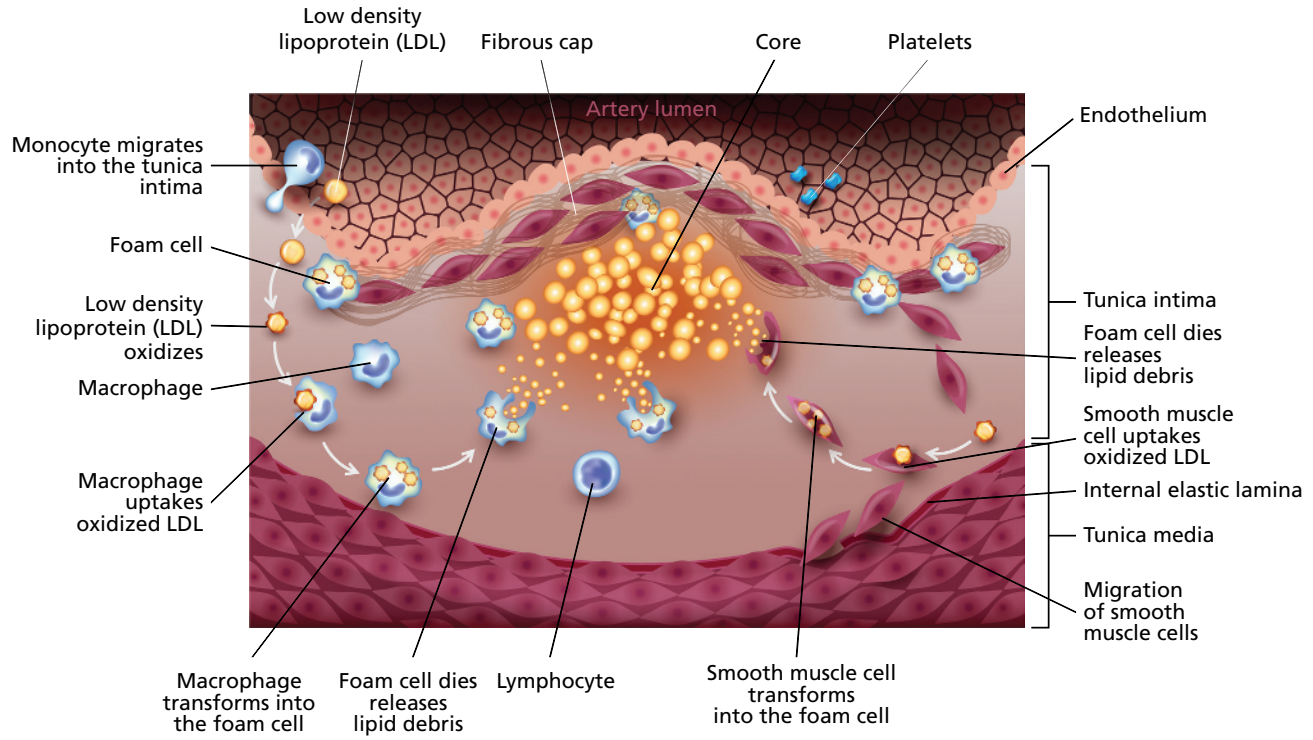


Figure 1.7 Schematic diagram of a stable atherosclerotic plaque characterized by the presence of a low inflammatory infiltrate. This type of lesion is constituted by a lipid core (extracellular lipid, cholesterol crystals and necrotic debris) covered by a ***thick*** fibrous cap consisting principally of smooth muscle cells in a collagenous-proteoglycan matrix, with varying degrees of infiltration by macrophages and T lymphocytes.

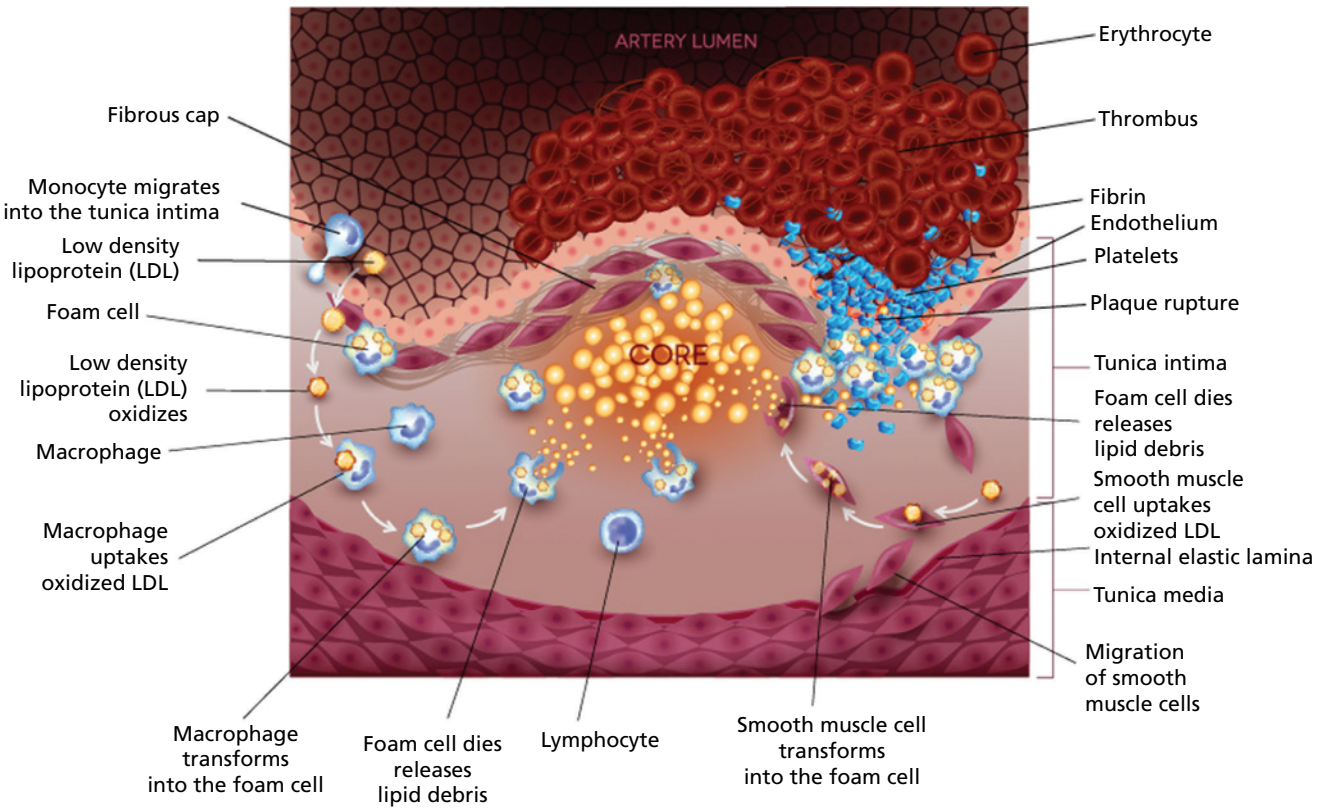


Figure 1.8 Unstable atherosclerotic plaque characterized by the presence of a *thin* fibrous cap rich in inflammatory macrophagic foam cells and T lymphocytes. Rupture of the fibrous cap at the shoulder region has resulted in thrombus formation.

reproducible method, allowing the identification of a patient subgroup with a high cerebrovascular risk, before its clinical event manifestation.

Jaffer *et al*. have published a detailed review on different techniques for detection of vulnerable plaque based on several biomarkers that have been implemented in recent years [213]. In this context, plaques with active inflammation may be identified directly by extensive macrophage accumulation [214]. Possible intravascular diagnostic techniques [215] based on inflammatory infiltration determination within the plaque include thermography [216] contrast-enhanced MRI [217] fluorodeoxyglucose positron emission tomography [218] and immunoscintigraphy [219]. In addition, non-invasive techniques include MRI with superparamagnetic iron oxide [220, 221], and gadolinium fluorine compounds [222, 223].

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 [224]. Vascular endothelium is subjected to complex mechanical stresses resulting from its 3-D 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 the endothelial cells [225, 226]. High wall shear stress (WSS) (>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 [225]. It has been shown that the plaques and wall thickenings are localized mostly on the outer wall of one or both daughter vessels at bifurcations and along the inner wall of curved segments [224]. In the PREDICTION study, Stone *et al*. 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. 74% patients had follow-up studies at 6 to 10 months to relate the effects of local haemodynamic milieu on plaque changes. Authors reported that decrease in lumen area was independently predicted by baseline large plaque burden and low endothelial shear stress [227]. Other investigators have reported that high wall shear stress is associated with transformation of plaques into high risk phenotypes prone to instability and rupture [228,229].

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 plays 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 haemorrhages 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.

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 [84, 87]. 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, pregnancy-associated plasma protein-A, pentraxin-3, LpPLA2 are currently the best candidates for diffuse active plaque detection. In order to achieve this aim, a coordinate 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**

References

1 Herrington W, Lacey B, Sherliker P, *et al*. Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. *Circ Res*. 2016; **118**:535–46.
2 Pahwa R and Jialal I. Atherosclerosis *StatPearls* Treasure Island (FL); 2020.
3 Wu MY, Li CJ, Hou MF and Chu PY. New Insights into the Role of Inflammation in the Pathogenesis of Atherosclerosis. *Int J Mol Sci*. 2017; **18**.
4 Insull W, Jr. The pathology of atherosclerosis: plaque development and plaque responses to medical treatment. *Am J Med*. 2009; **122**:S3–S14.
5 Forstermann U, Xia N, Li H. Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis. *Circ Res*. 2017; **120**:713–735.

6 Libby P, Ridker PM, Hansson GK, Leducq Transatlantic Network on A. Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol.* 2009; **54**:2129–38.
7 Libby P, Buring JE, Badimon L, Atherosclerosis. *Nat Rev Dis Primers.* 2019; **5**:56.
8 Gimbrone MA, Jr., Garcia-Cardena G. Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis. *Circ Res.* 2016; **118**:620–36.
9 Ghattas A, Griffiths HR, Devitt A, Monocytes in coronary artery disease and atherosclerosis: where are we now? *J Am Coll Cardiol.* 2013; **62**:1541–51.
10 Gimbrone MA, Jr., Garcia-Cardena G. Vascular endothelium, hemodynamics, and the pathobiology of atherosclerosis. *Cardiovasc Pathol.* 2013; **22**:9–15.
11 Davies PF. Flow-mediated endothelial mechanotransduction. *Physiol Rev.* 1995; **75**:519–60.
12 Gimbrone MA, Jr., Topper JN, Nagel T, Anderson KR and Garcia-Cardena G. Endothelial dysfunction, hemodynamic forces, and atherogenesis. *Ann N Y Acad Sci.* 2000; **902**:230–9; discussion 239–40.
13 Pober JS, Cotran RS. The role of endothelial cells in inflammation. *Transplantation.* 1990; **50**:537–44.
14 Egan K, FitzGerald GA. Eicosanoids and the vascular endothelium. *Handb Exp Pharmacol.* 2006:189–211.
15 Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. *Nat Rev Immunol.* 2007; **7**:803–15.
16 Stevens T, Garcia JG, Shasby DM, Bhattacharya J and Malik AB. Mechanisms regulating endothelial cell barrier function. *Am J Physiol Lung Cell Mol Physiol.* 2000; **279**:L419–22.
17 De Caterina R, Libby P, Peng HB, *et al.* Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest.* 1995; **96**:60–8.
18 Frangogiannis NG. Targeting the inflammatory response in healing myocardial infarcts. *Curr Med Chem.* 2006; **13**:1877–93.
19 Gainetdinov RR, Premont RT, Bohn LM, *et al.* Desensitization of G protein-coupled receptors and neuronal functions. *Annu Rev Neurosci.* 2004; **27**:107–44.
20 Pober JS. Effects of tumour necrosis factor and related cytokines on vascular endothelial cells. *Ciba Found Symp.* 1987; **131**:170–84.
21 Petrache I, Birukova A, Ramirez SI, *et al.* The role of the microtubules in tumor necrosis factor-alpha-induced endothelial cell permeability. *Am J Respir Cell Mol Biol.* 2003; **28**:574–81.
22 Cybulsky MI, Gimbrone MA, Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science.* 1991; **251**:788–91.
23 Osborn L, Hession C, Tizard R, *et al.* Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell.* 1989; **59**:1203–11.
24 Lambert JM, Lopez EF, Lindsey ML. Macrophage roles following myocardial infarction. *Int J Cardiol.* 2008; **130**:147–58.
25 Frangogiannis NG, Mendoza LH, Ren G, *et al.* MCSF expression is induced in healing myocardial infarcts and may regulate monocyte and endothelial cell phenotype. *Am J Physiol Heart Circ Physiol.* 2003; **285**:H483–92.
26 Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol.* 2006; **6**:508–19.
27 Hansson GK, Libby P, Schonbeck U, Yan ZQ. Innate and adaptive immunity in the pathogenesis of atherosclerosis. *Circ Res.* 2002; **91**:281–91.
28 Verma S, Devaraj S, Jialal I. Is C-reactive protein an innocent bystander or proatherogenic culprit? C-reactive protein promotes atherothrombosis. *Circulation.* 2006; **113**:2135–50; discussion 2150.
29 Rader DJ, Daugherty A. Translating molecular discoveries into new therapies for atherosclerosis. *Nature.* 2008; **451**:904–13.
30 Herder M, Arntzen KA, Johnsen SH, *et al.* Long-term use of lipid-lowering drugs slows progression of carotid atherosclerosis: the Tromso study 1994 to 2008. *Arterioscler Thromb Vasc Biol.* 2013; **33**:858–62.
31 Puri R, Nissen SE, Shao M, *et al.* Antiatherosclerotic effects of long-term maximally intensive statin therapy after acute coronary syndrome: insights from Study of Coronary Atheroma by Intravascular Ultrasound: Effect of Rosuvastatin Versus Atorvastatin. *Arterioscler Thromb Vasc Biol.* 2014; **34**:2465–72.
32 Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. *J Lipid Res.* 2002; **43**:1363–79.
33 Rader DJ, Hovingh GK. HDL and cardiovascular disease. *The Lancet.* 2014; **384**:618–625.
34 Barter PJ, Caulfield M, Eriksson M, *et al.* Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med.* 2007; **357**:2109–22.
35 Toth PP, Barter PJ, Rosenson RS, *et al.* High-density lipoproteins: a consensus statement from the National Lipid Association. *J Clin Lipidol.* 2013; **7**:484–525.
36 Degoma EM, Rader DJ. Novel HDL-directed pharmacotherapeutic strategies. *Nat Rev Cardiol.* 2011; **8**:266–77.
37 Kratzer A, Giral H, Landmesser U. High-density lipoproteins as modulators of endothelial cell functions: alterations in patients with coronary artery disease. *Cardiovasc Res.* 2014; **103**:350–61.
38 Boren J, Williams KJ. The central role of arterial retention of cholesterol-rich apolipoprotein-B-containing lipoproteins in the pathogenesis of atherosclerosis: a triumph of simplicity. *Curr Opin Lipidol.* 2016; **27**:473–83.
39 Miller YI, Choi SH, Wiesner P *et al.* Oxidation-specific epitopes are danger-associated molecular patterns recognized by pattern recognition receptors of innate immunity. *Circ Res.* 2011; **108**:235–48.
40 Navab M, Ananthramaiah GM, Reddy ST, *et al.* The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. *J Lipid Res.* 2004; **45**:993–1007.
41 Linton MRF, Yancey PG, Davies SS, *et al.* The Role of Lipids and Lipoproteins in Atherosclerosis. In: KR Feingold, B Anawalt, A Boyce, *et al.* eds. *Endotext* South Dartmouth (MA); 2000.
42 Gistera A, Hansson GK. The immunology of atherosclerosis. *Nat Rev Nephrol.* 2017; **13**:368–380.
43 Witztum JL, Steinberg D. The oxidative modification hypothesis of atherosclerosis: does it hold for humans? *Trends Cardiovasc Med.* 2001; **11**:93–102.
44 Stocker R, Keaney JF, Jr. Role of oxidative modifications in atherosclerosis. *Physiol Rev.* 2004; **84**:1381–478.
45 Quinn MT, Gauss KA. Structure and regulation of the neutrophil respiratory burst oxidase: comparison with nonphagocyte oxidases. *J Leukoc Biol.* 2004; **76**:760–81.
46 Lambeth JD. NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol.* 2004; **4**:181–9.
47 Octavia Y, Brunner-La Rocca HP, Moens AL. NADPH oxidase-dependent oxidative stress in the failing heart: From pathogenic roles to therapeutic approach. *Free Radic Biol Med.* 2012; **52**:291–7.
48 Opitz N, Drummond GR, Selemidis S, *et al.* The 'A's and 'O's of NADPH oxidase regulation: a commentary on "Subcellular localization and function of alternatively spliced Noxo1 isoforms". *Free Radic Biol Med.* 2007; **42**:175–9.
49 Lyle AN, Griendling KK. Modulation of vascular smooth muscle signaling by reactive oxygen species. *Physiology (Bethesda).* 2006; **21**:269–80.
50 Briones AM, Tabet F, Callera GE, *et al.* Differential regulation of Nox1, Nox2 and Nox4 in vascular smooth muscle cells from WKY and SHR. *J Am Soc Hypertens.* 2011; **5**:137–53.
51 Akki A, Zhang M, Murdoch C, *et al.* NADPH oxidase signaling and cardiac myocyte function. *J Mol Cell Cardiol.* 2009; **47**:15–22.
52 Kattoor AJ, Pothineni NVK, Palagiri D, Mehta JL. Oxidative Stress in Atherosclerosis. *Curr Atheroscler Rep.* 2017; **19**:42.
53 Vendrov AE, Hakim ZS, Madamanchi NR, *et al.* Atherosclerosis is attenuated by limiting superoxide generation in both macrophages and vessel wall cells. *Arterioscler Thromb Vasc Biol.* 2007; **27**:2714–21.
54 Forstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *Eur Heart J.* 2012; **33**:829–37, 837a–837d.
55 Khan BV, Harrison DG, Olbrych MT, *et al.* Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. *Proc Natl Acad Sci U S A.* 1996; **93**:9114–9.
56 Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci U S A.* 1991; **88**:4651–5.
57 Zeiher AM, Fisslthaler B, Schray-Utz B, Busse R. Nitric oxide modulates the expression of monocyte chemoattractant protein 1 in cultured human endothelial cells. *Circ Res.* 1995; **76**:980–6.
58 Teplyakov AI. Endothelin-1 involved in systemic cytokine network inflammatory response at atherosclerosis. *Journal of cardiovascular pharmacology.* 2004; **44** Suppl 1:S274–5.
59 Lerman A, Edwards BS, Hallett JW, *et al.* Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. *N Engl J Med.* 1991; **325**:997–1001.
60 Dang A, Wang B, Li W, *et al.* Plasma endothelin-1 levels and circulating endothelial cells in patients with aortoarteritis. *Hypertension research: official journal of the Japanese Society of Hypertension.* 2000; **23**:541–4.
61 McCarron RM, Wang L, Stanimirovic DB, Spatz M. Endothelin induction of adhesion molecule expression on human brain microvascular endothelial cells. *Neuroscience letters.* 1993; **156**:31–4.
62 Halim A, Kanayama N, el Maradny E, *et al.* Coagulation in vivo microcirculation and in vitro caused by endothelin-1. *Thrombosis research.* 1993; **72**:203–9.
63 Li H, Forstermann U. Uncoupling of endothelial NO synthase in atherosclerosis and vascular disease. *Curr Opin Pharmacol.* 2013; **13**:161–7.
64 Antoniades C, Shirodaria C, Crabtree M, *et al.* Altered plasma versus vascular biopterins in human atherosclerosis reveal relationships between endothelial nitric oxide synthase coupling, endothelial function, and inflammation. *Circulation.* 2007; **116**:2851–9.
65 Porkert M, Sher S, Reddy U, *et al.* Tetrahydrobiopterin: a novel antihypertensive therapy. *J Hum Hypertens.* 2008; **22**:401–7.
66 Stroes E, Kastelein J, Cosentino F, *et al.* Tetrahydrobiopterin restores endothelial function in hypercholesterolemia. *J Clin Invest.* 1997; **99**:41–6.

67 Thum T, Fraccarollo D, Schultheiss M, *et al*. Endothelial nitric oxide synthase uncoupling impairs endothelial progenitor cell mobilization and function in diabetes. *Diabetes*. 2007; **56**:666–74.

68 Ueda S, Matsuoka H, Miyazaki H, *et al*. Tetrahydrobiopterin restores endothelial function in long-term smokers. *J Am Coll Cardiol.* 2000; **35**:71–5.

69 Madamanchi NR and Runge MS. Mitochondrial dysfunction in atherosclerosis. *Circ Res*. 2007; **100**:460–73.

70 Nishino T, Okamoto K, Eger BT, *et al*. Mammalian xanthine oxidoreductase – mechanism of transition from xanthine dehydrogenase to xanthine oxidase. *FEBS J*. 2008; **275**:3278–89.

71 Patetsios P, Song M, Shutze WP, *et al*. Identification of uric acid and xanthine oxidase in atherosclerotic plaque. *Am J Cardiol.* 2001; **88**:188–91, A6.

72 Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. *Circ Res*. 2014; **114**:1852–66.

73 Kragel AH, Reddy SG, Wittes JT, Roberts WC. Morphometric analysis of the composition of atherosclerotic plaques in the four major epicardial coronary arteries in acute myocardial infarction and in sudden coronary death. *Circulation*. 1989; **80**:1747–56.

74 Clinton SK, Underwood R, Hayes L, *et al*. Macrophage colony-stimulating factor gene expression in vascular cells and in experimental and human atherosclerosis. *The American Journal of Pathology*. 1992; **140**:301–16.

75 Saigusa R, Winkels H, Ley K. T cell subsets and functions in atherosclerosis. *Nat Rev Cardiol.* 2020; **17**:387–401.

76 Fernandez DM, Rahman AH, Fernandez NF, *et al*. Single-cell immune landscape of human atherosclerotic plaques. *Nat Med*. 2019; **25**:1576–1588.

77 Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest*. 1994; **94**:2493–503.

78 Schwartz RS, Topol EJ, Serruys PW, *et al*. Artery size, neointima, and remodeling: time for some standards. *J Am Coll Cardiol.* 1998; **32**:2087–94.

79 Falk E, Nakano M, Bentzon JF, *et al*. Update on acute coronary syndromes: the pathologists' view. *Eur Heart J*. 2013; **34**:719–28.

80 Burke AP, Farb A, Malcom GT, *et al*. Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. *The N Engl J Med*. 1997; **336**:1276–82.

81 Wang JC, Normand SL, Mauri L, Kuntz RE. Coronary artery spatial distribution of acute myocardial infarction occlusions. *Circulation*. 2004; **110**:278–84.

82 Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes (1). *N Engl J Med*. 1992; **326**:242–50.

83 Davies MJ. Stability and instability: two faces of coronary atherosclerosis. The Paul Dudley White Lecture 1995. *Circulation*. 1996; **94**:2013–20.

84 Mauriello A, Sangiorgi G, Fratoni S, *et al*. Diffuse and active inflammation occurs in both vulnerable and stable plaques of the entire coronary tree: a histopathologic study of patients dying of acute myocardial infarction. *J Am Coll Cardiol.* 2005; **45**:1585–93.

85 Spagnoli LG, Bonanno E, Mauriello A, *et al*. Multicentric inflammation in epicardial coronary arteries of patients dying of acute myocardial infarction. *J Am Coll Cardiol.* 2002; **40**:1579–88.

86 Buffon A, Biasucci LM, Liuzzo G, *et al*. Widespread coronary inflammation in unstable angina. *N Engl J Med*. 2002; **347**:5–12.

87 Goldstein JA, Demetriou D, Grines CL, *et al*. Multiple complex coronary plaques in patients with acute myocardial infarction. *N Engl J Med*. 2000; **343**:915–22.

88 Kolodgie FD, Burke AP, Farb A, *et al*. The thin-cap fibroatheroma: a type of vulnerable plaque: the major precursor lesion to acute coronary syndromes. *Curr Opin Cardiol.* 2001; **16**:285–92.

89 Kolodgie FD, Gold HK, Burke AP, *et al*. Intraplaque hemorrhage and progression of coronary atheroma. *N Engl J Med*. 2003; **349**:2316–25.

90 van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation*. 1994; **89**:36–44.

91 Mittleman MA, Mostofsky E. Physical, psychological and chemical triggers of acute cardiovascular events: preventive strategies. *Circulation*. 2011; **124**:346–54.

92 Widlansky ME, Gokce N, Keaney JF, Jr., Vita JA. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol.* 2003; **42**:1149–60.

93 Benagiano M, D'Elios MM, Amedei A, *et al*. Human 60-kDa heat shock protein is a target autoantigen of T cells derived from atherosclerotic plaques. *Journal of Immunology*. 2005; **174**:6509–17.

94 Ludwig A, Berkhout T, Moores K, *et al*. Fractalkine is expressed by smooth muscle cells in response to IFN-gamma and TNF-alpha and is modulated by metalloproteinase activity. *Journal of Immunology*. 2002; **168**:604–12.

95 Fong AM, Robinson LA, Steeber DA, *et al*. Fractalkine and CX3CR1 mediate a novel mechanism of leukocyte capture, firm adhesion, and activation under physiologic flow. *The Journal of Experimental Medicine*. 1998; **188**:1413–9.

96 Pasterkamp G, den Ruijter HM, Libby P. Temporal shifts in clinical presentation and underlying mechanisms of atherosclerotic disease. *Nat Rev Cardiol.* 2017; **14**:21–29.

97 Quillard T, Franck G, Mawson T, *et al*. Mechanisms of erosion of atherosclerotic plaques. *Curr Opin Lipidol.* 2017; **28**:434–441.

98 Hao H, Gabbiani G, Camenzind E, Bacchetta M, *et al*. Phenotypic modulation of intima and media smooth muscle cells in fatal cases of coronary artery lesion. *Arterioscler Thromb Vasc Biol.* 2006; **26**:326–32.

99 Finn AV, Otsuka F. Neoatherosclerosis: a culprit in very late stent thrombosis. *Circ Cardiovasc Interv.* 2012; **5**:6–9.

100 Nakazawa G, Otsuka F, Nakano M, *et al*. The pathology of neoatherosclerosis in human coronary implants bare-metal and drug-eluting stents. *J Am Coll Cardiol.* 2011; **57**:1314–22.

101 Park SJ, Kang SJ, Virmani R, *et al*. In-stent neoatherosclerosis: a final common pathway of late stent failure. *J Am Coll Cardiol.* 2012; **59**:2051–7.

102 Inoue K, Abe K, Ando K, *et al*. Pathological analyses of long-term intracoronary Palmaz-Schatz stenting; Is its efficacy permanent? *Cardiovasc Pathol.* 2004; **13**:109–15.

103 Nakazawa G, Vorpahl M, Finn AV, *et al*. One step forward and two steps back with drug-eluting-stents: from preventing restenosis to causing late thrombosis and nouveau atherosclerosis. *JACC Cardiovasc Imaging.* 2009; **2**:625–8.

104 Kang SJ, Lee CW, Song H, *et al*. OCT analysis in patients with very late stent thrombosis. *JACC Cardiovasc Imaging.* 2013; **6**:695–703.

105 Virmani R, Burke AP, Kolodgie FD, Farb A. Vulnerable plaque: the pathology of unstable coronary lesions. *J Interv Cardiol.* 2002; **15**:439–46.

106 Shishikura D, Kataoka Y, Di Giovanni G, *et al*. Progression of ultrasound plaque attenuation and low echogenicity associates with major adverse cardiovascular events. *Eur Heart J*. 2020.

107 Wu X, Mintz GS, Xu K, *et al*. The Relationship Between Attenuated Plaque Identified by Intravascular Ultrasound and No-Reflow After Stenting in Acute Myocardial Infarction. *The HORIZONS-AMI (Harmonizing Outcomes With Revascularization and Stents in Acute Myocardial Infarction) Trial*. 2011; **4**:495–502.

108 Yamagishi M, Terashima M, Awano K, *et al*. Morphology of vulnerable coronary plaque: insights from follow-up of patients examined by intravascular ultrasound before an acute coronary syndrome. *J Am Coll Cardiol.* 2000; **35**:106–111.

109 Ehara S, Kobayashi Y, Yoshiyama M, *et al*. Spotty calcification typifies the culprit plaque in patients with acute myocardial infarction: an intravascular ultrasound study. *Circulation*. 2004; **110**:3424–3429.

110 Lee T, Kakuta T, Yonetsu T, *et al*. Assessment of echo-attenuated plaque by optical coherence tomography and its impact on post-procedural creatine kinase-myocardial band elevation in elective stent implantation. *JACC: Cardiovascular Interventions*. 2011; **4**:483–491.

111 Kataoka Y, Wolski K, Uno K, *et al*. Spotty calcification as a marker of accelerated progression of coronary atherosclerosis: insights from serial intravascular ultrasound. *J Am Coll Cardiol.* 2012; **59**:1592–1597.

112 Pu J, Mintz GS, Biro S, *et al*. Insights into echo-attenuated plaques, echolucent plaques, and plaques with spotty calcification: novel findings from comparisons among intravascular ultrasound, near-infrared spectroscopy, and pathological histology in 2,294 human coronary artery segments. *J Am Coll Cardiol.* 2014; **63**:2220–2233.

113 Nair A, Kuban BD, Tuzcu EM, *et al*. Coronary plaque classification with intravascular ultrasound radiofrequency data analysis. *Circulation*. 2002; **106**:2200–6.

114 Farb A, Burke AP, Tang AL, *et al*. Coronary plaque erosion without rupture into a lipid core. A frequent cause of coronary thrombosis in sudden coronary death. *Circulation*. 1996; **93**:1354–63.

115 Virmani R, Kolodgie FD, Burke AP, *et al*. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol.* 2000; **20**:1262–75.

116 Hong MK, Mintz GS, Lee CW, *et al*. A three-vessel virtual histology intravascular ultrasound analysis of frequency and distribution of thin-cap fibroatheromas in patients with acute coronary syndrome or stable angina pectoris. *Am J Cardiol.* 2008; **101**:568–72.

117 Stone GW, Maehara A, Lansky AJ, *et al*. A prospective natural-history study of coronary atherosclerosis. *N Engl J Med*. 2011; **364**:226–35.

118 Calvert PA, Obaid DR, O'Sullivan M, *et al*. Association between IVUS findings and adverse outcomes in patients with coronary artery disease: the VIVA (VH-IVUS in Vulnerable Atherosclerosis) Study. *JACC Cardiovasc Imaging*. 2011; **4**:894–901.

119 Cheng JM, Garcia-Garcia HM, de Boer SP, *et al*. in vivo detection of high-risk coronary plaques by radiofrequency intravascular ultrasound and cardiovascular outcome: results of the ATHEROREMO-IVUS study. *Eur Heart J*. 2014; **35**:639–47.

120 Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the vulnerable plaque. *J Am Coll Cardiol.* 2006; **47**:C13–8.

121 Stone PH, Saito S, Takahashi S, *et al*. Prediction of progression of coronary artery disease and clinical outcomes using vascular profiling of endothelial shear stress and arterial plaque characteristics: the PREDICTION Study. *Circulation*. 2012; **126**:172–181.

122 Ino Y, Kubo T, Tanaka A, *et al*. Difference of culprit lesion morphologies between ST-segment elevation myocardial infarction and non-ST-segment elevation acute coronary syndrome: an optical coherence tomography study. *JACC Cardiovasc Interv.* 2011; **4**:76–82.

123 Vergallo R, Ren X, Yonetsu T, *et al*. Pancoronary plaque vulnerability in patients with acute coronary syndrome and ruptured culprit plaque: a 3-vessel optical coherence tomography study. *Am Heart J*. 2014; **167**:59–67.

124 Virmani R, Kolodgie FD, Burke AP *et al*., Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol.* 2000; **20**:1262–1275.

125 Soeda T, Higuma T, Abe N, *et al*. Morphological predictors for no reflow phenomenon after primary percutaneous coronary intervention in patients with ST-segment elevation myocardial infarction caused by plaque rupture. *European Heart Journal-Cardiovascular Imaging.* 2017; **18**:103–110.

126 Satogami K, Ino Y, Kubo T, *et al*. Impact of plaque rupture detected by optical coherence tomography on transmural extent of infarction after successful stenting in ST-segment elevation acute myocardial infarction. *JACC: Cardiovascular Interventions.* 2017; **10**:1025–1033.

127 Burke AP, Farb A, Malcom GT, *et al*. Effect of risk factors on the mechanism of acute thrombosis and sudden coronary death in women. *Circulation.* 1998; **97**:2110–6.

128 Tearney GJ, Regar E, Akasaka T, *et al*. Consensus standards for acquisition, measurement, and reporting of intravascular optical coherence tomography studies: a report from the International Working Group for Intravascular Optical Coherence Tomography Standardization and Validation. *J Am Coll Cardiol.* 2012; **59**:1058–72.

129 Campbell IC, Suever JD, Timmins LH, *et al*. Biomechanics and inflammation in atherosclerotic plaque erosion and plaque rupture: implications for cardiovascular events in women. *PLoS One.* 2014; **9**:e111785.

130 Chandran S, Watkins J, Abdul-Aziz A, *et al*. Inflammatory Differences in Plaque Erosion and Rupture in Patients With ST-Segment Elevation Myocardial Infarction. *J Am Heart Assoc.* 2017; **6**.

131 Kramer MC, Rittersma SZ, de Winter RJ, *et al*. Relationship of thrombus healing to underlying plaque morphology in sudden coronary death. *J Am Coll Cardiol.* 2010; **55**:122–32.

132 Jia H, Abtahian F, Aguirre AD, *et al*. in vivo diagnosis of plaque erosion and calcified nodule in patients with acute coronary syndrome by intravascular optical coherence tomography. *J Am Coll Cardiol.* 2013; **62**:1748–58.

133 Prati F, Uemura S, Souteyrand G, *et al*. OCT-based diagnosis and management of STEMI associated with intact fibrous cap. *JACC Cardiovasc Imaging.* 2013; **6**:283–7.

134 Jia H, Dai J, Hou J, *et al*. Effective anti-thrombotic therapy without stenting: intravascular optical coherence tomography-based management in plaque erosion (the EROSION study). *Eur Heart J.* 2017; **38**:792–800.

135 Greenland P, Bonow RO, Brundage BH, *et al*. American College of Cardiology Foundation Clinical Expert Consensus Task F, Society of Atherosclerosis I, Prevention and Society of Cardiovascular Computed T. ACCF/AHA 2007 clinical expert consensus document on coronary artery calcium scoring by computed tomography in global cardiovascular risk assessment and in evaluation of patients with chest pain: a report of the American College of Cardiology Foundation Clinical Expert Consensus Task Force (ACCF/AHA Writing Committee to Update the 2000 Expert Consensus Document on Electron Beam Computed Tomography) developed in collaboration with the Society of Atherosclerosis Imaging and Prevention and the Society of Cardiovascular Computed Tomography. *J Am Coll Cardiol.* 2007; **49**:378–402.

136 Vengrenyuk Y, Carlier S, Xanthos S, *et al*. A hypothesis for vulnerable plaque rupture due to stress-induced debonding around cellular microcalcifications in thin fibrous caps. *Proc Natl Acad Sci U S A.* 2006; **103**:14678–83.

137 Kume T, Okura H, Kawamoto T, *et al*. Assessment of the coronary calcification by optical coherence tomography. *EuroIntervention.* 2011; **6**:768–72.

138 Yabushita H, Bouma BE, Houser SL, *et al*. Characterization of human atherosclerosis by optical coherence tomography. *Circulation.* 2002; **106**:1640–5.

139 Hao H, Fujii K, Shibuya M, *et al*. Different findings in a calcified nodule between histology and intravascular imaging such as intravascular ultrasound, optical coherence tomography, and coronary angioscopy. *JACC Cardiovasc Interv.* 2014; **7**:937–8.

140 Alfonso F, Cuesta J, Bastante T, *et al*. Calcified nodule mimicking red thrombus on optical coherence tomography. *JACC Cardiovasc Interv.* 2015; **8**:120–1.

141 Alfonso F, Gonzalo N, Nunez-Gil I, Banuelos C. Coronary thrombosis from large, nonprotruding, superficial calcified coronary plaques. *J Am Coll Cardiol.* 2013; **62**:2254.

142 Kume T, Akasaka T, Kawamoto T, *et al*. Measurement of the thickness of the fibrous cap by optical coherence tomography. *Am Heart J.* 2006; **152**:755 e1–4.

143 Kubo T, Imanishi T, Kashiwagi M, *et al*. Multiple coronary lesion instability in patients with acute myocardial infarction as determined by optical coherence tomography. *Am J Cardiol.* 2010; **105**:318–22.

144 Fujii K, Kawasaki D, Masutani M, *et al*. OCT assessment of thin-cap fibroatheroma distribution in native coronary arteries. *JACC Cardiovasc Imaging.* 2010; **3**:168–75.

145 Uemura S, Ishigami K, Soeda T,*et al*. Thin-cap fibroatheroma and microchannel findings in optical coherence tomography correlate with subsequent progression of coronary atheromatous plaques. *Eur Heart J.* 2012; **33**:78–85.

146 Takarada S, Imanishi T, Kubo T, *et al*. Effect of statin therapy on coronary fibrous-cap thickness in patients with acute coronary syndrome: assessment by optical coherence tomography study. *Atherosclerosis.* 2009; **202**:491–7.

147 Komukai K, Kubo T, Kitabata H, *et al*. Effect of atorvastatin therapy on fibrous cap thickness in coronary atherosclerotic plaque as assessed by optical coherence tomography: the EASY-FIT study. *J Am Coll Cardiol.* 2014; **64**:2207–17.

148 Fuster V. Lewis A. Conner Memorial Lecture. Mechanisms leading to myocardial infarction: insights from studies of vascular biology. *Circulation.* 1994; **90**:2126–46.

149 Lendon CL, Davies MJ, Born GV, Richardson PD. Atherosclerotic plaque caps are locally weakened when macrophages density is increased. *Atherosclerosis.* 1991; **87**:87–90.

150 Moreno PR, Falk E, Palacios IF, *et al*. Macrophage infiltration in acute coronary syndromes. Implications for plaque rupture. *Circulation.* 1994; **90**:775–8.

151 Tearney GJ, Yabushita H, Houser SL, *et al*. Quantification of macrophage content in atherosclerotic plaques by optical coherence tomography. *Circulation.* 2003; **107**:113–9.

152 Raffel OC, Tearney GJ, Gauthier DD, *et al*. Relationship between a systemic inflammatory marker, plaque inflammation, and plaque characteristics determined by intravascular optical coherence tomography. *Arterioscler Thromb Vasc Biol.* 2007; **27**:1820–7.

153 Duewell P, Kono H, Rayner KJ, *et al*. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature.* 2010; **464**:1357–61.

154 Abela GS, Aziz K, Vedre A, *et al*. Effect of cholesterol crystals on plaques and intima in arteries of patients with acute coronary and cerebrovascular syndromes. *Am J Cardiol.* 2009; **103**:959–68.

155 Abela GS, Aziz K. Cholesterol crystals cause mechanical damage to biological membranes: a proposed mechanism of plaque rupture and erosion leading to arterial thrombosis. *Clin Cardiol.* 2005; **28**:413–20.

156 Kataoka Y, Puri R, Hammadah M, *et al*. Cholesterol crystals associate with coronary plaque vulnerability in vivo. *J Am Coll Cardiol.* 2015; **65**:630–2.

157 Dai J, Tian J, Hou J, *et al*. Association between cholesterol crystals and culprit lesion vulnerability in patients with acute coronary syndrome: An optical coherence tomography study. *Atherosclerosis.* 2016; **247**:111–7.

158 Barger AC, Beeuwkes R, 3rd. Rupture of coronary vasa vasorum as a trigger of acute myocardial infarction. *Am J Cardiol.* 1990; **66**:41G–43G.

159 Tenaglia AN, Peters KG, Sketch MH, Jr. Annex BH. Neovascularization in atherectomy specimens from patients with unstable angina: implications for pathogenesis of unstable angina. *Am Heart J.* 1998; **135**:10–4.

160 Kitabata H, Tanaka A, Kubo T, *et al*. Relation of microchannel structure identified by optical coherence tomography to plaque vulnerability in patients with coronary artery disease. *Am J Cardiol.* 2010; **105**:1673–8.

161 Caplan JD, Waxman S, Nesto RW, Muller JE. Near-infrared spectroscopy for the detection of vulnerable coronary artery plaques. *J Am Coll Cardiol.* 2006; **47**:C92–6.

162 Moreno PR, Lodder RA, Purushothaman KR, *et al*. Detection of lipid pool, thin fibrous cap, and inflammatory cells in human aortic atherosclerotic plaques by near-infrared spectroscopy. *Circulation.* 2002; **105**:923–7.

163 Puri R, Madder RD, Madden SP, *et al*. Near-Infrared Spectroscopy Enhances Intravascular Ultrasound Assessment of Vulnerable Coronary Plaque: A Combined Pathological and in vivo Study. *Arterioscler Thromb Vasc Biol.* 2015; **35**:2423–31.

164 Madder RD, Smith JL, Dixon SR, Goldstein JA. Composition of target lesions by near-infrared spectroscopy in patients with acute coronary syndrome versus stable angina. *Circ Cardiovasc Interv.* 2012; **5**:55–61.

165 Madder RD, Goldstein JA, Madden SP, *et al*. Detection by near-infrared spectroscopy of large lipid core plaques at culprit sites in patients with acute ST-segment elevation myocardial infarction. *JACC Cardiovasc Interv.* 2013; **6**:838–46.

166 Madder RD, Husaini M, Davis AT, *et al*. Detection by near-infrared spectroscopy of large lipid cores at culprit sites in patients with non-ST-segment elevation myocardial infarction and unstable angina. *Catheter Cardiovasc Interv.* 2015; **86**:1014–21.

167 Kini AS, Baber U, Kovacic JC, *et al*. Changes in plaque lipid content after short-term intensive versus standard statin therapy: the YELLOW trial (reduction in yellow plaque by aggressive lipid-lowering therapy). *J Am Coll Cardiol.* 2013; **62**:21–29.

168 Oemrawsingh RM, Cheng JM, Garcia-Garcia HM, *et al*. Near-infrared spectroscopy predicts cardiovascular outcome in patients with coronary artery disease. *J Am Coll Cardiol.* 2014; **64**:2510–8.

169 Kini AS, Baber U, Kovacic JC, *et al*. Changes in plaque lipid content after short-term intensive versus standard statin therapy: the YELLOW trial (reduction in yellow plaque by aggressive lipid-lowering therapy). *J Am Coll Cardiol.* 2013; **62**:21–9.

170 Oemrawsingh RM, Garcia-Garcia HM, van Geuns RJ, *et al*. Integrated Biomarker and Imaging Study 3 (IBIS-3) to assess the ability of rosuvastatin to decrease necrotic core in coronary arteries. *EuroIntervention.* 2016; **12**:734–9.

171. Naghavi M, Libby P, Falk E, *et al*. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part II. *Circulation.* 2003; **108**:1772–8.

172 Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med.* 1999; **340**:115–26.

173 Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. *Circulation.* 2006; **113**:2335–62.

174 Creemers EE, Tijsen AJ, Pinto YM. Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? *Circulation research.* 2012; **110**:483–495.

175 Fuster V, Fayad ZA, Moreno PR, *et al*. Atherothrombosis and high-risk plaque: Part II: approaches by noninvasive computed tomographic/magnetic resonance imaging. *J Am Coll Cardiol.* 2005; **46**:1209–18.

176 Fuster V, Moreno PR, Fayad ZA, *et al*. Atherothrombosis and high-risk plaque: part I: evolving concepts. *J Am Coll Cardiol.* 2005; **46**:937–54.

177 Tsimikas S, Willerson JT and Ridker PM. C-reactive protein and other emerging blood biomarkers to optimize risk stratification of vulnerable patients. *J Am Coll Cardiol.* 2006; **47**:C19–31.

178 Wang G-K, Zhu J-Q, Zhang J-T, *et al*. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *Eur Heart J.* 2010; **31**:659–666.

179 D'Alessandra Y, Devanna P, Limana F, *et al*. Circulating microRNAs are new and sensitive biomarkers of myocardial infarction. *Eur Heart J.* 2010; **31**:2765–2773.

180 Tijsen AJ, Creemers EE, Moerland PD, *et al*. MiR423-5p as a circulating biomarker for heart failure. *Circulation Research.* 2010; **106**:1035.

181 Fichtlscherer S, De Rosa S, Fox H, *et al*. Circulating microRNAs in patients with coronary artery disease. *Circulation Research.* 2010; **107**:677–684.

182 Li S, Zhu J, Zhang W, *et al*. Signature microRNA expression profile of essential hypertension and its novel link to human cytomegalovirus infection. *Circulation.* 2011; **124**:175–184.

183 Zampetaki A, Kiechl S, Drozdov I, *et al*. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circulation Research.* 2010; **107**:810–817.

184 Du Clos TW. Function of C-reactive protein. *Annals of Medicine.* 2000; **32**:274–8.

185 Calabro P, Willerson JT, Yeh ET. Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. *Circulation.* 2003; **108**:1930–2.

186 Verma S, Wang CH, Li SH, *et al*. A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. *Circulation.* 2002; **106**:913–9.

187 Ridker PM, Cushman M, Stampfer MJ, *et al*. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med.* 1997; **336**:973–9.

188 Haverkate F, Thompson SG, Pyke SD, *et al*. Production of C-reactive protein and risk of coronary events in stable and unstable angina. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *The Lancet.* 1997; **349**:462–6.

189 Ridker PM, Rifai N, Pfeffer MA, *et al*. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. *Circulation.* 1998; **98**:839–44.

190 Lindahl B, Toss H, Siegbahn A, *et al*. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. *N Engl J Med.* 2000; **343**:1139–47.

191 Liuzzo G, Biasucci LM, Gallimore JR, *et al*. The prognostic value of C-reactive protein and serum amyloid a protein in severe unstable angina. *N Engl J Med.* 1994; **331**:417–24.

192 Ridker PM, Rifai N, Rose L, *et al*. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med.* 2002; **347**:1557–65.

193 Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: A comparison of c-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. *JAMA.* 2001; **285**:2481–2485.

194 Pai JK, Pischon T, Ma J, *et al*. Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med.* 2004; **351**:2599–610.

195 Burke AP, Tracy RP, Kolodgie F, *et al*. Elevated C-reactive protein values and atherosclerosis in sudden coronary death: association with different pathologies. *Circulation.* 2002; **105**:2019–2023.

196 Ridker PM, Everett BM, Thuren T, *et al.* Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med.* 2017; **377**:1119–1131.

197 Everett BM, Donath MY, Pradhan AD, *et al*. Anti-inflammatory therapy with canakinumab for the prevention and management of diabetes. *J Am Coll Cardiol.* 2018; **71**:2392–2401.

198 Ridker PM, MacFadyen JG, Thuren T, *et al*. Effect of interleukin-1β inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: exploratory results from a randomised, double-blind, placebo-controlled trial. *The Lancet.* 2017; **390**:1833–1842.

199 Tardif J-C, Kouz S, Waters DD, *et al.* Efficacy and safety of low-dose colchicine after myocardial infarction. *N Engl J Med.* 2019; **381**:2497–2505.

200 Ridker PM, Everett BM, Pradhan A, *et al*. Low-dose methotrexate for the prevention of atherosclerotic events. *N Engl J Med.* 2019; **380**:752–762.

201 Davies MJ, Gordon JL, Gearing AJ, *et al*. The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. *The Journal of pathology.* 1993; **171**:223–9.

202 Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation.* 2000; **101**:1767–72.

203 Peter K, Nawroth P, Conradt C, *et al*. Circulating vascular cell adhesion molecule-1 correlates with the extent of human atherosclerosis in contrast to circulating intercellular adhesion molecule-1, E-selectin, P-selectin, and thrombomodulin. *Arterioscler Thromb Vasc Biol.* 1997; **17**:505–12.

204 Libby P, Aikawa M. New insights into plaque stabilisation by lipid lowering. *Drugs.* 1998; **56** Suppl 1:9–13; discussion 33.

205 Urbich C, Dernbach E, Aicher A, *et al*. CD40 ligand inhibits endothelial cell migration by increasing production of endothelial reactive oxygen species. *Circulation.* 2002; **106**:981–6.

206 Varo N, de Lemos JA, Libby P, *et al*. Soluble CD40L: risk prediction after acute coronary syndromes. *Circulation.* 2003; **108**:1049–52.

207 Heeschen C, Dimmeler S, Hamm CW, *et al*. Soluble CD40 ligand in acute coronary syndromes. *N Engl J Med.* 2003; **348**:1104–11.

208 Semb AG, van Wissen S, Ueland T, *et al. et al*. Raised serum levels of soluble CD40 ligand in patients with familial hypercholesterolemia: downregulatory effect of statin therapy. *J Am Coll Cardiol.* 2003; **41**:275–9.

209 Mallat Z, Corbaz A, Scoazec A, *et al*. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. *Circulation.* 2001; **104**:1598–603.

210 Bayes-Genis A, Conover CA, Schwartz RS. The insulin-like growth factor axis: A review of atherosclerosis and restenosis. *Circ Res.* 2000; **86**:125–30.

211 Bayes-Genis A, Conover CA, Overgaard MT, *et al*. Pregnancy-associated plasma protein A as a marker of acute coronary syndromes. *N Engl J Med.* 2001; **345**:1022–9.

212 Sangiorgi G, Mauriello A, Bonanno E, *et al*. Pregnancy-associated plasma protein-a is markedly expressed by monocyte-macrophage cells in vulnerable and ruptured carotid atherosclerotic plaques: a link between inflammation and cerebrovascular events. *J Am Coll Cardiol.* 2006; **47**:2201–11.

213 Jaffer FA, Libby P, Weissleder R. Molecular and cellular imaging of atherosclerosis: emerging applications. *J Am Coll Cardiol.* 2006; **47**:1328–38.

214 Constantinides P. Cause of thrombosis in human atherosclerotic arteries. *Am J Cardiol.* 1990; **66**:37g–40g.

215 Fayad ZA, Fuster V. Clinical imaging of the high-risk or vulnerable atherosclerotic plaque. *Circ Res.* 2001; **89**:305–16.

216 Stefanadis C, Toutouzas K, Tsiamis E, *et al*.Thermal heterogeneity in stable human coronary atherosclerotic plaques is underestimated in vivo: the "cooling effect" of blood flow. *J Am Coll Cardiol.* 2003; **41**:403–8.

217 Ruehm SG, Corot C, Vogt P, *et al*. Magnetic resonance imaging of atherosclerotic plaque with ultrasmall superparamagnetic particles of iron oxide in hyperlipidemic rabbits. *Circulation.* 2001; **103**:415–22.

218 Lederman RJ, Raylman RR, Fisher SJ, *et al*. Detection of atherosclerosis using a novel positron-sensitive probe and 18-fluorodeoxyglucose (FDG). *Nuclear Medicine Communications.* 2001; **22**:747–53.

219 Ciavolella M, Tavolaro R, Taurino M, *et al*. Immunoscintigraphy of atherosclerotic uncomplicated lesions in vivo with a monoclonal antibody against D-dimers of insoluble fibrin. *Atherosclerosis.* 1999; **143**:171–5.

220 Schmitz SA, Coupland SE, Gust R, *et al*. Superparamagnetic iron oxide-enhanced MRI of atherosclerotic plaques in Watanabe hereditable hyperlipidemic rabbits. *Investigative Radiology.* 2000; **35**:460–71.

221 Schmitz SA, Taupitz M, Wagner S, *et al*. Iron-oxide-enhanced magnetic resonance imaging of atherosclerotic plaques: postmortem analysis of accuracy, interobserver agreement, and pitfalls. *Investigative Radiology.* 2002; **37**:405–11.

222 Yuan C, Kerwin WS. MRI of atherosclerosis. *Journal of Magnetic Resonance Imaging: JMRI.* 2004; **19**:710–9.

223 Yuan C, Kerwin WS, Ferguson MS, *et al*. Contrast-enhanced high resolution MRI for atherosclerotic carotid artery tissue characterization. *Journal of magnetic resonance imaging: JMRI.* 2002; **15**:62–7.

224 Asakura T, Karino T. Flow patterns and spatial distribution of atherosclerotic lesions in human coronary arteries. *Circ Res.* 1990; **66**:1045–66.

225 Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. *JAMA.* 1999; **282**:2035–42.

226 Chatzizisis YS, Coskun AU, Jonas M, *et al*. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. *J Am Coll Cardiol.* 2007; **49**:2379–93.

227 Stone PH, Saito S, Takahashi S, *et al*. Prediction of progression of coronary artery disease and clinical outcomes using vascular profiling of endothelial shear stress and arterial plaque characteristics: the PREDICTION Study. *Circulation.* 2012; **126**:172–81.

228 Samady H, Eshtehardi P, McDaniel MC, Coronary artery wall shear stress is associated with progression and transformation of atherosclerotic plaque and arterial remodeling in patients with coronary artery disease. *Circulation.* 2011; **124**:779–88.

229 Gijsen FJ, Wentzel JJ, Thury A, *et al*. Strain distribution over plaques in human coronary arteries relates to shear stress. *Am J Physiol Heart Circ Physiol.* 2008; **295**:H1608–14.