Risk stratification of individual coronary lesions using local endothelial shear stress: a new paradigm for managing coronary artery disease

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Purpose of review

The purpose of this review is to summarize the role of endothelial shear stress in the natural history of coronary atherosclerosis, and to propose an individualized risk-stratification strategy of atherosclerotic lesions based on the in-vivo characterization of local endothelial shear stress and wall morphology.

Recent findings

Low endothelial shear stress promotes the development of early fibroatheromas, which subsequently follow an individualized natural history of progression. This individual natural history is critically dependent on the magnitude of low endothelial shear stress, which subsequently regulates the severity of inflammation within the wall and ultimately the vascular remodeling response. Very low endothelial shear stress enhances plaque inflammation, leading to excessive expansive remodeling. Excessive expansive remodeling leads to perpetuation, or even exacerbation, of the local low endothelial shear stress environment, thereby setting up a self-perpetuating vicious cycle among low local endothelial shear stress, inflammation, and excessive expansive remodeling, which transforms an early fibroatheroma to a high-risk plaque.

Summary

In-vivo assessment of the local endothelial shear stress environment, severity of inflammation and vascular remodeling response, all responsible for individual plaque behavior and natural history, in combination with systemic biomarkers of vulnerability, may allow for detailed risk stratification of individual early atherosclerotic plaques, thereby guiding both systemic and local, lesion-specific therapeutic strategies.

Keywords

coronary atherosclerosis, inflammation, remodeling, risk stratification, shear stress

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Abbreviations

ECM	extracellular matrix
ESS	endothelial shear stress
IEL	internal elastic lamina
LDL-C	low-density lipoprotein-cholesterol
MMP	matrix metalloproteinase
NF-κB	nuclear factor-κB
ROS	reactive oxygen species
TCFA	thin cap fibroatheroma
VSMC	vascular smooth muscle cell

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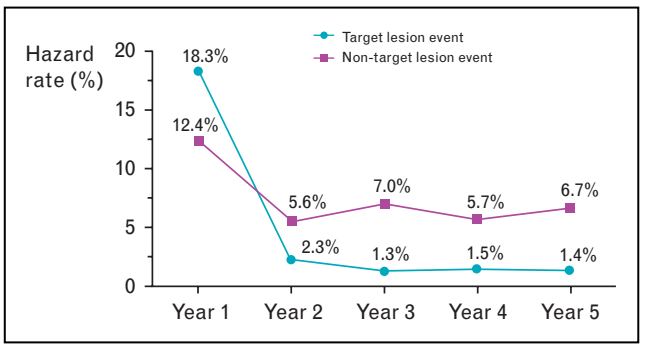
Introduction

Atherosclerosis is a chronic inflammatory, fibroproliferative disease primarily of large and medium-sized conduit arteries, whose clinical manifestations constitute the primary cause of morbidity and mortality in the industrialized world [1]. Despite the systemic nature of atherosclerosis, its distribution is multifocal and heterogeneous, such that multiple atherosclerotic lesions at a different stage of progression coexist in the same individual, indeed, in the same artery at a single point in time [2,3]. The significance of the multifocal and heterogeneous nature of coronary atherosclerosis is underscored by a recent 5-year follow-up study which demonstrated the development of new major cardiac events in almost 40% of patients in portions of the coronary arteries that were not perceived to exhibit atherosclerosis of sufficient severity to warrant revascularization at the time of an index procedure for another artery (Fig. 1) [4].

Although all the coronary artery lesions are exposed to the same systemic risk factors, each of these atherosclerotic lesions presents its own potential for progression and risk. A portion of atherosclerotic lesions are thin cap fibroatheromas (TCFAs; also called high-risk or thrombosis-prone or vulnerable plaques) prone to acute

Figure 1 Hazard rates per year for stented target-lesion and nonstented (not-target) lesion major acute coronary events



Beyond 1 year after implementation of bare metal stent a substantial number of events occurs due to nonstented lesions, whereas the event rate attributed to the stented target-lesions remains relatively stable and low. These findings confirm the multifocal and heterogeneous nature of coronary atherosclerosis. Adapted with permission [4].

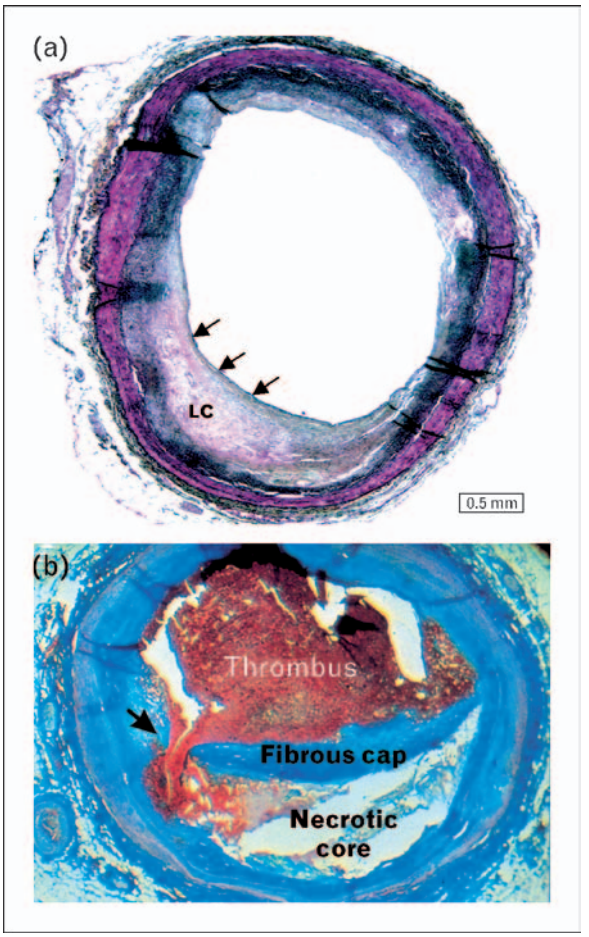
disruption, and consequent acute coronary syndrome (Fig. 2a). Histopathology studies by Virmani *et al.* indicate that more than 75% of TCFAs obstruct the coronary lumen by less than 50% prior to rupture, and do not limit coronary flow nor produce angina [5,6••]. These lesions are currently neither identified nor treated before plaque rupture. Even though we know that atherosclerotic lesions preferentially develop in regions of low endothelial shear stress (ESS) [7,8], the critical question is why only few among many individual local plaques rupture and lead to acute coronary syndrome (Fig. 2b). The magnitude of low ESS is likely to be a critical determinant of the individual natural history of atherosclerotic lesions [9••].

The purpose of this review is to summarize the role of ESS in the natural history of coronary atherosclerosis, and to propose an individualized risk-stratification strategy of atherosclerotic lesions based on the in-vivo characterization of local ESS and wall morphology. Knowledge of the local ESS environment of a given plaque, in combination with the individual histopathologic characteristics of that plaque, and the nature of the vascular remodeling response may be essential in predicting the location and rate of atherosclerosis progression, and may help predict specific sites of plaque rupture by identifying lesions evolving toward vulnerability. Early identification of lesions, which are likely to evolve to high-risk plaque may justify more intensive systemic treatment or local interventions prior to plaque rupture.

Definition of endothelial shear stress and endothelial shear stress patterns

ESS is the tangential stress derived from the friction of the flowing blood on the endothelial surface of the arterial wall [9••,10] (Fig. 3a). The pulsatile nature of the coronary

Figure 2 Thin cap fibroatheroma and ruptured plaque

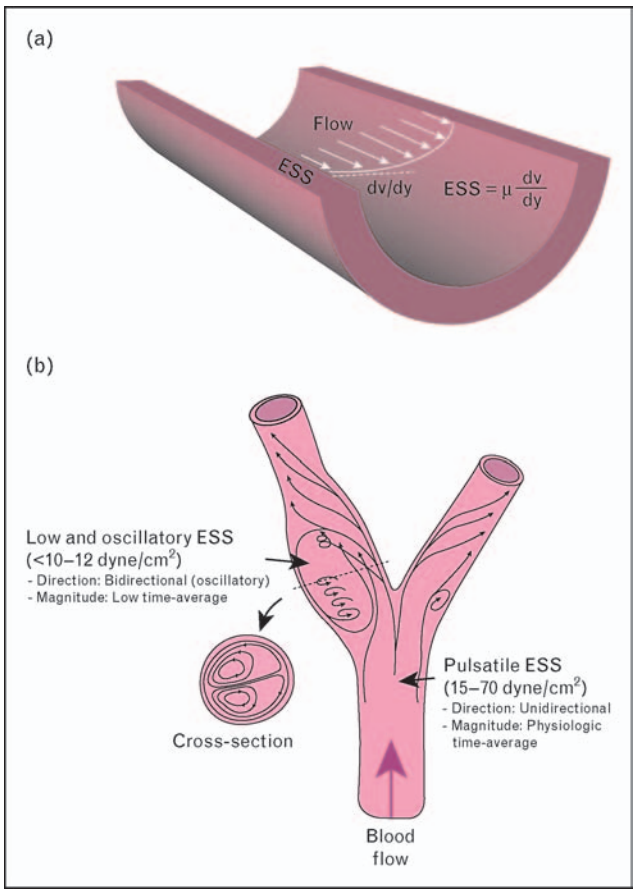


(a) Histologic appearance of an eccentric thin cap fibroatheroma (TCFA); arrows denote the thin cap. The lumen is preserved because of the expansive remodeling accommodating the enlarging plaque. LC, lipid core. Reprinted with permission [67]. (b) Histologic appearance of a ruptured plaque (arrow indicates the site of rupture) implicated with acute luminal thrombus formation and obstruction. Adapted with permission from P. Constantinides. Plaque fissures in human coronary thrombosis. *Journal of Atherosclerosis Research*. Published by Elsevier, 1966.

blood flow in combination with the blood's rheological properties and the complex geometric configuration of the coronary arteries determines the ESS patterns, which are characterized by direction and magnitude (Fig. 3b) [9••,11,12]. In relatively straight arterial segments, ESS is pulsatile and unidirectional with a magnitude that varies within a range of 15–70 dyne/cm^2 over the cardiac cycle and yields a positive time-average.

In contrast, in geometrically irregular regions, where disturbed laminar flow occurs, pulsatile flow generates low or oscillatory ESS. Low ESS refers to ESS, which is unidirectional at any given point with a fluctuating magnitude during the cardiac cycle that results in a significantly low time-average (<10–12 dyne/cm^2)

Figure 3 Definition of endothelial shear stress and endothelial shear stress patterns



(a) Endothelial shear stress (ESS) is proportional to the product of blood viscosity (μ) and the spatial gradient of blood velocity at the wall (dv/dy). It is expressed in units of force per unit area [N/m^2 or Pascal (Pa) or $dyne/cm^2$; $1\ N/m^2 = 1\ Pa = 10\ dyne/cm^2$]. Reprinted with permission [9••]. (b) Definition of ESS patterns. Adapted with permission [68].

(Fig. 3b) [13]. Low ESS typically occurs at the inner areas of curvatures, as well as upstream of stenoses.

Oscillatory ESS is characterized by significant changes in both direction (i.e. it is bidirectional) and magnitude between systole and diastole, resulting in a very low time-average, usually close to zero (Fig. 3b). Oscillatory ESS occurs primarily downstream of stenoses, at the lateral walls of bifurcations and in the vicinity of branch points. Beside the temporal oscillations, ESS exhibits significant spatial oscillations over short distances, especially in geometrically irregular regions, resulting in high spatial gradients, which are also involved in atherosclerosis [14,15•].

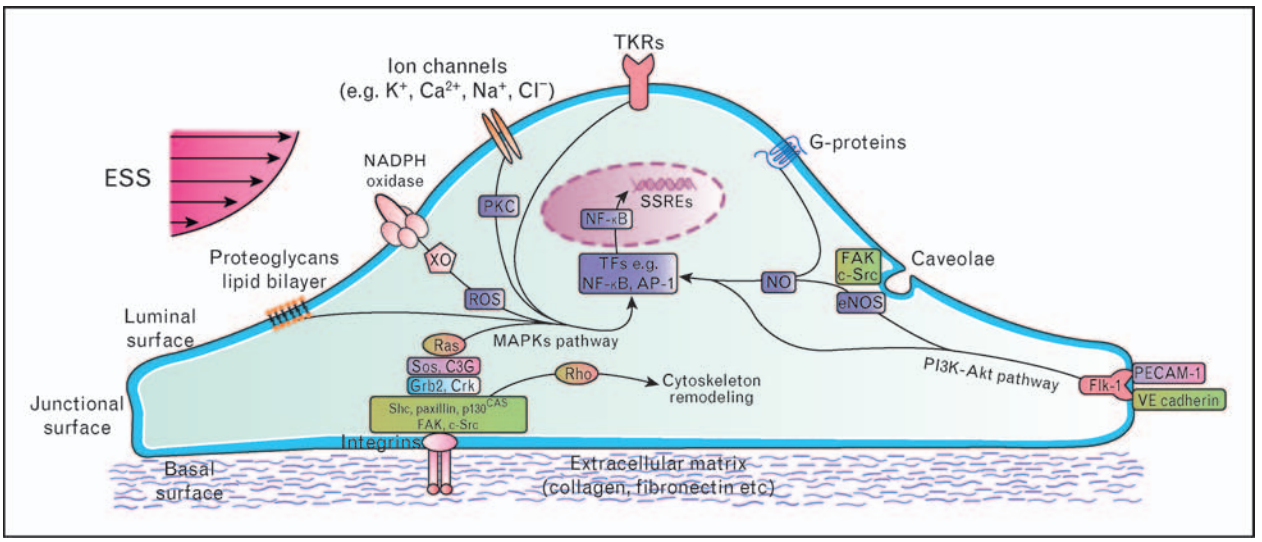
Role of endothelial shear stress in endothelial gene expression

Endothelial cells are capable of sensing the local ESS stimuli through several types of mechanoreceptors, located on the luminal, junctional and basal endothelial surfaces [9••,16]. Following activation of mechanoreceptors, a complex network of intracellular pathways is activated, a process known as mechanotransduction (presented in detail in Fig. 4) [9••,17]. These pathways lead to phosphorylation of several transcription factors, which in turn bind positive or negative shear stress responsive elements at promoters of mechanosensitive genes, inducing or suppressing gene expression, and ultimately modulating cellular function and morphology [7]. In arterial regions with nondisturbed flow, where ESS varies within a physiologic range, endothelial cells express various atheroprotective genes, and suppress several pro-atherogenic ones, leading eventually to stability and quiescence in that region. In contrast, in regions with low and disturbed flow where low or oscillatory ESS occur, the atheroprotective genes are suppressed, while the pro-atherogenic genes are upregulated, thereby promoting the atherosclerotic process [18,19].

Role of low endothelial shear stress in the pathophysiology of atherosclerosis

The role of ESS in the pathophysiology of atherosclerosis is summarized in Fig. 5. In arterial regions with disturbed flow, low ESS reduces the bioavailability of nitric oxide by decreasing endothelial nitric oxide synthase mRNA and protein expression [20,21•], while upregulating endothelin-1 [22], a potent vasoconstrictive and mitogenic molecule, thereby inducing endothelial dysfunction and exposing the endothelium to the atherogenic effect of local and systemic risk factors. Low ESS also promotes low-density lipoprotein-cholesterol (LDL-C) uptake and synthesis by the endothelium through upregulation of specific transcription factors, primarily sterol regulatory elements binding proteins, leading to subendothelial accumulation of LDL-C [23]. The increased mitotic and apoptotic activity of endothelial cells induced by the local low ESS [24], as well as the conformational changes of endothelial cells from fusiform to polygonal shape [25] promote the widening of the junctions between endothelial cells, thereby accentuating the subendothelial deposition of LDL-C. Within the intima LDL-C is associated with proteoglycans [26••] and undergoes oxidative modification by the increased reactive oxygen species (ROS). Low ESS mediates the production of ROS within the intima by enhancing gene expression and posttranscriptional activity of the major oxidative enzymes (i.e. nicotinamide adenine dinucleotide phosphate oxidase and xanthine oxidase) at endothelial cell membranes [27]. Low ESS also downregulates the intracellular ROS scavengers, such as manganese superoxide dismutase and glutathione, further augmenting local oxidative stress [18]. The minimal intimal accumulations of oxidized LDL-C constitute the earliest histopathologic stage of atherosclerosis, so-called fatty streaks (Fig. 6) [26••,28].

Figure 4 Mechanotransduction of endothelial shear stress

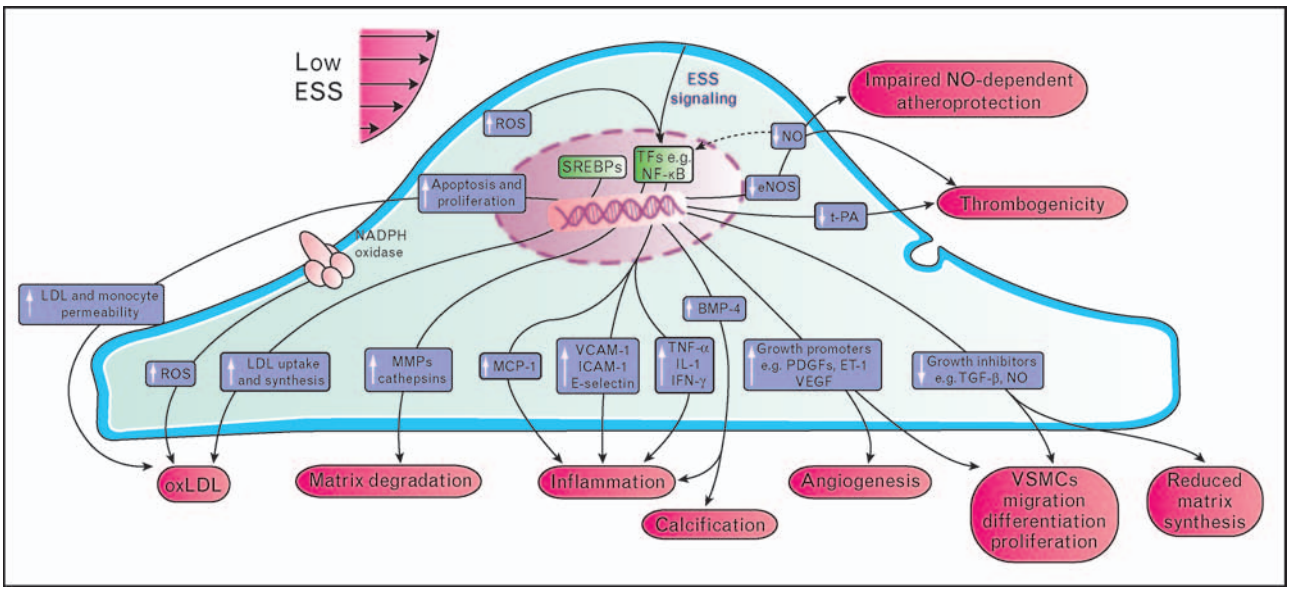


Local endothelial shear stress (ESS) is sensed by luminal endothelial mechanoreceptors, such as ion channels, G-proteins, caveolae, tyrosine kinase receptors (TKRs), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase (XO), plasma membrane lipid bilayer, and heparan sulfate proteoglycans. Also, ESS signals are transmitted through the cytoskeleton to the basal or junctional endothelial surface, where certain integrins or a mechanosensory complex consisting of platelet endothelial cell adhesion molecule-1 (PECAM-1) and Flk-1 are activated, respectively, and initiate a downstream signaling cascade. Activated integrins phosphorylate and activate a multiple complex of nonreceptor tyrosine kinases (FAK, c-Src, Shc, paxillin, and p130CAS), adaptor proteins (Grb2, Crk), and guanine nucleotide exchange factors (Sos, C3G), thereby activating Ras family GTPase. Active Ras plays a pivotal role in intracellular transduction of ESS signals as it triggers various parallel downstream cascades of serine kinases; each of these kinases phosphorylates and hence activates the next one downstream, ultimately activating mitogen-activated protein kinases (MAPKs). Also, integrins promote the activation of Rho family small GTPases, which mediate the remodeling of cytoskeleton resulting in permanent structural changes of endothelial cells. Beside integrin-mediated mechanotransduction, ESS activates a number of other downstream signaling pathways initiated by luminal or junctional mechanoreceptors. These pathways include the production of reactive oxygen species (ROS) from NADPH oxidase and XO, activation of protein kinase C (PKC), release of endothelial nitric oxide synthase (eNOS) and other signaling molecules from caveolae, and activation of phosphoinositide-3 kinase (PI3K)-Akt cascade. Ultimately, all these signaling pathways lead to phosphorylation of several transcription factors (TFs), such as nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1). These TF proteins bind positive or negative shear stress responsive elements (SSREs) at promoters of mechanosensitive genes inducing or suppressing their expression, thereby modulating cellular function and morphology. Reprinted with permission [9••].

Through activation of nuclear factor-κ B (NF-κB) low ESS upregulates the expression of several adhesion molecules, such as vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and E-selectin; chemoattractant chemokines, such as monocyte chemoattractant protein-1; and pro-inflammatory cytokines, such as tumor necrosis factor-α, interleukin-1, and interferon-γ [29•,30]. Adhesion molecules are expressed by endothelial cells and mediate the rolling and adhesion of circulating leukocytes (e.g. monocytes, T-cells, mast cells) on the endothelial surface, whereas monocyte chemoattractant protein-1 and other chemoattractant chemokines promote transmigration of leukocytes into the intima [1]. Local inflammatory cell accumulation is further enhanced by low ESS through upregulation of bone morphogenic protein-4, a member of the transforming growth factor-β (TGF-β) superfamily of cytokines [31]. Once monocytes infiltrate beneath the endothelium they undergo structural and functional alterations and differentiate to macrophages, which express scavenger receptors (CD36), phagocytize the oxidized LDL-C and transform to foam cells. Foam cells produce cytokines, growth factors [e.g. platelet-derived growth factor (PDGF)], ROS (e.g. myeloperoxidase) and matrix degrading enzymes [e.g. matrix metalloproteinases (MMPs), cathepsins], sustaining the local inflammation, oxidative stress, dynamic matrix remodeling, and ultimately atherosclerosis progression [1]. Overexpression and increased activity of MMPs, particularly MMP-2 and 9, and cathepsins (e.g. cathepsins K, L), which are the major proteases associated with extracellular matrix (ECM) degradation, are also mediated directly by low ESS, probably through NF-κB-dependent pathways [32••,33,34•,35•]. The accumulations of lipid-laden foam cells constitute the intermediate lesions or pathologic intimal thickening, which evolve through several stages of progression (Fig. 6) [26••].

A portion of intermediate lesions, at some point during their natural history, acquire a fibrous cap and evolve to early fibroatheromas. Although the pathophysiologic events occurring during the transition of intermediate lesions to early fibroatheromas are not well understood, it appears that the regional disruption of the internal elastic lamina (IEL), which separates the diseased

Figure 5 Role of endothelial shear stress in the pathophysiology of atherosclerosis



In arterial regions with disturbed laminar flow, low endothelial shear stress (ESS) shifts the endothelial function and structure toward an atherosclerotic phenotype, thereby promoting atherogenesis, atherosclerotic plaque formation and progression, and vascular remodeling. BMP, bone morphogenic protein; ET, endothelin; eNOS, endothelial nitric oxide synthase; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; LDL, low-density lipoprotein cholesterol; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; NO, nitric oxide; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; SREBP, sterol regulatory element binding protein; TF, transcription factor; TGF, transforming growth factor; TNF, tumor necrosis factor; t-PA, tissue plasminogen activator; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell. Reprinted with permission [9••].

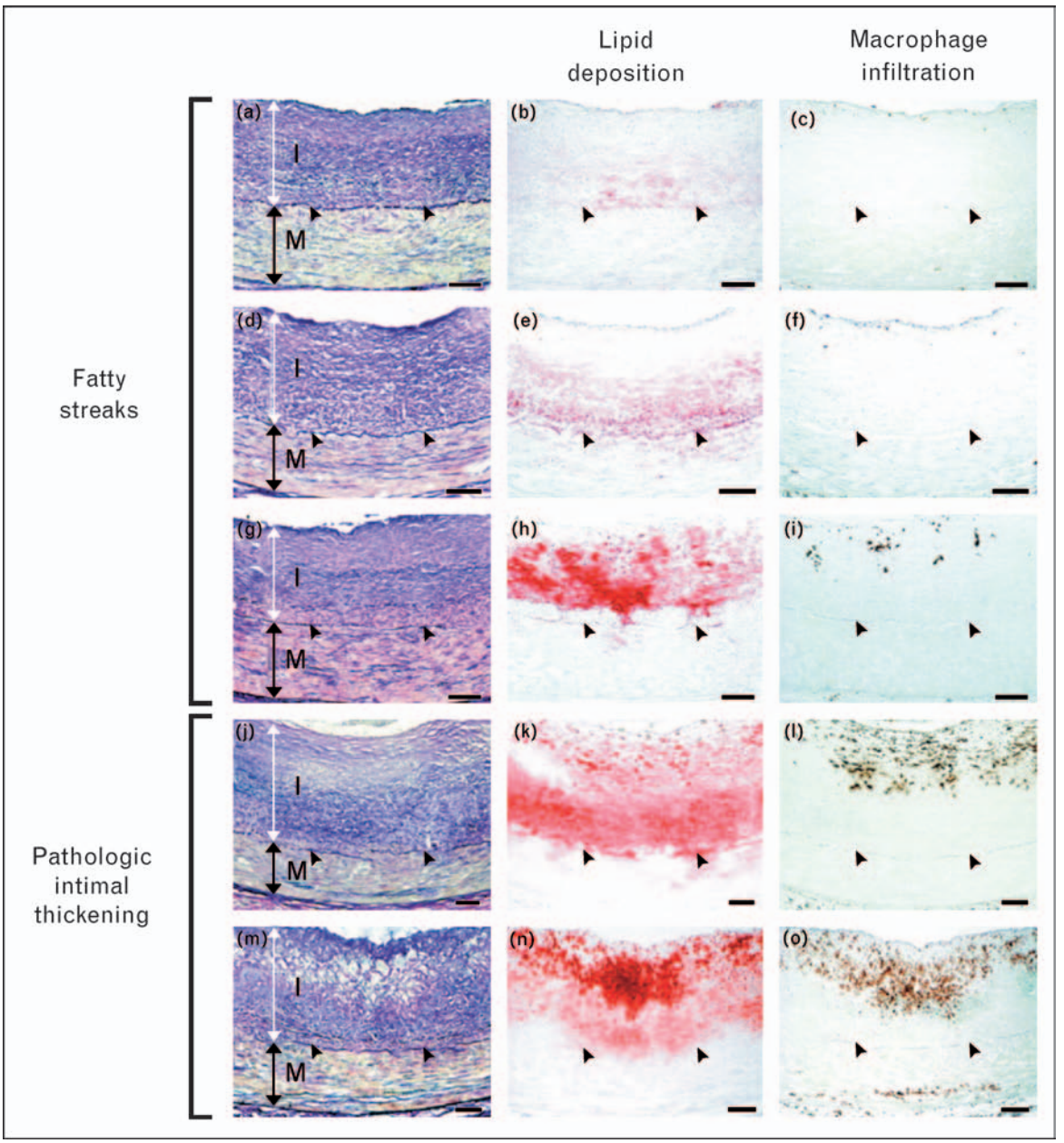
intima from the media, constitutes the key event that drives the formation of fibroatheromas. Histopathology studies in a diabetic, hyperlipidemic swine model of native atherosclerosis have shown that in the setting of very low levels of local ESS, and subsequently intense accumulation of inflammatory cells within the intima, the part of the IEL beneath the plaque undergoes local degradation by the foam cell-derived proteases (e.g. MMP-2, 9 and cathepsins) [36••,37••]. These IEL breaks constitute the gateway for vascular smooth muscle cells (VSMCs), which are originally located into the media, to enter the intima (Fig. 7a) [38••,39]. Low ESS further promotes VSMC migration to the intima by enhancing the endothelial gene and protein expression of growth promoters, such as PDGF, endothelin-1, and vascular endothelial growth factor (VEGF) [32••], and blunting the expression of growth inhibitors, such as nitric oxide, TGF-β, and plasminogen activator inhibitor-1 [9••]. Within the intima the VSMCs differentiate to a more synthetic phenotype to elaborate ECM proteins (e.g. collagen and elastin), and proliferate under the effect of growth factors secreted by endothelial cells and foam cells, thereby promoting plaque progression. The VSMCs, for yet unknown reasons, encompass the core of the lipid-laden foam cells (lipid core), produce ECM and create the fibrous cap, separating the thrombogenic lipid material from the circulating platelets and other pro-thrombotic factors. The fibrous cap along with the underlying lipid core forms the early fibroatheroma (AHA type IV lesion) [28,40].

A second key factor, in addition to IEL degradation, that critically determines the natural history of atherosclerosis is neovascularization (angiogenesis). Dense neovessels developed within the atherosclerotic plaque from the existing adventitial vasa vasorum supply the lesion with LDL-C, inflammatory cells, ROS and matrix proteases from the 'back door', thereby reinforcing the inflamed status of the plaque and promoting plaque progression [41•,42]. Strong support for an active role of angiogenesis in the natural history of atherosclerosis is provided by studies which showed that inhibition of neovascularization prevents the progression of atherosclerotic lesions [43]. Low ESS facilitates intimal neovascularization by inducing intimal thickening and thus ischemia, upregulating the expression of VEGF and other angiogenic factors [19], enhancing local inflammation, oxidative stress, and expression of matrix degrading enzymes, and accentuating endothelial cell and VSMC migration and proliferation.

Low endothelial shear stress modulates the natural history of atherosclerotic plaques

Following their formation early fibroatheromas follow an individualized natural history, which is critically dependent on the balance of two competing processes:

Figure 6 Histopathologic stages of early atherosclerosis



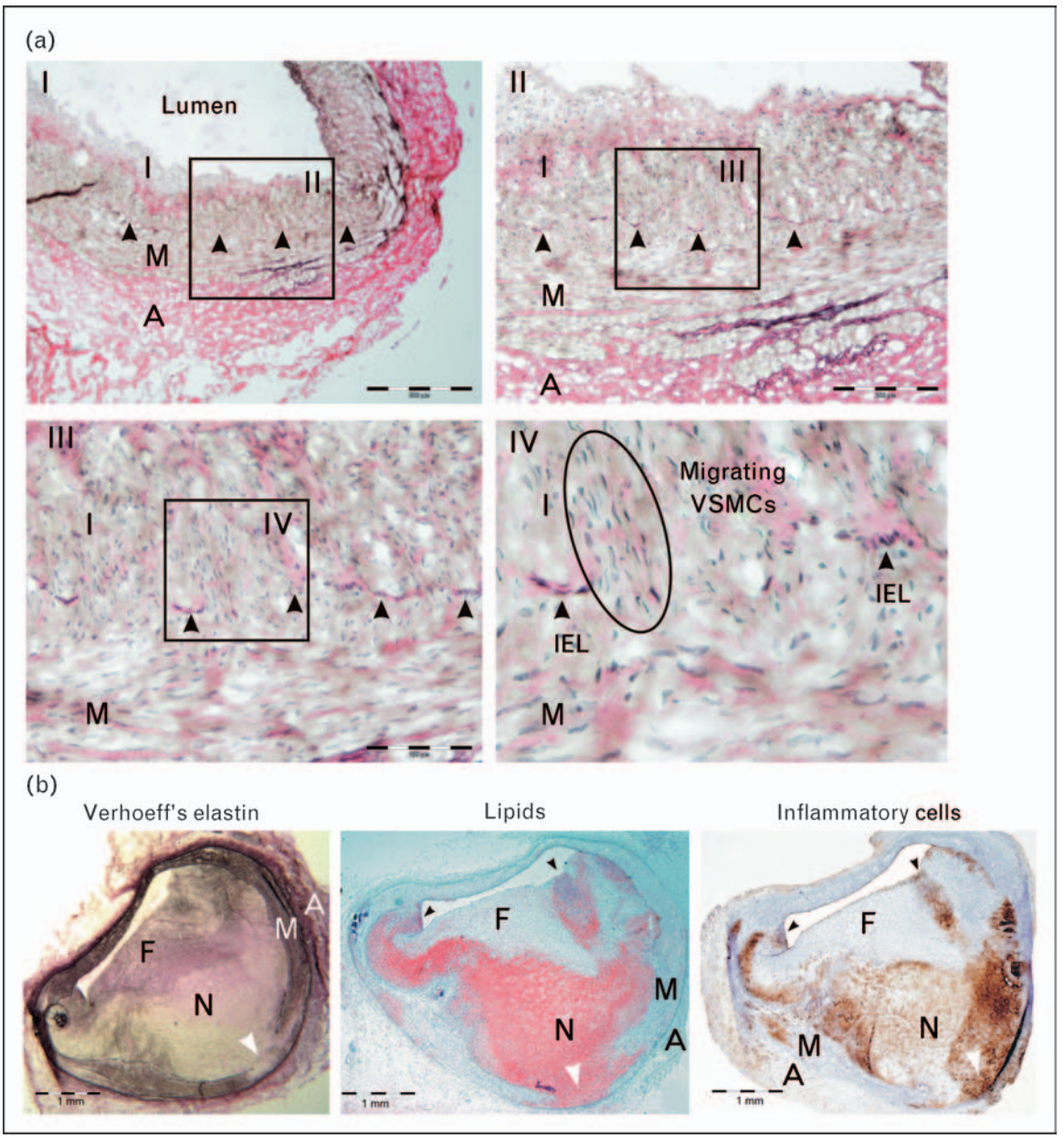
Figures in the first column show the intima (I) and media (M), figures in the second column denote lipid deposition and figures in the third column depict macrophage infiltration. (a to i) Fatty streaks representing intimal depositions of lipids at several stages of progression. (j to o) Pathological intimal thickening or intermediate lesions representing accumulations of lipid-laden macrophages at several stages of progression; arrowheads indicate internal elastic lamina, bars = 100 μm. Adapted with permission [26••].

inflammation with concomitant ECM degradation versus fibroproliferation with ECM synthesis. Experimental histopathology studies in a diabetic, hyperlipidemic swine model of native atherosclerosis showed that the magnitude of low ESS is a key regulator of the balance between inflammation/ECM degradation and fibroproliferation/ECM synthesis, thereby determining the remodeling response of the vascular wall to the growing plaque [36••,37••,44••]. A portion of early fibroatheromas will evolve into high-risk plaques, whereas others will remain quiescent and still others will evolve to fibrous stenotic plaques [9••] (Fig. 8).

High-risk plaques

High-risk plaques are typically TCFAs, characterized by a thin, highly inflamed fibrous cap overlying a large necrotic lipid core, rich in neovessels [28] (Figs 2 and 8). These plaques are usually minimally stenotic lesions associated

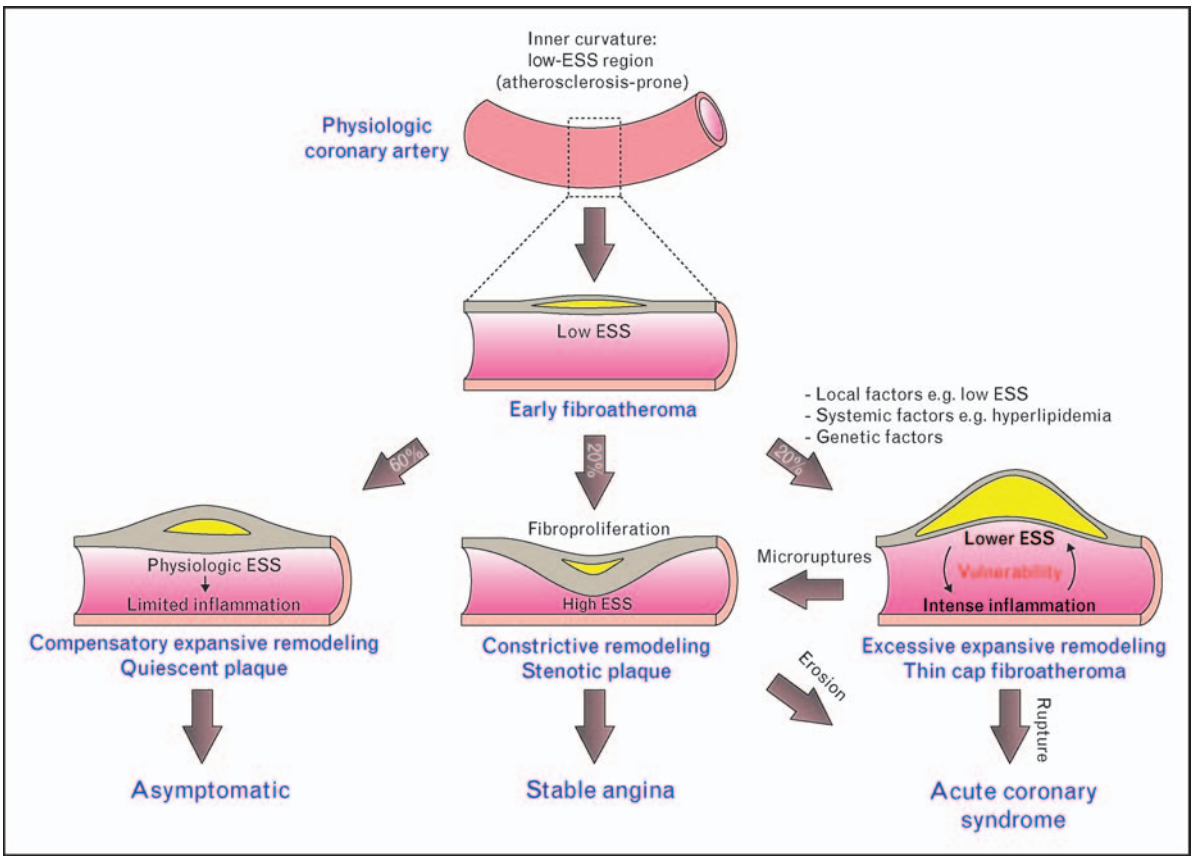
Figure 7 Role of internal elastic lamina in atherosclerosis



(a) A single histopathologic section from a swine coronary artery at varying magnifications (I–IV) depicting internal elastic lamina (IEL) breaks (arrowheads) through which vascular smooth muscle cells (VSMCs) migrate into the intima. Note the IEL remnants in IV and the migrating VSMCs encompassed with a circle; I, intima; M, media; A, adventitia. (b) Vehoeff's elastin, oil red-O (lipids) and CD45 (inflammatory cells) staining of a single histopathologic section from a swine coronary artery depicting a fibroatheroma with a large necrotic lipid core (N) and a highly inflamed thin fibrous cap (F) at the shoulders (black arrowheads). Note the severe IEL disruption at the base of the plaque accompanied by severe degradation of the underlying media (white arrowheads).

with expansive vascular remodeling, and an increased risk of sudden rupture that precipitates 60–70% of acute coronary syndromes [28,45–47]. Experimental studies showed that TCFAs develop in arterial areas with the lowest values of ESS, which enhance plaque inflammation, especially at the base of the plaque [36••,37••]. In this setting, the underlying IEL undergoes severe degradation (Fig. 7b), the media becomes severely inflamed and acquires the enzymatic products that shift the ECM balance towards intensive degradation, thereby promoting excessive (aneurysm-like) wall expansion and accommodation of the enlarging plaque [48••]. Excessive expansive remodeling leads to perpetuation, or even exacerbation, of the local low-ESS environment, thereby fostering continued lipid accumulation and inflammation, which lead to additional matrix protease expression, intensive matrix

Figure 8 Proposed natural history of coronary atherosclerosis



The initiating process of atherosclerosis in an atherosclerosis-prone region is a low-endothelial-shear-stress (ESS) environment, leading to the formation of an early fibroatheroma. The magnitude of low ESS, the severity of plaque inflammation and the vascular remodeling response to the presence of the plaque are key regulators of the subsequent natural history of the fibroatheroma. In regions with very low ESS plaque inflammation is exacerbated and promotes severe degradation of the internal elastic lamina (IEL) at the base of the plaque. In this setting, the media becomes severely inflamed and acquires the enzymatic products that shift the extracellular matrix balance towards intensive degradation, thereby promoting excessive (aneurysm-like) wall expansion and accommodation of the enlarging plaque. Excessive expansive remodeling leads to perpetuation, or even exacerbation, of the local low-ESS environment, thereby fostering continued lipid accumulation and inflammation, which lead to additional matrix protease expression, intensive matrix degradation within the inflamed vascular wall and the fibrous cap shoulders, additional wall expansion and fibrous cap thinning. This self-perpetuating vicious cycle among low local ESS, inflammation, and excessive expansive remodeling exacerbates the inflammatory status of the plaque and may rapidly transform an early fibroatheroma to a thin cap fibroatheroma. In arterial regions with slightly low ESS the severity of lipid accumulation and inflammation is limited and subsequently the IEL degradation and wall expansion are limited. In this setting, the growing plaque slightly narrows the lumen (i.e. compensatory expansive remodeling), thereby restoring the adverse ESS stimulus to less pathologic levels and establishing quiescence. Fibrous stenotic plaques either directly evolve with a phenotype promoting fibroproliferation consistently throughout their natural history course, or represent an end-stage of scarring in the setting of prior inflamed thin cap fibroatheromas through repetitive microruptures and subsequent healing. Stenotic plaques are associated with constrictive remodeling and infrequently undergo local erosion, which leads to local thrombus formation and precipitation of an acute coronary syndrome. Reprinted with permission [9••].

degradation within the inflamed vascular wall and the fibrous cap shoulders, additional wall expansion and fibrous cap thinning. This self-perpetuating vicious cycle among low local ESS, inflammation, and excessive expansive remodeling exacerbates the inflammatory status of the plaque and may rapidly transform an early fibroatheroma to a TCFA.

The presence and severity of systemic factors (e.g. magnitude of hyperlipidemia, hyperglycemia, hypertension), as well as genetic factors also interplay with the low-ESS microenvironment and modulate the excessive expansion of the arterial wall.

Quiescent plaques

A portion of early fibroatheromas evolve to quiescent plaques, which are nonstenotic or minimally stenotic lesions with a thick fibrous cap and a small lipid core (Fig. 8). These plaques are characterized by limited inflammation, remain biologically quiescent, and cause no symptoms [49]. Quiescent lesions appear to develop in arterial regions with slightly low ESS and do not acquire the severity of lipid accumulation and inflammation, as do areas with lower ESS in which TCFAs develop; hence a stable balance between inflammation and fibroproliferation is established [36••,37••]. As a result of the limited inflammation that is stimulated, the IEL degradation and

wall expansion are limited. The growing plaque leads to limited enlargement of the vessel wall and then starts to slightly protrude into the lumen (i.e. compensatory expansive remodeling), thereby restoring the adverse low-ESS stimulus to higher, less pathologic, levels [37••,50]. In the setting of the attenuated low-ESS stimulus for exacerbation of inflammation, plaque progression and arterial expansion, the inflammatory process is limited and quiescence is established.

The long-term stability or quiescence of these plaques, however, is unknown. If local vascular conditions later change, such that a low ESS microenvironment is recreated, or the systemic atherosclerotic stimuli are enhanced (e.g. increased magnitude of hyperlipidemia), then the process of inflammation, progressive atherosclerosis, and excessive expansive remodeling may again re-emerge, and may transform the quiescent lesion to a TCFA.

Fibrous plaques

Stenotic plaques are stable fibroproliferative lesions with limited inflammation, characterized morphologically by a relatively thick, collagen-rich fibrous cap, overlying a small lipid core [28,46] (Fig. 8). These lesions are associated with constrictive vascular remodeling, and over time become occlusive, resulting in chronic stable angina. Many stenotic lesions represent an end-stage of scarring in the setting of prior inflamed TCFA undergoing repetitive microruptures, VSMC proliferation, local deposition of collagen and subsequent healing [51]. Stenotic lesions may also directly evolve from early fibroatheromas with a phenotype promoting fibroproliferation versus inflammation throughout its natural history course [52••]. The pathophysiologic factors involved in this process are currently unknown.

Stenotic plaques infrequently undergo local erosion or develop calcified nodules, which lead to local thrombus formation precipitating 20–40% of acute coronary syndromes [28]. Low ESS does not appear to play a role in the pathophysiology of plaque erosion. High ESS, however, which occurs at the throat of highly stenotic plaques, may be responsible for local endothelial erosion and induction of acute coronary thrombosis.

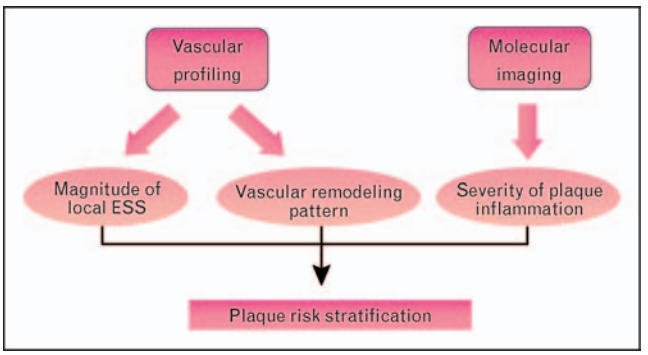
Risk stratification of individual atherosclerotic lesions

Given the marked heterogeneity of natural history trajectories of coronary atherosclerosis, development of a comprehensive approach for risk stratification of each individual plaque at early stages would be invaluable. The major characteristics of individual atherosclerotic plaques contributing to the ongoing process of the respective natural history trajectories include the magnitude of ESS, which constitutes the stimulus for ongoing inflammation and plaque progression, the severity of inflammation that plaque acquires over its development and progression, and the nature of vascular remodeling response to the presence of the plaque; both the magnitude of inflammation and the vascular remodeling response are directly determined by the local ESS environment. Measurement of local ESS, complemented by the assessment of the severity of inflammation and vascular remodeling at early stages of the natural history of a given lesion, would allow for detailed risk stratification of that lesion to evolve to high-risk plaque based on the following conceptual scheme (Fig. 9):

(1) high-risk early lesion with very low ESS, intense inflammation and excessive expansive remodeling;
(2) medium-risk early lesion with low/moderate ESS, moderate inflammation and less excessive expansive remodeling;
(3) low-risk early lesion with physiologic ESS, limited inflammation and compensatory expansive remodeling.

Integration into the above scheme of patient-specific systemic characteristics (e.g. magnitude of hyperlipidemia), as well as of the information provided by traditional and novel systemic biomarkers (e.g. high sensitivity C-reactive protein, lipoprotein-associated phospholipase A_2) [53•,54•] and genomics [55•] would increase our ability to predict the future natural history of individual plaques. The ability, however, of such stratification strategies to predict the clinical outcomes needs to be tested in the clinical arena. Several natural history studies are now underway [56••]; a large natural history study in patients with coronary artery disease (PREDICTION Trial) investigates the incremental value of characterizing the local ESS and remodeling environment to predict the development of new acute cardiac events.

Figure 9 Proposed scheme for risk stratification of individual atherosclerotic lesions based on the magnitude of low endothelial shear stress, the severity of inflammation and the nature of the vascular remodeling response to the presence of the plaque



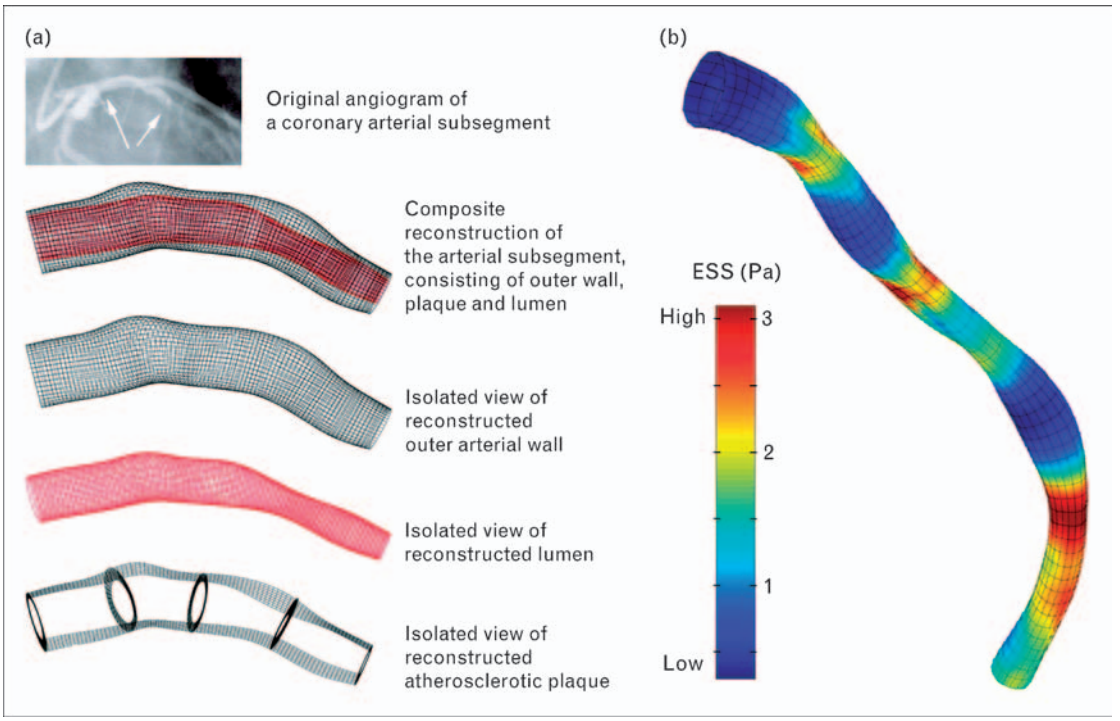
Vascular profiling for assessment of endothelial shear stress and vascular remodeling

Several in-vivo technologies for the assessment of functional and morphologic characteristics of a particular plaque now exist, including intravascular ultrasound (IVUS)-based virtual histology and palpography, thermography, optical coherence tomography, near-infrared spectroscopy, intravascular magnetic resonance imaging, and angioscopy [56••]. Although these modalities may be useful to characterize a particular plaque, they may be insufficient to optimally predict future risk because they provide a snapshot of the plaque at only a single point in time. Thus, these modalities are most useful to identify only the ends of the spectrum between a stenotic plaque and a high-risk plaque, but they cannot address the stimuli responsible for the subsequent natural history of that plaque. Incorporation of an in-vivo assessment of local ESS stimuli and local remodeling behavior of a particular plaque may substantially enhance the prognostic significance of these imaging modalities because one can then have insight into both the existing nature and the future natural history of that plaque. Technologies now exist for in-vivo assessment of ESS. The most comprehensive technique for investigating the relationship between ESS and vascular pathobiology is a methodology known as vascular profiling [13], a highly accurate [57••,58•,59] and reproducible [60] imaging technique, which utilizes routine IVUS and coronary angiography to create an accurate three-dimensional representation of the coronary artery (Fig. 10). Computational fluid dynamics is then used to determine the flow patterns within the artery, which in turn form the basis of identifying both the magnitude of local ESS and vascular remodeling behavior within a given arterial subsegment. Future technologies may be able to noninvasively assess local ESS and remodeling behavior with multislice computed tomography [61•], magnetic resonance imaging [62], or other imaging approaches.

Molecular imaging for assessment of inflammation

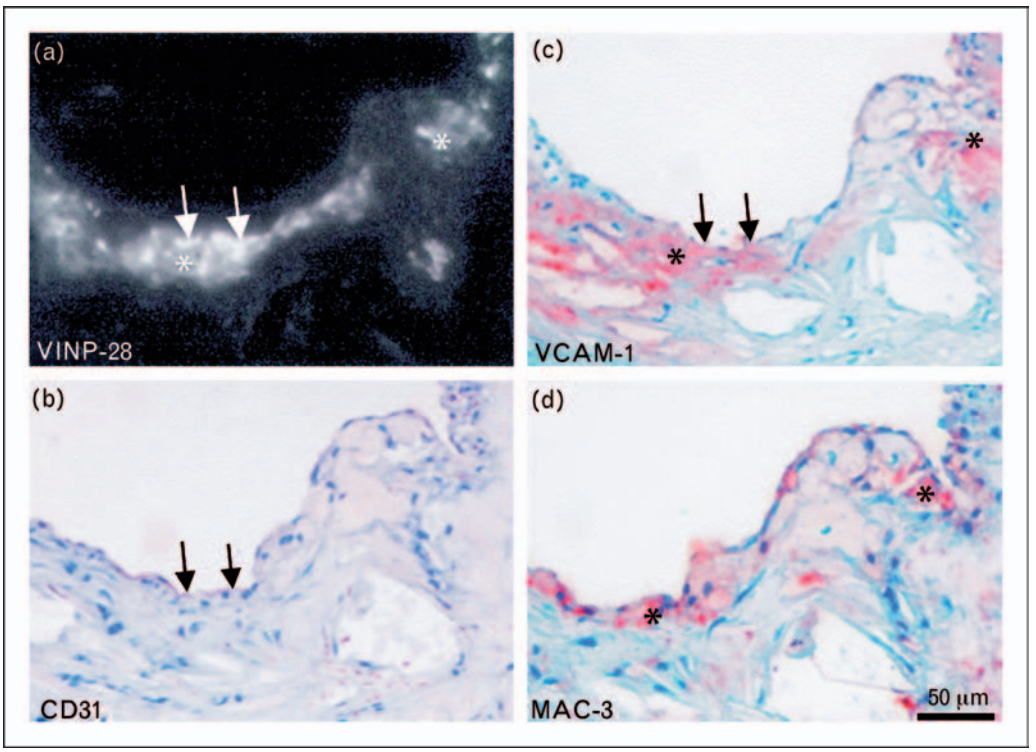
As a potential aid for the direct assessment of the severity of plaque inflammation new molecular imaging nanotechnologies have been developed within the last few years (Fig. 11). Molecular imaging of inflammation in atherosclerosis is an emerging field that is based on the strategy that certain molecular imaging agents (tracking agents) carry an affinity ligand (e.g. peptide, engineered antibody, or other small molecules), which binds specific molecular mediators of inflammation within the plaque (e.g. VCAM-1) [63••–66••]. Using appropriate, preferably noninvasive, molecular imaging systems, such as magnetic resonance imaging [63••], computed tomography [64••], ultrasound [65••], nuclear imaging [66••], or optical imaging [63••], the tracking agents are detected and the

Figure 10 Vascular profiling



(a) Example of a three-dimensional reconstructed coronary arterial segment. Reprinted with permission [13]. (b) Example of endothelial shear stress (ESS) profiling along the three-dimensional reconstructed lumen of a left anterior descending artery. Blue denotes regions with low ESS. Reprinted with permission [9••].

Figure 11 Molecular imaging of inflammation in atherosclerosis



(a) Fluorescent microscopy of cellular uptake of VCAM-1-targeted nanoparticles (VINP-28) in a histologic section derived from a murine atherosclerotic plaque. VINP-28 binds the endothelial cells as indicated by arrows and macrophages as indicated by asterisks. (b–d) Histopathologic confirmation of VCAM-1 binding with CD31 immunostaining for endothelial cells (b), VCAM-1 immunostaining for VCAM-1 expression (c), and MAC-3 immunostaining for macrophages (d). Reprinted with permission [63••].

signal intensity, and indirectly the magnitude of inflammation, are assessed.

Lesion-specific therapeutic opportunities

Risk stratification of early atherosclerotic lesions and identification of their subsequent natural history may permit the development of novel lesion-specific therapeutic strategies. Identification of a high-risk plaque at its early stages of development would potentially justify highly selective, prophylactic local interventions, such as implantation of stents or targeted nanoparticle-based delivery of anti-inflammatory drugs, supplemented by an intensive systemic pharmacologic approach to limit the severity of inflammation, stabilize the plaque, and therefore avert a future acute coronary event. Newer stents associated with reduced rates of late thrombosis and other complications are anticipated to contribute substantially towards this direction. Application of less aggressive strategies, such as moderate antiatherosclerotic therapies combined with regular follow-up, could be justified for early lesions with the potential to evolve to quiescent plaques. The clinical and economic implications of identifying and treating high-risk individual coronary lesions before an adverse cardiac event can occur are anticipated to be enormous.

Conclusion

The magnitude of low ESS is a critical factor that determines the severity of inflammation within a given early atherosclerotic plaque, and subsequently the nature of vascular remodeling response and the individual natural history of that plaque. In the setting of very low ESS plaque inflammation is intense, the artery undergoes local excessive expansive remodeling and the lesion evolves to high-risk plaque. In the setting of slightly low ESS the inflammation is limited, the artery undergoes compensatory expansive remodeling, which restores the local ESS to less pathologic levels, and the plaque remains quiescent. In-vivo assessment of the local ESS environment, the severity of inflammation and the vascular remodeling response, in combination with the information provided by systemic biomarkers of vulnerability, may allow for detailed risk stratification of individual early coronary plaques, thereby guiding both systemic and local, preemptive, lesion-specific therapeutic strategies. Current imaging techniques have the potential to enable risk stratification of early plaque and predict the progression to high-risk plaque. Future natural history studies will clarify whether prediction of progression of vulnerability, culminating in plaque rupture, can be accomplished.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 601).

1 Libby P. Inflammation in atherosclerosis. Nature 2002; 420:868–874.

2 VanderLaan PA, Reardon CA, Getz GS. Site specificity of atherosclerosis: site-selective responses to atherosclerotic modulators. Arterioscler Thromb Vasc Biol 2004; 24:12–22.

3 Rioufol G, Finet G, Ginon I, *et al.* Multiple atherosclerotic plaque rupture in acute coronary syndrome: a three-vessel intravascular ultrasound study. Circulation 2002; 106:804–808.

4 Cutlip DE, Chhabra AG, Baim DS, *et al.* Beyond restenosis: five-year clinical outcomes from second-generation coronary stent trials. Circulation 2004; 110:1226–1230.

5 Kolodgie FD, Virmani R, Burke AP, *et al.* Pathologic assessment of the vulnerable human coronary plaque. Heart 2004; 90:1385–1391.

6 •• Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the vulnerable plaque. J Am Coll Cardiol 2006; 47:C13–C18.
This review summarizes the current knowledge regarding the histopathology of rupture-prone plaques.

7 Gimbrone MA Jr, Topper JN, Nagel T, *et al.* Endothelial dysfunction, hemodynamic forces, and atherogenesis. Ann N Y Acad Sci 2000; 902:230–239.

8 Stone PH, Coskun AU, Yeghiazarians Y, *et al.* Prediction of sites of coronary atherosclerosis progression: In vivo profiling of endothelial shear stress, lumen, and outer vessel wall characteristics to predict vascular behavior. Curr Opin Cardiol 2003; 18:458–470.

9 •• 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; 49:2379–2393.
This is a review of the role of low endothelial shear stress in the natural history of coronary atherosclerosis at the molecular, cellular and vascular levels.

10 Slager CJ, Wentzel JJ, Gijsen FJ, *et al.* The role of shear stress in the generation of rupture-prone vulnerable plaques. Nat Clin Pract Cardiovasc Med 2005; 2:401–407.

11 Feldman CL, Stone PH. Intravascular hemodynamic factors responsible for progression of coronary atherosclerosis and development of vulnerable plaque. Curr Opin Cardiol 2000; 15:430–440.

12 Chatzizisis YS, Giannoglou GD. Pulsatile flow: a critical modulator of the natural history of atherosclerosis. Med Hypotheses 2006; 67:338–340.

13 Stone PH, Coskun AU, Kinlay S, *et al.* Effect of endothelial shear stress on the progression of coronary artery disease, vascular remodeling, and in-stent restenosis in humans: in vivo 6-month follow-up study. Circulation 2003; 108:438–444.

14 Giannoglou GD, Soulis JV, Farmakis TM, *et al.* Haemodynamic factors and the important role of local low static pressure in coronary wall thickening. Int J Cardiol 2002; 86:27–40.

15 • Soulis JV, Giannoglou GD, Chatzizisis YS, *et al.* Spatial and phasic oscillation of non-Newtonian wall shear stress in human left coronary artery bifurcation: an insight to atherogenesis. Coron Artery Dis 2006; 17:351–358.
This study described the spatial variation of endothelial shear stress over the cardiac cycle in the left main coronary artery bifurcation.

16 Traub O, Berk BC. Laminar shear stress: mechanisms by which endothelial cells transduce an atheroprotective force. Arterioscler Thromb Vasc Biol 1998; 18:677–685.

17 Li YS, Haga JH, Chien S. Molecular basis of the effects of shear stress on vascular endothelial cells. J Biomech 2005; 38:1949–1971.

18 Brooks AR, Lelkes PI, Rubanyi GM. Gene expression profiling of human aortic endothelial cells exposed to disturbed flow and steady laminar flow. Physiol Genomics 2002; 9:27–41.

19 Dai G, Kaazempur-Mofrad MR, Natarajan S, *et al.* Distinct endothelial phenotypes evoked by arterial waveforms derived from atherosclerosis-susceptible and -resistant regions of human vasculature. Proc Natl Acad Sci U S A 2004; 101:14871–14876.

20 Cheng C, van Haperen R, de Waard M, *et al.* Shear stress affects the intracellular distribution of eNOS: direct demonstration by a novel in vivo technique. Blood 2005; 106:3691–3698.

21 • Gambillara V, Chambaz C, Montorzi G, *et al.* Plaque-prone hemodynamics impair endothelial function in pig carotid arteries. Am J Physiol Heart Circ Physiol 2006; 290:H2320–H2328.
This was an experimental histopathology study on the molecular mechanisms of low endothelial shear stress-induced endothelial dysfunction.

22 Ziegler T, Bouzourene K, Harrison VJ, *et al.* Influence of oscillatory and unidirectional flow environments on the expression of endothelin and nitric oxide synthase in cultured endothelial cells. Arterioscler Thromb Vasc Biol 1998; 18:686–692.

23 Liu Y, Chen BP, Lu M, *et al.* Shear stress activation of SREBP1 in endothelial cells is mediated by integrins. Arterioscler Thromb Vasc Biol 2002; 22:76–81.

24 Tricot O, Mallat Z, Heymes C, *et al.* Relation between endothelial cell apoptosis and blood flow direction in human atherosclerotic plaques. Circulation 2000; 101:2450–2453.

25 Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. JAMA 1999; 282:2035–2042.

26 •• Nakashima Y, Fujii H, Sumiyoshi S, *et al.* Early human atherosclerosis: accumulation of lipid and proteoglycans in intimal thickenings followed by macrophage infiltration. Arterioscler Thromb Vasc Biol 2007; 27:1159–1165.
This study investigated the histopathologic stages of early atherosclerosis in humans from fatty streaks to pathologic intimal thickening.

27 McNally JS, Davis ME, Giddens DP, *et al.* Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. Am J Physiol Heart Circ Physiol 2003; 285:H2290–H2297.

28 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.

29 • Cheng C, Tempel D, van Haperen R, *et al.* Shear stress-induced changes in atherosclerotic plaque composition are modulated by chemokines. J Clin Invest 2007; 117:616–626.
This in-vivo experimental study explored the molecular mechanisms responsible for the pro-inflammatory effect of low endothelial shear stress.

30 Orr AW, Sanders JM, Bevard M, *et al.* The subendothelial extracellular matrix modulates NF-kappaB activation by flow: a potential role in atherosclerosis. J Cell Biol 2005; 169:191–202.

31 Sorescu GP, Song H, Tressel SL, *et al.* Bone morphogenic protein 4 produced in endothelial cells by oscillatory shear stress induces monocyte adhesion by stimulating reactive oxygen species production from a nox1-based NADPH oxidase. Circ Res 2004; 95:773–779.

32 •• Cheng C, Tempel D, van Haperen R, *et al.* Atherosclerotic lesion size and vulnerability are determined by patterns of fluid shear stress. Circulation 2006; 113:2744–2753.
This in-vivo experimental study showed that low endothelial shear stress induces a vulnerable plaque phenotype.

33 Gambillara V, Montorzi G, Haziza-Pigeon C, *et al.* Arterial wall response to ex vivo exposure to oscillatory shear stress. J Vasc Res 2005; 42:535–544.

34 • Platt MO, Ankeny RF, Jo H. Laminar shear stress inhibits cathepsin L activity in endothelial cells. Arterioscler Thromb Vasc Biol 2006; 26:1784–1790.
This in-vitro study was the first to show that endothelial shear stress regulates the expression and activity of cathepsin L.

35 • Platt MO, Ankeny RF, Shi GP, *et al.* Expression of cathepsin K is regulated by shear stress in cultured endothelial cells and is increased in endothelium in human atherosclerosis. Am J Physiol Heart Circ Physiol 2007; 292:H1479–H1486.
This in-vitro study was the first to show that oscillatory endothelial shear stress promotes the expression of cathepsin K.

36 •• Chatzizisis YS, Jonas M, Coskun AU, *et al.* Prediction of the localization of high-risk coronary atherosclerotic plaques based on low endothelial shear stress: A serial IVUS and histopathology natural history study [abstract]. Arterioscler Thromb Vasc Biol 2007; 27:E95.
This histopathology investigation in swine coronary arteries showed that the magnitude of low endothelial shear stress determines the severity of high-risk plaque characteristics.

37 •• Chatzizisis YS, Jonas M, Coskun AU, *et al.* Low endothelial shear stress (ESS) leads to excessive expansive remodeling of coronary atherosclerotic subsegments [abstract]. Arterioscler Thromb Vasc Biol 2007; 27:E73–E74.
This histopathology investigation in swine coronary arteries showed that arterial regions with the lowest values of endothelial shear stress exhibit excessive expansive remodeling and host high-risk plaques.

38 Bentzon JF, Weile C, Sondergaard CS, *et al.* Smooth muscle cells in •• atherosclerosis originate from the local vessel wall and not circulating progenitor cells in ApoE knockout mice. Arterioscler Thromb Vasc Biol 2006; 26:2696–2702.
This investigation revealed that the vascular smooth muscle cells within the atherosclerotic plaque originate from the media.

39 Sukhova GK, Wang B, Libby P, *et al.* Cystatin C deficiency increases elastic lamina degradation and aortic dilatation in apolipoprotein E-null mice. Circ Res 2005; 96:368–375.

40 Stary HC, Chandler AB, Dinsmore RE, *et al.* A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 1995; 92: 1355–1374.

41 Langheinrich AC, Michniewicz A, Sedding DG, *et al.* Correlation of vasa • vasorum neovascularization and plaque progression in aortas of apolipoprotein E(−/−)/low-density lipoprotein(−/−) double knockout mice. Arterioscler Thromb Vasc Biol 2006; 26:347–352.
An experimental study which showed that the neovessels within the plaque originate from the adventitial vasa vasorum.

42 Moreno PR, Purushothaman KR, Fuster V, *et al.* Plaque neovascularization is increased in ruptured atherosclerotic lesions of human aorta: implications for plaque vulnerability. Circulation 2004; 110:2032–2038.

43 Moulton KS, Vakili K, Zurakowski D, *et al.* Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. Proc Natl Acad Sci U S A 2003; 100:4736–4741.

44 Stone PH, Coskun AU, Kinlay S, *et al.* Regions of low endothelial shear stress •• are sites where coronary plaque progress and vascular remodeling occurs in humans: An in-vivo serial study. Eur Heart J 2007; 28:705–710.
This human study showed that low endothelial shear stress is associated with expansive remodeling in coronary atherosclerotic subsegments.

45 Burke AP, Kolodgie FD, Farb A, *et al.* Morphological predictors of arterial remodeling in coronary atherosclerosis. Circulation 2002; 105:297–303.

46 Varnava AM, Mills PG, Davies MJ. Relationship between coronary artery remodeling and plaque vulnerability. Circulation 2002; 105:939–943.

47 Schoenhagen P, Ziada KM, Kapadia SR, *et al.* Extent and direction of arterial remodeling in stable versus unstable coronary syndromes: an intravascular ultrasound study. Circulation 2000; 101:598–603.

48 Sipahi I, Tuzcu EM, Schoenhagen P, *et al.* Paradoxical increase in lumen •• size during progression of coronary atherosclerosis: observations from the REVERSAL trial. Atherosclerosis 2006; 189:229–235.
This human study described that a portion of atherosclerotic lesions are associated with excessive expansive remodeling.

49 MacIsaac AI, Thomas JD, Topol EJ. Toward the quiescent coronary plaque. J Am Coll Cardiol 1993; 22:1228–1241.

50 Wentzel JJ, Janssen E, Vos J, *et al.* Extension of increased atherosclerotic wall thickness into high shear stress regions is associated with loss of compensatory remodeling. Circulation 2003; 108:17–23.

51 Burke AP, Kolodgie FD, Farb A, *et al.* Healed plaque ruptures and sudden coronary death: evidence that subclinical rupture has a role in plaque progression. Circulation 2001; 103:934–940.

52 Feldman CL, Coskun AU, Yeghiazarians Y, *et al.* Remodeling characteristics •• of minimally diseased coronary arteries are consistent along the length of the artery. Am J Cardiol 2006; 97:13–16.
An intravascular ultrasound study in human coronary arteries introduced a new approach for the assessment of the remodeling behavior of atherosclerotic coronary arteries.

53 Lavi S, McConnell JP, Rihal CS, *et al.* Local production of lipoprotein- • associated phospholipase A2 and lysophosphatidylcholine in the coronary circulation: association with early coronary atherosclerosis and endothelial dysfunction in humans. Circulation 2007; 115:2715–2721.
A human study that demonstrated the association of lipoprotein-associated phospholipase A_2 with early atherosclerosis.

54 Ridker PM. C-reactive protein and the prediction of cardiovascular events • among those at intermediate risk: moving an inflammatory hypothesis toward consensus. J Am Coll Cardiol 2007; 49:2129–2138.
A review of the role of high sensitivity C-reactive protein on the prediction of acute cardiovascular events.

55 Miller DT, Ridker PM, Libby P, Kwiatkowski DJ. Atherosclerosis: the path from • genomics to therapeutics. J Am Coll Cardiol 2007; 49:1589–1599.
This review focuses on the value of gene expression profiling for the understanding of the natural history of atherosclerosis and the customization of antiatherosclerotic therapies.

56 Waxman S, Ishibashi F, Muller JE. Detection and treatment of vulnerable •• plaques and vulnerable patients: novel approaches to prevention of coronary events. Circulation 2006; 114:2390–2411.
This comprehensive review summarizes the current knowledge regarding the detection and treatment of high-risk plaque.

57 Giannoglou GD, Chatzizisis YS, Sianos G, *et al.* In-vivo validation of spatially •• correct three-dimensional reconstruction of human coronary arteries by integrating intravascular ultrasound and biplane angiography. Coron Artery Dis 2006; 17:533–543.
This study validated *in vivo* the accuracy of geometrically correct three-dimensional reconstruction of human coronary arteries.

58 Chatzizisis YS, Giannoglou GD, Matakos A, *et al.* In-vivo accuracy of geome- • trically correct three-dimensional reconstruction of human coronary arteries: is it influenced by certain parameters? Coron Artery Dis 2006; 17:545–551.
This study investigated the arterial geometric factors that influence the accuracy of the geometrically correct three-dimensional reconstruction of human coronary arteries.

59 Slager CJ, Wentzel JJ, Schuurbiers JC, *et al.* True 3-dimensional reconstruction of coronary arteries in patients by fusion of angiography and IVUS (ANGUS) and its quantitative validation. Circulation 2000; 102:511–516.

60 Coskun AU, Yeghiazarians Y, Kinlay S, *et al.* Reproducibility of coronary lumen, plaque, and vessel wall reconstruction and of endothelial shear stress measurements in vivo in humans. Catheter Cardiovasc Interv 2003; 60:67–78.

61 Frauenfelder T, Boutsianis E, Schertler T, *et al.* In-vivo flow simulation in • coronary arteries based on computed tomography datasets: feasibility and initial results. Eur Radiol 2007; 17:1291–1300.
This study tested the feasibility of using computed tomography for accurate 3D reconstruction of human coronary arteries.

62 Wentzel JJ, Corti R, Fayad ZA, *et al.* Does shear stress modulate both plaque progression and regression in the thoracic aorta? Human study using serial magnetic resonance imaging. J Am Coll Cardiol 2005; 45:846–854.

63 Nahrendorf M, Jaffer FA, Kelly KA, *et al.* Noninvasive vascular cell adhesion •• molecule-1 imaging identifies inflammatory activation of cells in atherosclerosis. Circulation 2006; 114:1504–1511.
This was an experimental study on noninvasive molecular imaging of the expression of vascular cell adhesion molecule-1 in atherosclerotic lesions using magnetic resonance.

64 Hyafil F, Cornily JC, Feig JE, *et al.* Noninvasive detection of macrophages •• using a nanoparticulate contrast agent for computed tomography. Nat Med 2007; 13:636–641.
This was an experimental study on noninvasive molecular imaging of macrophages in atherosclerosis using computed tomography.

65 Kaufmann BA, Sanders JM, Davis C, *et al.* Molecular imaging of inflammation •• in atherosclerosis with targeted ultrasound detection of vascular cell adhesion molecule-1. Circulation 2007; 116:276–284.
This was an experimental study on noninvasive molecular imaging of the expression of vascular cell adhesion molecule-1 in atherosclerotic lesions using ultrasound.

66 Tawakol A, Migrino RQ, Bashian GG, *et al.* In vivo 18F-fluorodeoxyglucose •• positron emission tomography imaging provides a noninvasive measure of carotid plaque inflammation in patients. J Am Coll Cardiol 2006; 48:1818–1824.
This was a human study on molecular imaging of inflammation in atherosclerosis using positron emission tomography.

67 MacNeill BD, Lowe HC, Takano M, *et al.* Intravascular modalities for detection of vulnerable plaque: current status. Arterioscler Thromb Vasc Biol 2003; 23:1333–1342.

68 Ku DN, Giddens DP, Zarins CK, Glagov S. Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low oscillating shear stress. Arteriosclerosis 1985; 5:293–302.